SERG International
2018 Workshop
Proceedings

Coast Edmonton Plaza Hotel
Edmonton, AB
February 6th - 8th, 2018

Host:

Alberta Agriculture and Forestry

Sponsors

AEF Global	**Forest Protection Limited**
Agrifor Biotechnical Services	**GDG Environment**
BioForest Technologies Inc	**Sylvar Technologies Inc**
	Valent BioSciences Corp.

SERG-I Executive Committee

Government of Saskatchewan

SOPFIM
Société de protection des forêts contre les insectes et maladies

NOVA SCOTIA
Natural Resources

Newfoundland Labrador

Forêts, Faune et Parcs
Québec

Ontario

Alberta
Government

BRITISH COLUMBIA

FPL
FOREST PROTECTION LIMITED
ISO 14001:2004 Certified

Manitoba

FOREST SERVICE
U S
DEPARTMENT OF AGRICULTURE

Natural Resources Canada	**Ressources naturelles Canada**
Canadian Forest Service	**Service canadien des forêts**

SERG International Workshop

Feb 6th - Feb 8th, 2018
Coast Edmonton Plaza Hotel, Edmonton, AB

Proceedings

Tuesday, February 6, 2018

Emerald Ash Borer

SERG-I Graduate Student Award Presentations

Advances and Implementation of New Technologies

Silviculture Practices

Wednesday, February 7, 2018

Early Intervention Strategies To Suppress A Spruce Budworm Outbreak

Thursday, February 8, 2018

Beetle Population Detection and Suppression

Investigations into the Chemical Ecology of the Emerald Ash Borer (EAB), *Agrilus planipennis*: development of tools for sampling and management of EAB.

Principal investigators
Krista L. Ryall, Canadian Forest Service, Great Lakes Forestry Centre,
1219 Queen Street East, Sault Ste. Marie, ON, P6A 2E5
Phone 705-541-5549; krista.ryall@nrcan.gc.ca Contact person

Peter Silk, Canadian Forest Service, Atlantic Forestry Centre, Fredericton, NB
Phone 506-451-6084; peter.silk@nrcan.gc.ca

Collaborators : Jeff Fidgen, CFS; Peter Mayo, Gaetan LeClair CFS (synthesis, analytical chemistry); Robert Lavallée (CFS); David MaGee (Chemistry Dept., UNB)

Abstract

Our research has demonstrated the attraction of male emerald ash borer (*Agrilus planipennis*, EAB) to a female-produced lactone pheromone, the first pheromone to be demonstrated for any Buprestidae. Our objectives this year were to continue to develop a "push-pull" strategy for EAB using traps baited with the lactone pheromone, to increase attraction of males (the "pull"), as compared to traps baited with one of various non-host volatiles (citronella, butternut, ylang-ylang) to "push" EAB away. We evaluated mean trap captures on traps baited with our attractant lure ((3Z-hexenol + (3Z)-lactone), placed on trees with one of four treatments: citronella, butternut, ylang-ylang, and a control tree. Our results demonstrated that traps placed on trees with citronella had marginal reductions in captures as compared to control traps. Captures on traps co-baited with ylang-ylang or butternut were slightly, but not significantly reduced. In the second field trial, we conducted a preliminary test of four fluorinated analogs to evaluate their impact on mean trap captures; while not statistically significant due to highly variable trap captures, at least one of the new analogs shows promise for increasing captures of male EAB. Ongoing work is continuing to explore responses of EAB to pheromones, semiochemicals, and non-host volatiles.

Résumé

Notre recherche a montré que l'agrile du frêne mâle (*Agrilus planipennis*) est attiré par la phéromone lactone produite par la femelle, la première phéromone mise en évidence pour tout buprestidé. Notre objectif cette année était de poursuivre l'élaboration d'une stratégie « repousser-attirer » visant l'agrile du frêne au moyen d'arbres pièges appâtés avec la phéromone lactone, comparativement à des pièges appâtés avec des volatiles non hôtes (mélange de terpène d'épinette, citronnelle et bois de cèdre) pour « repousser » l'agrile. Nous avons évalué les captures moyennes dans les pièges contenant notre appât attractif ((3Z-hexenol + (3Z)-lactone) que nous avions placés sur des arbres selon l'un de quatre traitements : dose élevée de volatiles de l'épinette; appât à la citronnelle; appel au bois de cèdre; arbre témoin. Nos résultats ont montré que les pièges placés sur des arbres ayant reçu une dose élevée de volatiles de l'épinette présentaient un nombre de captures moyen sensiblement inférieur aux pièges témoins. Les pièges coappâtés avec de la citronnelle présentaient aussi des diminutions légèrement significatives de captures comparativement aux pièges témoins. Les captures dans les pièges coappâtés avec du bois de cèdre ont présenté une légère diminution, quoique non significative. Nous pensons que le taux de libération de la citronnelle et du bois de cèdre n'était pas suffisamment élevé pour abaisser les captures d'agriles du frêne. Dans d'autres essais sur le terrain, nous avons fait des essais préliminaires avec deux analogues fluorés pour évaluer leur incidence sur les captures moyennes dans les pièges; bien que les résultats ne soient pas statistiquement significatifs en raison de la petite taille des échantillons, au moins un des deux nouveaux analogues semble prometteur pour augmenter les captures de l'insecte mâle. Les travaux se poursuivent pour explorer les réactions de l'agrile du frêne aux phéromones, aux substances sémiochimiques et aux volatiles non hôtes.

EXECUTIVE SUMMARY

This project pertains to the following areas of research for SERG International: Forest Pest Management Products and Developing Forest Pest Management Strategies.

1. Objective:

To develop and improve tools for early detection and management of the emerald ash borer, by chemically identifying, synthesizing, and field testing semiochemicals that elicit sex-based mate location. First, we intend to further test the synthetic lactone analogs to increase our understanding of the plasticity in the pheromone ecology of this species, as well as for use as a lure in early detection surveys; our research has shown that traps baited with the saturated analog capture similar numbers of EAB as compared to traps baited with the lactone pheromone. Most recently, additional fluorinated analogs have been synthesized for testing. In addition we propose to determine the feasibility of slowing the spread of EAB through the application of semiochemical-based strategies, such as a 'push-pull' strategy. Finally we want to integrate these tools with others (e.g., stem-injected insecticides) in the context of an IPM strategy.

2. Background:

The emerald ash borer (EAB) *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is an invasive beetle species originating from Asia that has killed tens of millions of ash trees (*Fraxinus* spp.) in the USA and Canada (Cappaert, et al. 2005). Monitoring of its rapid spread has proven very difficult. Improved detection methods and tools are urgently required; a pheromone-baited trap would provide a species-specific detection and monitoring tool for this devastating insect pest.

Female *A. planipennis* produce a volatile pheromone, (3*Z*)-dodecen-12-olide [(3*Z*)-lactone] (Bartelt et al. 2007; Silk et al. 2011). A series of field trapping experiments demonstrated that addition of (3*Z*)-lactone to a dark green sticky trap significantly increases trap captures of male *A. planipennis* (Silk et al. 2011; Ryall et al. 2012, 2013; Silk and Ryall 2014), when deployed in combination with the host volatile, (3*Z*)-hexenol (de Groot et al. 2008; Crook and Mastro 2010 and references therein; Grant et al. 2010, 2011; Crook et al. 2012; Silk and Ryall 2014). The potential for use of (3*Z*)-lactone in detection surveys has also been demonstrated: significant increases in both trap captures and detection rates (proportion of traps positive for *A. planipennis*) occurred when (3*Z*)-lactone (3.0 mg) was added to traps baited with the host volatile, (3*Z*)-hexenol in low-density populations (Ryall et al. 2013). Numerous questions remain, however, regarding optimal deployment of (3*Z*)-lactone in detection surveys, including whether any additional female-produced compounds have been overlooked. Our recent research has demonstrated that a synthetic analog effects similar trap captures, as compared to (3*Z*)-lactone, but at a higher dose (5.0 mg analog to 3.0 mg (3*Z*)-lactone) (Silk et al., 2015). Further optimization of the analog, which represents a less-expensive, easier to synthesize option, is required. Moreover, recently additional analog structures have been identified, which might elicit attraction by male EAB. Testing of these fluorinated analog structures increases our understanding of the pheromone ecology of this species.

Developing management options to reduce or slow losses by this devastating insect is of critical importance. Can semiochemicals be used to disrupt EAB and help slow its spread in Canada, particularly in the urban environment? We propose that a "push-pull" strategy may be possible, based on several experiments: the first experiment conducted in 2013 demonstrated significant increases in trap captures when high release rate (3*Z*)-lactone dispensers were deployed throughout the canopy of ash trees (Ryall et al, in prep). Three experiments in 2014 to 2016 demonstrated *reductions* in trap captures of EAB when non-host volatiles (spruce terpene blend) were deployed around traps placed in infested ash trees (Ryall et al, in prep). We propose that (3*Z*)-lactone (or the synthetic analog) could be deployed to 'pull' EAB to trap-trees injected with insecticides, alongside trees containing non-host volatile dispensers to 'push' EAB away. These tactics could be developed into a "slow the ash mortality" management strategy.

Semiochemicals may not only provide effective tools for detection and monitoring spread of EAB but may have potential for mitigating spread via mating disruption, mass trapping, or a combination of the two. There is now an opportunity to combine the expertise, equipment and quarantine facilities from both CFS/AFC and GLFC to tackle this difficult task.

3. Methodology for 2017:

3a. Insects: Adult EAB were collected in Ontario and infested material was placed in the quarantine facility at GLFC. Emerged beetles were separated by sex and placed in a constant temperature (25°C) and light regime, 16:8 L/D photoperiod and fed ash foliage. These insects were made available for bioassays during this project.

3b. Synthetic Chemistry: We required 3Z-lactone and the fluorinated analogs for field testing. These were synthesized in Silk's lab using new synthetic pathways.

3c. Trapping Experiments, 2017:

Further testing of trap disruption using non-host volatiles: Can non-host volatiles be used to "push" EAB from ash trees? Three previous experiments conducted in 2014 to

2016 demonstrated significant reductions in captures of EAB in high density populations when high doses of non-host volatiles were deployed around baited traps. This experiment compares trap captures on traps placed on ash trees with vs. without non-host volatiles (a high release rate of citronella, butternut essential oils, and ylang-ylang essential oil) in low-moderate populations to evaluate potential reductions in trap captures.

Four treatments were tested to evaluate their potential as a "push- or repel" strategy. Ten replicates of four treatments (control, citronella, butternut, and ylang-ylang) were established. Traps baited with (3Z)-lactone and (3Z)-hexenol were placed in the mid-canopy of all 40 trees. For the three "push" treatments, high doses of the non-host volatiles (citronella, butternut, and ylang-ylang), were placed on the trap and within the canopy of the tree within 1-2 m of the trap. Ash trees growing on the edge of woodlots were used in the experiment; trap trees were approximately 25-30 m apart. Half of the blocks were placed in trees showing some signs of EAB infestation, whereas the remaining blocks were placed in trees which were largely asymptomatic for EAB infestation but were located within 500-m. Control trees contained only the pheromone-baited trap. Traps were deployed from late May through late July and sampled bi-weekly.

Testing (3Z)-lactone- vs. fluorinated analogs: Previous studies demonstrated significant increases in mean trap captures with addition of (3Z)-lactone to traps baited with (3Z)-hexenol. Recent laboratory analysis identified potentially attractive fluorinated analogs. In the 2017 experiment there were six treatments: (3Z)-hexenol alone, (3Z)-hexenol + (3Z)-lactone, (3Z)-hexenol + 5,5-fluorinated analog, (3Z)-hexenol + 6,6-fluorinated analog, (3Z)-hexenol + 7,7-fluorinated analog, and (3Z)-hexenol + 8,8-fluorinated analog (3Z)-hexenol + 5,5-fluorinated analog We installed eight replicate blocks with inter-trap spacing of 20 m within block. Traps were deployed from mid-June through early July and sampled bi-weekly.

Results 2017

Push-pull experiment

In the push-pull experiment, there was no significant difference in mean trap captures among treatments (add stats) (Fig. 1).

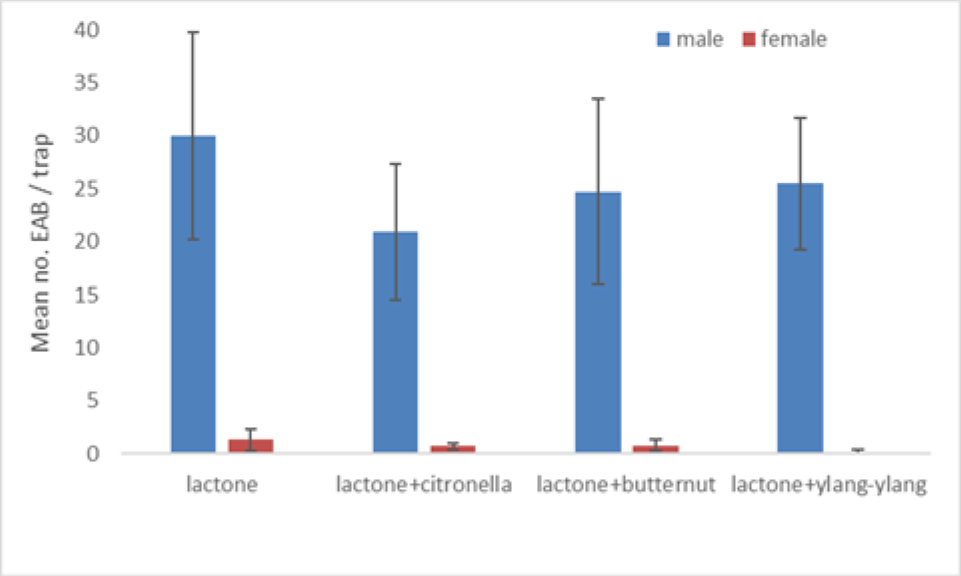


Fig. 1. Mean number of EAB captured per trap with addition of non-host volatiles

Testing (3Z)-lactone- vs. fluorinated analogs:
In the test of the fluorinated analogs, mean trap captures were highly variable (Fig. 2), with no significant differences among treatments (add stats).

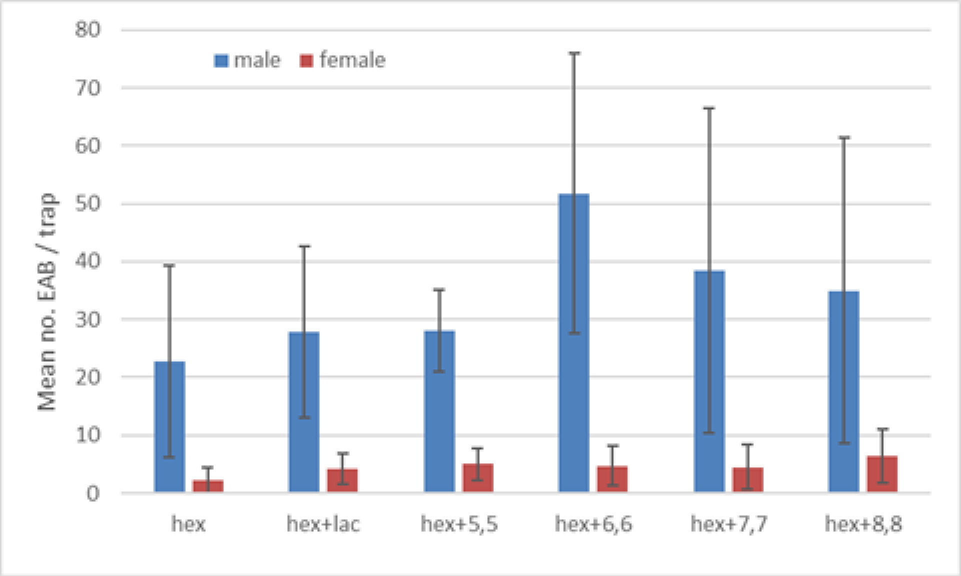


Fig. 2: Mean number of EAB captured per trap with the addition of (3*Z*)-lactone or one of four fluorinated analogs to traps co-baited with (3*Z*)-hexenol.

Discussion

We evaluated mean trap captures on traps baited with our attractant lure ((3Z-hexenol + (3Z)-lactone), placed on ash trees with one of four treatments: citronella, butternut, ylang-ylang, and a control tree. Our results demonstrated that traps placed on trees with citronella had marginal reductions in captures as compared to control traps..Captures on traps co-baited with ylang-ylang or butternut were slightly, but not significantly reduced. Spruce blend from previous work, therefore, remains the strongest “push” component. In the second field trial, we conducted a preliminary test of four fluorinated analogs to evaluate their impact on mean trap captures; while not statistically significant due to highly variable trap captures, at least one of the new analogs shows promise for increasing captures of male EAB. Ongoing work is continuing to explore responses of EAB to pheromones, semiochemicals, non-host volatiles and acoustic signals.

REFERENCES

Barlelt R, Cossé AA, Zilkowski BW, and Fraser I (2007) Antennally active macrolide from the emerald ash borer *Agrilus planipennis* emitted predominantly by females. J Chem Ecol 33:1299-1302

Cappaert, D, Mccullough, DG, Poland TM, and Siegert, NW (2005) Emerald Ash borer in North America: a research and regulatory challenge. Am Entomol 51: 152-165.

de Groot, P, Grant GG, Poland TM, Scharbach R, Buchan L, Nott RW, Macdonald L, and Pitt D (2008) Electrophysiological response and attraction of emerald ash borer to green leaf volatiles (GLVs) emitted by host foliage. J Chem Ecol 34: 1170-1179.

Grant, GG, Ryall KL, Lyons DB, and Abou-Zaid MM (2010) Differential response of male and female emerald ash borers (Col., Buprestidae) to (*Z*)-3-hexenol and Manuka oil. J Appl Entomol 134: 26-33.

Grant, GG, Poland TM, Ciaramitaro T, Lyons DB, and Jones GC (2011) Comparison of male and female emerald ash borer (Coleoptera: Buprestidae) responses to phoebe oil and (*Z*)-3-hexenol lures in light green prism traps. J Econ Entomol 104: 173-179.

Ryall, KL, Fidgen, JG, and Turgeon, JJ (2011) Detectability of the emerald ash borer (Coleoptera: Buprestidae) in asymptomatic urban trees by using branch samples. Env Entomol 40: 679–688.

Ryall K, Silk P, Mayo P, Crook D, Krimian A, Cossé A, Sweeney J, and Scarr T (2012) Attraction of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) to a volatile pheromone: effects of release rate, host volatile and trap placement. Env Entomol 41: 648-656.

Ryall, K, Fidgen J, Silk P, and Scarr T (2013) Efficacy of the pheromone (3Z)-lactone and the host kairomone (3Z)-hexenol at detecting early infestation of the emerald ash borer, *Agrilus planipennis*. Entomol Exp Appl 147: 126-131.

Ryall, KL, Silk PJ, Fidgen, J, Mayo, P, Lavallée, R, Guertin, C, and T Scarr. (2015) Effects of pheromone release rate and trap placement on trapping of *Agrilus planipennis* (Coleoptera: Buprestidae) in Canada. Env Entomol 44: 734-745.

Silk, PJ, Ryall K, Mayo, P, MaGee, DI, LeClair G, Fidgen, J, and Lavallée, R. (2015) A biologically active analog of the sex pheromone of the emerald ash borer, *Agrilus planipennis*. J Chem Ecol 41: 294-302.

Silk, PJ and K Ryall. (2014) The Chemical Ecology of The Emerald Ash Borer, *Agrilus planipennis*. A review. Can. Entomol. doi:10.4039/tce.2014.58

Silk, PJ, Ryall K, Mayo P, Lemay M, Grant G, Crook D, Cossé A, Fraser I, Sweeney JD, Lyons DB, Pitt D, Scarr T, and Magee D (2011) Evidence for a volatile pheromone in *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) that increases attraction to a host foliar volatile. Env Entomol 40: 904-916.

Funding FPL $9.0K

QMFFP $2.0K

OMNRF $10.0K

MB-CWS $4.0K

SK-MOE $1.0K

USDA-FS $1.0K

CFS C-base $12K (Ryall) $20K (Silk)

CFS / ISC SPA $10.2K (Ryall)

Individual-based approach to monitor potentially invasive Europena *Agrilus*: an update

Marc Rhainds [1*]*,and Troy Kimoto*

[1] *Natural Resources Canada, Canadian Forest Service - AFC, P.O. Box 4000, Fredericton, New Brunswick, E3B 5P7.* [*] *Corresponding author: marc.rhainds@canada.ca*

[2] *Canadian Food Inspection Agency, 4321 Still Creek Drive, Burnaby, British Columbia, V5C 6S7*

Abstract

This report provides an update on a multi-year study aimed at developing risk-indices based on the potential reproductive output of early invading females *Agrilus*. To date, about 60% of specimens captured at Slovakian oak forests in 2015 - 2016 have been processed to determine the sex of specimens, body size measurements, and (for females) number of eggs. Data on abundance and body size are provided for seven species: *A. angustulus*, *A. biggutatus*, *A. graminis, A. laticornis, A. obscuricollis, A. olivicolor* and *A. sulcicollis.*

Résumé

Ce rapport procure une mise à jour d'un projet de recherche visant à évaluer le risque des invasions biologiques en fonction du potentiel reproducteur des femelles pour sept espèces européennes de *Agrilus* : *A. angustulus, A. biggutatus, A. graminis, A. laticornis, A. obscuricollis, A. olivicolor* and *A. sulcicollis.* À ce jour, 60% des specimens collectés en Slovaquie en 2015 – 2016 ont été disséqués afin de déterminer le sexe des individus, leur taille corporelle, et (pour les femelles) le nombre d'oeuds dan la cavité abdominale.

Background and Objectives

During the last decade, public funding of monitoring programs for potentially invasive insects has vastly increased, justifiably so in light of great risks associated with biological invasion. In most of these trials, the experimental unit of observation is a given trap with known number of specimens of different species; counts of beetles are tabulated but specimens are usually not sexed or measured. The approach is somehow 'flawed' as individual beetles are the active agents of biological invasion. For example, a trap that would capture predominantly males (as opposed to egg-carrying females) may not be informative in the context of biological invasion. Similarly, captures of a single reproductively mature mated females may pose a higher invasion risk than captures of multiple virgin females. Because body size of females is tightly linked with potential fecundity in insects, introduction of small versus large females may pose different risk in terms of establishment of invasive species shortly after their introduction.

With the exception of emerald ash borer (EAB), almost nothing is known about population dynamics in *Agrilus* species. In essence, population dynamics is conditioned by four parameters: (1) birth rate; (2) emigration; (3) immigration; (4) death rate. Reproductive output of females is equivalent to birth rate and thus provides an entry into population dynamics. Morphometric / volumetric measurements of the abdominal cavity of female *Agrilus*, and how they vary in space and time, provides a direct gateway into understanding population dynamics.

Materials and Methods

As part of a larger investigation to develop reliable monitoring tools for potentially invasive *Agrilus* species in Slovakian oak forests, the abundance of specimens at traps baited with different volatile combination was evaluated in Slovakia in 2015 and 2016. In many *Agrilus* species, specimens need to be dissected to determine their sex. Because only 60% of specimens have been dissected to date (i.e. sex remains unknown for a large portion of specimens; Table 1), and also because the response of males and females to visual – olfactory stimuli is known to often diverge along sex lines, the data are not analyzed in the context of which trapping protocol is most effective to detect a given species.

We report body size measurements of males and females for 7 *Agrilus* species: *A. angustulus* (ANGUS), *A. biggutatus* (BIG), *A. graminis* (GRAM), *A. laticornis* (LATI), *A. obscuricollis* (OBSC), *A. olivicolor* (OLIVE), and *A. sulcicollis* (SULC). Three morphometric measurements are reported for males and females of different species (measured to the nearest 0.05 mm): (1) length of elytra, (2) width of first abdominal sternite, and (3) width of third abdominal sternite.

Results

With the exception of SULC (and possibly OLIVE), females were either equally or more abundant than males (Table 1).

Body size of specimens varied in the following order (from smallest to largest): OBSC < OLIVE < LATI < ANGUS < GRAM < SULC < BIG (Table 2).

Females were slightly larger than males in all species with the exception of SULC where males appeared the largest sex; differences between sexes were always statistically significant (generalized linear model including year and sex as class variables)

Discussion

The mechanisms underlying high abundance and large body size of males relative to females in SULC (as opposed to opposite trends in other species) need to be explored.

The ongoing study includes measurements not reported in this report (tickness of abdomen at the point of attachment with thorax, widths and lenghts of all three sternites) that can be use to estimate the volume of abdominal cavities. Estimates of size-related potential fecundity can be used to quantify probability of establishment of a given *Agrilus* species at the early stage of invasion (risk calibration based on the potential reproductive output of early invading females). In future studies, abdomen of EAB will be estimated at some locations to determine if intraspecific variation in reproductive output among females (= birth rate) influences the population dynamics of what has arguably become the most severe tree pest in North America.

Long-term objective of the study is to improve monitoring of individuals that pose greatest biological invasion risks (large, mated females). This novel approach has broad application as a complementary tool to implementation of monitoring protocols for invasive species.

Funding

SERG:	$ 5 000
CFS-AFC	$ 40 000 (including casual salary dollars)

Acknowledgements.

We are grateful to R Graves. G Brodersen, V Webster, and M Giasson for assisting with measurements of *Agrilus* specimens. Funding was provided by DNR-ON and CFS-AFC. My manager K Porter has been very supportive of the project.

Table 1. Abundance of different *Agrilus* species captured in oak forests in Slovakia. Species as follows: *A. angustulus* (ANGUS), *A. biggutatus* (BIG), *A. graminis* (GRAM), *A. laticornis* (LATI), *A. obscuricollis* (OBSC), *A. olivicolor* (OLIVE), and *A. sulcicollis* (SULC)

Species	Year	# captured	# processed	Sex-ratio
ANGUS	2015	1293	536	336 ♀ : 200 ♂ (1.68)
	2016	2355	1314	724 ♀ : 590 ♂ (1.23)
BIG	2015	72	18	11 ♀ : 7 ♂ (1.57)
	2016	46	44	22 ♀ : 22 ♂ (1.00)
GRAM	2015	47	33	18 ♀ : 15 ♂ (1.20)
	2016	212	67	41 ♀ : 26 ♂ (1.58)
LATI	2015	415	330	217 ♀ : 113 ♂ (1.92)
	2016	1077	597	353 ♀ : 242 ♂ (1.46)
OBSC	2015	278	155	97 ♀ : 58 ♂ (1.67)
	2016	441	221	116 ♀ : 105 ♂ (1.10)
OLIVE	2015	236	214	119 ♀ : 95 ♂ (1.25)
	2016	562	99	31 ♀ : 68 ♂ (0.46)
SULC	2015	338	203	70 ♀ : 133 ♂ (0.53)
	2016	470	300	97 ♀ : 203 ♂ (0.48)

Table 2. Body size measurements of different *Agrilus* species captured in oak forests in Slovakia. Species as follows: *A. angustulus* (ANGUS), *A. biggutatus* (BIG), *A. graminis* (GRAM), *A. laticornis* (LATI), *A. obscuricollis* (OBSC), *A. olivicolor* (OLIVE), and *A. sulcicollis* (SULC)

Species	Year	Sex	Elytra length (mm)	Sternite width (mm)	
				3rd	1st
ANGUS	2015	♀	4.12 ± 0.02	1.43 ± 0.01	0.96 ± 0.01
		♂	3.96 ± 0.02	1.32 ± 0.01	0.94 ± 0.01
	2016	♀	4.29 ± 0.01	1.47 ± 0.01	0.94 ± 0.00
		♂	4.03 ± 0.01	1.31 ± 0.01	0.90 ± 0.00
BIG	2015	♀	8.67 ± 0.28	2.70 ± 0.11	1.86 ± 0.09
		♂	7.88 ± 0.24	2.38 ± 0.03	1.56 ± 0.04
	2016	♀	8.56 ± 0.24	2.68 ± 0.06	1.68 ± 0.03
		♂	8.42 ± 0.16	2.46 ± 0.05	1.58 ± 0.03
GRAM	2015	♀	4.56 ± 0.13	1.54 ± 0.05	0.98 ± 0.03
		♂	4.13 ± 0.08	1.34 ± 0.03	0.91 ± 0.03
	2016	♀	4.60 ± 0.06	1.58 ± 0.03	0.99 ± 0.02
		♂	4.34 ± 0.06	1.40 ± 0.03	0.92 ± 0.02
LATI	2015	♀	3.95 ± 0.03	1.40 ± 0.01	0.96 ± 0.01
		♂	3.84 ± 0.02	1.32 ± 0.01	1.01 ± 0.01
	2016	♀	4.04 ± 0.02	1.40 ± 0.01	0.96 ± 0.01
		♂	3.83 ± 0.02	1.30 ± 0.01	0.98 ± 0.01
OBSC	2015	♀	3.24 ± 0.03	1.12 ± 0.01	0.77 ± 0.01
		♂	3.09 ± 0.04	1.06 ± 0.02	0.74 ± 0.01
	2016	♀	3.33 ± 0.02	1.14 ± 0.01	0.75 ± 0.01
		♂	3.14 ± 0.02	1.02 ± 0.01	0.70 ± 0.01
OLIVE	2015	♀	3.71 ± 0.03	1.34 ± 0.01	0.92 ± 0.01
		♂	3.51 ± 0.03	1.23 ± 0.01	0.90 ± 0.01
	2016	♀	3.80 ± 0.05	1.37 ± 0.02	0.90 ± 0.02
		♂	3.58 ± 0.03	1.23 ± 0.01	0.91 ± 0.01
SULC	2015	♀	5.23 ± 0.07	1.82 ± 0.02	1.24 ± 0.02
		♂	5.49 ± 0.03	1.82 ± 0.01	1.36 ± 0.01
	2016	♀	5.40 ± 0.05	1.83 ± 0.02	1.21 ± 0.01
		♂	5.60 ± 0.03	1.79 ± 0.01	1.37 ± 0.01

Validation of emerald ash borer branch sampling and estimation of 2 year life cycle in northern populations.

C J K MacQuarrie[1], K Ryall[1]

Abstract:

This report presents the results of sampling to assess the frequency of two-year life cycle in new infestations of emerald ash borer (EAB) in Canada, and an assessment of the accuracy of the branch sampling method in northern EAB populations. A preliminary analysis of samples taken in late 2016 from Thunder Bay, Ontario validated the branch sampling method. Regardless, in 2017 we again sampled trees in Thunder Bay, Ontario and also sampled trees from a wood lot north of Barrie, Ontario near the leading edge of EAB infestation in Ontario to begin the characterization of populations along a north-south gradient of infestation. To these sites we added achieved data collected near Dutton, Ontario in 2005 and from the 2017 detection of EAB in Quebec City, Quebec. As of January 2018 processing of the 2017 Ontario samples was completed and processing of the Quebec samples was ongoing. Data from all sites will be used to determine the frequency of two year life cycle based on the developmental stage of EAB larvae at the time of sampling. Data from the Thunder Bay and Quebec City sites will be used to assess the accuracy of the branch sampling method.

Validation de l'échantillonnage de branches et estimation du cycle de vie de deux ans dans les populations nordiques de l'agrile du frêne

Résumé

Ce rapport présente les résultats d'un échantillonnage effectué pour évaluer la fréquence d'un cycle de vie de deux ans dans les nouvelles infestations d'agrile du frêne au Canada, ainsi qu'une évaluation de l'exactitude de la méthode d'échantillonnage de branches dans les populations nordiques d'agrile du frêne. Une analyse préliminaire des échantillons recueillis à la fin de 2016 à Thunder Bay (Ontario) a validé la méthode d'échantillonnage de branches. En 2017, nous avons quand même de nouveau prélevé des échantillons sur des arbres à Thunder Bay ainsi que sur des arbres dans un boisé situé au nord de Barrie (Ontario) près de la lisière de l'infestation

ontarienne de l'agrile du frêle afin d'entreprendre la caractérisation des populations retrouvées le long d'un gradient nord-sud de l'infestation. Nous avons ajouté à ces sites les données recueillies près de Dutton (Ontario) en 2006 et celles de la détection de l'agrile du frêne en 2017 à Québec (Québec). En date de janvier 2018, le traitement des échantillons de 2017 recueillis en Ontario était achevé, et le traitement des échantillons du Québec était en cours. Les données de tous les sites serviront à déterminer la fréquence du cycle de deux ans sur la base du stade de développement des larves de l'agrile du frêne au moment de l'échantillonnage. Les données des sites de Thunder Bay et de Québec seront utilisées pour évaluer l'exactitude de la méthode d'échantillonnage de branches.

Introduction

Emerald ash borer was discovered in Thunder Bay Ontario in the summer of 2016, and in Sherbrooke Quebec, Quebec City, Quebec and Winnipeg, Manitoba in 2017. Together these represent the most northerly locations EAB has been found in North America, and the farthest west in Canada. At all sites estimates suggest the insect has been present for 2-3 years, if not longer. Anecdotal reports indicate two potentially unique phenomena of the Thunder Bay population 1) the insect may be infested the trunk and bole of the trees sooner than in more southern populations (i.e., while populations are younger); and 2) that a higher proportion of the population may be in a two year life cycle compared to southern populations. If these annecdoal accounts are true then these phenomena would have to potential impact on how EAB is managed. First, if EAB infests the trunk earlier than it does in southern populations that behaviour would decrease the utility of branch sampling (Ryall et al 2011) to detect populations and may require an adjustment to the method. Second, a higher frequency of a two-year life cycle would slow the growth rate of populations and influence how fast populations spread and the rate at which trees die. Moreover, a slower population growth rate could increase the frequency of overwintering mortality as a higher percentage of insects spend the winter in unsuitable stages. Resolving these two issues is imperative as it may influence the ability to detect and assess the risk of future invasions in western Canada, other regions of Quebec and the Maritimes.

In 2016 we undertook sampling to obtain samples of early stage EAB populations from Thunder Bay. In 2017 we planned sampling to occur in Thunder Bay and at new EAB infestations in Ontario. The detection of EAB in Quebec City presented an opportunity to obtain samples from this site as well, so we expanded our sampling to include trees from areas around the new detection. Finally we augmented these our new data with samples collected in Dutton, Ontario in 2005 from a new infestation. Most of these samples were collected in late summer and early fall and processing is still on going, as is the identification and characterization of larvae. Here we report on methods, and preliminary trends in the larval collection data.

Methods

Validate branch sampling

We collected branches and bole sections from infested or putatively infested ash at three sites (Thunder Bay, Ontario; Quebec City, Quebec; Oro Medonte township, Ontario) in 2016 and 2017. The Thunder Bay and Quebec City trees were city trees growing either in parks or along boulevards. The trees from Oro Medonte township were planted in a woodlot. Each branch and bole section was cut to be ca. 1 m long when the samples were taken. At least 1 bole section and multiple branches were taken from each tree. The trees at some sites in Quebec City were particularly large and the landowner (Parks Canada) wished to retain some of the wood for other purposes, so the bole sections were slabbed using a portable sawmill and only the slabs were retained. Previous work (MacQuarrie et al, unpublished) has shown that slabbing EAB-infested trees to a minimum depth of 10 cm captures a significant portion of the resident EAB.

All ash material was peeled using draw knives and the resident EAB were extracted. During sampling we recorded the stage of EAB collected (larvae, prepupae, pupae, adult) and the number of old galleries and exit holes. We also recorded signs and symptoms of EAB attack (e.g., epicormics branches, woodpecker feeding). All samples collected were retained in 80% EtOH for further analysis (see below).

Analysis The data were analysed using the same methods as in Ryall et al. 2011. In short we determined parameters for the relationship between the number of EAB in branches to those in the bole of the tree for samples collected in the four new infestations. We then used these parameters to estimate the probability of detection of an infested Thunder Bay ash tree. We also used the parameters of these new models to compare to that in Ryall et al 2011 to determine if there exists a different relationship than previously determined.

Frequency of two-year life cycle

Samples collected during the sampling in the first study were measured to determine the age of each larvae. The age of EAB larvae can be determined using the width of the head capsule and the conformation of the larvae when it was collected (prepupae are J-shaped and located within a pupal chamber). Based on both when the sample was collected and the age of the larvae we determined if it was likely in a one year or two year life cycle using criteria from MacQuarrie and Scharbach (2015) (i.e., third and fourth instar larvae, prepupae, pupae and adults collected before 15 August were assigned to the previous years' generation. All other insects were assumed to be from the current generation; based on an assumed flight period of ca. mid May to mid July).

Analysis We analyzed the frequency of each life stage at each site relative to the total distribution of ages within in the population, using each sampling site as a replicate. If a two year life cycle is more common we expect a higher proportion of the population to be at a stage consistent with taking two years to complete development. To expand the data available for this analysis, we included a data set collected in Dutton, Ontario in 2015 (Lyons and Jones, unpublished). At the time, this site was a new population of emerald ash borer, similar to those sampled for this study in 2016 and 2017.

Results

Sampling for both experiments was completed in fall of 2017. Processing of samples is for some sites is either complete (Thunder Bay, Oro Medonte) or ongoing (Quebec City). We present summary data below for the samples collected to date and a preliminary analysis of the relationship between sample sizes in branches and those in bole samples for three of the four sites (Thunder Bay, Oro Medonte, Quebec City).

In general the trees used in these experiments were the same diameter and height (Figure 1). However trees in Thunder Bay were slightly shorter and with lower crowns. This may reflect the shorter growing season and more stressful conditions expected at this northern site. Trees in the

Oro Medonte township woodlot had higher crowns (i.e., distance between ground level and the first branch) which is typical of ash grown in woodlots and forests.

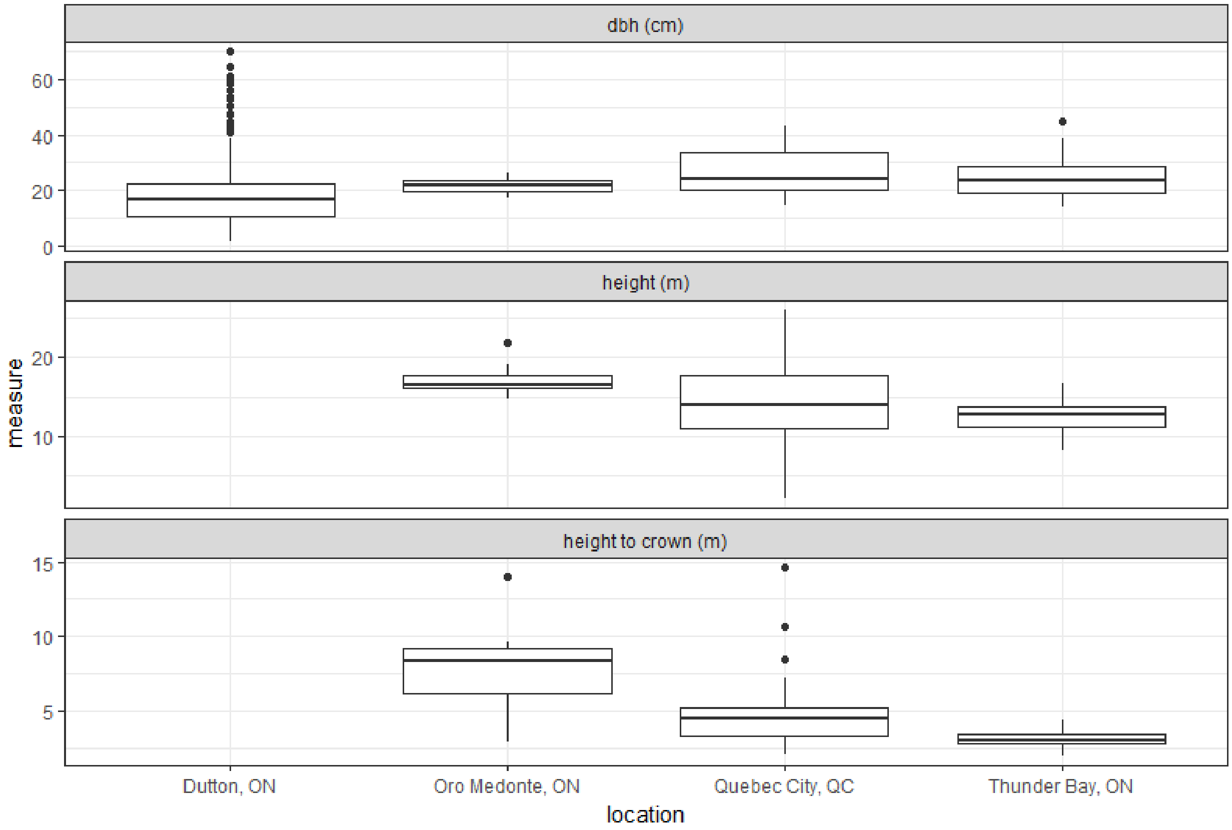


Figure 1: Dimensions of trees sampled in the experiment

When we examine the numbers of individuals in each sample we found that, in general there were larger numbers of individuals or galleries in bole samples than in branches (Figure 2). However, bole samples are typically larger (i.e., 1 m long vs. 50 cm and larger diameter) and so typically more individuals are found.

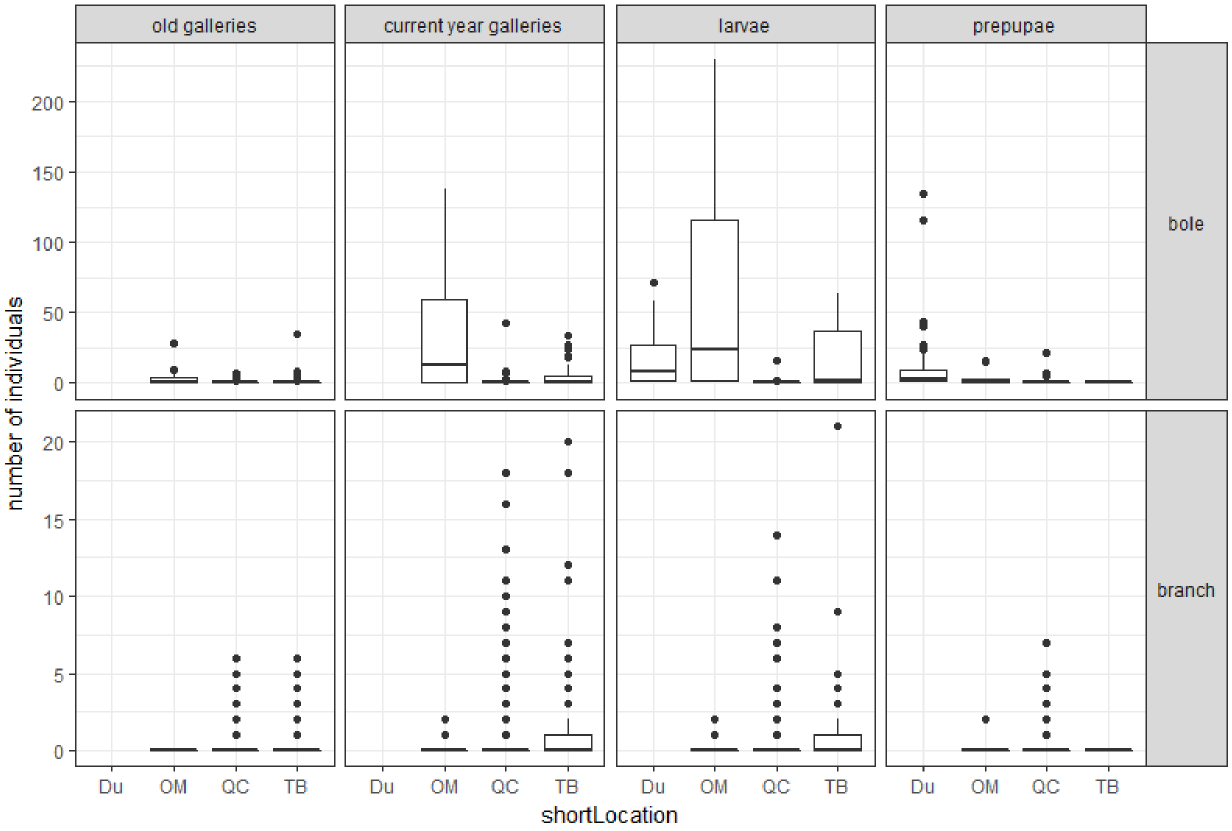


Figure 2: Absolute number of samples of four emerald ash borer stages in samples from four sites in eastern Canada (Du = Dutton, Ontario; OM = Oro Medonte, Ontario; QC = Quebec City, Quebec; TB = Thunder Bay, Ontario)

When we examine density of individuals we see a similar pattern, with slightly higher densities of EAB in bole sections compared to branches (Figure 3). In this figure we are unable to show density of EAB from the Dutton site because the sample lengths were not recorded and thus we are not able to calculate the sampled area.

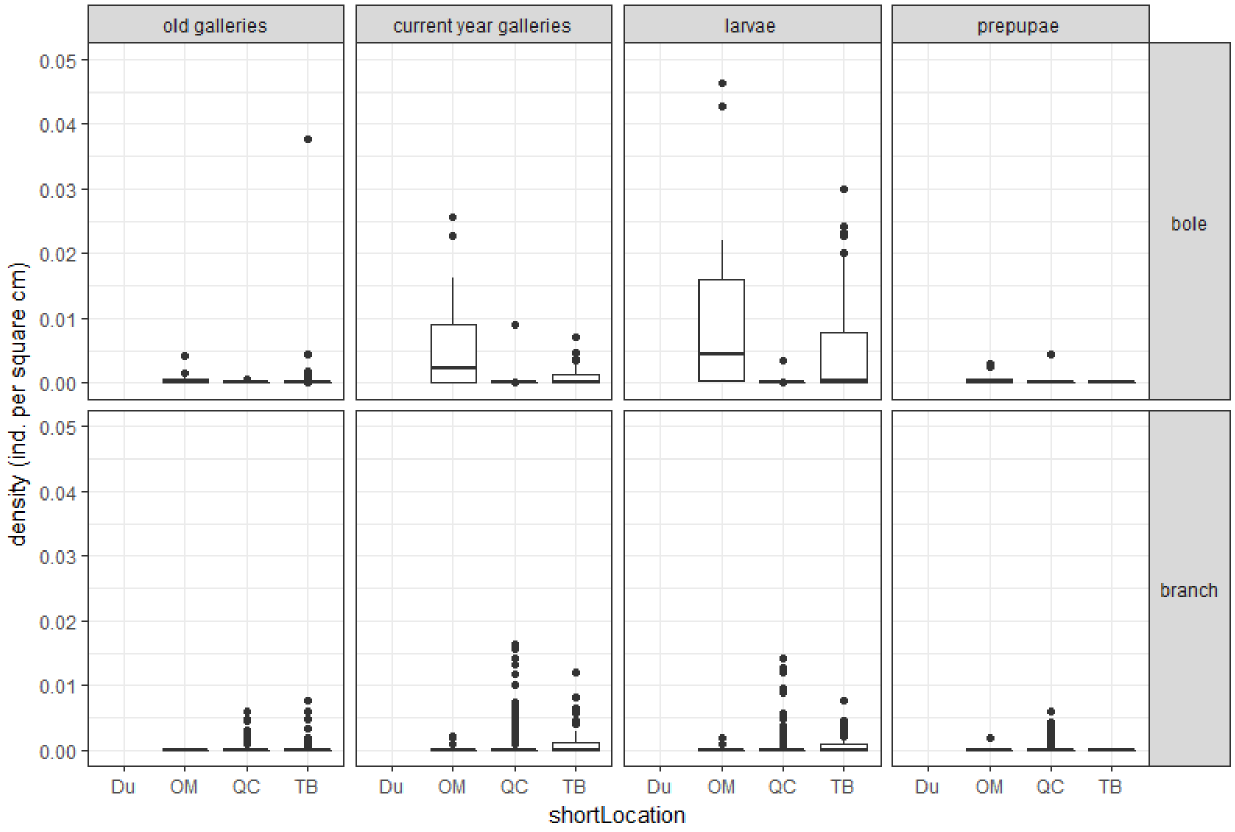


Figure 3: Density of four emerald ash borer stages in samples from four sites in eastern Canada (Du = Dutton, Ontario; OM = Oro Medonte, Ontario; QC = Quebec City, Quebec; TB = Thunder Bay, Ontario)

We completed a preliminary analysis of the relationship between EAB density on branches, and the presence of EAB in a tree (as indicated by the presence of EAB in the bole sample). This analysis showed that even at very low EAB densities (5.0×10^{-7} EAB per m^2) in branches can detect an infested tree at a probability > 50 %. This supports the previous finding of Ryall et al. 2011 regarding the accuracy of branch sampling to detect incipient EAB populations.

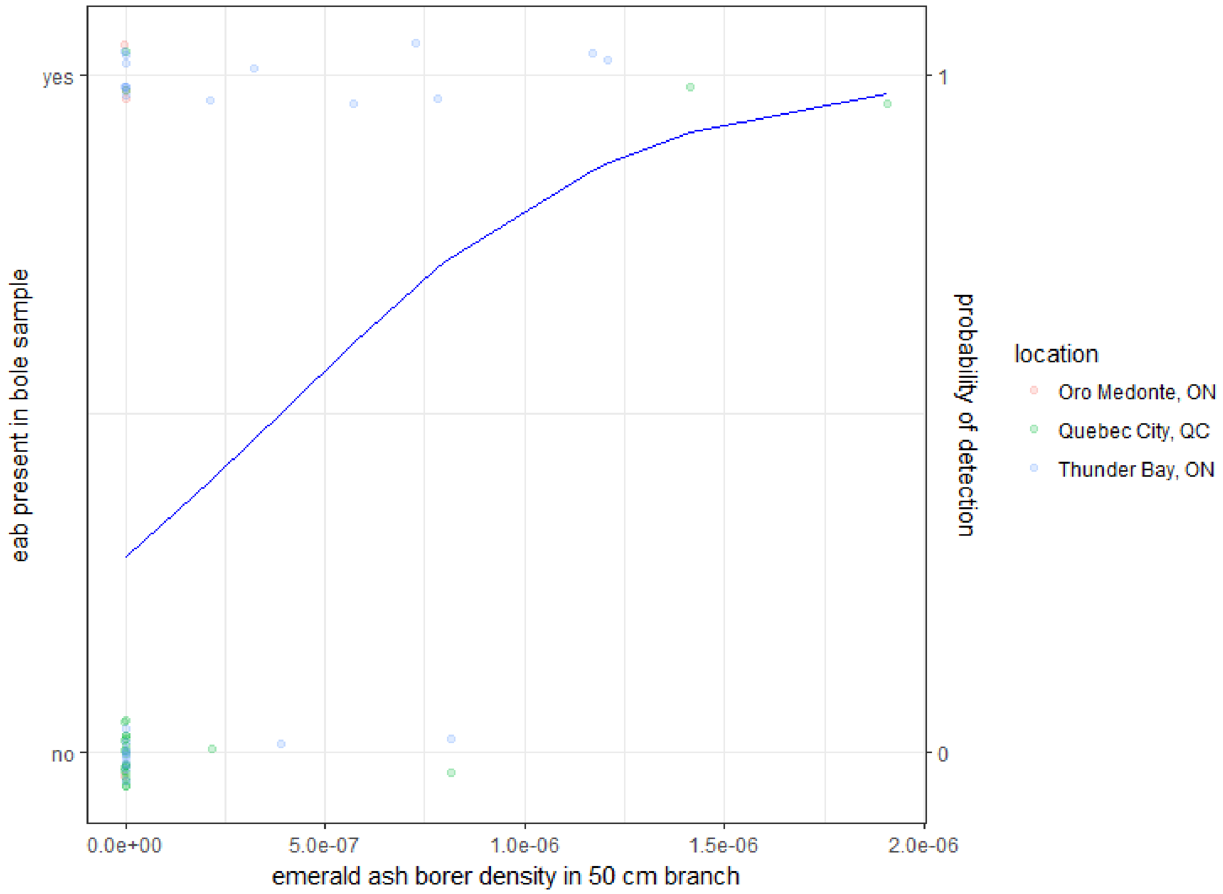


Figure 4: Probability of detecting emerald ash borer in a tree, as a function of density in one, 50 cm branch. Probability of detection is > 0.5 at densities above 5 x 10^{-7} EAB per m^{-2}, based on a single sample.

Discussion

A preliminary analysis of the data collected to date would seem to support previous conclusions and advice regarding the effectiveness of branch sampling for EAB. Analysis of these data will continue by examining how detectability is improved by the addition of branch samples. Data collection for the second part of the study is ongoing and will be completed by the end of February 2018. Our preliminary look at these data suggest that a significant portion of the larvae collected are consistent with a two year life cycle. We will repeat sampling at some of these sites in 2018 to determine if these patterns are shifting as infestations begin to age.

References

Financial report 2017-2018

Funding sources	**requested**	**received**
NRCan CFS*	$5,000	**$5,000**
SERG-I	$7,500	**$7,500**
Total	$12,500	**$12,500**

*Includes in-kind (travel expenses, staff time, administrative support and lab supplies).

Modelling the development of emerald ash borer in its expanding range.

C J K MacQuarrie[1], *D Inward*[2], *N Straw*[2], *S Smith*[3], *K Dearborn*[3]

1) NRCan Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario.
2) Forest Research, Alice Holt Lodge, Farnham, Surrey, United Kingdom.
3) University of Toronto, Faculty of Forestry, Toronto, Ontario

Abstract:

This report presents work towards a study of development rates in emerald ash borer reared under laboratory conditions. The goal of this study is to construct development rate models for all emerald ash borer life stages for use in risk assessments and improving the timing of control tactics against the insect. Work towards this project planed for 2017 was deferred and will now begin in early 2018.

Résumé

Le rapport présente des travaux effectués en vue d'étudier le rythme de développement de l'agrile du frêne élevé en laboratoire. Le but de cette étude est de mettre au point des modèles du rythme de développement à toutes les étapes de vie de l'agrile du frêne pour évaluer le risque et améliorer le moment de la réalisation des tactiques de lutte contre l'insecte. Les travaux prévus pour 2017 dans le cadre du projet ont été reportés et sont maintenant censés commencer au début de 2018.

Introduction

The relationship between emerald ash borer (EAB) development and temperature remains unclear. There are no published estimates for the developmental rates of EAB adults, larvae and pupae. Developmental thresholds have been estimated to predict the timing of adult emergence, but these are based on a minimum temperature threshold of 10 C that has not been verified. Anecdotal evidence suggests that populations in northern sites (e.g., Sault Ste. Marie, Thunder Bay) emerge later and develop slower than populations further south, suggesting that development varies across the landscape. Populations have also been observed to have a certain percentage of individuals that exhibit a 2 year life-cycle, and that these percentages are higher at cooler locations (e.g., in Sault Ste. Marie).

With information about EAB's developmental rates we can predict many important processes, for instance: the timing of adult emergence; when vulnerable life stages are likely to be present in the population (e.g., for the timing of control measures); the geographic range within which a population can complete its life cycle and; the population dynamics and rate of increase of populations.

Methods

Study 1 - Developmental rates

Adult EAB will be obtained as they emerge from infested ash trees collected in southern Ontario. The beetles will be fed to maturation on ash foliage and then provided with an oviposition substrate. Eggs will be collected and randomised, and then placed in petri dishes for rearing at a range of temperatures (e.g., 7, 10, 13, 17, 21, 25 and 30 °C) and monitored until hatching. Newly hatched larvae will be similarly reared across the same range of temperatures, but will be divided into two further cohorts. One cohort will be fed on the bark of North American green ash (*Fraxinus pennsylvanica*), a highly susceptible host, whereas the second cohort will be fed upon European Ash (*Fraxinus excelsior*). The results will indicate how rates of development are influenced by host species as well as temperature. Development will be monitored regularly at each phase of the study. For larvae, this will include measurements of mass, length and transition (moulting) between instars. The subsequent development of pre-pupal and pupal life stages will be similarly monitored up to adult emergence.

Study 2 – Modelling.

Data developed in Study 1 will be analysed to determine developmental threshold temperatures for each life stage, and a measure of the accumulated heat required for each stage to complete development (day-degrees). The effect of temperature on development rate will be determined using nonlinear mixed modelling. The effect of host type (European vs. North American ash) on development rate will also be determined, as well as any influence that diet may have on growth rate, mortality and final body mass. The data will also be used to model how development and how life cycle length varies according to local temperatures and host species. In particular, we will determine 1) how the shorter growing season and cooler climate of western Canada and the Maritimes influences the growth and potential range of EAB; and 2) how the cooler summer temperatures typical of the UK and a diet of *Fraxinus excelsior* may extend the life cycle and reduce the projected rate of increase and spread of EAB.

Results

There are no results to report for 2017.

Discussion

A meeting was held between the collaborators to discuss project planning in the fall of 2017, but technical work towards the project's two studies was otherwise delayed in 2017. Funds requested from SERGi are used to support a graduate student at the University of Toronto. This student (KD) began his program in January of 2018 and thus no work was completed before that date. With the agreement of the SERG funding partners, those funds allocated to support the student have been transferred and will be used to augment the stipend of KD for work in 2018.

Financial report 2017-2018

Funding sources	**requested**	**received**
NRCan CFS	$3,000*	**$ 3,000**
Forest Research (UK)*	$9,700	**$ 9,700**
DEFRA	$38,000	**$38,000**
University of Toronto	$15,000	**$15,000**
SERG-I	$10,000	**$10,000**
Total		

*Includes in-kind (travel expenses, staff time, administrative support and lab supplies).

Release of exotic parasitoids for biological control of the emerald ash borer in Canada.

Progress Report

Principal investigator: Krista Ryall, Natural Resources Canada, Canadian Forest Service; 1219 Queen Street East, Sault Ste. Marie, Ontario P6A 2E5; Krista.ryall@Canada.ca

Collaborators : Dr C. MacQuarrie and Dr. A Roe, Natural Resources Canada, Canadian Forest Service

Abstract:

During 2017, we established eight new release sites, seven in Ontario and one in Quebec. We released over 36, 000 *Tetrastichus planipennisi* at these sites. In addition, over 13, 500 *Oobius agrili* were released at the sites. For the first time, *Spathius galinae* was released in Canada, with over 700 individuals released at two of the Ontario sites. In total, there are now 19 sites where over 97, 000 *T. planipennisi* have been released over the past five years. In addition, over 38, 500 *O. agrili* have been released at 16 sites over the past three years. In fall 2017, we sampled trees from three of the completed sites to evaluate recovery of *T. planipennisi*; rearing of log bolts has begun as of December 2017 and will continue through April 2018 to attempt to recover the parasitoid at these sites. Sampling to recover *O. agrili* will begin in summer/fall 2018. An initiative to conduct rearing *T. planipennisi* at the Insect Production and Quarantine Laboratory, Great Lakes Forestry Centre was initiated in December 2016; as a result, over 3500 “made in Canada” wasps were released at one site in Ontario in 2017. Most recently, in December 2017, technicians travelled to the USDA lab in Michigan to receive training on how to rear *Oobius agrili*; rearing of this species will begin in 2018. The objective of this rearing initiative is to augment numbers provided by USDA-APHIS for research purposes.

Résumé

Au cours de l’année 2017, nous avons établi huit nouveaux sites de relâchement, soit sept en Ontario et un au Québec. Nous y avons relâché plus de 36 000 *Tetrastichus planipennisi*. Il y a aussi eu relâchement de plus de 13 500 *Oobius agrili*. Pour la première fois, *Spathius galinae* a été relâché au Canada - plus de 700 individus dans deux des sites ontariens. Au total, on compte maintenant 19 sites où plus de 97 000 *T. planipennisi* ont été relâchés depuis cinq ans. Plus de 38 500 *O. agrili* ont aussi été relâchés dans 16 sites depuis trois ans. À l’automne 2017, nous avons échantillonné des arbres dans trois des sites traités pour évaluer la détection de *T. planipennisi*; l’élevage dans des blocs de bois a commencé en décembre 2017 et se poursuivra jusqu’en avril 2018 pour tenter de détecter le parasitoïde dans ces sites. L’échantillonnage visant à détecter *O. agrili* commencera à l’été/l’automne 2018. Un projet d’élevage de *T. planipennisi* au laboratoire de production d’insectes et de quarantaine du Centre de foresterie des Grands Lacs a été entrepris en décembre 2016, ce qui a permis de

relâcher plus de 3 500 guêpes « produites au Canada » dans un site ontarien en 2017. Plus récemment, en décembre 2017, des techniciens se sont rendus au laboratoire du USDA au Michigan pour suivre une formation sur l'élevage de *Oobius agrili*; l'élevage de cette espèce débutera en 2018. L'objectif de ce projet d'élevage est de suppléer aux spécimens fournis par le USDA-APHIS aux fins de recherche.

Background:

The emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), is a devastating invasive beetle which has killed tens of millions of ash trees. Few management or control options are available, particularly in natural forest habitats. The United States began a classical biological control program against this devastating insect pest in 2007, by searching for effective natural enemies in its home range (Bauer et al. 2015). Four species are currently being released across the range of EAB in the U.S.

In 2013, three petitions were submitted to the Director of Plant Biosecurity and Forestry Division, Canadian Food Inspection agency (CFIA) to begin releases of parasitoids within Canada. Petitions were for: 1. *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae); 2. *Spathius agrili* Yang (Hymenoptera: Braconidae); and 3. *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae). Permission to release *T. planipennisi* and *S. agrili* was granted, but initially denied for *O. agrili*. Some members of the Biological Control Review Committee were concerned that host specificity testing indicated that *O. agrili* might attack native species of *Agrilus* that had eggs comparable in size to *A. planipennis*. A revised petition for release of *O. agrili* was resubmitted to CFIA and approval was granted in 2015. Although some reviewers still had reservations about host specificity, it was concluded that "the release of this species in the United States means the encyrtid is likely to enter Canada on its own accord". Additionally, the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) was no longer recommending release of *S. agrili* north of 40° latitude excluding that species for release in Canada. Finally, in 2017, a petition to release a fourth species, *Spathius galinae*, was approved by CFIA.

Within Canada, releases of the three species of parasitoid are being conducted in an attempt to establish populations of these wasps in the region infested with EAB. These wasps are originally from China and Russia and are highly specific to EAB with high parasitism levels in their native range. The first, *Tetrastichus planipennisi,* is a larval parasitoid. Adults emerge in late May and produce multiple generations per year. Females parasitize late instar EAB larvae, with up to 57 wasps produced per EAB larva. This species has been reported to have parasitism rates up to 50% in China. It has been released since 2013 in Canada. Next, *Oobius agrili* is an egg parasitoid, with two generations per year. Up to 80 EAB eggs are attacked per female with one egg laid per EAB egg. This species has up to 60% parasitism in its native range. It has been released since 2015. The third species, *Spathius galinae*, is another larval parasitoid originally found in Russia causing high levels of parasitism to EAB on green ash. All parasitoids are generously provided by USDA-APHIS Biocontrol production laboratory in Brighton, Michigan which has been in full-time production since January 2009.

Methods

Site Selection:

Site selection criteria are specified in the USDA-APHIS *Emerald Ash Borer Biological Control Release and Recovery Guidelines*. Release sites should be naturally forested areas at least 40 acres (16.2 ha) in area, or if smaller than 40 acres the sites should contain a high ash density and ash corridors to other wooded areas. Ideally the site should contain a high healthy ash component (>25%) over 4 cm DBH and distributed over a variety of size classes. The population density of emerald ash borer should be low to moderate, and must be confirmed at each site by removing sections of bark. Sites should have easy access and permission must be obtained from the landowner. An agreement must be negotiated that the sites will not be harvested or developed for 5 years. The release point must be >100 m from the forest edge and human activity (e.g., insecticide treatments) should be avoided.

The number of sites has increased from three in 2013 to a total of 19 in 2017. In 2013, two sites within a contiguous forest called Hay Swamp in Huron Co. and owned by the Ausable Bayfield Conservation Authority (ABCA) were selected for release of *T. planipennisi* (Tract 1 (Hay Swamp) and Tract 6 (Ausable Line)) (Table 1). The third site was an ash plantation on private property (owner Robert Haig) on Brooke Line, Lambton County. In 2014, it was our intention to secure six additional release sites distributed across southern Ontario and Quebec. Unfortunately, sites in southcentral Ontario (Peterborough to Kingston area) that met the selection criteria were difficult to find at that time due to low/absent EAB populations. Thus, in 2014, four sites (Table 1) were selected for release of *T. planipennisi*: 1) Silver Creek Conservation Authority (Credit Valley Conservation Authority); 2) Middleton McConkey Tract (Long Point Region Conservation Authority); 3) Gatineau Park (National Capital Commission); and 4) Conroy Road, Ottawa (National Capital Commission). A fifth site, Wildwood Conservation Authority (Upper Thames River Conservation Authority), consisting of an ash plantation was selected for the later round of releases. Four new sites were selected in 2015 (Table 1): three in Montreal (Jardin Botanique, Parc Nature Bois-de-Liesse and Bois Summit) and the fourth site was Metro Tract in York Region. Forest inventory data was collected in each plot in each year as required by the USDA-APHIS release guidelines. In 2016, no additional new sites were added; instead releases were completed at all current sites to provide two consecutive years of releases. In 2017, eight new sites were added; seven in Ontario and one in Quebec. The locations of all Canadian release sites are listed in Table 1.

Collaborators and Cooperators:

The United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) generously provided us with parasitoids in each year from 2013-2017. The Ontario Ministry of Natural Resources and Forestry assisted with the selection of release sites in Ontario. The Quebec Ministry of Natural Resources and Energy selected a release site in Quebec, completing the plot assessment and assisting with the field releases. The Ausable Bayfield Conservation Authority, Long Point Region Conservation Authority, Upper Thames

River Conservation Authority, and the Credit Valley Conservation Authority granted permission for us to work on their properties. Releases in 2013 were also made on property owned by Mr. Robert Haig. An additional two release sites were properties managed by the National Capital Commission, one in Ottawa and one in Gatineau, Quebec. Since 2015, staff from the Ville de Montréal, Environnement, have assisted with site selection and releases. In 2015, York region assisted with site selection. In 2017, sites were selected on conservation authority properties, municipalities, and a Parks Canada site.

Table 1. Locations of parasitoid release sites in Ontario and Quebec in 2013 to 2016.

Release sites				GPS coordinates of plot centre		
Site name	**Site location**	**Province**	**Owner**	**Latitude**	**Longitude**	**Release year(s)**
Ausable Line	Huron Co.	Ontario	ABCA[1]	43.38295	-81.54295	2013
Hay Swamp	Huron Co.	Ontario	ABCA	43.35646	-81.55526	2013
Brooke Line	Lambton Co.	Ontario	Bob Haig	42.84389	-81.85438	2013
Middleton-McConkey Tract	Norfolk Co.	Ontario	LPRCA[2]	42.81081	-80.62296	2014- 2016
Silver Creek CA	Halton Hills	Ontario	CVCA[3]	43.68049	-79.97522	2014- 2016
Wildwood CA	Oxford Co.	Ontario	UTRCA[4]	43.24619	-81.05774	2014- 2016
Conroy Road	Ottawa	Ontario	NCC[5]	45.34123	-75.60871	2014- 2016
Metro Tract	York Region	Ontario	York Region	44.31144	-79.39548	2015- 2017
Gatineau Park	l'Outaouais region	Quebec	NCC	45.63861	-75.95150	2014- 2016
Jardin Botanique	l'île de Montréal	Quebec	Ville de Montréal	45.56271	-73.56406	2015-2016
Parc Nature Bois-de-Liesse	l'île de Montréal	Quebec	Ville de Montréal	45.50550	-73.76253	2015-2016
Bois Summit	l'île de Montréal	Quebec	Ville de Montréal	45.49130	-73.60575	2015- 2016
Wilmot Creek	Aurora District	Ontario	MNRF	43.91355	-78.61118	2017
Baxter C.A.	City of Ottawa	Ontario	RVCA[6]	45.09381	-75.63126	2017
Monkland	Stormont, Dundas & Glengarry Co.	Ontario	South Nation Conservation	45.24773	-74.92613	2017
Iroquois	Stormont, Dundas & Glengarry Co.	Ontario	Private land	44.87007	-75.26279	2017
Renfrew	Renfrew	Ontario	Town of Renfrew	45.48478	-76.68911	2017

Fort St. Joseph	Algoma District	Ontario	Parks Canada	46.06605	-83.94609	2017
Barrie	Barrie	Ontario	City of Barrie	44.35766	-79.72049	2017
L'Assomption	L'Assomption Co.	Quebec	Private land	45.82047	-73.45377	2017

[1] ABCA = Ausable Bayfield Conservation Authority
[2] LPRCA = Long Point Region Conservation Authority
[3] CVCA = Credit Valley Conservation Authority
[4] UTRCA = Upper Thames River Conservation Authority
[5] NCC = National Capital Commission
[6] RVCA = Rideau Valley Conservation Authority

Releases:

For each of the release periods in all five years, CFS staff drove to the USDA-APHIS parasitoid rearing facility in Brighton, Michigan and picked up our prearranged allocation of *T. planipennisi*, *O. agrili*, or *S. galinae*. The insects were provided as pre-emergent pupae/adults in or on host larvae (*T. planipennisi* and *S. galinae*) in small bolts from ash trees in which the host larvae have been reared or within host eggs (*O. agrili* is a solitary endoparasitoid). The parasitoids will finish development and begin emergence soon after deployment in the field. Over 97, 000 *T. planipennisi* have been released in total over the past five years. Over 38, 500 *O. agrili* have been released since 2015. The estimated numbers of *T. planipennisi* and of *O. agrili* released from 2013 through 2017 are listed in Table 2. Over 700 *S. galinae* were released at two sites (Iroquois and Monkland) in 2017. In 2017, parasitoids were shipped to co-operators to assist with timely releases.

Table 2. Estimated number of females of *T. planipennisi* and *O. agrili* released at the various sites in Ontario and Quebec from 2013-2016.

		Tetrastichus planipennisi					***Oobius agrili***		
Site name	**Prov**	**2013**	**2014**	**2015**	**2016**	**2017**	**2015**	**2016**	**2017**
Ausable Line	ON	6240							
Hay Swamp	ON	6070							
Brooke Line	ON	2820							
Wildwood CA	ON		1436	2703	2581		1500	1700	
Middleton-McConkey Tract	ON		3164	2703			1500	1700	
Silver Creek CA	ON		3316	2703			1500	1700	
Conroy Road	ON		3551	2703			1500	1700	

Gatineau Park	QC	3283	2703			1500	1700	
Bois de Liesse	QC		2703	2379		1500	1700	
Bois Summit	QC		1281	2385		1500	1700	
Jarden Botanique	QC		2703	2486		1500	1700	
Metro Tract	ON		1281	2516	5069		1700	1500
Wilmot Creek					4984			1400
Baxter C.A.					4932			1400
Monkland					5151			1500
Iroquois					2958			
Renfrew					5050			1400
Fort St. Joseph					3516			1400
Barrie								3500
L'Assomption					5030			1500

Release dates for the three species in each year are listed in Table 3. Releases of *T. planipennisi* were undertaken six times per year during 2013-2017. From 2013 to 2017, BioForest Technologies Inc. graciously provided us with phenology forecast maps generated using BioSIM, to estimate our phenological targets for release of *T. planipennisi*. Initial estimated release dates were produced using historical temperature normals and were updated as real time temperature data became available. The first target release phenological date was 167 degree days above base 10°C (targeting the overwintering larvae). Two additional releases were made two and four calendar weeks after this phenological date. The second target release phenological date was at 1000 degree days above base 10°C (targeting the new generation larvae). Additional releases were again made at 2 and 4 calendar weeks after this phenological date. Releases of *S. galinae* occurred during the second target release dates, based on their availability from the USDA laboratory. The small ash bolts containing the parasitoids were hung from some of the 12 release trees at each release date. Release dates of *O. agrili* are also listed in Table 3. BioForest Technologies also provided us with phenology maps predicting the target dates for release of *O. agrili* of 444 degree days above base 10°C which coincides with the appearance of eggs of EAB.

Table 3. Release dates for the two parasitoids per year.

	2013	**2014**	**2015**	**2016**	**2017**
T. planipennisi					
Spring	4 June	3-5 June	26-28 May	25-26 May	30 May-1 June
spring	18 June	17-19 June	9-11 June	7-8 June	12-15 June
Spring	3 July	1-3 July	23-25 June	21-23 June	27-29 June

Summer	20 Aug	19-21 Aug	18-20 Aug	16-19 Aug	22-24 Aug
Summer	4 Sept	3-5 Sept	1-3 Sept	31 Aug-2 Sept	7-11 Sept
Summer	18 Sept	16-18 Sept	15-17 Sept	7-8 Sept	19-21 Sept
O. agrili					
Summer			21-23 July	28-30 June	4-7 July
Summer			28-30 July	12-14 July	18-21 July
summer			5-7 Aug	26-28 July	1-3 Aug

Plot Assessments:

Following site selection, a group of EAB infested ash trees a minimum of 100 m from roadways was chosen as the site centre. A single ash tree in the group was designated the site epicentre and was marked accordingly. Three ash trees > 4 cm DBH in each of the four cardinal directions around the epicentre were selected as the 12 release trees. These trees were numbered with aluminum tree tags, and their crown class, DBH and GPS coordinates were recorded. Signs and symptoms of EAB attack were also recorded for these trees. A 200 by 200 m grid, with 50 by 50 m grid cells was set up around the site epicentre. Within each of the 16 grid cells, a tree inventory was conducted in a 10 by 10 m subsample plot. Data on all trees over 4 cm DBH was collected including species, DBH, and crown position. For *Fraxinus* spp., EAB signs and symptoms were also noted (i.e., woodpecker feeding holes, bark deformities, epicormic shoots, EAB exit holes). All data to date has been uploaded to EAB Parasitoid Release and Recovery Database (www.mapbiocontrol.org) to make it available for analysis in the future to determine optimum release strategies.

Release methods:

Figure 1a and b. The *Oobius agrili* release device and the mini-bolts containing *T. planipennisi* .deployed on an ash tree.   S. galinae were deployed as live adults rather than emerging from mini bolts.

Post-Release Assessment:

Following release of the parasitoids, overwintering survival and establishment must be assessed. The USDA-APHIS *Emerald Ash Borer Biological Control Release and Recovery Guidelines* provides techniques to evaluate parasitoid establishment. One technique and the one most highly recommended required the felling of a number of ash trees within the release area and the subsequent dissection (i.e., bark removal) of the trees or the placement of log bolts from the trees into rearing containers to observe parasitoid emergence.

Assessment to evaluate recovery of the released parasitoids was generally conducted one-year following the last release, or at least after a winter season has occurred. Sampling for recovery began in spring 2014 with eight trees sampled at Brooke Line (Table 4). In fall 2014, four more trees were sampled from Brooke Line and four trees were sampled from Ausable Line (Table 4). Next, four trees were harvested from the Wildwood site in spring 2015. In fall 2015, four trees were felled at the Middleton-McConkey Tract, five trees were felled at the Gatineau Park site, and four trees were felled at the Conroy Road site. In Spring 2016, four trees were cut from Wildwood CA. Finally, in Fall 2016, an additional four trees were cut at Wildwood CA, Middleton-McConkey Tract, and Conroy Road. In fall 2017, four trees were harvested at Bois Summit and Jardin Botanique and 5 trees at Bois-de-Liesse. Rearing to recover parasitoids from these trees has begun as of December 2017. It is too early to sample at Metro Tract and sapling peeling is scheduled for spring 2018 at Silver Creek CA.

Trees were cut into 40-cm long bolts and transported to SSM. Logs collected in spring were placed into rearing shortly after collection; logs cut in fall were stored at cold temperatures for several months prior to being brought into the laboratory and placed in rearing chambers. In the laboratory, log bolts were placed in 1.3 m-high by 40 cm diameter -wide fibre drum with a sealed top and a modified funnel emptying into a capture container. Ash bolts from a given tree were haphazardly placed in drums to maximize the use of internal space (for 2014-2016). In 2017 bolts were placed sequentially (base to branches) in the tubes. Each drum could fit between 2-16 bolts depending on their diameters. Complete tree rearing required anywhere from 1 to 15 drums. Drums were placed horizontally on shelves in a well-lit rearing room with a temperature of 24-26°C, RH of 40-50% and a photoperiod of L:D 16:8 h. The rearing containers were examined daily for insect emergence.

The second recommended technique involves the placement of yellow pan traps on the release ash trees or nearby ash trees. Yellow pan traps as described in the *Emerald Ash Borer Biological Control Release and Recovery Guidelines* were deployed on the 12 release trees on 18 August 2014 at Ausable Line and Brooke Line. Only 5 traps were deployed at Hay Swamp due to the high ash tree mortality at the release epicentre. Traps were sampled on 3, 16 September and 1 October 2014. This method was discontinued after 2014.

Recovery results

Recovery of adult *T. planipennisi* has been highly successful: 100% of sites (six of six) where sampling has been completed. Emergence of adult *T. planipennisi* occurred from trees collected from Ausable Line, Brooke Line, Wildwood CA, Gatineau Park, Middleton-McConkey Tract, and Conroy Road (Table 4). In 2014, initially only 10 bolts per tree were reared from the harvested trees at Brooke Line; however an additional ten bolts total from the 8 trees were also dissected by bark peeling, and did not reveal any evidence of *T. planipennisi*. The approx. mean number of adult *T. planipennisi* reared per tree has ranged from a low of 6.5 adults (26 individuals from four trees at Middleton) to a high of 187.4 adults (937 individuals from five trees at Gatineau) (Table 4). The percentage of trees per site from which adult *T. planipennisi* were detected ranged from 33 to 100%, with an average of 65.9% of trees producing at least one adult *T. planipennisi*. This is based on rearing that has been completed to-date (i.e., not included trees collected fall 2017). The sex ratio has ranged from 0-7 females per 1 male (Table 4).

The number of EAB per tree ranged from only 7 to over 600 (for eventual analysis, this mean number per tree needs to be adjusted by tree size to estimate # EAB per square metre of bark surface area). Based on results from rearing alone, it is not possible to calculate a precise estimate of percentage parasitism because the number of *T. planipennisi* adults per EAB larva ranges considerably. For example, Wang et al. (2015) estimated brood size to be approx. 33, 42 or 48 *T. planipennisi* wasps per EAB larva in three separate experiments; similarly, Ulyshen et al. (2010) reported 32-91 *T. planipennisi* progeny per EAB larva, whereas Ulyshen (unpublished data) suggested a wider range between 4 to 172 wasps emerging per EAB larva. Next, Yang et al. (2013) reported between 18.9-31.2 *T. planipennisi* progeny per EAB larva. Finally, based on the information provided by USDA-APHIS regarding emergence of wasps from the mini-bolts, an estimated range of 6-45 *T. planipennisi* emerge per EAB larva. Based on these previous results, if we assume a minimum of 25 *T. planipennisi* wasps emerging per EAB larva, then the estimated percentage parasitism level can be calculated to range from 0 to 15% per tree (calculated as number of EAB larvae estimated to be parasitized / (sum number of EAB larvae estimated to be parasitized + number of EAB adults emerging) times 100). At the site level, parasitism ranged from 0 to 8.4%. Again, these are preliminary data estimating percentage parasitism and these values should be interpreted with caution as the exact number of adult wasps per EAB larva are currently unknown and are estimated from previous research.

There was a negative relationship between tree DBH and percentage parasitism (Figure 2). Again, these are preliminary data regarding percentage parasitism and these values should be interpreted with caution; regardless lower parasitism levels would still be associated with larger trees.

Finally, no *T. planipennisi* adults were recovered from any of the yellow-pan traps, so this method was not continued.

Table 4. Recovery of *Tetrastichus planipennisi*: number and timing of trees sampled, number of years post-release trees were sampled, percentage of trees infested, total number of females: males reared per site, and estimated percentage parasitism levels

Site name	# trees/season / year	Mean tree dbh	Mean no. EAB / tree	% trees with TP	F : M	Est. % parasitism
Ausable Line	4 / fall /2014	16.3	158.8	50	332 : 164	3.0
Hay Swamp	*					
Brooke line	8 /spring / 2014	12.8	0.1	0	0	0
	4 / fall / 2014	12.9	37.5	50	23: 17	1.1
Wildwood	4 / spring / 2015	11.5	29.0	0	0	0
	4 / spring / 2016	14.3	28.5	100	17: 36	1.8
	4 / fall / 2016	15.8	174.5	75	366:52	2.3
Middleton	4 / fall / 2015	19.8	401.5	75	13: 13	0.06
	4 / fall / 2016	22.1	450.3	100	0:18	0.04
Silver Creek	Too early					
Conroy Road	4 / fall / 2015	16.0	377.0	75	28: 14	0.11
	4 / fall / 2016	18.5	380.5	100	130:35	0.4

Gatineau Park	5 / fall / 2015	9.5	82.4	100	713: 224	8.4
Bois de Liesse	5 / fall / 2017	15.7				
Bois Summit	4 / fall / 2017	18.2				
Jarden Botanique	4 / fall / 2017	21.0				
Metro Tract	Too early					

*ash mortality too high at this site to conduct follow-up sampling to recover *T. planipennisi*.

TBD = To Be Determined.

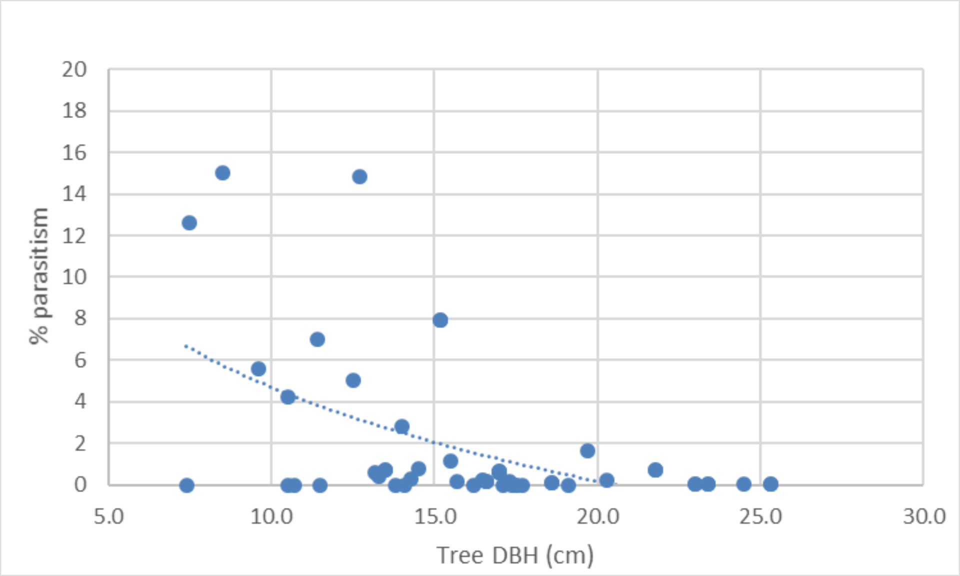


Figure 2. Percentage parasitism by *Tetrastichus planipennisi* of emerald ash borer larvae by tree DBH. (N=53 trees)

Discussion

The USDA-APHIS has generously provided parasitoids for release against EAB in Canada since 2013. To date, over 96,000 *T. planipennisi* have been released in total over the past five years and over 35, 000 *O. agrili* have been released since 2015. In 2017 over 700 *Spathius galinae* have been released. These three parasitoids have been released at 19, 16 and 2 sites, respectively, to date, across southern Ontario and Quebec. It is still very early in the development of the biological control program for EAB in Canada; no results on any potential impact of the parasitoids are yet available. Initial follow-up sampling to evaluate if the parasitoids can be recovered has been conducted in six sites so far. Early establishment of *T. planipennisi* appears to be quite high (six of six sites sampled to date). In the U.S., reports show that *T. planipennisi* has established permanently in at least 50% of all release sites

(Mapbiocontrol 2014). Jennings et al. (2016) also reported that establishment and dispersal of this parasitoid was generally quite successful in Maryland, US. It is too early yet to conduct recovery sampling for the egg parasitoid, *O. agrili*; this sampling will commence in 2018. However, established populations of the egg parasitoid have also been detected in >50% of sites in the U.S. (Mapbiocontrol 2014).

The numbers of adult parasitoids recovered appears to be low, considering the large numbers that were released. Similarly, however, in one published report, over 191 506 *T. planipennisi* were released at 32 sites, and only 1856 were recovered from 12 release sites and seven control sites (Jennings et al. 2016). This involved sampling of over 400 trees. Most of these recovered parasitoids (1796) were collected through peeling of the bark from sample trees, with only 60 collected from rearing. This suggests that rearing of log bolts may be adequate for detection per se of the adult parasitoids, but maybe does not give a sufficiently accurate estimate of number of parasitoids. Additional sampling of trees infested with EAB will be necessary over the next several years to determine if the parasitoids have become permanently established (typically requiring at least three consecutive years of recovery following releases).

Of those trees sampled, approx. 66% contained at least one brood of *T. planipennisi*, indicating that the wasps are dispersing throughout the site and locating additional trees infested by EAB. In the first year, the trees cut for rearing were the release trees; in subsequent years, harvested trees were typically within 40 m of the epicentre of the release trees. Duan et al. (2013) reported over 90% of infesed trees to have one or more brood of *T. planipennisi* within four years following releases conducted in Michigan. In Maryland, Jennings et al. (2016) reported approx. 20% of trees to have at least one brood in the second and third year, increasing to over 40% in year four following releases. Their study had a much larger sample size than ours, with over 450 trees sampled from 27 sites. In another study, Duan et al. (2015) reported that parasitism levels by *T. planipennisi* were the same in both their release and 'control' plots, within the first 2-4 years following releases. Given that their control plots were 1-6km from the release points, this suggests that the wasps are capable of dispersing at least 1.5 km per year. Similarly, Jennings et al. (2016) recovered adult parasitoids from seven control plots up to 5 km away from the releases within the first few years following release. Laboratory bioassays have demonstrated that *T. planipennisi* can dispers up to 7 km (Fahrner et al. 2014). Additional sampling of trees at greater distances from the epicentre in the focal release stand and in neighboring stands is necessary to evaluate dispersal distances by the parasitoids over time throughout the current release range in southern Ontario and Quebec.

Parasitism levels by *T. planipennisi* can only be estimated at this point, given that we only conducted rearing of log bolts and that there is considerable variability in number of wasps emerging from an individual EAB larva (see details in methods). Conservative estimates of 25 wasps per larva were used in the current calculations, resulting in estimates of parasitism under 10% at the site-level. This is similar to reports of parasitism levels in the first couple of years following releases (Duan et al. 2013), *but again values estimated from the current study must be interpreted with caution*. Duan et al. (2013) reported increases in parasitism levels by *T. planipennisi* from an initial 1-5% to 12-21% over four years following releases at the same study sites. In another study, Duan et al. (2014 and 2015) reported that native North American parasitoids and the introduced *T. planipennisi* caused significant decreases in the rate of population growth of EAB in Michigan. While EAB populations were still increasing in their study, they hypothesized that continued release and establishment of exotic parasitoids would increase mortality levels over time, leading to a reduction in the growth rate of EAB earlier in the outbreak cycle. Jennings et al. (2016) also reported parasitism levels to increase from <1% to over 11% during the 1 to 4 years following releases. Additional sampling will be conducted in future years, including peeling of bark to provide more precise proportions of parasitized vs non-parasitized EAB. It is also possible that brood sizes vary depending on parasitoid:host ratios (Wang et al. 2015); thus further detailed data collection is necessary. To-date, the objective of the research was to evaluate establishment and recovery, hence this additional time-consuming sampling was not previously conducted.

Based on data from 53 trees reared to date, there appeared to be a negative relationship between percent parasitism and tree diameter at breast height. This is very similar to that reported by Abel et al. (2012) and Johnson et al. (in press), who reported that the success of *T. planipennisi* at parasitizing EAB larvae is significantly reduced when trees are >12 cm DBH. Similarly, Jennings et al. (2016) reported >95% of parasitized EAB larvae were detected in trees with <16cm DBH. Thicker bark and larger log size also negatively affected *T. planipennisi* parasitism rates in Wang et al. (2015). These data suggest that releases should be directed towards sites with smaller tree sizes and sampling efforts to recover adult parasitoids could possibly be focused towards smaller diameter logs (<16cm). More specific data on preferred tree and log diameter needs to be collected for this species to enable researchers to develop a sub-sampling technique to minimize rearing of log while maximizing detection rates of the parasitoid.

Further research is necessary to continue to evaluate initial and permanent establishment of one or both parasitoids, to quantify percent parasitism levels and how they may increase over time, to quantify dispersal of parasitoids from the initial release plots, and to evaluate site requirements for maximizing establishment rates Finally, a rearing initiative has been started with the Insect Production and Quarantine Laboratory at the Great Lakes Forestry Centre, in collaboration with Dr. Amanda Roe and John Dedes as well as the USDA-APHIS rearing facility in Brighton, Michigan. Rearing of *T. planipennisi* has recently begun (Dec 2016) to attempt to augment numbers of parasitoids available for release within Canada; this initiative led to the release of over 3500 “made in Canada” T. planipennisi at one site in 2017. Most recently, technicians at GLFC have received training and will begin a colony of *O. agrili* at our GLFC laboratory.

Budget

CFS funding $13 000; in-kind salary contribution (0.5 FTE research scientist; 0.5 FTE two technicians)

USDA-APHIS: in-kind contribution of >85 000 parasitoids to date

SPA ISC $1800

SERG funding : NSDNR $1.0K OMNRF $6.0K MCWS $4.0K SK MOE $2.0K USFS $2.0K

References

Abell, KH, Duan, JJ, Bauer, LS, Lelito, JP, and RG Van Driesche. 2012. The effect of bark thickness on host partitioning between *Tetrastichus planipennisi* (Hymen: Eulophidae) and *Atanycolus* spp. (Hymen: Braconidae), two parasitoids of emerald ash borer (Coleop: Buprestidae). Biological Control. 63: 320-325.

Bauer, LS, Duan, JJ, Gould, JR, and R Van Driesche. 2015. Progress in the classical biological control of

Agrilus planipennis Fairmaire (Coleoptera: Buprestidae) in North America. Can Entomol. 147-300-317.

Duan, JJ, Bauer, LS, Abell, KJ, Lelito, J, and RG Van Driesche. 2013. Establishment and abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Michigan: potential for success in classical biocontrol of the invasive emerald ash borer (Coleoptera: Buprestidae). J Econ Entomol. 106: 1145-1154.

Duan, JJ, Abell, KJ, Bauer, LS, Gould, J, and R Van Driesche. 2014. Natural enemies implicated in the regulation of an invasive pest: a life table analysis of the population dynamics of the emerald ash borer. Agr For Entomol. 16: 406-416.

Duan, JJ, Bauer, LS, Abell, KJ, Ulyshen, MD, and RG Van Driesche. 2015. Population dynamics of an invasive forest insect and associated natural enemies in the aftermath of invasion: implications for biological control. J Appl Entomol. 52: 1246-1254.

Fahrner, SJ, JP, Lelito, Blaedow, K, Heimpel, GE, and BH Aukema. 2014. Factors affecting the flight capacity of *T. planipennisi* (Hymenoptera: Eulophidae), a classical biological control agent of *Agrilus planipennis* (Coleoptera: Buprestidae). Environ Entomol. 43: 1603-1612.

Jennings, DE, Duan, JJ, Bean, D, Gould, JR, Rice, KA, and PM Shrewsbury. 2016. Monitoring the establishment and abundance of introduced parasitoids of emerald ash borer larvae in Maryland, U.S.A. Biological Control. 101: 138-144.

Johnson, TD, Lelito, JP, Pfammatter, JA, and KF Raffa. In press. Evaluation of tree mortality and parasitoid recoveries on the contiguous western invasion front of emerald ash borer. Agric For Entomol. http://dx.doi.org/10.111/afe.12164.

Ulyshen, MD, Duan, JJ, and LS Bauer. 2010. Interactions between *Spathius agrili* (Hymenoptera: Braconidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), larval parasitoids of *Agrilus planipennis* (Coleoptera: Buprestidae). Biological Control 52: 188-193.

Wang, X-Y, Jennings, DE, and JJ Duan. 2015. Trade-offs in parasitism efficiency and brood size mediate parasitoid co-existence, with implications for biological control of the invasive emerald ash borer. J Appl Ecol. 52: 1255-1263.

Yang, S, Duan, JJ, Lelito, J, R Van Driesche. 2013. Multiparasitism by *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) and *Spathius agrili* (Hymenoptera: Braconidae): Implication for biological control of the emerald ash borer (Coleoptera: Buprestidae). 65: 118-123.

FraxiProtec™ a new biological way to control EAB populations

Réjean Bergevin

G.D.G. Environnement Ltée, 430 St-Laurent Street, Trois-Rivières, Quebec, G8T6H3

Abstract

FraxiProtec™ solution is a new biological way of controlling EAB populations. The system uses an autodissemination device to release a pathogenic fungus into the adult EAB population. We will present the new technology along with the preliminary results of the 2017 research projects conducted in 12 municipalities in the vicinity of Montreal. These research projects are part of the PMRA registration process. The research will be carried over three years and will lead to the most efficient methodology which will be used for the directions on the pesticide label**.**

Résumé

FraxiProtec^{MC} est un nouveau système de lutte biologique contre l'agrile du frêne. Grâce à un dispositif d'autodissémination, il répand un champignon pathogène parmi les agriles adultes. Nous présenterons aujourd'hui cette nouvelle technologie ainsi que les résultats préliminaires de projets pilotes réalisés en 2017 dans 12 municipalités de la grande région de Montréal. Ces projets de recherche sur trois ans, qui s'inscrivent dans le processus d'homologation de l'ARLA, permettront d'établir la méthodologie la plus efficiente, qui servira de prescription pour l'étiquette du pesticide.

The FraxiProtec™ solution

The solution is deployed through an assisted autodissemination device comprised of a fungal coated pouch of the active ingredient which is placed in a contamination chamber high up in an infected tree. During the EAB flight period, adults get attracted by the control device and become infected by the fungus. Contaminated males and females circulate in the surrounding trees and become vectors of the deadly fungal disease.

Efficacy

Both laboratory and field studies have demonstrated that FraxiProtec™ is an effective solution in controlling EAB adults. EAB adults that come into contact with the conidia (asexual, non-motile spore of a fungus) at the pouch surface were successfully contaminated, resulting in a mortality rate of up to 100% within 5 days. Current research has also demonstrated promising results of horizontal transmission of the pathogen from male to female during the mating period. One of the major aspects of the effectiveness of FraxiProtec™ is that it contributes to a significant reduction in oviposition in the surrounding area where the device is deployed. These aspects make FraxiProtec™ a very promising control tool since it directly impacts the adult EAB population where it is set up and indirectly allows the ash trees to better resist larvae invasion on an ongoing basis.

Safety information

The FraxiProtec™ active ingredient is a fungus, *Beauveria bassiana*, isolate CFL-A. After many lab tests, the efficacy of that isolate against EAB has been demonstrated. Furthermore, the fungus does not represent a threat to other insects since the active ingredient is hidden in the contamination chamber and not sprayed in the environment. There is no impact on Hymenoptera (bees and other insects of that order). Lab experiments have been conducted to document its safety. The safe use of *B. bassiana* is widely known and accepted in organic farming with documentation supporting the safety of its use. There is extensive literature demonstrating how it poses no harm to mammals, fish, humans and other elements of the environment. Pertinent literatures and commentary on these aspects can be provided upon request. FraxiProtec™ is currently undergoing an EPA registration process and no issues have been raised so far.

Research project

The purpose of the research project is to generate additional information on the effectiveness of the FraxiProtec™ solution in different environmental conditions. It will also provide potential users with the appropriate information related to the deployment site distribution pattern required for obtaining optimal effectiveness in EAB population reduction.

Proposed protocol

The actual protocol that has been set for all participating municipalities are the following:

1-Parks or street alignment. At least 30 trees are required in a park to show appropriate results. The ash trees must have a DBH of at least 15 cm. Street scape trees also require more than 30 ash trees with similar characteristics as those in the park scenario. Every ash tree in the sample is georeferenced and characterised using different physical traits. Any available information on EAB trapping results or branch sampling is helpful in selecting potential trial sites and designing the control device network. The final selection of trees for the test(s) are done on site in collaboration with the relevant operation managers.

2-Assisted autodissemination device and prism sticky traps. The deployment requires a network of 15 infected ash trees being on the test site. Each deployment site will have 15 green sticky traps. The installation of the control device network would start at the beginning of the EAB flight period. Under your regional weather conditions, it would most likely be between mid-May to early July. Fungal coated-pouches will be changed after 2 weeks. This procedure will assure a maximum efficacy of the assisted autodissemination device. During the peak flight period, EAB adults on the green sticky traps will be collected at the same time. Communities can deploy more than one control device network for testing.

Analysis is performed on all collected EAB. A molecular analysis will also be applied to establish a causative relationship between the fungus CFL-A and the EAB disease observed on collected samples. Progress reports are part of the information available to the participating municipalities.

Results:
Season 2017 is the first of three year experiment

-FraxiProtec™ shows an average of 32% contamination of the EAB that had contact with the fungi
-Contamination has been observed as far as 150 meters from the network protocol.
-Contamination is effective in low EAB population as well as in very high.
-Preliminary EAB survival table analysis show that FraxiProtec™ has a real potential for significant oviposition reduction.

Participating municipalities;
Boucherville, Contrecoeur, Repentigny, Granby, Québec City, National Parks Commission, Montreal Sud-Ouest, Mont-Royal Park, Beaconsfield, Pointe-Claire, Laval, Rosemère.

Fonctionnement de FraxiProtec^{MC}

Le système repose sur un dispositif d'autodissémination assistée comprenant une pochette imprégnée d'un champignon qui agit comme ingrédient actif, qui est placée dans une chambre de contamination près de la cime de l'arbre contaminé. Ce dispositif attire les agriles adultes en période de vol, qui sont alors contaminés par le champignon. Les femelles et les mâles contaminés deviennent ainsi, en circulant dans les arbres voisins, les vecteurs d'une maladie fongique mortelle.

Efficacité

Des études en laboratoire et sur le terrain ont démontré l'efficacité de FraxiProtec^{MC} comme système de lutte contre l'agrile du frêne adulte. En effet, les agriles adultes en contact avec les conidies (spores non mobiles assurant la reproduction asexuée du champignon) à la surface du sachet sont bel et bien contaminés : le taux de mortalité après cinq jours peut même atteindre 100 %. Les travaux en cours ont par ailleurs produit des résultats encourageants quant à la transmission horizontale du champignon pathogène du mâle à la femelle pendant l'accouplement. L'efficacité de FraxiProtec^{MC} réside notamment dans la réduction considérable de l'oviposition observée dans les environs du dispositif. FraxiProtec^{MC} semble donc très prometteur, puisqu'il agit directement sur la population d'agriles adultes près du système et améliore indirectement la résistance des frênes aux infestations de larves.

Innocuité

L'ingrédient actif de FraxiProtec^{MC} est l'isolat CFL-A du champignon *Beauveria bassiana*, dont l'efficacité contre l'agrile du frêne a été démontrée lors de nombreux essais en laboratoire. Par ailleurs, le champignon ne pose aucun danger pour les autres insectes, puisque l'ingrédient actif est dissimulé dans une chambre de contamination au lieu d'être pulvérisé dans l'environnement. Les hyménoptères (abeilles et autres insectes du même ordre) ne sont donc aucunement touchés. L'innocuité de *B. bassiana* a été démontrée en laboratoire, et plusieurs sources confirment que ce champignon peut être utilisé sans risque dans les cultures biologiques, où il est très répandu. De nombreuses études montrent qu'il est sans danger pour les mammifères, les poissons, les humains et d’autres éléments de l’environnement. Des documents et des analyses à cet effet peuvent être fournis sur demande. FraxiProtec^{MC} est en cours d’homologation par l’Agence américaine de protection de l’environnement (EPA), qui n’a signalé aucun problème à ce jour.

Projets pilotes

Les projets pilotes servent à générer des données supplémentaires sur l’efficacité de FraxiProtec^{MC} dans différentes conditions ambiantes. Ils aideront également les utilisateurs potentiels à déterminer le meilleur endroit où installer les dispositifs sur le site visé pour obtenir une réduction optimale de la population d’agriles du frêne.

Protocole proposé

Voici le protocole établi pour toutes les municipalités participantes :

1) Parcs ou bordures de rue. Pour obtenir des résultats satisfaisants dans un parc, il faut au moins 30 frênes ayant un diamètre à hauteur de poitrine d’au moins 15 cm. Un minimum de 30 frênes présentant un diamètre comparable est également nécessaire pour les aménagements en bordure de rue. Chaque frêne de l’échantillon est géoréférencé et caractérisé selon différents traits physiques. Toute donnée existante issue de pièges à agriles ou d’échantillons de branches est prise en compte dans le choix des sites potentiels et la conception du réseau de dispositifs. Le choix des arbres qui serviront au projet est arrêté sur place, de concert avec les responsables concernés.

2) Dispositif d’autodissémination assistée et pièges prismes collants. Le projet pilote doit être déployé dans un endroit comportant au moins 15 frênes contaminés. Chaque site de déploiement reçoit 15 pièges collants verts. L’installation du réseau de dispositifs débute lorsque les agriles commencent à voler, généralement entre la mi-mai et le début de juillet selon les conditions météorologiques locales. Les sachets enduits du champignon sont remplacés après deux semaines pour maximiser l’efficacité du dispositif d’autodissémination assistée. Au plus fort de la période de vol, les agriles adultes capturés sur les pièges collants verts sont recueillis. Une même municipalité peut déployer plusieurs réseaux de dispositifs dans le cadre du projet.

Tous les agriles recueillis sont analysés. Une analyse moléculaire est également effectuée pour établir une causalité entre le champignon CFL-A et la maladie observée chez les insectes recueillis. Les municipalités participantes reçoivent entre autres des rapports périodiques.

Résultats

La saison 2017 était la première de l’expérience sur trois ans.

– FraxiProtec^{MC} produit un taux de contamination moyen de 32 % chez les agriles adultes en contact avec le champignon.
– Des agriles contaminés ont été observés jusqu’à 150 mètres du réseau de dispositifs.
– La contamination est efficace autant dans les petites populations d’agriles que dans les très grandes.

– L’analyse préliminaire de la table de survie de l’agrile montre que FraxiProtec^{MC} présente un potentiel réel de réduction considérable de l’oviposition.

Municipalités et organisations participantes
Boucherville, Contrecœur, Repentigny, Granby, Québec, Commission des champs de bataille nationaux, Montréal – arrondissement du Sud-Ouest, parc du Mont-Royal, Beaconsfield, Pointe-Claire, Laval, Rosemère

A hint left by mountain pine beetle on anatomical defenses of lodgepole pine trees: larger resin ducts enhance tree resistance

Shiyang (Violet) Zhao and Nadir Erbilgin

Department of Renewable Resources, University of Alberta

Abstract

The mountain pine beetle (*Dendroctonus ponderosa*) has caused a wide range of tree mortality in lodgepole pine (*Pinus contorta* var. *latifolia*) forests in western Alberta since 2006. Besides beetle-killed pine trees, there are also a low number of live lodgepole pine trees in post-outbreak pine stands. These residual trees will face potential attacks from additional disturbances such as forest pathogens and other pest insects. Understanding the mechanism underlying the survival of residual trees from mountain pine beetle outbreaks will help us to predict the future of lodgepole pine forests following insect outbreaks. To address the issue, we sampled 140 beetle-killed and 213 residual trees from 31 sites with varying degrees of beetle-caused mortality and compared their growth and resin duct characteristics. Among residual trees, we categorized them into three groups: healthy, declining (trees showed symptoms or signs of agents other than mountain pine beetle), and survived trees (trees attacked by mountain pine beetle, but survived). The several major findings were: (1) no differences were found on average radial growth between beetle-killed trees and residual trees within 10-year preceding outbreak; (2) beetle-killed trees produced a greater number of xylem resin ducts but the size of individual duct was smaller than those in residual trees; (3) survived trees responded to outbreaks the fast and the most, which expressed lower growth rate, higher resin duct production, density and relative resin duct area, versus healthy trees seldom respond to beetle outbreaks all the time; (4) healthy trees had larger resin ducts than declining trees, but not different from survived trees. Through our research we determined that the size of resin ducts may play a more crucial role than the number of resin ducts on the survival of pine trees not only during the mountain pine beetle outbreaks, but also during the post-outbreak phases. The resulting information suggests that we should also focus on tree anatomical characteristics when we classify trees as resistant to insects and pathogens.

Un indice laissé par le dendroctone du pin ponderosa sur les défenses anatomiques du pin tordu latifolié : des canaux résinifères plus larges renforcent la résistance de l'arbre

Shiyang (Violet) Zhao et Nadir Erbilgin

Département des ressources renouvelable, Université de l'Alberta

Résumé

Depuis 2006, le dendroctone du pin ponderosa (*Dendroctonus ponderosa*) a causé divers taux de mortalité dans les forêts de pins tordus latifoliés (*Pinus contorta* var. *latifolia*) de l'ouest de l'Alberta. Outre les pins tués par l'insecte, on trouve aussi un faible nombre de pins tordus latifoliés vivants dans les peuplements de pin après l'infestation. Ces arbres rémanents pourraient subir l'attaque d'autres facteurs perturbateurs comme des pathogènes forestiers et autres insectes ravageurs. Comprendre le mécanisme qui sous-tend la survie des arbres rémanents après des infestations de dendroctone du pin ponderosa nous aidera à prédire l'avenir des forêts de pins tordus latifoliés après des infestations d'insectes. Pour étudier la question, nous avons échantillonné 140 arbres tués par l'insecte et 213 arbres rémanents dans 31 sites qui avaient subi divers degrés de mortalité causée par le dendroctone, et nous avons comparé leur croissance et les caractéristiques de leurs canaux résinifères. Nous avons réparti les arbres rémanents dans trois groupes : arbres sains, arbres dépérissants (arbres montrant des symptômes ou des signes provoqués par des agents autres que le dendroctone du pin ponderosa) et arbres survivants (arbres attaqués par le dendroctone du pin ponderosa, mais ayant survécu). Les principales conclusions sont les suivantes : 1) aucune différence n'a été observée dans la croissance radiale moyenne entre les arbres tués par le dendroctone et les arbres rémanents dans les 10 ans précédant l'infestation; 2) les arbres tués par le dendroctone ont produit plus de canaux résinifères de xylène, mais la taille des canaux individuels était plus petite que dans les arbres rémanents; 3) les arbres survivants réagissaient aux infestations le plus rapidement et en plus grand nombre, ce qui se traduisait par un taux de croissance plus lent, une production de canaux résinifères plus élevée, une densité et une zone de canaux résinifères relative plus élevées comparativement aux arbres sains, qui réagissent rarement aux infestations de l'insecte à chaque fois; 4) les arbres sains avaient des canaux résinifères plus grands que les arbres dépérissants, mais ne différaient pas des arbres survivants à cet égard. Grâce à notre recherche, nous avons déterminé que la taille des canaux résinifères pourrait jouer un rôle plus important que le nombre de canaux résinifères sur la survie des pins, non seulement durant les infestations de dendroctone du pin ponderosa, mais aussi durant les phases post-infestation. Les données obtenues semblent montrer que nous devrions nous concentrer sur les caractères anatomiques des arbres quand nous les classons comme des arbres résistants aux insectes et aux pathogènes.

Background

Mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins [Coleoptera: Curculionidae]) populations have increased in Alberta since they have crossed the Rocky Mountains and colonized pine forests in western Alberta in 2006. Millions of mature lodgepole pine (*Pinus contorta* var. *latifolia*) trees have been killed, leaving only a low number of residual overstory mature lodgepole pine trees and non-host tree species, such as spruce and *Populus*, remaining in beetle-killed stands (Taylor et al. 2006). The reason behind the survival of residual trees remains unknown. The change in nutrient cycling, underground soil communities, forest structure and other factors in post-MPB stands can certainly affect the health condition of residual pine trees and potentially change their suitability to other insects and tree pathogens (McIntosh and Macdonald 2013b, Cigan et al. 2015, Karst et al. 2015, Pec et al. 2015). Following forest disturbances, it is very common that insects and pathogens can take advantage of dead trees and may cause additional disturbances on the neighbouring live trees (Cerezke 1977, 1994, Sibold et al. 2007, Boone et al. 2008, Dhar et al. 2016). Considering the low pine recruitment and regeneration in MPB-affected stands (Astrup et al. 2008, McIntosh and Macdonald 2013a), the residual overstory pine trees might be the only seed source for the future pine regeneration in Alberta. Thus, studies on the future health conditions of residual pine trees in these disturbed landscapes are urgently needed. While chemical compounds in tree phloem can vary between resistant and susceptible lodgepole pine trees (Erbilgin et al. 2017), anatomical defense represents tree defense capacities over a longer time. Here we analyzed patterns in anatomical defenses and tree increment growth to understand 1) how residual trees survived the MPB outbreak, 2) whether the outbreak altered growth/defense relationships, and 3) identify relationships with current health conditions of residual trees.

Methodology

We identified 31 sites in post-MPB stands in western Alberta, Canada. At each site we established 2 plots (n = 61 plots). In sites, we sampled the wood from 140 beetle-killed trees using wedges and 213 residual trees using increment cores at breast height (1.4 m). Samples were collected in 2016. All trees had a diameter at breast height (DBH) over 15 cm and had a crown class of intermediate, codominant or dominant. We confirmed MPB-killed tree by the presence of MPB attack signs such as beetle entrance holes (pitch tubes) and extensive beetle galleries under bark. The sampled residual trees were classified into three groups based on tree health conditions and included 76 healthy, 62 declining due to biotic agents other than MPB, and 75 trees that survived MPB with signs of attack but appeared vigorous.

A master chronology was developed based on the ring width series of cores from healthy residual trees. The strength of cross-dating was confirmed by COFECHA (Grissino-Mayer 2001). This master chronology was used to justify any missing or false rings on cores before any calendar year was assigned to each ring. The year of death for beetle-killed trees was adjusted by visual cross-dating due to lack of tree rings on wedges. Since most sites experienced beetle mortality at multiple years, the start of an outbreak was considered as the year of the earliest death caused by MPB that occurred in a site.

A sampling area of 0.9 mm for cores and wedges was used to count and measure resin ducts. The resin duct characteristics that were measured from the sampling area included annual resin duct production (no. year^{-1}) and individual resin duct area (mm^{2}), which were determined by ImageJ (version 1.50i, National institutes of Health, USA. Two standardized variables of resin duct characteristics were also calculated on each core or wedge: resin duct density (no. mm^{-2} year^{-1}) and relative resin duct area (% annual ring). Annual growth rate and all resin duct measures were averaged over for certain time periods.

Result and discussion

There were no differences on mean growth rate between beetle-killed and residual trees within the first 10-year preceding outbreak. However, beetle-killed trees produced 127-132% more xylem axial resin ducts than residual trees, but the size of individual duct in residual trees was 22% larger than those in MPB-killed trees. Both resin duct density and relative resin duct area in beetle-killed trees were higher (105-131% and 59-79% respectively) than residual trees, but the two characteristics were greatly affected by the massive resin duct production in beetle-killed trees. Therefore, both standardized variables of resin duct development might not be good perimeters of predicting tree survivals during beetle outbreaks. Meanwhile, trees that survived attack responded to MPB outbreaks in the stand with a lower mean growth rate in the first 3- and 5-year following outbreak, higher mean resin duct production for up to 10-year after outbreak, and higher resin duct density and relative resin duct area in both 5-year and up to 10-year following outbreak. Declining trees had higher resin duct density and relative resin duct area in the total growth period following outbreak, while healthy trees only showed increased resin duct production in the total growth period after MPB outbreak. We also found that healthy trees had larger individual resin ducts than declining trees, but did not differ from trees that survived attacks.

Accomplishment

All samples have been collected and processed, and the data has been analysed. The manuscript is in preparation.

Acknowledgement

Funding for this research was provided by fRI Research, Alberta innovates and forest resource improvement association of Alberta. Caroline Whitehouse, Devin Letourneau, Ryan Hermanutz, Allison Brown and Geoff Atkinson from Alberta Agriculture and Forestry provided us with help on site selection and permitting. We also want to thank Tolko Industries Ltd., Daishowa-Marubeni International Ltd., Blue Ridge Lumber Inc., Weyerhaeuser Company Ltd. and Canadian Forest Products Ltd. who gave us permission to set up sites in their forest management units.

Reference

Astrup, R., K. D. Coates, and E. Hall. **2008**. Recruitment limitation in forests: Lessons from an unprecedented mountain pine beetle epidemic. For. Ecol. Manage. 256: 1743–1750.

Boone, C. K., D. L. Six, and K. F. Raffa. **2008**. The enemy of my enemy is still my enemy: Competitors add to predator load of a tree-killing bark beetle. Agric. For. Entomol. 10: 411–421.

Cerezke, H. F. 1977. Characteristics of damage in tree-length white spruce logs caused by the white-spotted sawyer, Monochamus scutellatus. Can. J. For. Res. 7: 232–240.

Cerezke, H. F. 1994. Warren rootcollar weevil, Hlobius warreni Wood (Coleoptera: curculionidae), in Cnada: ecology, behavior, damage relationships, and management. Can. Entomol. 126: 1383–1442.

Cigan, P. W., J. Karst, J. F. Cahill, A. N. Sywenky, G. J. Pec, and N. Erbilgin. **2015**. Influence of bark beetle outbreaks on nutrient cycling in native pine stands in western Canada. Plant Soil. 390: 29–47.

Dhar, A., L. Parrott, and C. D. B. Hawkins. **2016**. Aftermath of mountain pine beetle outbreak in british columbia: stand dynamics, management response and ecosystem resilience. Forests. 7: 1–19.

Erbilgin, N., J. A. Cale, A. Hussain, G. Ishangulyyeva, G. J. Klutsch, A. Najar, and S. Zhao. **n.d.** Weathering the storm: How lodgepole pine trees survive mountain pine beetle outbreaks. Oecologia.

Hood, S., and A. Sala. **2015**. Ponderosa pine resin defenses and growth: Metrics matter. Tree Physiol. 35: 1223–1235.

Kane, J. M., and T. E. Kolb. **2010**. Importance of resin ducts in reducing ponderosa pine mortality from bark beetle attack. Oecologia. 164: 601–609.

Karst, J., N. Erbilgin, G. J. Pec, P. W. Cigan, A. Najar, S. W. Simard, and J. F. Cahill. **2015**. Ectomycorrhizal fungi mediate indirect effects of a bark beetle outbreak on secondary chemistry and establishment of pine seedlings. New Phytol. 208: 904–914.

McIntosh, A. C. S., and S. E. Macdonald. **2013a**. Potential for lodgepole pine regeneration after mountain pine beetle attack in newly invaded Alberta stands. For. Ecol. Manage. 295: 11–19.

McIntosh, A., and S. Macdonald. **2013b**. Short-term resistance of ecosystem properties and processes to simulated mountain pine beetle attack in a novel region. Ecosphere. 4: 1–28.

Pec, G. J., J. Karst, A. N. Sywenky, P. W. Cigan, N. Erbilgin, S. W. Simard, and J. F. Cahill. **2015**. Rapid increases in forest understory diversity and productivity following a mountain pine beetle (Dendroctonus ponderosae) outbreak in pine forests. PLoS One. 10: 1–16.

Sibold, J. S., T. T. Veblen, K. Chipko, L. Lawson, E. Mathis, and J. Scott. **2007**. Influences of secondary disturbances on lodgepole pine stand development in rocky mountain national park. Ecol. Appl. 17: 1638–1655.

Taylor, S. W., A. L. Carroll, R. I. Alfaro, and L. Safranyik. **2006**. Forest, climate and mountain pine beetle outbreak dynamics in western Canada, pp. 67–94. *In* Les Safranyik, Wilson, B. (eds.), Mt. Pine Beetle A Synth. Biol. Manag. Impacts Lodg. Pine. Pacific Forestry Centre, Victoria, British Columbia, Canada.

Investigating the effects of gut-associated microbiota on the growth of spruce budworm

Melbert Schwarz[1], Daniel Kneeshaw[1],Steven Kembel[1]
[1] Département des sciences biologiques, Université du Québec à Montréal, Montréal (Québec) Canada H2X 1YA

Microbial communities have been shown to play an important role for host health in mammals, especially humans. It is thought that microbes could play an equally important role in other animals such as insects. A growing body of evidence seems to support this, however most of the research effort in understanding host-microbe interactions in insects has been focused on a few well studied groups such as bees and termites. The effects of host-associated microbial communities in *Lepidoptera* remains relatively unstudied. We studied the effects of the gut-associated microbial community in the eastern spruce budworm, *Choristoneura fumiferana*, an economically important forest pest in eastern Canada by studying the effects of an antibiotic treatment and diet on larval *C. fumiferana* growth. We show that the antibiotic treatment was sufficient to cause shifts in the microbial communities associated with balsam fir and black spruce foliage as well as in the guts of *C. fumiferana* larvae under laboratory conditions. Contrary to our expectations, we found that the observed antibiotic treatment did not significantly alter larval growth. We did find, however, that under laboratory conditions *C. fumiferana* larvae performed better when feeding on black spruce foliage compared to balsam fir which is widely understood to be the preferred food of *C. fumiferana* due to phenology. Finally we show that although most bacteria originating on foliage appear to be transient through the gut, there is evidence that some bacteria may thrive better in the *C. fumiferana* gut. However, the functional role that these bacteria fill within the *C. fumiferana* gut remains unclear.

Keywords : Spruce budworm, microbiome, host-microbe interactions, lepidoptera

Les communautés microbiennes jouent un rôle important pour la santé de leurs hôtes chez les mammifères, en particulier pour les humains. Les microorganismes pourraient jouer un rôle important pour les autres animaux, dont les insectes. Plusieurs études ont montré l'importance du microbiome pour les insectes, mais la majorité des recherches sur les interactions entre les insectes et la vie microbienne s'est focalisée sur certains groupes taxonomiques, dont les abeilles et les termites. Les effets des communautés microbiennes sur leurs hôtes chez les lépidoptères sont cependant encore mal compris. Dans le cadre de notre projet, nous avons étudié les effets du microbiote associés à l'intestin de la tordeuse des bourgeons de l'épinette (*Choristoneura fumiferana*), un insecte ravageur important au Canada. Nous avons quantifié en laboratoire les effets des traitements antibiotiques et les différentes diètes sur la croissance et la santé des larves de *C. fumeriferana*. Nos résultats ont montré que les traitements antibiotiques n'avaient pas modifié la croissance des larves. La croissance des larves de *C. fumeriferana* était augmentée lorsque leur diète était composée d'épinette noire par rapport à lorsqu'elle était composée de sapin baumier, malgré le fait que le sapin baumier est supposé être la diète préférée de *C. fumeriferana* en raison de sa phénologie. Finalement, nous avons montré que la majorité des bactéries dans l'intestin de la tordeuse sont des bactéries transitoires originaires des feuilles, mais il y a également certains taxons bactériens avec une bonne performance dans l'intestin de la tordeuse. Le rôle fonctionnel de ces bactéries reste inconnu.

Introduction:

Host-microbe interactions have been studied extensively in humans and it is largely accepted that microbes, particularly those associated with the gut, are critical for human health. They interact with the immune system and aid in host nutrient acquisition through metabolic pathways. There is evidence to support this assumption in a number of insects as well (Engel and Moran, 2013; Kwong et al., 2017; De Souza et al., 2013; Koch and Schmid-Hempel, 2011; Prado et al., 2010; Rosengaus et al., 2011; Emery et al., 2017; Engel et al., 2012). Gut microbiota have been shown previously to aid in the digestion of hard to process compounds. For example, bacteria in honey bee guts have been shown to aid in the degradation of the pectin found in pollen (Engel et al., 2012) and bacteria have been shown to aid a number of xylophagous insects in the degradation of cellulose (Engel and Moran, 2013). In addition to microbially mediated digestion of food, gut microbes aid some herbivorous insects in processing secondary compounds which would otherwise be toxic (Hammer and Bowers, 2015). Spruce budworm must digest conifer needles which are high in lignin and terpenes and thus symbiosis with bacteria could be beneficial.

That being said, it is also possible for pathogenic bacteria to have a negative interaction with the host. One example of this that is particularly relevant to the spruce budworm system is the interaction of *Bacillus thuringensis* and *Enterococcus faecalis* in lepidopteran species (Caccia et al., 2016; Graf, 2011; Mason et al., 2011). There is also some evidence suggesting that not all insects are nutritionally dependent on microbial communities, for example a study on dung beetles found that the disturbance of the gut microbial community via indirect exposure to antibiotics did not alter host growth (Hammer et al., 2016)

The primary objective of this study was to determine if the gut microbiota associated with spruce budworm larvae influence larval growth. We also sought to quantify the effects that antibiotics would have on gut microbial diversity and community composition. We hypothesized that the eastern spruce budworm must maintain a microbial community in the larval gut to facilitate the digestion of conifer needles and to promote larval growth. We also hypothesized that antibiotic treatment would significantly alter the composition of the spruce budworm gut microbial community and that the use of antibiotics would significantly reduce diversity of the microbial communities associated with diet, spruce budworm guts, and spruce budworm frass. Thus we further hypothesized that the disturbance of microbial communities with antibiotics would negatively influence spruce budworm survival and growth. Finally we hypothesized that composition of the spruce budworm gut microbial community would be significantly different between fast and slow growing larvae.

Methods:

We raised spruce budworm larvae in a sterile environment in order to study their associated microbial community. Spruce budworm larvae were raised on black spruce (*Picea mariana*) foliage, balsam fir (*Abies balsamea*) foliage, or artificial diet (McMorran diet) from the second instar to the sixth instar. Prior to the start of experimental treatments, all larvae were fed on the artificial diet for one week. Half of the larvae feeding on foliage were fed foliage that was treated with antibiotics. All of the artificial diet contained antibiotics. Throughout the course of the experiment, larval growth was tracked by recording the mass of each larva every two days. Microbial DNA was extracted from spruce budworm guts as well as a random subset of foliage and frass. The 16S rRNA gene was sequenced using an illumina MiSeq and sequences were binned into operational taxonomic units (OTUs) based on 97% sequence similarity. Using OTUs we were able to calculate the relative abundance of microbial taxa associated with the foliage, budworm guts, and budworm frass.

Our analysis was twofold; first using the relative abundances of bacterial taxa (OTUs) to quantify the effects of antibiotic treatment on the spruce budworm microbial community and second by using the growth rate of individual larvae as a proxy for health to quantify the importance of the gut microbial community to the eastern spruce budworm. In order to quantify the effects of antibiotic treatment on the spruce budworm microbial community we calculated Shannon diversity of bacterial taxa to test for differences in microbial diversity. We tested for differences in community composition among diet types and antibiotic treatment using permutational multivariate ANOVA (PERMANOVA) and visualized any difference in community composition using non-metric multidimensional scaling (NMDS). For these analyses we used two complementary distance measures, UniFrac and unweighted UniFrac distance. UniFrac distance is based on the phylogenetic relatedness of taxa and weighted UniFrac is weighted by the relative abundances of taxa. We chose to use both distance measures because they inform us about different aspects of the community, for example unweighted UniFrac informs us about the overall membership of the community while weighted UniFrac is sensitive to changes in the relative abundance of taxa making it more sensitive to rare taxa.

To better understand the role of the gut microbial community on spruce budworm health we first compared the weights of larvae over time using a mixed effects model with time, diet type, and antibiotic treatment along with their interactions as fixed effects and using time nested within individual samples as a random effect to control for repeated measures. We also tested for differences in larval survival using a logistic regression with survival as a binary response variable and antibiotic treatment, diet, and their interaction as main effects. As an additional analysis we compared the composition of the microbial communities associated with the fastest and slowest growing larvae (defined as the top 25% and bottom 25% of growth rates respectively) in each treatment group using the methods described above.

Results and discussion:

We observed changes in microbial diversity due to both diet type (spruce versus fir versus artificial diet) and antibiotic treatment in foliage and gut samples suggesting that the antibiotic treatment applied to the food was sufficient to alter the microbial community in the spruce budworm gut. We also observed a significant effect of antibiotic treatment on the composition of foliage- and gut-associated microbial communities (Fig 1). Contrary to our hypothesis we observed no significant effect of antibiotic treatment on either larval growth (Fig 2) or survival (Fig 3). There was, however, a significant effect of foliage type on larval growth. Larvae feeding on black spruce foliage grew more than larvae feeding on balsam fir foliage (Fig 2). Because all larvae were fed artificial diet for the first week of the experiment, larvae feeding on artificial diet were used as a control group for microbiome composition and were not included in the growth and survival analysis due to the differences in nutritional quality between foliage and the artificial diet.

Our findings suggest that spruce budworm does not rely on the aid of gut-associated bacteria to facilitate the digestion of conifer needles or the growth of the insect. Furthermore we were unable to detect differences in the microbial community of larvae in the top 25% of growth or the bottom 25% of growth, thus we were unable to identify any bacteria which may have a negative impact on spruce budworm health. This study only tested the effects of the whole microbial community on spruce budworm growth so it is possible that there were unmeasured effects of the microbial community on host health. Further testing of how gut-associated microbes affect spruce budworm development or parasitism rates could be useful avenues of research in terms of spruce budworm control. That being said, other studies have suggested that lepidopteran larvae in general do not maintain a resident microbial community and that the tobacco hornworm *Manduca sexta* does not require gut-microbes to promote growth (Hammer et al., 2017; Whitaker et al., 2016). Because of the number of lepidopteran species that are considered pests, this research could have significant implications in terms of the control of various lepidopteran pest species in that control options targeting the host-associated bacterial community may not be effective. Further research could also explore the effects of microbial communities from across the range of spruce budworm and its hosts or to compare the microbial communities associated with areas varying in spruce budworm outbreak severity.

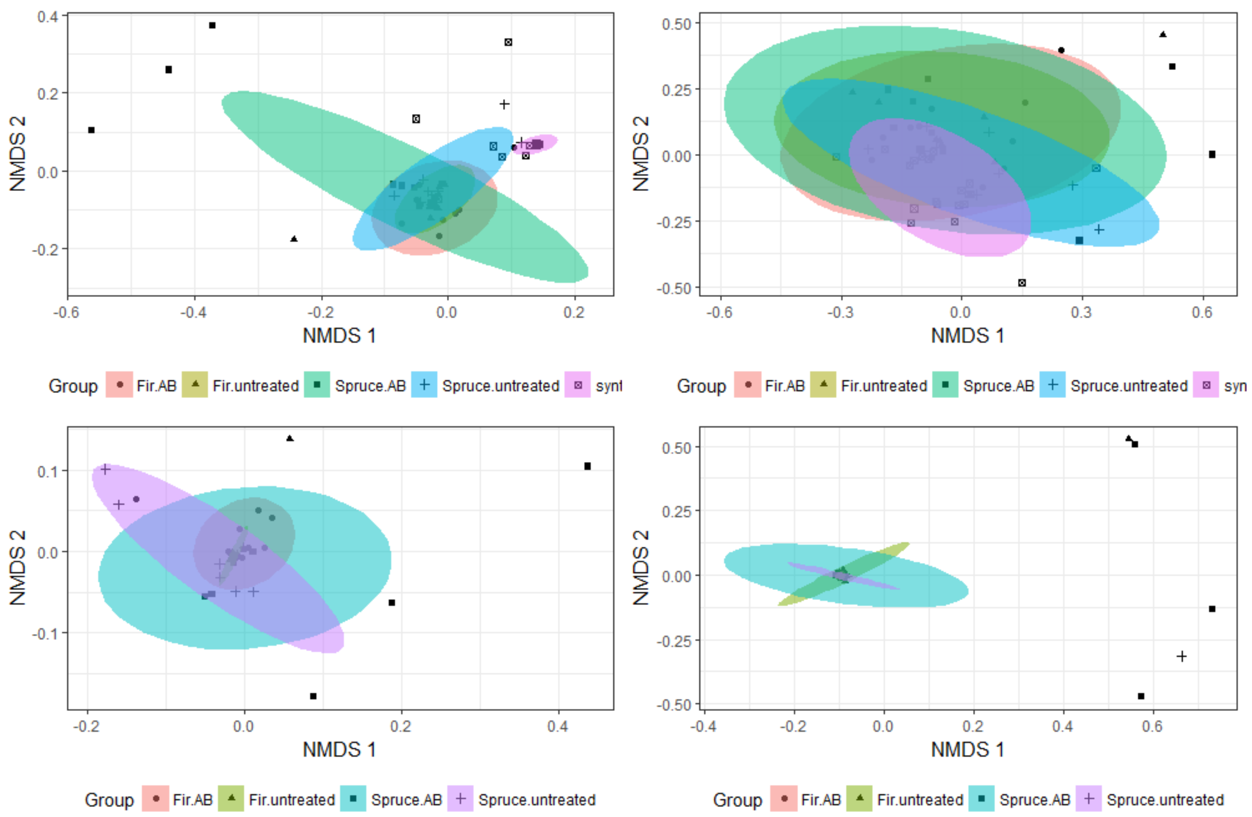


Figure 1. NMDS ordinations of gut-associated microbial communities based on weighted and unweighted UniFrac distances. (A: all guts weighted UniFrac stress =0.07, B: All guts UniFrac stress =0.21, C: guts of larvae feeding on foliage weighted UniFrac stress =0.07, D: guts of larvae feeding on foliage unweighted UniFrac stress =0.10). Ellipses represent 95% confidence intervals around samples from different treatments (Fir.AB = antibiotic treated fir, Spruce.AB = antibiotic spruce, Fir.untreated = untreated fir, and Spruce.untreated = untreated spruce).

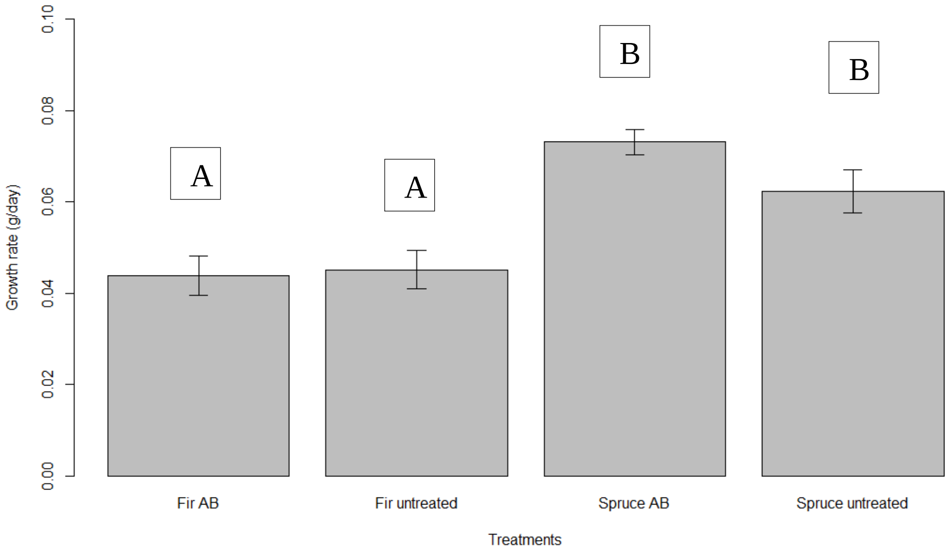


Figure 2. Growth rate (grams/day, ± S.E) of spruce budworm larvae among different diets (spruce versus fir foliage) and antibiotic treatments (AB = antibiotic treated). Letters indicate treatment combinations that differed significantly ($p < 0.05$) according to a Tukey's Honest Significant Difference post-hoc test, based on a mixed model.

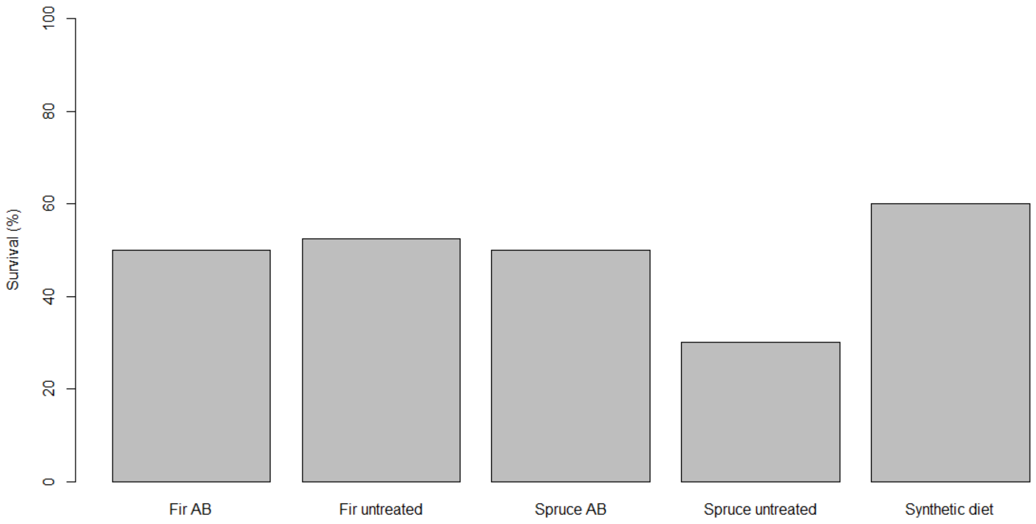


Figure 3. Percent survival of spruce budworm larvae among treatment diets (spruce versus fir foliage and synthetic diet) and antibiotic treatments (AB = antibiotic treatment).

References:

Caccia S, Di Lelio I, La Storia A, Marinelli A, Varricchio P, Franzetti E, et al. (2016). Midgut microbiota and host immunocompetence underlie Bacillus thuringiensis killing mechanism. Proc Natl Acad Sci 113: 9486–9491.

Emery O, Schmidt K, Engel P. (2017). Immune system stimulation by the gut symbiont Frischella perrara in the honey bee (Apis mellifera). Mol Ecol 26: 2576-2590, doi: 10.1111/mec.14058.

Engel P, Martinson VG, Moran NA. (2012). Functional diversity within the simple gut microbiota of the honey bee. Proc Natl Acad Sci USA 109: 11002-11007, doi: 10.1073/pnas.1202970109

Engel P, Moran NA. (2013). The gut microbiota of insects - diversity in structure and function. FEMS Microbiol Rev 37: 699–735.

Graf J. (2011). Shifting paradigm on Bacllus thuringiensis toxin and a natural model for enterococcus faecalis septicemia. MBio 2: 1–2.

Hammer TJ, Bowers MD. (2015). Gut microbes may facilitate insect herbivory of chemically defended plants. Oecologia 179: 1–14.

Hammer TJ, Fierer N, Hardwick B, Simojoki A, Slade E, Taponen J, et al. (2016). Treating cattle with antibiotics affects greenhouse gas emissions, and microbiota in dung and dung beetles. Proc R Soc B Biol Sci 283: 20160150, doi: 10.1098/rspb.2016.0150.

Hammer TJ, Janzen DH, Hallwachs W, Jaffe SL, Fierer N. (2017). Caterpillars lack a resident gut microbiome. PNAS 249: 84–89.

Koch H, Schmid-Hempel P. (2011). Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. Proc Natl Acad Sci U S A 108. e-pub ahead of print, doi: 10.1073/pnas.1110474108.

Kwong WK, Mancenido AL, Moran NA. (2017a). Immune system stimulation by the native gut microbiota of honey bees. R Soc Open Sci 4: 170003, doi: 10.1098/rsos.170003.

Mason KL, Stepien T a, Blum JE, Holt JF, Labbe NH, Rush JS, et al. (2011). From Commensal to Pathogen: Translocation of. 2: 1–7.

Prado SS, Hung KY, Daugherty MP, Almeida RPP. (2010). Indirect effects of temperature on stink bug fitness, via maintenance of gut-associated symbionts. Appl Environ Microbiol 76:1261-1266, doi: 10.1128/AEM.02034-09.

Rosengaus RB, Zecher CN, Schultheis KF, Brucker RM, Bordenstein SR. (2011). Disruption of the termite gut microbiota and its prolonged consequences for fitness. Appl Environ Microbiol 77: 4303–4312 doi: 10.1128/AEM.01886-10.

De Souza DJ, Lenoir A, Kasuya MCM, Ribeiro MMR, Devers S, Couceiro JDC, et al. (2013). Ectosymbionts and immunity in the leaf-cutting ant Acromyrmex subterraneus subterraneus. Brain Behav Immun 28:182-187 doi: 10.1016/j.bbi.2012.11.014.

Whitaker MRL, Salzman S, Sanders J, Kaltenpoth M, Pierce NE. (2016). Microbial communities of lycaenid butterflies do not correlate with larval diet. Front Microbiol 7:1920, doi: 10.3389/fmicb.2016.01920.

GIS analyses of factors influencing spruce budworm outbreak initiation in northern New Brunswick

Mingke Li[1] and David A. MacLean[2]
[1] MF student; [2] Professor;
Faculty of Forestry and Environmental Management, University of New Brunswick,
P.O. Box 4400, Fredericton, NB, E3B 5A3, Canada

Abstract

Many studies have been carried out to determine potential relationships between environmental factors and spruce budworm (Choristoneura fumiferana [Clem.]; SBW) defoliation, tree growth loss or mortality, but few have focused on the influence on SBW populations. The objective of this study was to analyze the effect of four categories of factors (forest composition, climate, topography/location, and site quality) on SBW outbreak initiation in northern New Brunswick. Analyses were mainly based on the second-instar larvae (L2) per branch population data jointly collected by forest industry and NB Department of Energy and Resource Development from 2013 to 2017. GIS analyses, especially spatial overlay analyses, were applied on the geo-referenced datasets using ESRI ArcGIS. Descriptive statistics results suggested that the moderate and higher L2 samples tended to be located in colder area with lower elevation and closer to defoliated areas in Quebec. The outbreak in the Gaspe-Bas St. Laurent region moved south, closer to New Brunswick, over the study period. An autologistic model will be fit to explore the relationships in future.

De nombreuses études ont été menées pour déterminer s'il existe des liens entre les facteurs environnementaux et la défoliation par la tordeuse des bourgeons de l'épinette (Choristoneura fumiferana [Clem.]; TBE), la perte de croissance ou la mort de l'arbre, mais peu se sont penchées sur l'influence des populations de TBE. Le but de l'étude était d'analyser l'effet de quatre catégories de facteurs (structure de la forêt, climat, topographie/emplacement, et qualité du site) sur le début d'une infestation de TBE dans le nord du Nouveau-Brunswick. Les analyses partaient principalement des données sur les populations de larves de deuxième stade (L2) par branche, recueillies conjointement par l'industrie forestière et le ministère du Développement de l'énergie et des ressources du N.-B. entre 2013 et 2017. Les ensembles de données géo-référencées ont fait l'objet d'analyses fondées sur le SIG, en particulier des analyses de superposition spatiale, au moyen du logiciel ArcGIS d'ESRI. Les résultats des statistiques descriptives semblent indiquer que les échantillons modérés de L2 et plus avaient tendance à se situer dans des lieux plus froids, à une élévation moindre, et plus près des régions défoliées du Québec. L'infestation dans la région Gaspésie-Saint-Laurent s'est déplacée vers le sud, plus près du Nouveau-Brunswick, au cours de la période à l'étude. Un modèle autologistique pourra éventuellement explorer les liens.

Introduction

Spruce budworms (*Choristoneura fumiferana* [Clem.]; SBW) are native insects in North America that preferentially feed on the new foliage of balsam fir (*Abies balsamea* [L.] Mill.) and spruce (*Picea spp*. A. Dietr.) trees (MacLean 2005). SBW outbreaks occur every 30 to 40 years (Royama 1984). The current SBW outbreak, which began around 2006 in Quebec, has been expanding to northern New Brunswick since 2013. An increasing number of sampled points with higher second-instar larvae (L2) population levels have been observed in northern New Brunswick from 2013 to 2017 (Healthy Forest Partnership 2017). Thus, we are now at the beginning stage of a new SBW outbreak, and what forest management activities are applied to deal with the present situation will determine the forest dynamics, ecology as well as economic benefits from the forest in subsequent years.

Several studies have analyzed SBW outbreak initiations. Greenbank (1957) studied the outbreaks beginning in 1912 and 1949 in New Brunswick, concluded that larval survival increases in dry and sunny summers, and outbreaks tended to occur when eggs are deposited in dense and mature softwoods. An atlas of SBW defoliation showed that outbreaks in the 1970s in eastern North America typically began in a mix of balsam-fir and hardwood forest (Hardy et al. 1986). A recent study of outbreak initiation in Quebec found that elevation, abundance of balsam fir, distance from previous defoliation and number of days with degree-days >5° were the most significant factors of SBW defoliation initiations (Bouchard and Auger 2014). For western spruce budworm (*Choristoneura occidentalis* Freeman), Magnussen et al. (2004) determined that increasing stand volume, higher current needle biomass, and proximity to the nearest river or nearest defoliation led to increasing likelihood of an onset of SBW defoliation.

The focus of this research is to determine potential effects of environmental factors on SBW populations from 2013 to 2017, which is the period of outbreak initiation in northern New Brunswick. Based on the results of previous research, four categories of independent variables were hypothesized to potentially influence SBW outbreak initiation: 1) composition of tree species (volume proportion), including host species, i.e. balsam fir and spruce trees, and hardwood; 2) climate conditions (monthly average temperature and monthly cumulative degree-days); 3) topographic and location characteristics (proximity to moderate-severe defoliation areas, elevation, slope, and aspect), and 4) site quality (depth to water and biomass growth index; Hennigar et al. 2017). Some aerial application of insecticides, *Bacillus thuringiensis* variety kurstaki (Btk.), tebufenozide (Mimic), and pheromones has been carried out in an early intervention against spruce budworm research project (Healthy Forest Partnership 2016a) to protect the forest from spruce budworm infestation. Therefore, previous spray treatment was also included as an independent variable.

In addition, a series of calculated global Moran’s I suggested that L2 population observed at one location exhibited more similar values to neighboring locations than those further apart, which is defined as spatial autocorrelation (Sokal et al. 1998). When fitting models for response data like this, it is important to account for spatial autocorrelation in the analysis. Otherwise, the core assumption that “residuals of a model should be independent and identically distributed” will be violated (Dormann et al. 2007). For this reason, autologistic models (Besag 1974, Augustin et al. 1996, 1998), instead of ordinary logistic models, will be fitted to analyze possible relationships between environmental factors and the occurrence of moderate or higher L2 population levels.

Methods

Study area

To include all the sample points with mean L2 populations ≥ 6.5 per branch, the study area was defined as northern New Brunswick, from approximately 64° 30´ to 69° 0´ W, and from 46° 30´ to 48° 0´ N, amounting to 3,705,500 hectares. Within the study area, fir and spruce trees, the host species of SBW, are the largest species groups (Erdle and Ward 2008).

Data sources

SBW L2 data from 2013 to 2017 were provided by Healthy Forest Partnership Early Intervention Strategy (EIS) Research group. Forest industry and the NB Department of Energy and Resource Development (NBERD) jointly collected data from a large number of sample points around New Brunswick: 1136, 1349, 1923, 1649, and 1974 L2 points per year sampled from 2013 to 2017. Each point included one mid-crown branch sampled from each of three balsam fir or three spruce trees (2013-2015), or a mix of fir and spruce which represented the stand condition (2016-2017). NBERD processed the branch samples to determine the mean number of L2 per branch by branch washing (Healthy Forest Partnership 2016b). The average number of L2 per branch was classified into 6 classes: nil 0, trace 0.1-3.5, low 3.5-6.5, moderate 6.5-20.5, high 20.5-40.5, and extreme >40.5 L2 per branch. All L2 data were digitized and geo-referenced as point layers. Data sets of other environmental factors were acquired from various sources, and necessary preprocessing was done before analysis (Table 1).

Table 1. Data sources and related preprocessing for independent variables.

Factor	Data Sources	Preprocessing
Spray treatment	Healthy Forest Partnership Early Inventory Strategy (EIS) group	NA
Forest composition[1]	Enhanced Forest Inventory (EFI); NB Energy and Resource Development	Calculate 1) percent ratios of host species volume (surrogate for foliage biomass) and 2) ratio of host species volume to total volume surrounding each plot within a 20x20m buffer
Climate (Monthly average temperature & cumulative degree-days)	Canadian Climate Normals database; New Brunswick Fire Weather; New Brunswick Agriculture Weather; Ministère du Développement Durable, de l'Environnement et de la Lutte contre les Changements Climatiques (MDDELCC)	Under BioSIM (Régnière et al. 2014): 1) Enquiry daily weather data for NB 2) Transform daily data into monthly data 3) Generate climate data for sample points by interpolation
Proximity to moderate-severe defoliation areas	Annual reports of Québec government	Calculate distances between each sample and the edge of the nearest moderate-severe defoliation pattern
Topography (Elevation, slope, & aspect)	LiDAR Derived Bare Earth Digital Elevation Models; Service New Brunswick	Slope and aspect data sets are generated from DEM, and be smoothed by Focal Statistics
Depth to water	Forest Watershed Research Centre, UNB	NA
Biomass growth index	Site productivity; FORUS Research; Hennigar et al. 2017	NA

[1] This dataset will be available in the near future; related analyses are not included in this report.

GIS analyses

GIS analyses included a series of overlay analyses and summary statistics using ArcGIS. All GIS layers were georeferenced, and L2 data from 2013 to 2017 were analyzed separately against independent variables.

Prior to autologistic regression modeling (see below for details), L2 point layers were converted into grid systems with a 20x20m cell size. A threshold of 6.5 L2 per branch was set to transfer the raw response data to the binary (0/1) data required for logistic regression. Spray treatment data was also converted to binary (treated 0, untreated 1) areas. The values of environmental factors of points within each cell were averaged to generate representative values for each cell.

Statistical analysis

When developing ecological models, ignoring spatial autocorrelation could result in some independent variables appearing to have effects on a response while actually their relationships are not statistically significant (Legendre 1993, Syartinilia and Tsuyuki 2008). Also, if spatial autocorrelation remains in the residuals of a statistical model, it will violate one of the key assumptions of standard statistical analyses that residuals should be independent (Dormann et al. 2007). Therefore, when encountering spatial autocorrelation, autologistic models will be fitted to further understanding of what factors or what combinations of factors have effects on the occurrence of moderate or higher L2 population levels. Autologistic model is a basic logistic model adding in an autocovariate term that is calculated (Augustin et al. 1996, 1998) as:

$$autocov_i = \frac{1}{h_i}\sum_{j \epsilon N_i} \frac{1}{h_{ij}} y_i$$

where h_{ij} is the Euclidean distance between squares *i* and *j* measured from the square center, $h_i = \sum_{j \neq i} \frac{1}{h_{ij}}$ and N_i is the set of neighbors of square *i*. The autocovariate term is a distance-weighted average of all cells *j* in the neighborhood. Thus, the form of an autologistic model (Augustin et al. 1996, 1998) is:

$$ln\frac{p_i}{1-p_i} = \alpha + \beta_0 autocov_i + \sum_k \beta_k x_{ki} + \varepsilon_i$$

where x_{ki} are the values for *k* different environmental variables used to explain the occurrence of *y* in square *i*. Because the autocovariate term is computed based on a weigh matrix which is generated from a neighbor system by using Inverse Distance Weight method, different searching window sizes, i.e. sizes of neighborhood, ranging from 200x200m to 400x400m, will be tried. The statistical analysis will be done in R with the spdep package (Bivand et al. 2013, Bivand and Piras 2015). Eventually the best-fitted models will be selected out of the series of candidate models. Some combinations of variables will be avoided if they are collinear or related; e.g., including both depth to water (DTW) and biomass growth index (BGI), because DTW is one of the components to generate BGI (Hennigar et al. 2017). Performance of the model will be measured by Akaike Information Criteria (AIC; Akaike 1974), spatial autocorrelation remaining in residuals (Dormann et al. 2007) and Receiver Operating Characteristic (ROC; Fawcett 2006) curve. In the future, 2018 L2 data will be used to test the reliability of the model.

Results

Descriptive statistics

Within the study area, the number of L2 sample points was 1008, 1636, 1459, 1461 and 1653 in the 5 years from 2013 to 2017. Over these years, number of moderate-extreme L2 sample points increased. Specifically, the % of points in the nil-low and moderate-extreme L2 classes was 100 and 0, 97.6 and 2.4, 97.2 and 2.8, 94.1 and 5.9, and 93.2 and 6.8 from 2013 to 2017, respectively.

Spray treatment. Planning aerial application of insecticides were based on the previous year's fall and overwintering L2 data. In other words, the spray treatment in one year focused on the area in which samples of the moderate and higher L2 classes appeared in samples collected in the previous fall-winter. Figure 1a shows the count of sample points within ("treated") or outside ("untreated") of the spray treatment blocks in the same year as the L2 sampling, e.g., 2017 L2 data overlaid with the 2017 spray treatment blocks. Over the 5 years, 95.4% of L2 samples were outside, and 4.6% were inside of the current-year spray blocks. Overall, 87.4% of the moderate-

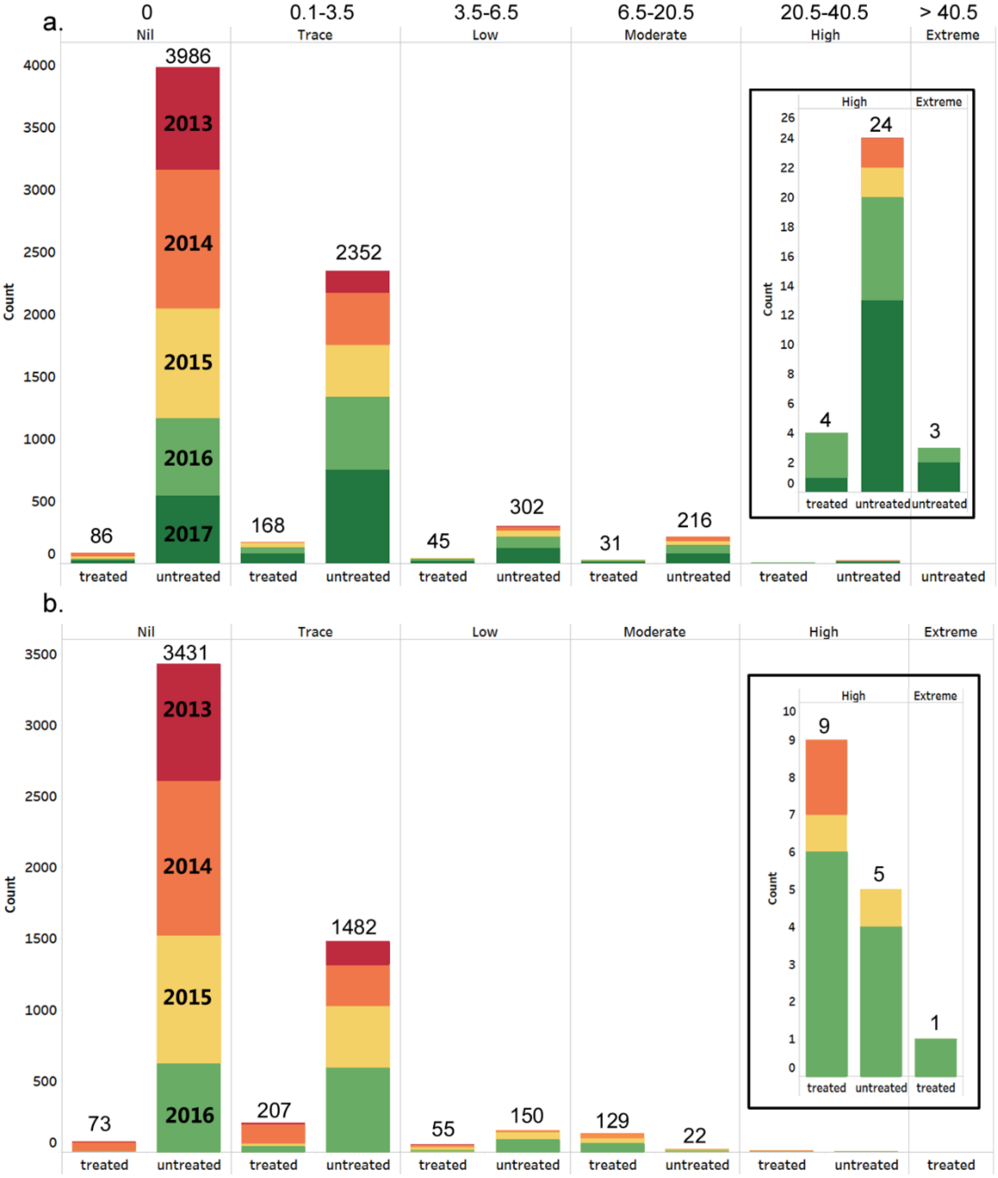


Figure 1. Number of SBW L2 samples each year from 2013 to 2017, which were within spray blocks ("treated") or not in spray blocks ("untreated"). a. L2 data compared to the sprayed blocks in the same year of L2 sampling. b. L2 data compared to the sprayed blocks in the subsequent year. 2017 L2 samples are the basis of 2018 spray treatment planning, and will not be sprayed until 2018 summer.

extreme L2 sample points (243 out of 278) were not in spray blocks in that year, so only 12.6% of the moderate and higher L2 sample points had been sprayed that year. Figure 1b compares L2 and higher L2 sample points in 2014, 2015, and 2016 that were sprayed in the following year. Over the years, a total of 84% of moderate and higher L2 points were treated in the subsequent year. 2017 L2 samples will not be sprayed until 2018 summer.

Forest composition. The dataset will be available in the near future, and the analysis will be completed then.

Climate. Monthly mean temperature showed a general indication that increasing L2 classes corresponded to decreasing mean temperature. For mean Apr.-Jun. temperature (°C), the values

Table 2. Summary of the mean values for all variables (except for forest composition) for nil-low L2 classes vs. moderate-extreme L2 classes from 2013 to 2017.

			2013	2014		2015		2016		2017	
		L2[1]	N-L	N-L	M-E	N-L	M-E	N-L	M-E	N-L	M-E
Climate	Avg. Temp. (℃)[2]	Apr. | Jun.	8.98[7] (0.02)	8.81 (0.01)	8.17 (0.05)	8.48 (0.02)	7.76 (0.08)	8.50 (0.02)	8.27 (0.06)	9.37 (0.01)	9.02 (0.03)
		Jul. | Sept.	16.03 (0.02)	16.42 (0.01)	16.34 (0.04)	17.31 (0.01)	16.85 (0.05)	16.53 (0.02)	16.25 (0.06)	16.53 (0.01)	16.38 (0.04)
	Accu. Degree days (℃•d)[3]	Apr. | Jun.	696 (2.9)	609 (1.6)	543 (7.0)	676 (1.8)	584 (9.5)	679 (1.9)	630 (6.0)	665 (1.6)	620 (4.0)
		Jul. | Sept.	3639 (7.8)	3745 (4.2)	3639 (18.3)	3718 (5.0)	3494 (23.1)	3722 (6.3)	3588 (20.8)	3677 (4.7)	3604 (12.4)
Topography & Location	Proximity (km)[4]		104 (1.2)	94 (1.1)	50 (0.8)	81 (1.2)	39 (3.6)	85 (1.2)	65 (4.2)	72 (1.1)	48 (3.2)
	Elevation (m)		314 (3.8)	284 (3.4)	253 (14.9)	282 (3.6)	312 (15.4)	273 (3.8)	245 (14.8)	272 (3.6)	254 (13.0)
	Slope (°)		4.41 (0.11)	4.17 (0.09)	4.16 (0.55)	3.98 (0.09)	4.14 (0.61)	3.71 (0.09)	3.36 (0.32)	3.84 (0.09)	3.44 (0.33)
	Aspect (°)		178 (3.0)	181 (2.4)	176 (17.0)	179 (2.5)	189 (18.9)	180 (2.5)	174 (10.7)	178 (2.3)	186 (8.9)
Site Quality	DTW (m)[5]		6.81 (0.26)	6.49 (0.19)	8.31 (1.12)	6.38 (0.21)	6.60 (0.91)	5.79 (0.19)	5.12 (0.66)	6.34 (0.21)	6.02 (0.87)
	BGI (kg•ha^{-1}•year^{-1})[6]		3620 (13.4)	3636 (10.2)	3916 (50.9)	3610 (11.2)	3609 (60.5)	3549 (12.0)	3472 (48.3)	3568 (11.0)	3516 (39.7)

[1] Spruce budworm L2 classes: N-L=nil-low (0-6.5 per branch); M-E= moderate-extreme (> 6.5 per branch).

[2] Average temperature.

[3] Accumulated degree days (>5℃).

[4] Proximity to the nearest moderate-severe defoliation areas in Quebec.

[5] Depth to water.

[6] Biomass growth index (Hennigar et al. 2017).

[7] Mean values and associated standard error of mean (in parentheses).

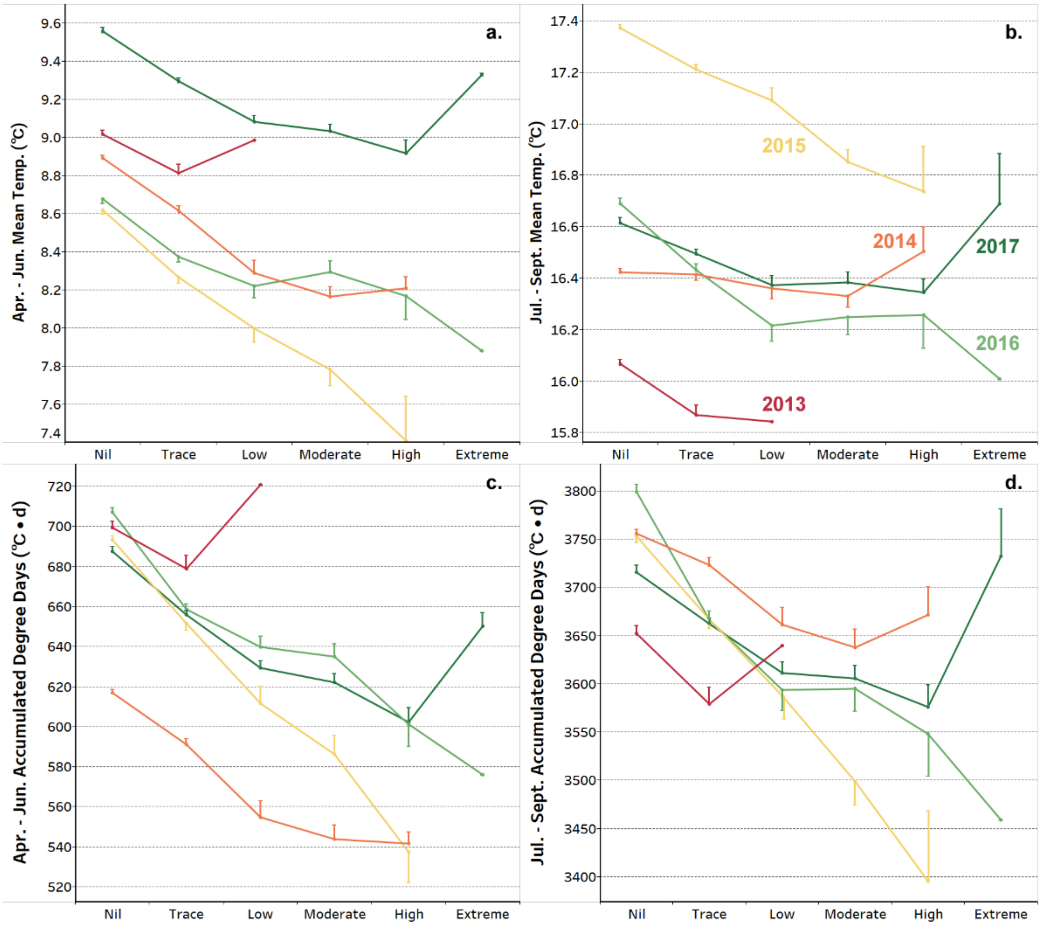


Figure 2. The relation of climate variables (a. Apr. to Jun. mean temperature; b. Jul. to Sept. mean temperature; c. Apr. to Jun. accumulated degree days; and d. Jul. to Sept. accumulated degree days) to six SBW L2 levels (nil to extreme) each year from 2013 to 2017.

of the moderate-extreme L2 classes were lower than those of the nil-low L2 classes: 8.2 versus 8.8 in 2014, 7.8 versus 8.5 in 2015, 8.3 versus 8.5 in 2016, and 9.0 versus 9.4 in 2017 (Table 2).

A similar pattern occurred for Jun.-Sept. monthly mean temperature, with the moderate and higher L2 classes having lower temperatures than nil-low classes: 16.3 versus 16.4 in 2014, 16.9 versus 17.3 in 2015, 16.3 versus 16.5 in 2016, and 16.4 versus 16.5 in 2017 (Table 2). Line graphs also showed this tendency, with the moderate-extreme L2 classes having lower mean temperatures than the nil-low L2 classes (Figure 2a, b).

Similar to the monthly mean temperature, the mean accumulative degree days (>5°C) also showed lower degree days in moderate-extreme L2 classes than in nil-low classes (Table 2; Figure 2c, d). For Apr.-Jun. degree days (°C·d), values of moderate-extreme L2 versus nil-low classes were 543 and 609 in 2014, 584 and 676 in 2015, 630 and 679 in 2016, and 620 and 665 in 2017 (Table 2). Jul. -Sept. degree days (°C·d) showed the same pattern; values of moderate-extreme L2 versus nil-low classes were 3639 and 3745 in 2014, 3494 and 3718 in 2015, 3588 and 3722 in 2016, and 3604 and 3677 in 2017 (Table 2).

Topography and location. The distance to the nearest moderate-severe defoliated areas in Quebec showed the strongest relationship with increasing L2 class among all the variables, with the higher L2 classes closer to defoliation areas (Table 2). The mean distances (km) for moderate-extreme and nil-low L2 classes were 50 versus 94 in 2014, 39 versus 81 in 2015, 65 versus 85 in 2016, and 48 versus 72 in 2017 (Table 2); that is, the moderate and higher L2 classes were located closer to defoliated areas Quebec than the nil-low L2 points. This was due to the fact that the outbreak in Quebec was moving farther south, particularly in 2016 and 2017. Contours (5, 10, 30, 50, 90, 150 km) of proximity to the nearest moderate-severe defoliation areas in Quebec were conspicuously moving toward the south over time (Figure 3). In particular, in 2016 and 2017, there were more moderate and higher L2 classes located within 40 km of defoliated areas in Quebec (Figure 4).

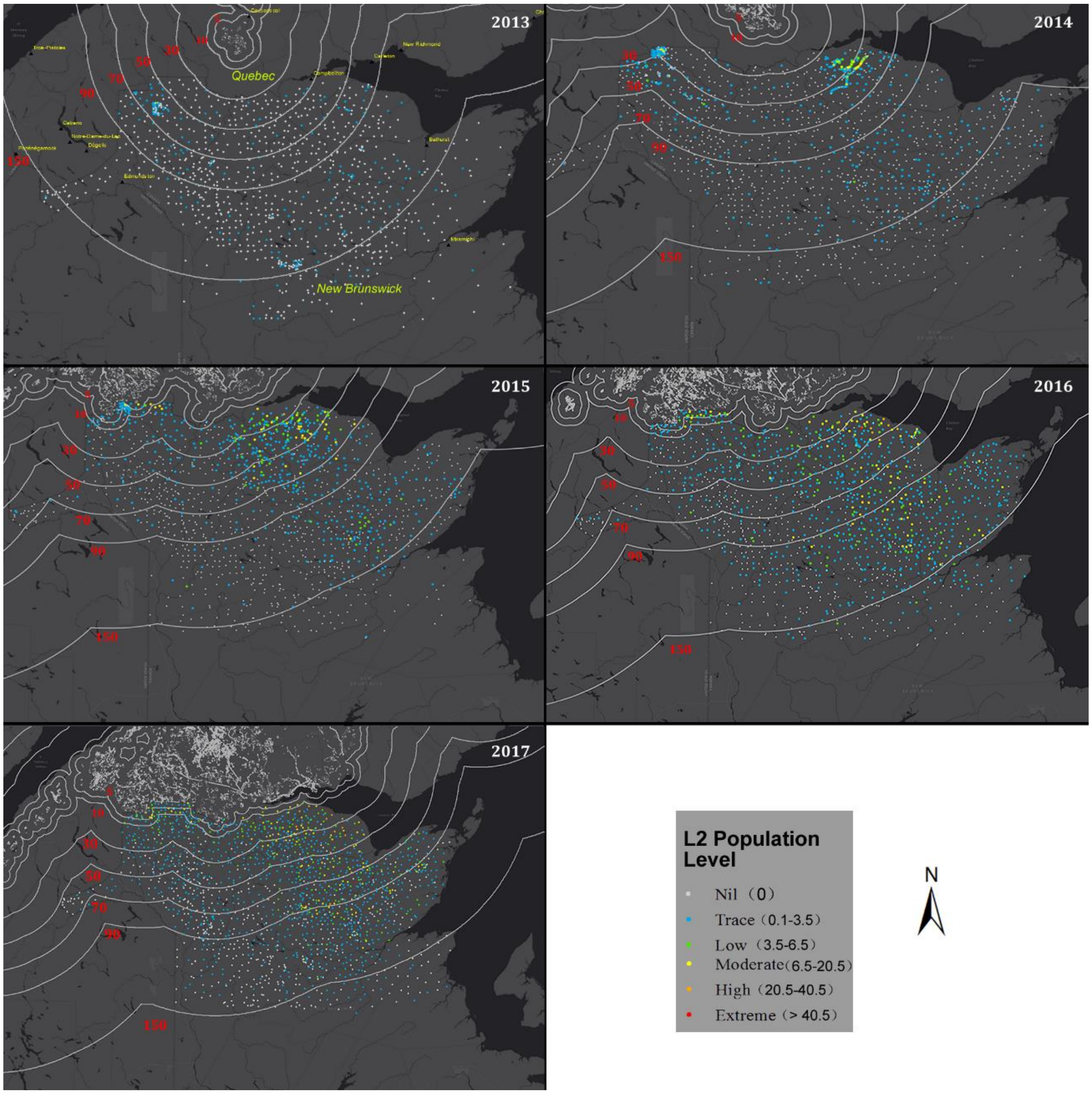


Figure 3. Contours for proximity (km) to the nearest moderate-severe defoliation areas in Quebec from 2013 to 2017.

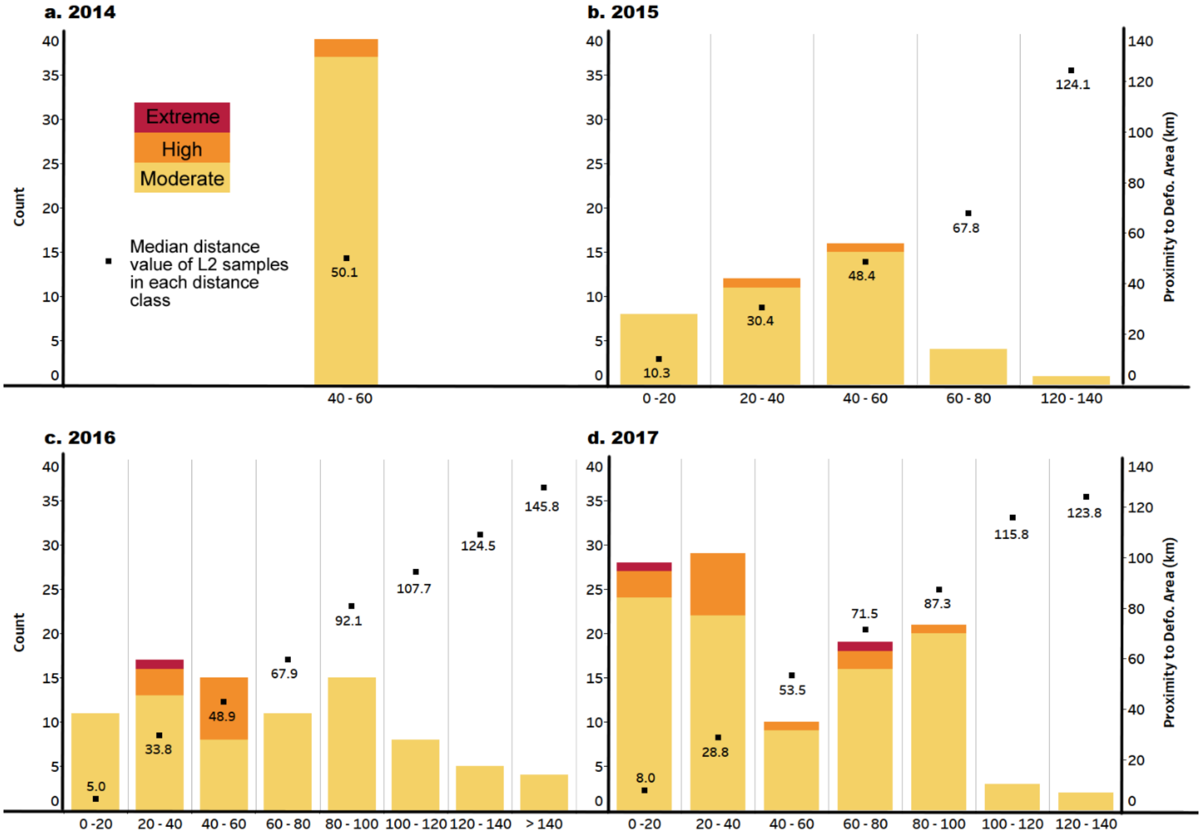


Figure 4. Number of SBW L2 moderate, high, and extreme points plotted against distance (km) to nearest defoliation areas in Quebec from 2013 to 2017. None of the moderate and higher L2 samples in 2015 fell in the 100-120 km distance class.

From 2014 to 2017, higher L2 classes generally occurred at lower elevations, as the mean elevations (m) of moderate-extreme L2 classes were lower than nil-low L2 classes: 253 versus 284 in 2014, 245 versus 273 in 2016, and 254 versus 272 in 2017 (Table 2). Although in 2015 the mean elevation of the moderate-extreme L2 classes (312 m) was higher than the nil-low L2 classes (282 m), it probably resulted from several outliers of the moderate and high L2 classes in 2015 with elevations over 400 m. Other samples of the moderate-extreme L2 classes each year were all lower than 300 m. Both slope and aspect were not related to higher L2 levels, although slope and aspect of the high and extreme L2 levels tended to vary dramatically and had high SEM values (Figure 5c, d).

Site quality. In 2014 and 2015, the average DTW (m) of the moderate-extreme L2 class was higher than the nil-low L2 (8.3 versus 6.5 in 2014 and 6.6 versus 6.4 in 2015; Table 2). In 2016 and 2017, however, mean DTW of the moderate-extreme L2 class was lower than the nil-low L2 (Table 2). Overall, DTW was not clearly related L2 populations (Figure 5e).

In 2016 and 2017, mean BGI (kg·ha^{-1}·year^{-1}) of the moderate-extreme L2 class was lower than the nil-lowL2 (3472 versus 3549 in 2016 and 3516 versus 3568 in 2017; Table 2). However, in 2014 and 2015, mean BGI of the moderate and higher L2 classes was higher than, or relatively similar to, the nil-low (3916 versus 3636 in 2014 and 3609 versus 3610 in 2015; Table 2). BGI values of the moderate-extreme L2 class in these 2 years were variable with large SEM values. Additionally, in 2014, mean BGI of the nil, trace, low, moderate, and high L2 class was 3605, 3698, 3889, 3905, and 4126, respectively, having an obvious trend with increasing L2 classes.

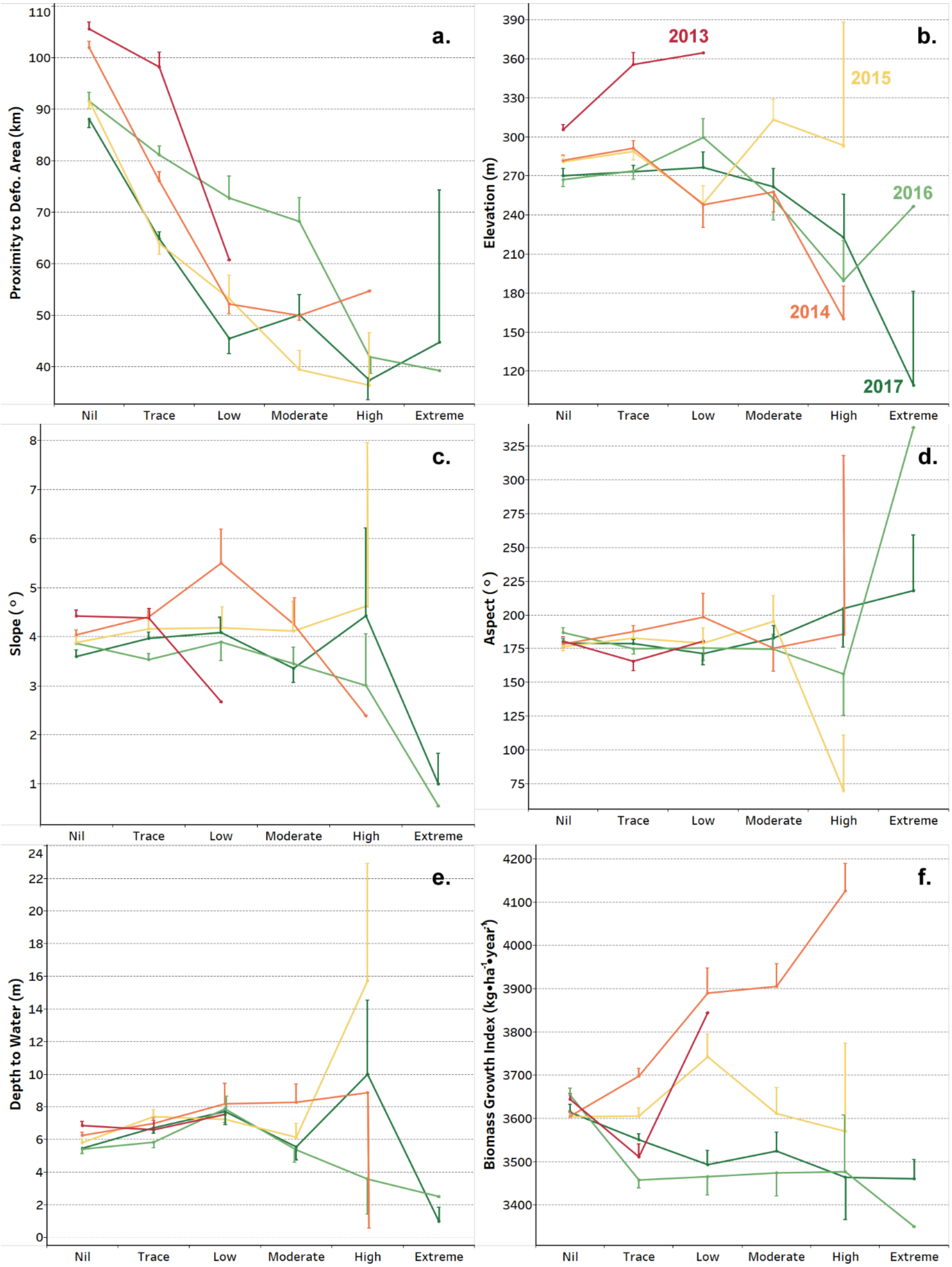


Figure 5. The relation of topography, location, and site quality variables (a. proximity to nearest defoliation; b. elevation; c. slope; d. aspect; e. depth to water; and f. biomass growth index) to six SBW L2 levels (nil to extreme) each year from 2013 to 2017.

However in 2017, BGI of each L2 class was 3633, 3551, 3493, 3525, 3464, and 3461 respectively, showing the opposite tendency from 2014.

Autologistic regression

This part of the research is still underway.

Discussion and conclusions

In general, analyses by the Healthy Forest Partnership (2016c) have indicated that spray treatments have been effective in reducing L2 numbers in treated blocks. According to the results of this study, 84% of moderate-extreme L2 points were treated in the following year, and only 13% of moderate-extreme L2 points were inside insecticide-treated blocks in the same year as the L2 sampling. Considering that spray treatment is finished in each summer and L2 sampling is carried out in the following fall and forms the basis for spray treatment planning for the subsequent year, these results demonstrate effectiveness of the insecticide-spraying strategies.

The numerical relationships between climate conditions and L2 classes showed that higher, moderate-extreme, L2 populations tended to occur in colder regions. In New Brunswick, the high L2 class samples tended to be located farther north, and thus the climate condition was colder. Considering that the outbreak in the Gaspe-Bas St. Laurent region adjacent to New Brunswick was moving farther south, it was understandable that the higher L2 class samples largely occurred in northern New Brunswick.

Location had the strongest influence on the L2 population in this study, with higher L2 classes located closer to moderate-severe defoliation areas. From 2013 to 2017, the mean distances from defoliation were lower for moderate-extreme L2 points than for nil-low points, and contours of proximity were noticeably moving southward over the years (especially 2015-2017). These results support previous studies that spruce budworm population has a positive relationship with proximity to other budworm outbreak areas (Magnussen et al. 2004, Bouchard and Auger 2014). The reason could be that spruce budworm population dynamics is affected by moths' dispersal from other outbreak areas (Royama 1984, Régnière and Nealis 2007).

Samples in 2014, 2016 and 2017 indicated that higher L2 levels tended to be located at lower elevations, which supported the conclusion of Bouchard and Auger's (2014) study. However, data in 2015 did not follow this pattern, probably because of some outliers in the data in that year. Neither slope nor aspect showed relationships with the L2 level in this study.

DTW was not consistently related to L2 levels, with sample points in 2014-2015 showing differing results to those in 2016-2017. In addition, only the moisture content of shallow soil layers influences site quality (Murphy et al. 2009), and the range of DTW of all samples over 4 years was quite wide. In related studies, it was found that stands with higher moisture and better nutrition suffered from more severe defoliation (MacKinnon and MacLean 2003). BGI data of samples in 2014 supported this conclusion, while data in other years showed relatively weak relationships or even a contrary relationship (2017).

Acknowledgements

We appreciate data supplied by the Healthy Forest Partnership, New Brunswick Department of Energy and Resource Development, and the Forest Watershed Research Centre at the University of New Brunswick. Luke Amos-Binks, Jeremy Gullison, and Jae Ogilvie provided advice and access to the datasets. We are grateful to Rémi Saint-Amant, Natural Resources Canada, for creating 2013-2017 New Brunswick climate databases for BioSIM. We appreciate

SERG-I for the Graduate Student Award to Mingke Li to attend the 2018 SERG-I Workshop.

References

Akaike, H. 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control **19**(6): 716–723.

Augustin, N.H., Mugglestone, M.A., and Buckland, S.T. 1996. An autologistic model for the spatial distribution of wildlife. J. Appl. Ecol. **33**(2): 339–347.

Augustin, N.H., Mugglestone, M.A., and Buckland, S.T. 1998. The role of simulation in modelling spatially correlated data. Environmetrics **9**(2): 175–796.

Besag, J. 1974. Spatial interaction and the statistical analysis of lattice systems. J. R. Stat. Soc. **36**(2): 192–236.

Bivand, R., and Piras, G. 2015. Comparing implementations of estimation methods for spatial econometrics. J. Stat. Softw. **63**(18): 1–36.

Bivand, R.S., Hauke, J., and Kossowski, T. 2013. Computing the Jacobian in Gaussian spatial models : an illustrated comparison of available methods. Geogr. Anal. **45**(2): 150–179.

Bouchard, M., and Auger, I. 2014. Influence of environmental factors and spatio-temporal covariates during the initial development of a spruce budworm outbreak. Landsc. Ecol. **29**(1): 111–126.

Dormann, C.F., Mcpherson, J.M., Arau, M.B., Bivand, R., Bolliger, J., Carl, G., Davies, R.G., Hirzel, A., Jetz, W., Kissling, W.D., Ohlemu, R., Peres-neto, P.R., Schurr, F.M., and Wilson, R. 2007. Methods to account for spatial autocorrelation in the analysis of species distributional data : a review. Ecography **30**(5): 609–628.

Erdle, T., and Ward, C. 2008. Management alternatives for New Brunswick's public forest. Fredericton, N.B., Dept. of Natural Resources, Fredericton, N.B.

Fawcett, T. 2006. An introduction to ROC analysis. Pattern Recognit. Lett. **27**(8): 861–874.

Greenbank, D.O. 1957. The role of climate and dispersal in the initation of outbreaks of the spruce budworm in New Brunswick Ⅱ. The role of dispersal. Can. J. Zool. **97**(331): 1077–1089.

Hardy, Y., Mainville, M., and Schmitt, D.M. 1986. Spruce budworms handbook: an atlas of spruce budworm defoliation in eastern North America, 1938-1980. USDA For. Serv., Coop. State Res. Serv., Washington D.C.

Healthy Forest Partnership. 2016a. Aerial application of pesticides and pheromones. Available from http://www.healthyforestpartnership.ca/en/research/research-projects [accessed 25 October 2016].

Healthy Forest Partnership. 2016b. What is branch washing and how does it relate to the "L2 Survey"? Available from http://www.healthyforestpartnership.ca/en/research/research-blog/what- is-branch-washing-and-how-does-it-relate-to-the-l2-survey [accessed 13 November 2016].

Healthy Forest Partnership. 2016c. Treatments having an effect in northern New Brunswick. Available from http://www.healthyforestpartnership.ca/en/research/research-blog/treatments-having-an-effect-in-northern-new-brunswick [accessed 19 April 2016].

Healthy Forest Partnership. 2017. Research area map. Available from http://www.healthyforestpartnership.ca/en/research/what-where-and-when [accessed 19 September 2017].

Hennigar, C., Weiskittel, A., Allen, H.L., and MacLean, D.A. 2017. Development and evaluation of a biomass increment-based index for site productivity. Can. J. For. Res. **47**(3): 400–410.

Legendre, P. 1993. Spatial autocorrelation: trouble or new paradigm ? Ecology **74**(6): 1659–1673.

MacKinnon, W.E., and MacLean, D.A. 2003. The influence of forest and stand conditions on spruce budworm defoliation in New Brunswick, Canada. For. Sci. **49**(5): 657–667.

MacLean, D.A. 2005. Insect outbreaks as determinants of the structure and function of Canada's forests. *In* Health and the Planet. *Edited by* W. Chesworth. Guelph, Ont.: Faculty of Environmental Sciences, University of Guelph, 2005. pp. 53–79.

Magnussen, S., Boudewyn, P., and Alfaro, R. 2004. Spatial prediction of the onset of spruce budworm defoliation. For. Chron. **80**(4): 485–494.

Murphy, P.N.C., Ogilvie, J., and Arp, P. 2009. Topographic modelling of soil moisture conditions: a comparison and verification of two models. Eur. J. Soil Sci. 60(1): 94–109.

Régnière, J., and Nealis, V.G. 2007. Ecological mechanisms of population change during outbreaks of the spruce budworm. Ecol. Entomol. **32**(5): 461–477.

Royama, T. 1984. Population dynamics of the spruce budworm *Choristoneura fumiferana*. Ecol. Monogr. **54**(4): 429–462.

Sokal, R.R.S., Oden, N.L., and Thomson, B.A. 1998. Local spatial autocorrelation in biological variables. Biol. J. Linn. Soc. **65**(1): 41–62.

Syartinilia, and Tsuyuki, S. 2008. GIS-based modeling of Javan Hawk-Eagle distribution using logistic and autologistic regression models. Biol. Conserv. **141**(3): 756–769.

Analysis of multimodal attractiveness cues in the invasive beech leaf mining weevil, *Orchestes fagi* L., in Nova Scotia, Canada

Joel Goodwin[*], *Simon Pawlowski*[*], *N. Kirk Hillier*[*], *Jon Sweeney*[**]

[*]Acadia University
[**]Natural Resources Canada, Canadian Forest Service

Abstract

The invasive beech leaf mining weevil*, Orchestes fagi* L. (Coleoptera:Curculionidae), is a common pest of beech trees in Europe which has recently become established in Nova Scotia, Canada. Adults overwinter and emerge in early spring to feed on and lay eggs in beech leaves. Developing larvae mine beech leaves and cause significant damage, eventually leading to defoliation and death. Larvae complete development and emerge in approximately 30 days. By using bioassays, field trapping experiments, and electroretinograms, this study will analyze auditory, chemical, and visual attractiveness cues in *O. fagi* to develop a multimodal monitoring system for this species. Results from field trapping trials in 2017 suggest significant preferences toward green, white, and yellow traps, and a significant preference toward triangular prism traps over intercept traps.

Résumé

Le charançon du hêtre invasif*, Orchestes fagi* L. (Coleoptera:Curculionidae), est un ravageur courant du hêtre en Europe qui s'est récemment établi en Nouvelle-Écosse, au Canada. Les adultes hibernent puis émergent au début du printemps pour se nourrir de feuilles de hêtre et y pondre leurs œufs. Les larves en développement creusent des galeries dans les feuilles de hêtre et causent des dommages importants qui finissent par mener à la défoliation et à la mort de l'arbre. Les larves se développent et émergent en 30 jours environ. À l'aide d'essais biologiques, d'expériences de piégeage sur le terrain et d'électrorétinogrammes, cette étude permettra d'analyser les signaux d'attraction auditifs, chimiques et visuels d'*O. fagi* afin de mettre au point un système de surveillance multimodal pour cette espère. Les résultats des essais de piégeage sur le terrain en 2017 donnent à penser que les pièges verts, blancs et jaunes sont les préférés, et que les pièges à prisme triangulaires sont passablement plus attirants que les pièges d'interception.

Introduction

Orchestes fagi, an invasive pest species, was first confirmed in Nova Scotia in 2011. Despite their primary food source being beech tree leaves, the impact of this species on European Beech, *Fagus sylvatica*, in their native European habitat has been minimal. However, since their discovery in Nova Scotia in 2012, these weevils have caused extensive damage to foliage of American beech, *Fagus grandifolia,* causing 95% tree mortality after more than five successive years of heavy infestation. North American beech has already suffered significant mortality from beech bark disease (another invasive species complex), but many beech have survived in the aftermath forest. Unfortunately, early indications from permanent sample plots suggest that the beech weevil is killing trees regardless of the severity of beech bark disease present (J. Sweeney, pers. communication). The weevil overwinters as an adult on the trunks of trees (beech, maples, spruce) so the risk of inadvertent spread by human transport of firewood and logs is high (Morrison et al., 2017). Evidence the weevil has already moved long distances by human transport are infestations in Cape Breton, Wolfville, Chester, and Upper Clements, Nova Scotia. The relatively rapid rate of spread of the weevil combined with significant tree mortality in infested stands suggests the weevil is a significant threat to American beech throughout its range in eastern North America.

Management of the beech weevil requires effective tools for survey and early detection. Previous trapping trials employing a traditional pheromone-based approach returned little evidence of baited traps returning more catch than nonbaited controls. Therefore, it is imperative we develop a more effective monitoring system for this species by incorporating multiple sensory cues. This project will investigate visual, olfactory, and auditory sensory cues which may influence *O. fagi* host attraction.

This project adheres to the SERG-I research areas in several ways. Firstly, we are working toward the development of a pest management strategy for an invasive insect for which no effective monitoring system has been developed to date. Secondly, this management strategy, should it prove to be effective, could easily be marketed throughout North America to track the spread of this invasive species; thus, our project is thereby working toward the development of a forest pest management product. Thirdly, for decades, pheromone- based trapping methods have been the predominant method for capturing forest pests. Therefore, by developing a multimodal enhanced trapping method for *O. fagi*, we will be working toward the improvement of pest management application technologies and techniques. Finally, by working toward the management of an invasive species, we will be mitigating the environmental impacts this insect could have on forested areas, thereby maintaining the natural biodiversity and ecology of forests and aiding in the conservation of beech trees in North America.

Objective

The overall objective of this research project is to develop multimodal enhanced trapping strategies for *O. fagi*. The most effective visual, olfactory, and auditory stimuli will be combined to develop a trap designed to best capture *O. fagi* by establishing a multimodal context of attraction. This will provide a critical, environmentally-friendly technology for monitoring the spread of this invasive species.

Methods

Visual Attraction

Preliminary laboratory bioassays at Acadia University found that *O. fagi* was attracted to the color blue. We are exploring color preference further by determining the effect of contrasting colors on attraction in *O. fagi* using laboratory behavioral bioassays, by determining the physiological sensitivity of weevils to differing wavelengths of light using electroretinography, and by comparing the efficacy of different trap designs (Figure 1) and colors at detecting beech weevil in field trapping bioassays.

Trap color preference was explored by deploying sticky triangular prism traps of six different colors (yellow, green, white, dark blue, light blue, and red), at shoulder height, in a 6x8 randomized block design in Oakfield, Nova Scotia. Trap color preference and sex differences in trap color preference were analyzed.

Trap type preference was explored by deploying two types of traps (intercept traps and sticky triangular prism traps) of three different colors (green, white, and light blue), at shoulder height, in a 6x8 randomized block design in Oakfield, Nova Scotia. Trap color preference, trap type preference, and sex differences were analyzed.

Trap height preference was explored by deploying two colors of sticky triangular prism traps (green and light blue) in an infected beech stand in Wolfville, Nova Scotia. Twenty traps were deployed in a random order. Trap color preference, trap height preference, and sex differences were analyzed.

Olfactory Attraction

Beech weevils are significantly attracted to geranyl-p-cymene, a plant volatile emitted from beech buds at the time of budburst, when adults are seeking hosts to mate and lay eggs (Silk et al., 2017). Volatile collections from beech buds and leaves at various stages of development suggest there are additional compounds that may be effective attractants (Pawlowski, 2017; P. Silk, pers.

communication). Promising attractants identified by Silk et al. will be tested in concert with the most attractive colors and practical trap designs to develop an effective survey trap.

Auditory Attraction

Sound production via stridulation has been documented in many weevil species, however the function of this behaviour is unknown. I will examine the function of stridulation in *O. fagi* by recording and analyzing sounds produced by the weevils under several conditions, and exposing male and female weevils to these sounds to determine their behavioural responses. If results suggest that certain stridulatory sounds attract the weevils, then these sounds will be reproduced and combined with attractive host volatiles and colors to enhance survey traps. Alternatively, if sounds repel weevils, they might have other management and control applications.

Data Analysis

The total seasonal catch data were analyzed using generalized linear mixed models in SAS (PROC GLIMMIX) using the distribution which had the best fit, which was determined by comparing AICc (Akaike Information Criterion) values. There was no obvious difference in the responses of males and females, so the sexes were pooled for ease of analysis. When analyzing the data from the trap height experiment, trap color was pooled to provide 10 repetitions of each height. Means were compared using the Tukey-Kramer test on least square means, controlling the overall experiment-wise error rate.

The most effective olfactory, visual, and auditory stimuli identified from each series of modality tests will be combined and field tested to develop an enhanced trap for survey of *O. fagi*. This will provide a useful, necessary tool for managing a damaging pest of both natural and urban forests in eastern Canada.

Results

Preliminary field trapping trials in 2017 assessed the effectiveness of trap color, trap type, and trap location in capturing *O. fagi*. Results from these trapping trials suggest significant preferences toward green, white, and yellow traps over blue and red traps (Figure 2). Additionally, the results suggest a significant preference toward sticky prism traps over intercept traps (Figure 3), and a slight preference toward high canopy traps over low canopy traps (Figure 4).

Conclusions

Trapping results suggest that yellow, green, and white traps are more attractive to *O. fagi* than blue and red traps, with yellow yielding highest catch overall. Additionally, the results suggest that sticky prism traps are preferred over intercept traps. No significant preference toward trap height was found. Therefore, based on these trapping trials, yellow sticky prism traps should form the base of our multimodal trap for *O. fagi*, to which additional attractive stimuli can be added as they are discovered.

Figure 1. Yellow sticky triangular prism trap (left) and white intercept trap (right), June 2017, Oakfield, Nova Scotia. (J. Goodwin photo).

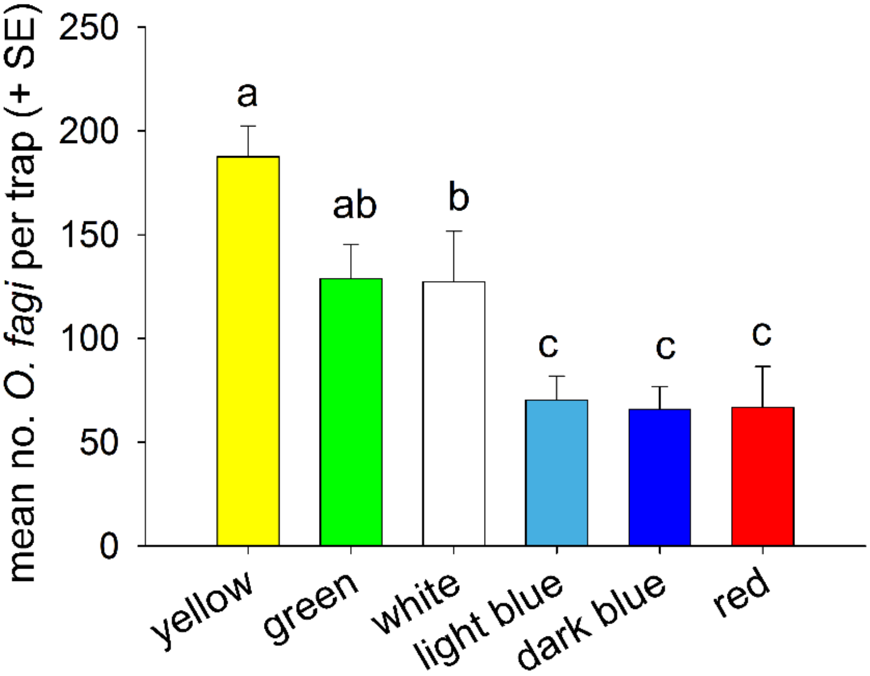


Figure 2. Effect of color on weekly mean trap catch of the beech leaf mining weevil, *Orchestes fagi*, in Oakfield, Nova Scotia. Traps were checked weekly from 11 May–28 July 2017. Sexes were pooled for ease of analysis. Bars labelled with different letters were significantly different (Tukey-Kramer test, $P < 0.05$).

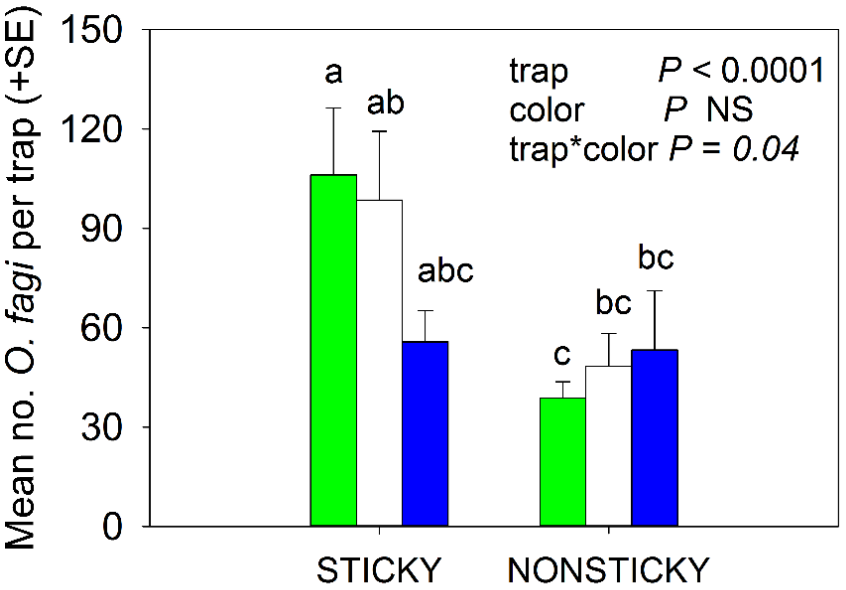


Figure 3. Effect of trap type and trap color on weekly mean trap catch of the beech leaf mining weevil, *Orchestes fagi*, in Oakfield, Nova Scotia. Traps were checked weekly from 25 May–28 July 2017. Sexes were pooled for ease of analysis. Bars labelled with different letters were significantly different (Tukey-Kramer test, P < 0.05).

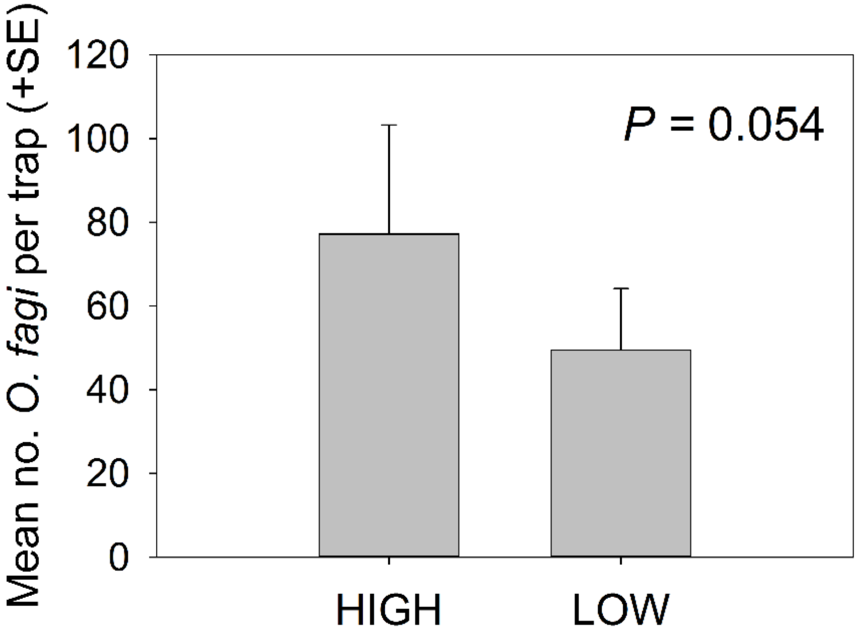


Figure 4. Effect of trap height on weekly mean trap catch of the beech leaf mining weevil, *Orchestes fagi*, in Wolfville, Nova Scotia. Traps were checked weekly from 7 June–27 July 2017. Sexes and colors were pooled for ease of analysis. Bars labelled with different letters were significantly different (Tukey-Kramer test, P < 0.05).

Acknowledgements

We thank the Atlantic Canada Opportunities Agency- Atlantic Innovation Fund (ACOA-AIF), the Nova Scotia Research and Innovation Graduate Scholarship Program, and Acadia University for funding this project. We also thank the Nova Scotia Department of Natural Resources and the K.C. Irving centre staff for allowing us to use their respective properties for trapping trials.

References

Morrison, A., Sweeney, J., Hughes, C., & Johns, R. C. (2017). Hitching a ride: firewood as a potential pathway for range expansion of an exotic beech leaf-mining weevil, Orchestes fagi (Coleoptera: Curculionidae). *The Canadian Entomologist*, *149*(01), 129–137. https://doi.org/10.4039/tce.2016.42

Pawlowski, S. P. (2017). *Comparative analyses on the phenology, electrophysiology, and chemistry of the beech leaf-mining weevil (Orchestes fagi L.) and its novel host American beech (Fagus grandifolia Ehrh.)* (master's thesis). Acadia University, Wolfville, N.S., Canada.

Silk, P. J., Mayo, P. D., LeClair, G., Brophy, M., Pawlowski, S. P., MacKay, C., … Sweeney, J. D. (2017). Semiochemical attractants for the beech leaf-mining weevil, Orchestes fagi (L.) (Curculionidae: Curculioninae: Rhamphini), *164*, 102–112.

Predation in endemic and outbreak forest tent caterpillar populations

E Despland[1], *C J K MacQuarrie*[2]

1) Concordia University, Montréal, Québec
2) NRCan Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario

Abstract

The forest tent caterpillar (FTC) is an important pest of Canadian hardwood forests, and is currently outbreaking across several provinces. We build on previous work investigating FTC larval mortality rates by placing FTC colonies in outbreaking and non-outbreaking quaking aspen (*Populus tremuloides*) stands in Abitibi`s boreal forest, with varying degrees of protection: free (no protection), tanglefoot (protection against walking predators only) and caged (protection against all predators and parasitoids). Results show that walking arthropod predators are significant sources of mortality in FTC caterpillar colonies. We also show that several species of ants are active early in the spring, at aspen leaf-out when FTC caterpillars eclose. Together, these results suggest that ants could play an important role in controlling FTC populations. Finally, no clear differences in mortality rates were observed between high- and low-density FTC populations, and the investigation will be on-going in 2018 in order to clarify this result.

Résumé

La livrée des forêts est un défoliateur important des forêts décidues Canadiennes, et des épidémies ont présentement lieu dans plusieurs provinces. Ce project se base sur nos travaux précédents sur les taux de mortalité larvaire chez la livrée : nous avons placé des colonies de livrée dans des peuplements de peuplier faux-tremble (*Populus tremuloides*) dans la forêt boréale en Abitibi avec des degrés de protection différents : colonies libres (aucune protection), `tanglefoot` (protection contre les prédateurs épigés), et en cage (protection contre tous les prédateurs et parasitoïdes). Les résultats démontrent que les prédateurs épigés sont des agents de mortalité importants pour les larves de livrée. Nous démontrons aussi que plusieurs espèces de fourmis sont actives tôt au printemps, quand le peuplier débourre et les livrées éclosent. Ensemble, ces résultats suggèrent que les fourmis pourraient jouer un rôle important dans le contrôle des populations de livrée. Finalement, nous n'avons pas détecté de différence significative de mortalité entre des livrées placées dans des peuplements subissant de fortes populations naturelles de livrée et dans d'autres qui ne sont pas affectés par l'épidémie en cours. Les travaux continueront en 2018 pour clarifier ce résultat.

Introduction

SERG-I areas addressed: Developing Forest Pest Management Strategies

SERG-I regions where this work could apply: Québec, Ontario, Manitoba, Saskatchewan, Alberta, northeastern United States

Completion date: 2019 (project), 2020 (deliverables)

Objectives:

1. Assess relative importance of walking (e.g. ants, spiders) and flying (e.g. beetles, stinkbugs, parasitoids, birds) natural enemies.
2. Characterize ant communities in FTC-affected boreal aspen stands.
3. Compare forest tent caterpillar mortality between low-density and outbreaking populations in boreal forest quaking aspen stands (*Populus tremuloides*).

Background

The forest tent caterpillar (FTC) is a frequent and significant defoliator of hardwood forests throughout Canada and can lead to decline or even to mortality of quaking aspen stands in boreal forests. One side effect of a changing climate is that outbreaks of FTC may become more severe, which could result in more significant mortality from a pest that previously was of little economic concern.

Outbreaking populations of insects experience dramatic variations in mortality rates between the endemic and epidemic state. Research often focuses on the increase of mortality that terminates outbreaks, but factors involved in keeping endemic populations low have rarely been considered.

The current project builds on previous research examining disease and predation on FTC across Canada, which showed that early-instar caterpillars in low-density populations can experience significant mortality from predators, often wiping out whole colonies. Specifically, we improve the experimental design to separate mortality due to walking and flying natural enemies and add pitfall traps to sample ground-dwelling predators. We also take advantage of the current upsurge to compare predation rates in low density and outbreaking populations.

Methods

We conducted experiments in low-density and outbreaking FTC populations in quaking aspen stands in the boreal forest FERLD (Forêt d'Enseignement et de Recherche du Lac Duparquet) field station in Abitibi, north-western Québec.

The experimental design was based on that used in a previous SERG-funded project (``Improving predictive models of forest tent caterpillar outbreak dynamics`` led by Chris MacQuarrie), adding an additional treatment (tanglefoot, a sticky resin that prevents walking arthropods from accessing the caterpillar colonies but permits access by flying predators and parasitoids). We monitored caterpillar groups placed on quaking aspen trees in 3 different treatments: open (exposed to all natural enemies), branches coated with tanglefoot (to exclude walking predators) and colonies caged (to exclude all natural enemies). The three treatments were combined in a triad design with one tree assigned to each treatment in each site, and 10 triads were set up both in aspen stands that had experienced significant FTC defoliation in 2015 and 2016 (high-density FTC populations) and in stands in which no defoliation has been recorded in recent years (low-density FTC).

Caterpillar colonies were monitored every 3 days after hatching, until they had all either disappeared or pupated. At the end of the experiment, eggs were counted to estimate hatch rate and initial colony size. Mortality in caged treatments (likely due to pathogens and/or egg parasitoids) is used as a baseline for non-predation related mortality.

Three pitfall traps were placed within each triad for 48h at approximately weekly intervals during FTC development (5 sampling dates between May 23rd and June 17th 2017) in order to monitor ground-based predator populations. All ants collected in the traps were identified.

Results

Results show higher survival in caged and tanglefoot colonies than in free colonies, suggesting that walking predators are important mortality agents in FTC populations.

The pitfall traps shows that several species of ants are present in these boreal aspen stands, and active early in the season, at budbreak when forest tent caterpillars eclose. The most common species include *Formica aserva*, *Formica podzolica*, *Camponotus herculaneus* and *Myrmica* spp. Ants were observed taking caterpillars in the trees and carrying them off to the nest, demonstrating that they do indeed act as mortality agents on FTC populations.

No significant difference in mortality rate was detected between low- and high-density populations. However, sample sizes were limited for logistical reasons, and we intend to pursue experiments in the 2018 field season in order to increase the power of our study and obtain a clearer result.

These results show that walking predators do play an important role in controlling FTC populations and suggest that ants are significant mortality agents that warrant further attention.

Deliverables and future plans

We plan to continue sampling at FERLD in 2018, following the same design (triads placed in both high- and low-density populations) in order to obtain clearer information about the relationship between FTC population density and mortality rate. Our collaborators will also purchase tree-climbing equipment on a recent CFI grant, which will permit us to access the forest canopy to compare FTC mortality rates between forest strata.

In order to complete the arthropod identifications associated with this project, we have applied for a Mitacs stage for the student involved. Dr Despland and a team of collaborators have recently been awarded provincial funding (MFFP) to continue studying trophic relationships in FTC outbreaks, including FTC mortality and predation rates as well as FTC effects on ground-based arthropod communities, soil nutrient cycling and effects on seedling regeneration. SERG funding has been instrumental to obtain the first year of data on which this larger proposal has been based. These funds have thus punched far above their weight in leveraging extra funds.

Results will be combined with those of the 2018 field season for publication in a peer-reviewed journal, and will be presented at international research conferences and at the Centre for Forest Studies annual colloquium that brings together actors in forest science in Québec. Finally, these results will be incorporated into on-going modelling of forest tent caterpillar population dynamics.

Financial report 2017-2018

	requested	**received**
CEF (reduced rate at FERLD and training for student)		In-kind
NSERC DG to ED		$ 6000
Concordia University (use of field vehicle)		In-kind
Mitacs (application under review)	$7500	
SERG-I		$6000
Total	$7500	**$12000**

Effect of dosage and treatment frequency on efficacy of TreeAzin® Systemic Insecticide for protecting foliage from the beech leaf-mining weevil and preventing mortality of American beech in high value urban environments.

Jon Sweeney[1], Cory Hughes[1], Tarryn Goble[2], Joe Meating[2], Ed Czerwinski[3], Joel Goodwin[4], Simon Pawlowski[4], N. Kirk Hillier[4], and Rob Johns[1]

[1]Natural Resources Canada, Canadian Forest Service, 1350 Regent Street, Fredericton, NB. E3B 5P7, Tel./Fax. 506 452 3499/506 452 3525, jon.sweeney@canada.ca ; rob.johns@canada.ca; cory.hughes@canada.ca
[2]BioForest, 59 Industrial Park Crescent, Unit 1, Sault Ste. Marie, ON P6B 5P3, Tel. 705-942-5824 jmeating@bioforest.ca; tgoble@bioforest.ca
[3]Forestreecare Company, Fredericton, NB, Tel. 506.262-8755; forestreecare@nexicom.net
[4]Acadia University, 33 Westwood Ave., Wolfville, NS, B4P 2R6, Tel. 902-585-1314; kirk.hillier@acadiau.ca

Abstract

The beech leaf-mining weevil, *Orchestes fagi,* is an invasive pest from Europe that has caused severe defoliation of American beech in its new range in Halifax, and Sydney, Nova Scotia, since at least 2008. Cumulative mortality of American beech increased from 20% in 2014 to 97% in 2016 in heavily infested plots near Halifax compared to <5% increase in mortality over the same period in plots with low weevil populations located 40 km from Halifax. Our goal is to develop a method of protecting high value street trees of American beech from beech weevil defoliation and reduce tree mortality. Field trials with TreeAzin® Systemic Insecticide (PCP No. 30559) was tested for control of the beech leaf-mining weevil in four separate trials in spring 2013, spring and fall of 2014, and fall of 2016, in collaboration between Canadian Forest Service, Acadia University, BioForest, and ForestreeCare. Stem injection of TreeAzin significantly reduced larval survival and foliar damage by European beech leaf-mining weevil in American beech trees when injected in early spring, prior to bud burst, at a rate of 3 mls per cm dbh, or when injected the previous fall at a rate of 3 mls or 5 mls per cm dbh. The higher dose was slightly more efficacious than the lower dose. Neither early spring nor fall treatments reduced damage from adult feeding nor the number of eggs laid per leaf. Efficacy data were submitted to PMRA in December 2017 as part of an application for emergency registration of TreeAzin for control of beech weevil in Nova Scotia. Additional efficacy data will be collected in 2018 and 2019 to determine whether annual or biennial injections are necessary to prevent tree mortality.

Résumé

Le charançon du hêtre, *Orchestes fagi*, est un ravageur invasif d’Europe qui cause une défoliation grave du hêtre à grandes feuilles dans sa nouvelle aire de répartition à Halifax et à Sydney, en Nouvelle-Écosse, depuis au moins 2008. La mortalité cumulative du hêtre à grandes feuilles est passée de 20 % en 2014 à 97 % en 2016 dans les parcelles lourdement infestées près d’Halifax, comparativement à une hausse de <5 % du taux de mortalité pour la même période dans des parcelles à faibles populations de charançon situées à 40 km d’Halifax. Notre objectif est de mettre au point une méthode pour protéger les hêtres à grandes feuilles de grande valeur en milieu urbain contre la défoliation causée par le charançon du hêtre et de réduire la mortalité des arbres. Des essais sur le terrain effectués avec l’insecticide systémique TreeAzin® (PCP No. 30559) ont eu lieu contre le charançon du hêtre lors de quatre essais distincts au printemps 2013, au printemps et à l’automne 2014 ainsi qu’à l’automne 2016 en collaboration avec le Service canadien des forêts, l’Université Acadia, BioForest et ForestreeCare. L’injection de TreeAzin dans le tronc de l’arbre a diminué de façon importante la survie des larves et les dommages au feuillage par le charançon du hêtre européen dans le hêtre à grandes feuilles lorsque

l’injection était faite au début du printemps avant l’éclosion des bourgeons à un taux de 3 ml par cm DHP, ou à l’automne précédent à un taux de 3 ml ou de 5 ml par cm DHP. La dose plus élevée a été légèrement plus efficace que la dose plus faible. Les traitements au début du printemps ou à l’automne n’ont pas réduit les dommages causés par les adultes qui se nourrissent ni le nombre d’œufs pondus par feuille. Les données sur l’efficacité ont été remises à l’ARLA en décembre 2017 dans le cadre d’une demande d’homologation urgente de TreeAzin pour lutter contre le charançon du hêtre en Nouvelle-Écosse. D’autres données sur l’efficacité seront recueillies en 2018 et en 2019 afin de déterminer si des injections annuelles ou bisannuelles sont nécessaires pour prévenir la mortalité de l’arbre.

Introduction

The beech leaf-mining weevil, *Orchestes fagi,* is an invasive pest from Europe that has caused severe defoliation of American beech in its new range in Halifax, and Sydney, Nova Scotia, since at least 2008. Cumulative mortality of American beech increased from 20% in 2014 to 97% in 2016 in heavily infested plots near Halifax compared to only a 4% increase in mortality over the same period in plots with low populations located 40 km distant from Halifax (Sweeney, Hughes et al. unpublished data). Residents of Halifax need a control method that will protect their high value street/yard trees. The beech weevil’s habit of overwintering in large numbers in crevices on the trunks of trees combined with growing numbers of dead beech in resident’s yards generates conditions for high risk of human-assisted spread of the weevil to cottages and campgrounds in logs and firewood. The weevil has already established satellite populations in Chester, Wolfville and Cape Breton, likely through anthropogenic movement, and is at risk of spreading rapidly to other parts of Canada that are home to American beech.

Field trials with TreeAzin® Systemic Insecticide (PCP No. 30559) in 2013-2015 showed that stem injection of beech in either fall or early spring killed most larvae and significantly reduced larval damage to foliage but did not reduce adult feeding damage. Further research was warranted to test whether increasing the dosage (from 3 ml per cm dbh to 5 ml per cm dbh) would increase foliage protection and prevent tree mortality. We also wanted to compare the efficacy of injecting trees every year versus every two years.

This proposal addresses the SERG-I research areas of development of new forest pest products and forest pest management strategies. Although this is primarily an urban forest problem, the importance of urban forestry and urban tree pest management has come to the forefront in recent years, with eradication programmes against the Asian longhorned beetle in greater Toronto and widespread mortality of ash trees by the emerald ash borer in many North American cities. This study was supported with $2,000 from NSDNR in each of 2016-17 and 2017-18, and with $2000 from OMNR in 2017-18, through SERG-International.

Objective: Determine the effect of dosage and frequency of treatment on the efficacy of TreeAzin for protecting foliage from the beech leaf-mining weevil and keeping American beech trees alive.

Methods

We tested two dosages of TreeAzin per cm dbh (3 mls, 5 mls) and two treatment frequencies (annual, biennial) for protecting American Beech from the beech leaf-mining weevil. This 2 x 2 factorial experiment was replicated on a total of 60 mature American beech trees (15 replicates per treatment) with trees injected 13 October 2016 (Fig. 1). Thirty of the trees were re-injected 18 October 2017 and thirty will not be re-injected until the fall of 2018; fifteen untreated trees are serving as controls. The following data were collected from each sample tree prior to injection, and were re-assessed again at 2 weeks post-budflush in spring and again after 2nd flush in August 2017: 1) crown condition was visually assessed using the CFS forest health method, and also by taking color photographs of tree crowns; 2) severity of beech bark disease was rated as absent, low, moderate, or heavy, according to the area of the main stem with cankers; 3) each tree was recorded as alive or dead, based on presence/absence of live buds and foliage.

Data on the effect of 2016 fall injections on egg lay and larval survival in spring and summer 2017 were collected as follows. One 30 cm-long branch tip was sampled from the mid-crown of each tree at the time of bud-flush in spring 2017, and again at weekly intervals until the end of July (when the new generation of weevil adults have completed emergence from leaves of untreated trees). Branch samples were frozen at -10C until processed to record the following data per branch: 1) total number of leaves; 2) number (%) of leaves with adult feeding damage; 3) number (%) of leaves with leaf mines; 4) total number of leaf mines. The apical leaf from each branch sample was dissected under a stereoscopic microscope and the following data recorded: 5) number of adult feeding holes; 6) number of unhatched eggs, hatched eggs and oviposition scars; 7) number of leaf mines initiated; 8) number of beech weevil larvae; 9) width of head capsule of each larva; 10) number of pupal cells (blotch mines); and 11) number of empty pupal cells (i.e., successful adult emergence).

Data analysis. Data were subject to analysis of variance using generalized linear models in SAS (PROC GLIMMIX). Proportion data, e.g., number of leaves with mines of the total number of leaves in a branch sample, or proportion of total eggs that hatched, were analyzed using the binomial distribution and logit link. Count data, e.g., number of eggs or larval mines per leaf, and data on the length of larval mines or width of head capsules were analyzed using generalized linear models that assumed either a normal, Poisson, or negative binomial distribution, and the goodness of fit assessed using the value of the corrected Aikaike Information Criterion. We report the results from the model that had the best fit (lowest AICc). Means were compared using the Tukey-Kramer test that controls the experiment-wise error rate.

Results

Injection of 3 or 5 mls of TreeAzin per cm DBH in fall of 2016 significantly reduced damage in spring of 2017 in the: 1) percentage of leaves with larval mines; 2) percent of hatched eggs that established larval mines; 3) mean length of larval mines; and 4) percentage of hatched eggs that developed to pupae or adults. The higher dose of 5 mls per cm dbh also significantly reduced: 5) the percentage of leaves with scorched tips; 6) the percentage of eggs that hatched; 7) the mean number of mines per leaf; and 8) the percentage of hatched eggs that developed to pupae. Neither dose reduced the percentage of leaves with adult feeding damage or number of eggs per leaf (Table 1).

Conclusions

Stem injection of TreeAzin reduced larval survival and damage by larvae of the European beech leaf-mining weevil in American beech trees either when injected in early spring, prior to bud burst, at a rate of 3 mls per cm dbh, or when injected the previous fall at a rate of either 3 mls per 5 mls per cm dbh. The higher dose was slightly more efficacious than the lower dose. Neither early spring nor fall treatments reduced damage from adult feeding nor the number of eggs laid per leaf. Efficacy data were submitted to PMRA in December 2017 as part of an application for emergency registration of TreeAzin for control of beech weevil in Nova Scotia. Additional data will be collected in 2018 and 2019 to determine whether annual or biennial injections are necessary to prevent tree mortality.

Table 1. Effect of TreeAzin fall injection (3 or 5 mls per cm dbh, *versus* untreated controls) on damage to foliage of American beech and survival of the European beech leaf-mining weevil, *Orchestes fagi,* In Halifax, Nova Scotia. Trees were injected 13 October 2016 and assessed 8 May–29 June 2017. Within rows, means followed by different letters were significantly different (Tukey-Kramer test, P < 0.05).

	Treatment										
Response variable	**Control**			**3mls/cm dbh**			**5mls/cm dbh**				
	mean	SE		mean	SE		mean	SE		**F**	**P**
% leaves with "scorched" tips*	41.7	6.1	a	38.6	4.8	a	29.8	3.4	b	10.9	**0.0001**
% leaves with adult shot holes	98.7	0.6	a	98.6	0.9	a	97.7	1.0	a	0.9	NS
number of eggs per leaf	4.4	0.5	a	5.1	0.5	a	4.4	0.4	a	0.6	NS
% eggs that hatched	53.9	3.5	a	45.4	2.2	ab	39.6	5.1	b	8.4	**<0.001**
% of hatched eggs that established larval mines	66.9	6.4	a	27.8	6.2	b	35.7	8.0	b	21.3	**<0.0001**
% leaves with mines	72.7	4.0	a	41.0	5.5	b	32	4.7	c	200	**<0.0001**
number of mines per leaf	1.87	0.29	a	0.87	0.22	ab	0.82	0.22	b	4.8	**<0.05**
length of larval mines	19.2	3.3	a	2.8	0.8	b	1.7	0.5	b	22.6	**<0.0001**
width of larval head capsule	0.331	0.038	a	0.216	0.041	a	0.205	0.033	a	3.0	**<0.05**
% of hatched eggs that developed to pupae	22	5.3	a	0.7	0.7	b	0.2	0.2	b	15.6	**<0.0001**
% of hatched eggs that developed to adults	11.9	3.2	a	0.7	0.7	b	0.2	0.2	b	9.7	**<0.001**

* > 30% of leaf was wilted and necrotic at the tip

Fig. 1 Ed Czerwinski injecting American Beech trees with TreeAzin, 13 October 2016, Fall River, Nova Scotia. (J. Sweeney photo)

Acknowledgements. We thank the Canadian Forest Service, Nova Scotia Department of Natural Resources, and the Ontario Ministry of Natural Resources, and SERG International for funding this project. We thank Halifax Regional Municipality, Ashburn Golf Course, St. Thomas Anglican Church, Fall River, Nova Scotia, and Murray and Roxanne Tate for permission to work on their property, and Brooke O'Conner, Andrew Morrison and Colin Mackay for technical support.

The Synthesis of a New Potential Beech Leaf-Mining Weevil (*Orchestes fagi* (L.)) Kairomone, 9-Geranylterpinolene

P. D. Mayo[1], P. J. Silk[1], S. D. Abeysekera[1] and D. I. MaGee[2]

[1]*Natural Resources Canada, Canadian Forest Service - Atlantic Forestry Centre, Fredericton, New Brunswick, Canada*

[2]*Department of Chemistry, University of New Brunswick, Fredericton, New Brunswick, Canada*

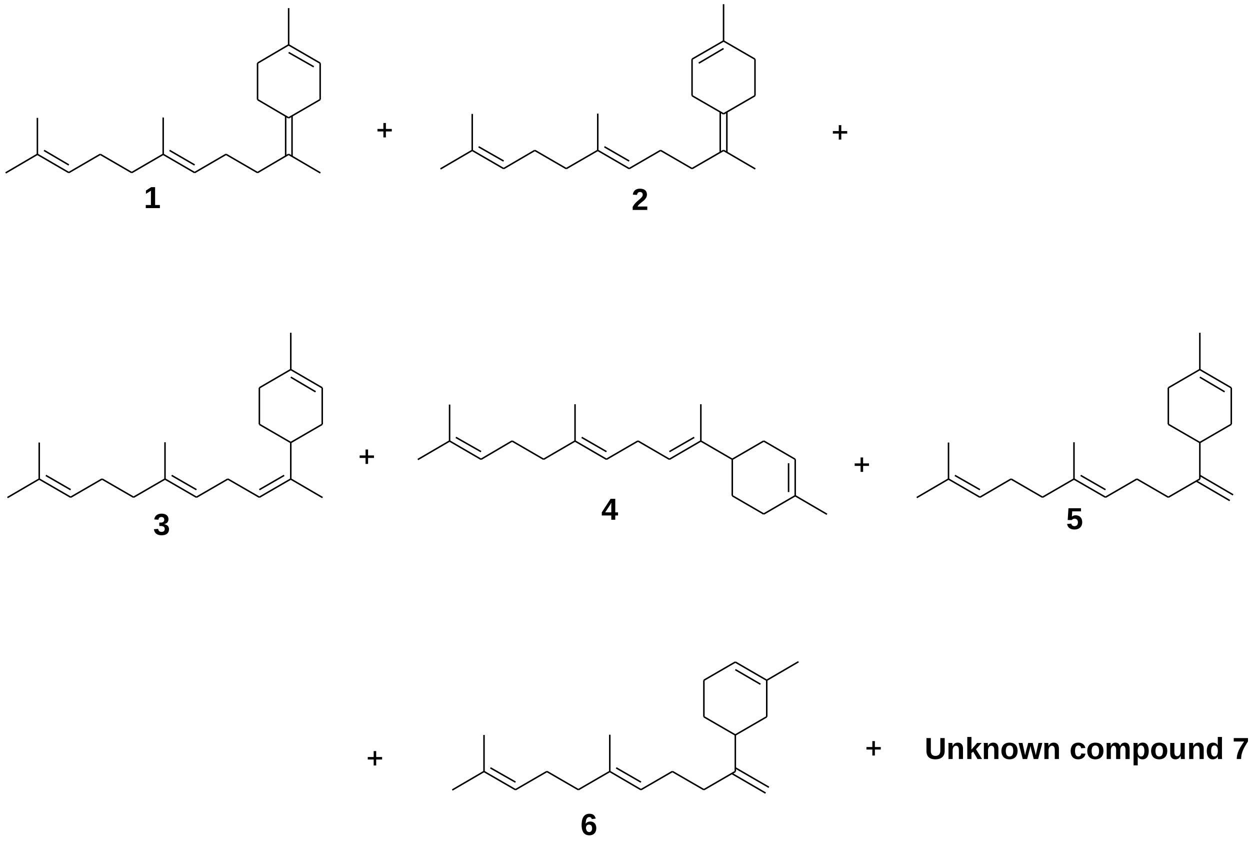
Abstract: The beech leaf-mining weevil, *Orchestes fagi (L.)*, also known as the beech flea weevil, is a common and widespread pest of beech, *Fagus sylvatica L.*, in its native Europe. It now appears to be well established in Nova Scotia, Canada. We have previously reported the synthesis of 9-geranyl-*p*-cymene and 9-geranyl-α-terpinene as they are both found in eclosing beech buds, and have researched the synthesis of other diterpenes which are isomers of 9-geranyl-α-terpinene. We now wish to report a synthesis of the diterpene 9-geranylterpinolene as a mixture of isomers, as a novel diterpene and as a possible kairomone of *O. fagi*. The synthesis of two other isomers of 9-geranyl-α-terpinene, α-springene and β-springene, was accomplished as well.

Résumé : L’orcheste du hêtre (*Orchestes fagi* (L.)), également nommé charançon du hêtre, est un ravageur du hêtre (*Fagus sylvatica* L.) commun et répandu dans son aire de répartition indigène, en Europe. Il semble être maintenant bien établi en Nouvelle-Écosse, au Canada. Nous avons déjà rapporté la synthèse du 9-géranyl-*p*-cymène et du 9-géranyl-α-terpinène, qui sont tous deux présents dans les bourgeons de hêtre en éclosion, et nous avons effectué une recherche sur la synthèse d’autres diterpènes qui sont des isomères du 9-géranyl-α-terpinène. Nous voulons signaler la synthèse du diterpène 9-géranylterpinolène en tant que mélange d’isomères, que

diterpène nouveau et que kairomone possible de l'*O. fagi*. De plus, nous sommes parvenus à la synthèse de deux autres isomères de 9-géranyl-α-terpinène, l'α-springène et le β-springène.

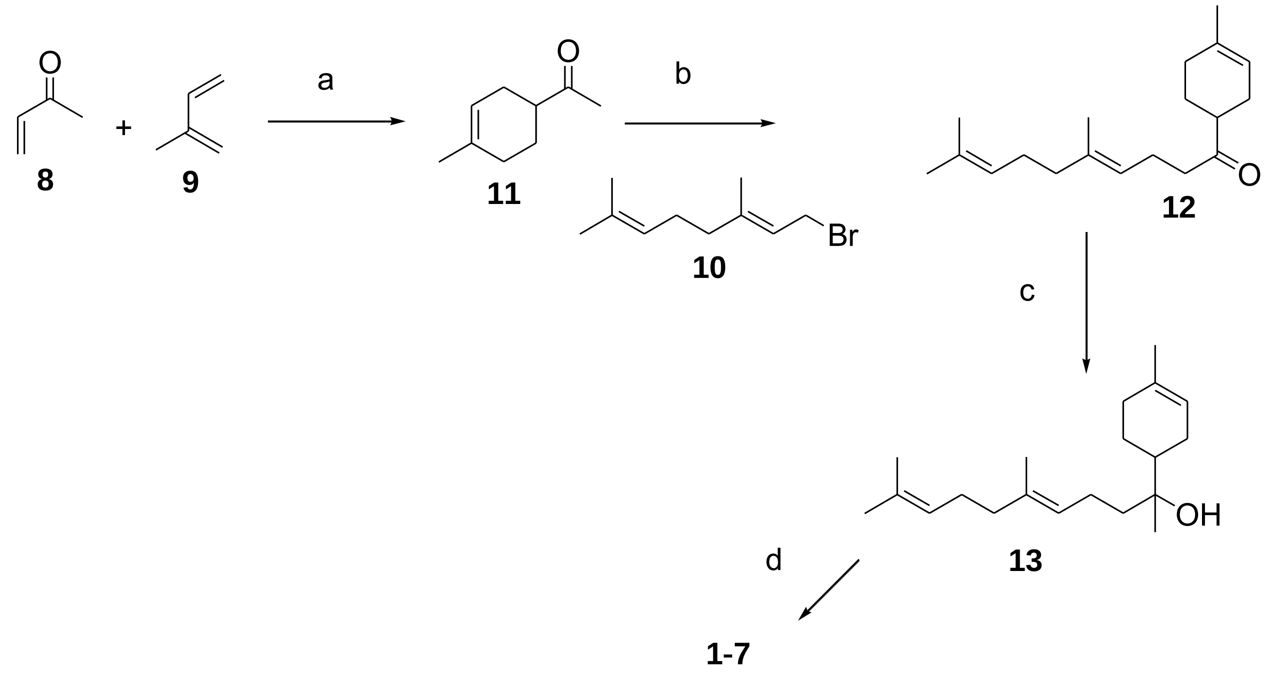
Project Details and Accomplishments:

The chief accomplishment of this project is the synthesis of 9-geranylterpinolene (2,6-dimethyl-10-(4-methyl-3-cyclohex-*Z*-ene-1-ylid-*E*-ene)-2,6*E*-undecadiene **1**) as a mixture of 7 detectable isomers (molecules **1** through **7**, see Scheme 1 below).



Scheme 1: 9-Geranylterpinolene **1** as a mixture of isomers **1**-**7**.

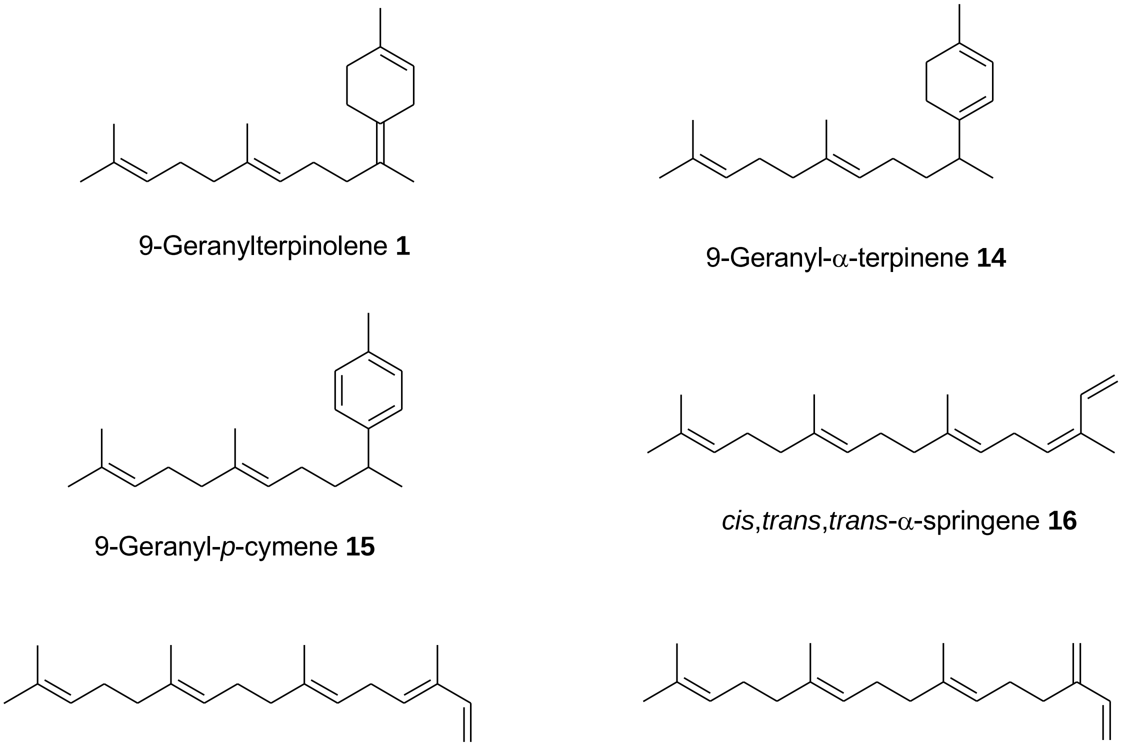
This is a novel synthesis as no synthesis of 9-geranylterpinolene has been discovered by myself in the literature to date. The synthesis starts from commercially available compounds (methyl vinyl ketone **8**, isoprene **9** and geranyl bromide **10**, see Scheme 2) and is only 4 steps long. Unfortunately, the target compound is in a mixture of 6 other isomers at the end of my synthesis.



Scheme 2: (a) $SnCl_4 \cdot 5H_2O$, benzene, rt, 10 %. (b) (i) LDA, THF, -78 °C; (ii) **10**, -78 °C – rt, yield not determined. (c) MeMgI, Et_2O, reflux, 18 % over 2 steps. (d) $POCl_3$, pyridine, 0 °C – rt, **1**-**7** in a combined yield of 92 %.

9-Geranylterpinolene **1** is relevant to the study of the semiochemistry of the beech leaf-mining weevil, *Orchestes fagi* (L.), as it is an isomer of 9-geranyl-α-terpinene **14** (synthesis previously reported by our group, see Scheme 3)[1] which we have found in the volatiles of eclosing beech buds. As well as 9-geranyl-α-terpinene **14**, smaller amounts of other isomers of **14** were found in eclosing beech buds but not identified. Also, 9-geranyl-*p*-cymene **15**, which we have found to be a kairomone of *O. fagi*,[2] is a product of air oxidation of 9-geranyl-α-terpinene **14**. Three other isomers of 9-geranyl-α-terpinene, namely *cis*,*trans*,*trans*-α-springene **16**, *trans*,*trans*,*trans*-α-springene **17** and all *trans*-β-springene **18** as a mixture were synthesized by means of a method known in the literature;[3] also all *trans*-β-springene **18** was synthesized in its pure form.[4] All 3 of these chemicals are known to semiochemistry and are isomeric with 9-geranylterpinolene **1**. For these reasons, our group feels that the mixture of 9-geranylterpinolene

1 and its isomers **2-7** is possibly worth field testing on *O. fagi* in the 2018 field season, along with the mixture of **16**-**18** and pure **18**.



9-Geranylterpinolene **1**

9-Geranyl-α-terpinene **14**

9-Geranyl-*p*-cymene **15**

cis,*trans*,*trans*-α-springene **16**

trans,*trans*,*trans*-α-springene **17**

all *trans*-β-springene **18**

Scheme 3: Isomers of **1** (**14** and **16**-**18**), as well as 9-geranyl-*p*-cymene **15**.

Also, the novel nature of the synthesis of **1** makes it of interest to natural product and terpene chemistry.

References

1. Mayo, P.D.; Silk, P.J.; Abeysekera, S.D.; MaGee, D.I. Synthet. Commun. 2016, 46(13), 1124–1132.
2. Silk, P.J.; Mayo, P.D.; LeClair, G.; Brophy, M.; Pawlowski, S.; MacKay, C.; Hillier, K.; Hughes, C.; Sweeney, J.D. Entomol. Exp. Appl., 2017, DOI: 10.1111/eea.12603.
3. Majetich, G.; Hicks, R.; Okha, F. New J. Chem. 1999, 23, 129–131.
4. Otera, J.; Niibo, Y.; Okuda, K. Chem. Lett., 1986, 15(11), 1829–1832.

Breakdown of Funding

2017 Fiscal year; CFIA - $17,000; AIF / ACOA - $22,000; CFS – in kind.

Total : $39,000.

Using the functional traits of soil fungi to improve post-disturbance pine regeneration

Nadir Erbilgin[1] *& Jonathan Cale*[1]

[1]Department of Renewable Resources, University of Alberta, Edmonton. Alberta, Canada

Summary: Projections of increased intensity and frequency of natural biotic (mountain pine beetle) and abiotic (wildfire) forest disturbances underscore a need for novel management strategies that accelerate healthy post-disturbance forest recovery in order to maintain dependable commodity supply. We have begun to investigate how biotic and abiotic disturbances individually and in combination affect the functional roles soil fungi play in forest regeneration. Utilizing field and greenhouse surveys and experiments we have made great progress toward achieving our goal of developing novel forest management methods through understanding how soil fungal communities mediate interactions between forest disturbances and forest regeneration through the functional benefits these communities provide developing seedlings. While work to meet our study objectives continues, we made substantial progress in 2017 toward meeting these objectives. We located and sampled soil, overstory and understory plant communities at 28 field sites representing 14 disturbed-undisturbed (control) site pairs. At each of the disturbed sites, lodgepole pine seeds were sown into four 1 m^2 plots to be used in assessing the feasibility of remediating post-disturbance fungal communities in order to accelerate forest regeneration in the coming years. After a comparative study, we identified an accurate and efficient method for extracting fungal DNA from soil samples. Two greenhouse experiments involving 756 lodgepole pine seedlings growing in pots inoculated with field soil have concluded. Data on seedling performance is being analyzed for one of the experiments, while tissue samples from the other experiment are being processed for analysis of defense related chemicals. With this progress and as work continues, we are well positioned to meet project milestones in 2018.

Résumé: L'accroissement attendu de l'intensité et de la fréquence des perturbations en milieu forestier attribuables à des facteurs naturels biotiques (dendroctone du pin ponderosa) ou abiotiques (incendies de forêt) nécessite des stratégies de gestion novatrices visant à accélérer le rétablissement de la forêt après une perturbation afin de maintenir un approvisionnement fiable en bois. Nous avons commencé à examiner comment les perturbations biotiques et abiotiques individuelles ou en combinaison affectent les rôles fonctionnels que jouent les champignons du sol dans la régénération forestière. Grâce aux relevés et aux expériences faites sur le terrain et en serre, nous avons grandement progressé vers la réalisation de notre objectif de mettre au point de nouvelles méthodes de gestion forestière grâce à une meilleure compréhension du rôle de médiation des communautés fongiques du sol dans les interactions entre les perturbations forestières et la régénération forestière du fait des avantages fonctionnels que ces communautés offrent aux jeunes semis en développement. Alors que les travaux pour réaliser les objectifs de l'étude se poursuivent, nous avons fait des progrès considérables en 2017. Nous avons repéré des communautés végétales du sol, de l'étage dominant et du sous-étage et prélevé des échantillons à 28 endroits pour obtenir 14 paires de sites perturbés-non perturbés. Chaque site perturbé a été ensemencé de pin tordu latifolié sur quatre parcelles de 1 m^2 afin d'évaluer la faisabilité de procéder à la remédiation des communautés fongiques après une perturbation pour accélérer la régénération de la forêt dans les années qui suivent. Une étude comparative nous a permis de déterminer une méthode précise et efficiente pour extraire l'ADN fongique des échantillons de sol. Deux expériences en serre comprenant 756 semis de pin tordu latifolié poussant dans des pots inoculés avec du sol provenant du terrain sont achevées. L'analyse des données sur le rendement des semis de l'une des expériences est en cours,

tandis que des échantillons de tissus de l'autre expérience sont en préparation en vue d'une analyse des substances chimiques de défense. Vu les progrès réalisés et le fait que les travaux se poursuivent, nous sommes en bonne posture pour atteindre les grands jalons de l'étude en 2018.

Introduction

National access to dependable forest commodities is increasingly at risk from increases in natural and anthropogenic disturbances, and forest pest outbreaks predicted under climate change scenarios. The forestry sector is an important component of the Canadian economy, contributing $19.8 million to national GDP in 2013 (Natural Resources Canada, 2014). Forestry revenues, however, are undermined by novel disturbance regimes, such as wildfire exceeding historic levels (Wotton & Flannigan 2010) and the unprecedented mountain pine beetle (*Dendroctonus ponderosae*; MPB) outbreaks (Parkins & MacKendrick 2007). Natural disturbances not only destroy timber, they disrupt forest ecosystem functions (e.g., carbon storage and nutrient cycling) (Bentz et al. 2010; Aukema et al. 2011; Boyd et al. 2013). These events variously threaten the regeneration—an integral part of forest recovery from disturbance—of desirable tree species and thus the sustainability of the forest commodities which bolster provincial and national economies (Natural Resources Canada, 2014). Regeneration of lodgepole pine (*Pinus contorta*) forests is especially critical as this is a dominant timber species in western Canada (Natural Resources Canada, 2014). Economic concerns and impacts of cumulative disturbances may become increasingly pronounced in coming years as stress in forests increases with climate change, creating conditions, for example, where biotic and abiotic disturbances are followed by wildfire and species invasions (Bentz et al. 2010; Wotton & Flannigan 2010; Bradley et al. 2012). Substantial pressure from cumulative disturbances may surpass the forest industry's capacity to maintain sustainable forest goods and services. To avoid this, new technologies promoting forest regeneration are needed to design efficient and innovative remediation, triage, and reforestation programs.

Tree seedling establishment is integral to forest regeneration and resilience following disturbance, a process dependent upon soil fungi. A critical aspect of forest resilience—the capacity to return to a pre-disturbance condition after disturbance—is the establishment of healthy seedlings of ecologically or economically suitable tree species following disturbance. Failed establishment can cause shifts in species dominance, and forest resilience capacity may be exceeded under frequent, successive, or compounded disturbance events (Boyd et al. 2013; Johnson et al. 2015). For many tree species, seedling establishment is also linked to co-occurring soil fungi (Nara 2006), which play important roles in forests including influencing plant nutrition, soil carbon sequestration, soil formation, and nutrient cycling (Fierer et al. 2010; Plassard & Dell 2010; Urbanova et al. 2015). Certain soil fungi colonize plant roots to form mycorrhizal symbioses, which increase root absorptive capacity, plant nutrition, and alter plant resistance to herbivores and pathogens (Campos-Soriano et al. 2012; Jung et al. 2012; Cameron et al. 2013; Vannette & Hunter 2013; Näsholm 2014). These symbioses often allow seedlings to thrive in conditions that would otherwise prohibit establishment (Karst et al. 2014). Forest regeneration is therefore mediated by the effects of soil fungi on seedling health. However, the functional benefits seedlings receive vary greatly among plant and fungal species combinations and thus variation in soil fungal communities can potentially alter seedling establishment, forest resilience, and the long-term reliability of forest product production.

Disturbance-impacted fungal communities can adversely affect seedling performance, but the underlying functional mechanism is unknown. Disturbance-induced plant stress and mortality reduces carbon allocation to soil fungi, causing loss of carbon-demanding species, and subsequent changes to fungal community composition (Holden et al. 2013; Karst et al. 2014; Štursová et al. 2014; Treu et al. 2014). In some cases, communities recover to pre-disturbance levels only after many years without disturbance, if at all (Cairney & Bastias 2007; Brown et al. 2013). Community response to individual disturbances is well studied (Bradbury et al. 1998; Dahlberg et al. 2001; Jones et al. 2003; Cullings et al. 2005; Cairney & Bastias 2007), but comparative examinations, largely restricted to two disturbances (e.g. wildfire and harvesting), suggest disturbance types differentially affect fungal communities (Dahlberg et al. 2001; Barker et al. 2013). However, a more comprehensive examination of soil fungal responses to disturbances is needed to identify functional groups consistently active in forest regeneration. For example, the recent MPB outbreak in Alberta is associated with shifts in fungal mutualists, and decreased seedling performance (Karst et al. 2015). How those changes compare to other common disturbances, and whether soil amendments can be used to mitigate harm, is both unknown and critical to understand.

***Objectives**:* Our overall goal was to determine how soil fungal communities mediate interactions between natural and anthropogenic forest disturbances and forest regeneration through alterations to the functional benefits these communities provide developing pine seedlings. This goal was constructed in order to develop new strategies for mitigating forest commodity loss and ecosystem degradation under a projected scenario of climate change-related increase in pressure from single or cumulative disturbance events. We are pursuing four objectives to achieve this goal:

1) To determine how soil fungal communities are affected by individual and cumulative forest disturbances,
2) To identify the components of fungal communities (e.g., saprotrophic, mycorrhizal, pathogenic) that promote forest recovery following disturbances as indicated by successful lodgepole pine regeneration,
3) To test if soil fungal communities can be amended to improve regeneration, and
4) To characterize how disturbances affect the functional roles soil fungi play in regeneration. Our work will identify new technologies to facilitate lodgepole pine regeneration in highly disturbed and invaded forests thereby avoiding adverse ecosystem and economic effects. Such technologies could include disturbance-specific reforestation or remediation protocols.

Methodology

Sites were selected in a paired manner, with each pair consisting of a disturbed and non-disturbed forest stand. Within a pair, stands had similar compositions, ages, and densities. Disturbed sites had been affected by one of four disturbance events within the past five years: wildfire, MPB outbreak, clearcut harvesting, or MPB followed by harvesting. Pairs of disturbed-undisturbed sites were used, with pairs being located at least 30 km from each other on the Alberta landscape. This design differed from the originally proposed design of independent sites consisting of nine disturbance scenarios for two primary reasons. First, we were unable to restrict site selection to the narrow range of forest conditions (e.g., composition, age, and density) needed to properly replicate and make reliable comparisons/inferences using independent-site design. Second, a lack of occurrence, records, spatial data, or access for field crews, forced us to limit the study to four disturbance scenarios (mentioned above). However, these four scenarios represent the most common disturbances affecting the western Canadian boreal forest. Furthermore, a paired design gives us stronger statistical power with which to identify patterns.

A 30 m x 30 m area was delineated at each site within which soil and overstory and understory plants were sampled. Soil cores (10 cm x 2.5 cm diameter) were collected from each of 25 points spaced 7.5 m apart along a grid occurring within the delineated area. Overstory sampling (to assess stand composition, density, and basal area) was conducted along two parallel belt transects (7.5 m x 30 m) spaced 7.5 m apart. Understory plant communities were sampled using three 1 m^2 quadrats nested within each belt transect. Within each transect the percent cover of each functional plant group (e.g., ferns, forbs, graminoids, etc) as well as woody debris and bare ground was estimated.

The extraction of fungal DNA from all 700 soil samples along with experimentation in order to optimize extraction and amplification methodologies was recently completed. Genomic analysis of soil fungal communities is underway using state-of-the-art next generation sequencing and bioinformatics techniques.

Four 2 m x 2 m plots were also established at each site approximately 10 m outside of the delineated area in 2016. In each of these four plots, the organic soil layer was removed and the remaining inorganic soil turned and sown with lodgepole pine seed. Within a site, plots were spaced 1 m apart. These plots were used to assess the potential to remediate soil fungal communities.

Two greenhouse projects were established using 6% field-collected soil to inoculate pots containing 3:1 sand to organic soil mixture sown with stratified lodgepole pine seed. Greenhouse project 1 addresses how variation in soil fungal communities influences seedling growth, biomass accumulation, nutrition, and carbon allocation to belowground symbionts. Furthermore, seedlings underwent a stable isotope probe in order to determine relative carbon allocation to symbiotic fungi. Greenhouse project 2 investigates how variation in these communities influences the produce phytochemicals involved seedling resistance to insects and pathogens. For this project, seedlings were either untreated or treated with defense-related hormones (methyl jasmonate and methyl salicylate), and above- belowground tissue was collected and are presently being prepared for chemical extraction of phytochemicals and subsequent chromatographic analysis.

Results

Field survey. All field work has concluded, while genomic analyses continue. DNA has been extracted from 700 samples of field soil. After much experimentation, working primers for DNA for ectomycorrhizal and arbuscular mycorrhizal fungi have been determined. Over the next few months, amplifications will be performed in series, and the products will be sequenced. Bioinformatics work will begin immediately after in January, 2018. Soil nutrients and texture has been quantified in order to characterize field sites. Specifically, we have measured soil percent clay, silt, and sand as well as soil ammonium, nitrate, phosphate, potassium, and pH. Biochar pouches buried in summer 2016 have been collected, and a subset of pouches is being used to quantify soil hyphal density for each field site.

Field experiment. The four 1x1 m plots at each disturbed site that were established and sown with lodgepole pine seed in 2016 were revisited in summer 2017. Sowings were successful in almost all plots as we observed 50–100 first-year seedlings. In order to amend soil fungal communities in these plots, soil cores were transferred from control sites paired with the disturbed sites. Cores were installed in each plot at different densities according to treatment (0, 1, 2, or 4 cores / m2). All soil transfers were completed. In summer 2018, first- and second-year seedlings as well as soil cores will be collected from each plot. Mycorrhizal colonization of seedlings, seedling biomass, and soil fungal communities will be compared among disturbance-amendment density treatments.

Greenhouse Experiment 1. Lodgepole pine seedlings (280) in pots inoculated with soil collected from the field sites began growing for ten months under greenhouse conditions in October, 2016. Seedling height was measured weekly over the growing period, and foliar chlorophyll content was measured just prior to harvesting. Three days prior to harvesting, half of the seedlings were pulse-labeled with C13-CO2. Measurements of growth, biomass, and nutrition are being analyzed.. Fungal DNA from the root trips of C13-treated seedlings will be extracted and fractionated in October-December, 2017 so that the C13-labeled DNA can be sequenced in early 2018. Furthermore, preliminary analysis suggests forest disturbances have differential effects on the survival, growth, and biomass accumulation of pine seedlings.

Greenhouse Experiment 2. Lodgepole pine seedlings (360) in pots inoculated with soil collected from the field sites began growing under greenhouse conditions in October, 2016. When ten-months old, seedlings were randomly assigned to three groups (methyl-jasmonate-treated, methyl-salicylates-treated, and non-treated controls) nested within site such that each group contained 2-4 seedlings (6-12 seedlings per site). Additional seedlings were used as greenhouse controls (6 seedlings; pots inoculated with sterile potting mix), Tween controls (15 seedlings), and induction controls (15 seedlings). Tween controls are needed to test potential effects of this surfactant, in which the phytohormones were suspended, on seedling phytochemicals. Induction controls are needed to test whether hormone-treated seedlings induce metabolite production in nearby non-treated seedlings. All seedlings were harvested in Sept., 2017. Seedlings are now being ground in preparation for phytochemical profiling, which will start in January, 2018.

Conclusions

No conclusions are presently available as progress toward meeting project objectives is ongoing.

Financials

Funding source	2017	2018
NERC-Strategic Partnership Grant. Covers salary for one post-doctoral fellow and two graduate students as well as salary for one summer student/yr, field truck rental, meals and accommodations, field and laboratory supplies, chemical and molecular analyses.	$198,390	$130,665
SERG-I – Partial funding for a second summer student; funding to attend annual SERG meeting.	$7,600	$14,000 (requested from SERG-I)
Total	$205,990	$144,665

References

Natural Resources Canada. 2014. The State of Canada's Forests. Annual Report 2014.
Wotton BM, Nock CA & Flannigan MD. 2010. Int. J. Wildl. Fire. 19:253–271.
Parkins JR & MacKendrick NA. 2007. Glob. Environ. Chang. 17:460–471.
Holmes TP, Aukema JE, Von Holle B, Liebhold A & Sills E. 2009. Ann. N. Y. Acad. Sci. 1162:18–38.
Aukema JE. et al. 2011. PLoS One 6:e24587.
Boyd IL, Freer-Smith PH, Gilligan CA & Godfray HCJ. 2013. Science 342:1235773.
Bentz BJ et al. 2010. Bioscience 60:602–613.
Bradley BA. et al. 2012. Front. Ecol. Environ. 10:20–28.
Johnson DJ, Flory SL, Shelton A, Huebner C & Clay K. 2015. J. Appl. Ecol. 52:210–219.
Nara K. 2006. New Phytol. 169:169–78.
Plassard C & Dell B. 2010. Tree Physiol. 30:1129–1139.
Urbanová M, Šnajdr J & Baldrian P. 2015. Soil Biol. Biochem. 84:53-64.
Fierer N, Nemergut D, Knight R & Craine JM. 2010. Res. Microbiol. 161:635–642.
Jung SC, Martinez-Medina A, Lopez-Raez JA & Pozo MJ. 2012. J. Chem. Ecol. 38:651–64.
Näsholm T. 2014. Tree Physiol. 34:331–333.
Vannette RL & Hunter MD. 2013. J. Ecol. 101:1019–1029.
Campos-Soriano L, García-Martínez J & Segundo BS. 2012. Mol. Plant Pathol. 13:579–592.
Cameron DD, Neal AL, van Wees SCM & Ton J. 2013. Trends Plant Sci. 18:539–545.
Karst J, Randall MJ & Gehring CA. 2014. Botany 92:855–865.
Štursová M. et al. 2014. ISME J. 8:1920–1931.
Treu R et al. 2014. Ecology 95:1096–1103.
Holden SR, Gutierrez A & Treseder KK. 2013. Ecosystems 16:34–46.
Cairney JWG & Bastias BA. 2007. Can. J. For. Res. 37:207–215.
Brown SP, Callaham MA, Oliver AK & Jumpponen A. 2013. FEMS Microbiol. Ecol. 86:557–566.
Dahlberg A, Schimmel J, Taylor AFS & Johannesson H. 2001. Biol. Conserv. 100:151–161.
Cullings K, Raleigh C & Vogler DR. 2005. Can. J. Bot. 83:1174–1180.
Bradbury SM, Danielson RM & Visser S. 1998. Can. J. Bot. 76:218–227.
Jones MD, Durall DM & Cairney JWG. 2003. New Phytol. 157:399–422.
Barker JS, Simard SW, Jones MD & Durall DM. 2013. Oecologia 172:1179–1189.
Karst J. et al. 2015. New Phytol. 208: 904–914.

SERG-I Progress Report: The impact of silviculture practices on the development of a recalcitrant understory layer: Are we creating a problem for future rotations?

presented by

Kierann Santala
Isabelle Aubin
Michael Hoepting
Natural Resources Canada, Canadian Forest Service

Great Lakes Forestry Centre
January 19th 2018

Abstract

Silviculture practices have well known direct positive effects on tree growth. However, such practices may release other forest components that may indirectly limit future seedling development through competition for light or nutrients. In this report, we will describe the foundations, objectives, methodology and preliminary results of the first and second year of a project examining the long-term impacts of vegetation management on understory development. This study consists of two components; the first take advantage of well documented ongoing silvicultural studies to explore long-term changes in forest understory using a legacy dataset. The second component includes a stratified field measurement to obtain a more refined assessment of successional dynamics operating within the understory community and vertical structure. By identifying vegetation management approaches that minimize the development of difficult to manage understory species and enhance crop tree productivity, this research will help optimize vegetation management investment in both the short- and long-term.

Résumé

Les pratiques sylvicoles ont des impacts directs positifs bien connus sur la croissance des arbres. Cependant, certaines pratiques sylvicoles peuvent favoriser des espèces pouvant entrer en compétition avec les semis pour la lumière et les nutriments, et ainsi limiter indirectement leur croissance. Dans ce rapport, nous présentons les prémisses, les objectives, la méthodologie et les résultats préliminaires de la première et deuxième année d’un projet qui évalue les impacts à long-terme de la gestion de la végétation compétitrice sur le développement du sous-bois. Cette étude comprend deux parties. La première, utilisant les données d’études sylvicoles bien documentées, explore les changements à long terme du sous-bois. La deuxième inclut un échantillonnage stratifié du sous-bois en vue d’obtenir une évaluation plus précise de la dynamique successionnelle du sous-bois et la structure verticale. Avec l’identification des techniques de gestion de la végétation qui minimisent le développement d’espèces récalcitrantes en sous-bois tout en améliorant la productivité des arbres commerciaux, cette étude aidera à l’optimisation de l’investissement dans la gestion de la végétation à court- et à long-terme.

Project Description

The current project aims to investigate the response of understory communities to current vegetation management practices. The management of vegetation that competes with desired crop trees is commonly used to increase survival and productivity of desired crop trees. The direct positive effects of these practices on tree growth have been well documented (Man *et al.* 2008; Pitt *et al.* 2009, 2010, 2011, 2015, 2016; Boucher *et al*. 2007). However, such practices may also have unintended long-term consequences, such as releasing other forest components that may indirectly limit future seedling development through competition for light or nutrients. Some studies have reported the development of a recalcitrant layer of competitive understory species with an intensification of silvicultural practices (e.g. Royo & Carson 2006; Aubin *et al.* 2014), which may stunt or delay tree growth of future rotations. This could lead to increased silvicultural efforts and costs needed to initiate the next rotation. It has been hypothesized that productive sites, usually considered the best candidates for silvicultural treatments, are at greater risk of invasion by recalcitrant species. Although the autecology of competitive species is well documented, most of these studies are localized and few have investigated the mechanisms at play following release. A large scale comparison is therefore necessary to overcome the issue of scaling up results from local studies to set regional or provincial management prescriptions.

This project was developed to inform management and silvicultural practices by 1) identifying patterns relevant at regional and provincial scales; and 2) improving mechanistic understanding of the processes involved in the development of a recalcitrant layer. When designing this project, we took advantage of ongoing silvicultural studies across Eastern Canada where a gradient of understory vegetation management treatments have been applied. This project consists of two components. The first took advantage of a legacy dataset of an ongoing white pine (*Pinus strobus* L.) silviculture study comprised of three sites in two silviculture systems (two shelterwood and one clearcut) and focused on the long-term functional response to vegetation control treatments[1]. The second component included a stratified floristic survey in two established long-term white pine shelterwood systems to investigate the effects of varying intensities of site preparation on understory composition[2]. These surveys will be used to investigate the structural and functional understory response to silviculture practices (i.e. site preparation and competition control treatments).

In the spring of 2015, we performed analysis on a dataset from the three white pine experiments to explore the effect of treatment over time on composition, functional assemblage of understory communities. This dataset allowed for comparison of patterns of understory development in distinct geographical locations (from New Brunswick to Ontario). Data used for analysis was collected over a 10 year period following planting, giving us a unique opportunity to explore how competition control treatments affect the development of recalcitrant species and the understory over time in uniform shelterwood and clearcut silviculture systems. Because this study had already investigated the effects of vegetation control treatments on wood volume and growth, we were able to compare gains in tree productivity with changes in understory community.

---

[1] SERG Project 2000/06: Effects of early herbaceous and woody vegetation control on eastern white pine (est. 2000).

[2] Meridian Road White pine crown spacing and site preparation study. Petawawa Research Forest, ON (est. 1994). Effects of selected intensities of silviculture on white pine growth and yield. Britt, ON (est. 1997)

In July of 2016, we sampled the Meridian study site at the Petawawa Research Forest and, in July 2017, sampled a similar study site near Britt, Ontario. The two study sites share common initial vegetation management treatments applied in white pine uniform shelterwoods over a range of site conditions (e.g. soil depth, moisture, and topography). We will use a trait-based approach for greater comparability between studies conducted over large geographical areas. Finally, we will apply a structural analysis using an innovative stratified field measurement technique to evaluate successional dynamics operating within the forest community more effectively. By identifying approaches that limit the development of difficult-to-manage understory vegetation and enhance crop tree productivity, this research will help optimize vegetation management investment in both the short- and long-term.

Participants

Main researchers: Dr. Isabelle Aubin (CFS) and Michael Hoepting (CFS)

Objectives

One key aspect of this project is to provide a large-scale assessment of the relationship between local understory development and forestry practices, in the search of global patterns that could inform silvicultural prescription development at the provincial and/or regional levels. Another key goal is to provide a mechanistic understanding of the phenomenon of recalcitrant layer development, leading to better informed management and mitigation options.

The questions that will be answered by these two studies are:

1) ***Assessing long-term changes in understory using a pre-existing dataset***
    1. How do vegetation management treatments alter community development trajectories over time?
    2. How does functional community composition respond to these treatments?
    3. What is the response on target species (recalcitrant species and interior forest herbs)?
    4. What is the effect of these treatments on tree growth and performance?

By answering these questions, we can discuss how the magnitude of change in community composition compares to that of gains in productivity.

2) ***Assessing impacts on understory structure using field measurements***
    1. Do some silvicultural practices negatively impact tree growth in the long-term by favoring the development of a recalcitrant understory layer?
    2. Are some practices more susceptible to the development of a recalcitrant layer?
    3. What are the mechanisms underlying the formation of such a layer and can we prevent it while still achieving enhanced crop tree production?
    4. Are there some sites that are more susceptible to layer formation (e.g. productive sites)?

Progress related to the Deliverables

In Year 1 (2016-2017)

- SERG-I interim report: COMPLETE
- Presentation to SERG-I workshop: COMPLETE

Component 1: Assessing long-term changes in community composition using a pre-existing dataset

- Collate existing datasets from Pitt & Hoepting silvicultural studies: COMPLETE
- Compile plant trait data from literature review: COMPLETE
- Analysis of vegetation response to silvicultural treatment: In progress

Component 2: Assessing impacts on forest structure using field measurements

- Perform a stratified floristic survey in the Meridian silvicultural study: COMPLETE
  - Vegetation Survey: COMPLETE
  - Data Entry: COMPLETE
  - Foliar nutrient Analysis: COMPLETE
  - Lab Measurements: In progress
  - Compile plant trait data from literature review: In progress
  - Preliminary analysis of vegetation response to silvicultural treatment: In progress

In Year 2 (2017-2018)

- SERG-I interim report: COMPLETE
- Presentation to SERG-I workshop: Anticipated delivery February 2018

Component 1: Assessing long-term changes in community composition using a pre-existing dataset

- Analysis of vegetation response to silvicultural treatment: COMPLETE
- Preparation of draft manuscript: COMPLETE

Component 2: Assessing impacts on forest structure using field measurements

- Perform a stratified floristic survey in the Meridian silvicultural study: COMPLETE
  - Vegetation Survey: COMPLETE
  - Data Entry: COMPLETE
  - Foliar nutrient Analysis: COMPLETE
  - Lab Measurements: COMPLETE
  - Compile plant trait data from literature review: In progress
  - Preliminary analysis of vegetation response to silvicultural treatment: COMPLETE
- Perform a stratified floristic survey in the Britt silvicultural study: COMPLETE
  - Vegetation Survey: COMPLETE
  - Data Entry: COMPLETE
  - Foliar nutrient Analysis: In progress
  - Lab Measurements: In progress
  - Compile plant trait data from literature review: In progress
  - Preliminary analysis of vegetation response to silvicultural treatment: COMPLETE

Component 1: Assessing long-term changes in understory using a pre-existing dataset

The following is a draft version of a manuscript anticipated to be submitted in spring of 2018. The draft presented in this report is currently in review by project collaborators.

Abstract:

Increased regulatory pressure to manage forest diversity requires management strategies that promote both sustainable fiber production and conserve biodiversity. Techniques to suppress competitive vegetation have become standard in eastern white pine plantations to release seedlings from competition and promote growth. We investigate the trade-offs between white pine performance and understory community composition under several vegetation suppression scenarios within two conventional overstory harvesting systems. We compared community and functional composition of understory species over 10 years in three white pine plantation experimental sites in Ontario and New Brunswick (Canada), one harvested using the clearcut system and two using the shelterwood system. Treatments included the suppression of either woody or non-woody species, or both and were applied over different durations of time. Ten years after planting, our results show that plots treated with a single broadcast application of herbicide (targeting both woody and non-woody vegetation) both favored rapid white pine growth and allowed the understory community to recover quickly and converge toward the functional attributes of a mature forest composition. Treatments that suppressed either only woody or non-woody vegetation caused major shifts in community composition that were still evident after 10 years. This was due to the prevalence of competitive species that formed stable and dominant layers. There was also a higher abundance of competitive open-habitat species in sites that were harvested by clearcut in comparison to the two shelterwood sites. Our findings suggest that a balance can in fact be struck between multiple management objectives. In the case of eastern white pine, it is likely that releasing seedlings early from competition and conserving enough canopy cover is necessary to (1) favour survival and growth; and (2) allow understory communities to recover.

Keywords: functional trait, conservation potential, vegetation management, understory vegetation, competition, silviculture, eastern white pine (*Pinus strobus* L.)

1. Introduction

Eastern white pine (*Pinus strobus* L.) has been historically one of the most important tree species in Eastern North America economically, socially, and culturally (Uprety et al., 2014). The abundance of this species has been decreasing across its natural range over the last few centuries due to harvesting, fire and pest damage (Beaulieu et al., 1996). Hence, there is an interest in the regeneration and management of this species (Carleton et al., 1996; Pitt et al., 2009). Early efforts to manage and regenerate white pine using silvicutural techniques produced inconsistent results due to poor growth and survival resulting from competition with herbaceous and woody vegetation, white pine blister rust inflection (*Cronartium ribicola* J.C. Fisch.), and white pine weevil attack (*Pissodes strobi* Peck) (Hosie, 1953; Stiell, 1985) and by the 1970's regeneration failures lead to experimentation with the uniform shelterwood system which was found to lower blister rust infection and reduce incidence of weevil attack (Campione, 2011; De Groot et al., 2005; Hannah, 1988; Lancaster and Leak, 1978; Ostry et al., 2010). More recently, tools have been developed to further reduce the effects of pathogens through genetic improvements of seedlings (Kinloch, 2003; Lu and Derbowka, 2009; Sniezko et al., 2014) and exposure to foliar entophytic fungi (Ganley et al., 2008). Increased productivity resulting from these advances has shifted the focus of research towards improving vegetation management strategies and its interaction with weevil attack to reduce the effects of competitive vegetation on white pine performance (Pitt et al 2016).

Vegetation management has been used for centuries (Hough, 1878; Rothkugel, 1907) to raise productivity of desired tree species and its direct positive effects on tree growth have been well documented (Wagner *et al.* 2006, Man *et al.* 2008; Pitt *et al.* 2009, 2010, 2011, 2015). This silviculture practice usually consists of the suppression of plants that compete with desired crop tree species through the use of herbicide application and/or manual removal of selected woody and non-woody species(Wagner et al., 2006). While providing desired crop trees with greater access to often limited site resources (light, soil water, nutrients), vegetation suppression practices can impact understory communities by disrupting assembly processes and resetting understory to an earlier developmental stage (Roberts et al., 2016). They can also be detrimental to interior forest species and can contribute to the loss of plant biodiversity (Gauthier et al., 2015; Miller et al., 1995; Noble and Dirzo, 1997). Intensive silvicultural practices have also been reported to favour the development of competitive species that can form recalcitrant layers (Horsley, 1987; Royo and Carson, 2006; Tappeiner et al., 1991). Dense recalcitrant layers (i.e. difficult to control vegetation that are more abundant than typical conditions) have the potential to inhibit or suppress forest regeneration and to prevent the colonization of more desirable late successional species (Mallik, 2003; Meier et al., 1995). The persistence of competitive species could lead to increased silvicultural efforts and costs needed to ensure success of the current crop and when initiating the next rotation.

Increased regulatory pressure to manage forests diversity (MacDicken et al., 2015) has increased the need to identify compatible management strategies that both promote sustainable fiber production and protect forest biodiversity (Eriksson and Hammer, 2006; Spence, 2001; Work et al., 2003). Although these two topics have been largely studied, often times this information is published as separate studies reducing the potential for joint comparison. Land managers would benefit from research that addresses the balance between these two objectives and would gain better insight into how best manage long-term sustainability and ecological integrity of commercial forests.

To explore these topics we took advantage of a well documented ongoing silviculture study that was initiated in 2000 to investigate the effects of a suite of vegetation suppression treatments on eastern white pine (*Pinus strobus* L.) regeneration and growth. We analysed the effects of a set of vegetation suppression treatments on both understory development and tree productivity. We are asking the following questions: 1) How do these treatments alter community development trajectories over time? 2) How do communities respond to these treatments? 3) What is the response on target species (competitive species and interior forest species) 4) What is the effect of these treatments on tree growth and performance? To provide greater comparability among study sites we assessed understory community development using a trait-based approach (Garnier et al., 2016). This approach is particularly useful in revealing mechanisms that drive successional development in plant communities (Knapp et al., 2016; Lavorel and Garnier, 2002; Naeem et al., 2012) (Diaz and Cabido, 2001; Miller et al. 1995).By answering these research questions, this paper aims to discuss how the magnitude of change in understory community compares to the gains in productivity. This knowledge will be useful in identifying vegetation suppression approaches and intensities that minimize the development of difficult to manage understory vegetation communities and lessen the affects on biodiversity while still enhancing crop tree productivity. This is important to allow for the optimization of vegetation management investment into managed stands both the short- and long-term.

2. Material and Methods

2.1. Study sites

Data was collected from three planted Eastern white pine (*Pinus strobus* L.) stands – two in Ontario, Canada (Clearcut-ON and Shelterwood-ON) and one in New Brunswick, Canada (Shelterwood-NB) (Table 1). These stands are the basis of a White Pine Competition Study that was originally developed to evaluate the effects of timing and duration of competitive vegetation suppression treatments on regenerating white pine performance (e.g. survival, growth). Pitt *et al*. (2016, 2011, 2009) and Parker *et al*. (2012, 2010, 2009) provided thorough descriptions of the study sites in reports of white pine growth responses and ecophysiological effects of treatments, so brief summaries will be provided herein. The three stands were harvested using two conventional silviculture systems (1) clearcut and (2) uniform shelterwood. Clearcut harvesting system involves the harvesting of all overstory trees from an area at one time whereas the uniform shelterwood system consists of harvesting all overstory trees in two or more successive stages to create understory conditions during the regeneration phase that favour white pine establishment and growth, and mitigate pests and disease (Hodge et al., 1989; Steill, 1985). Treatment history and stand characteristics of these three sites are summarized in Table 1.

The Ontario sites (Clearcut-ON and Shelterwood-ON) are located 2.2 km apart in the Great-Lakes –St. Lawrence forest region, northeast of North Bay. Both represent ecosite G033 (“dry, sandy: red pine – white pine conifer; tall tree” (OMNR 2009) and are typical white and red pine-dominated mixedwood forests. Fine loamy sands (first 10 cm) overlay deep, medium- to coarse-textured sands, with a rooting depth of about 60 cm and no signs of mottling or gleying. Mean annual, January, and July temperatures are 4.4°C, -12.2°C, and 18.8°C, respectively, with 1574 annual growing degree days and 475 mm of annual precipitation during May-September. Both stands were likely of fire-origin.

Clearcut-ON

The first study site (World Geodetic System; WGS 84: 46°42′44.3″ N; 79°22’14.4” W) was a 86 year old white and red dominated pine stand that was full-tree harvested in spring 2000 with subsequent manual felling of all remaining residual trees to emulate a true “clearcut” harvest condition (subsequently called Clearcut-ON). Treatment plots (18 x 18 m) were allocated over a 3 ha area in August 2000 with 3 m corridors between each. To ensure consistent regeneration and pre-treatment uniformity, each treatment plot was planted in May 2001 with Multipot 67, 1-0 containerized white pine at 2 m spacing.

Shelterwood-ON

The second study site (WGS 84: 46°43′50.7″ N; 79°22′46.1″ W) was a 100 year-old white and red pine dominated stand that was partially harvested in 1999 following a prescription for the regeneration cut phase of the uniform shelterwood system (hereafter called Shelterwood-ON). This harvest left an overstory of high quality white and red pine; however, many subordinate stems of white spruce, balsam fir, red maple, and trembling aspen that, while marked for harvest, were left standing due to poor market quality for these species. In fall of 2000, a subsequent thinning from below was completed using manual felling and a grapple skidder to remove most of these subordinate stems and reach the 18 $m^2 ha^{-1}$ residual basal area target for the stand. Over the two harvests, 66% of the density and 53% of the basal area was removed leaving a stand of relatively evenly spaced dominant and co-dominant trees, comprised of 75% white pine and 25% red pine by basal area (Table 1). To encourage natural regeneration, the entire study area was site prepared with two perpendicular passes of chains pulled by a skidder in spring of 2001. Treatment plots were established with 3 m corridors in between to facilitate future harvesting activities. The plots were planted in May 2002 with Multipot 67, 1-0 containerized white pine seedlings at a 2 m spacing.

Shelterwood - NB

The New Brunswick site (hereafter called Shelterwood-NB) is located in the Acadian forest region of central New Brunswick, near Doaktown (WGS 84: 46°24’30” N; 66°04’26” W), in the Eastern Lowlands Ecoregion (Ecological Region 6, District 4) and has an ecological strata of 12. It has mean annual, January, and July temperatures of 5.0°C, -10.5°C, and 18.9°C, respectively, with 1559 annual growing degree days and 480 mm precipitation during May–September. It is in the New Brunswick Lowlands geomorphological region, and its soils are fine, loamy sands (< 10 cm) underlain by formations of grey lithic and feldspar sandstone (Colpitts et al., 1995; Loucks, 1962). The 100 year old stand originated from fire. The site was subject to an operational regeneration harvest in 1998. Like the Shelterwood-ON site, the residual overstory was dominated by large white pine but they tended to be of lower overall quality (e.g. poor form) due to early white pine weevil damage. A single pass with light chains was used for site preparation in 1999 and the treatment plots were subsequently established similar to the Ontario site. Jiffy 36 white pine seedlings were planted in May 2002 at 2 m spacing.

Post-harvest stand volume, basal areas, and light transmittance were similar within the Shelterwood-ON and Shelterwood-NB sites but these metrics were more variable within Shelterwood-NB due to a more irregular spacing of fewer and larger diameter residual trees (Table 1).

Table 1. Stand treatment history and residual stand characteristics of three study sites

Site preparation				2002 Post-harvest stand characteristics					
Site	Harvesting System	Site preparation	Planting	Density (stems ha^{-1})	Height (m)	DBH (cm)	Basal Area^{a} (m^{2}ha^{-1})	Gross Total Volume^{b} (m^{3}ha^{-1})	Canopy Closure^{c} (%)
Clearcut-ON	2000: full-tree overstory harvest using clearcut system	None	2001: planted with white pine seedlings at 2m spacing	--	--	--	--	--	--
Shelterwood-ON	1998: regeneration harvest using Shelterwood system 2000: manual felling and grapple skidder used to remove subordinate stems to meet basal area targets	2001: chain scarification with two perpendicular passes	2002: planted with white pine seedlings at 2m spacing	133	27	40.4	18.1	217	61
Shelterwood-NB	1998: regeneration harvest using shelterwood system	1999: chain scarification with single pass	2002: planted with white pine seedlings at 2m spacing	100	25.3	45.1	17.5	197	60

2.2. Vegetation Suppression Treatments

The White Pine Competition Study was originally constructed as a response surface design to evaluate the impact on planted white pine growth of 4 levels of timing of woody species control (year 0, 2, 5, none) and 3 levels of duration of non-woody species control (none, first 2 years, first 4 years). Additionally, an “operational broadcast” treatment was included to emulate suppression of both woody and non-woody species following the second growing season. The study reported herein utilized the “four corners” of the response surface to evaluate responses from a wide gradient of treatments (n = 3), as well as the operational treatment (n = 3). The White Pine Competition study design also included an additional treatment factor comprised of three levels of initial hardwood regeneration density reflecting a density range that could naturally occur (5000, 10000, 15000 stems per ha). However, no significant effect of the hardwood densities on any of the vegetation and tree performance variables were found ($p > 0.05$) (Pitt et al. 2016). Therefore, hardwood density as a factor was not included in our statistical design so those plots were treated as additional replicates (n = 3 for each density) for their respective vegetation suppression treatments (Table 2).

In the present study we assess the impact of five treatments representing different duration and combination of woody and non-woody vegetation suppression. The first treatment Herb4 includes non-woody suppression from year zero to four. The Woody10 treatment was comprised of ten years of woody vegetation suppression. The Both4 treatment was a combination of woody and non-woody vegetation suppression for the first four years, followed by six years of only woody vegetation suppression. In the NoTrt treatment no vegetation suppression was performed. Lastly, the “operational broadcast” treatment (BroadOp) refer to a treatment intended to emulate typical vegetation suppression technique currently used. This treatment included non-woody and woody vegetation suppression in the second year of the study (see Table 2 for treatment details). For practical purposes, non-woody species include all plants that typically form a low-growing vegetation layer. Woody competition included tall shrubs (i.e. shrubs capable of achieving at least 2 m in height) and deciduous tree species. Treatments were applied in a total of thirty-three 18 x 18 m plots. Treatment replicates at each study site are presented in Table 2.

For treatments that suppressed only the non-woody vegetation (Herb4), and those that suppressed both woody and non-woody species (Both4, BroadOp) vegetation suppression treatments were achieved with directed foliar applications of glyphosate (2% Vision®) at the end of the growing season. Woody-only vegetation suppression (Woody10) was initially accomplished with a basal bark treatment (streamline method) of the tryclopyr herbicide Release® (25% product mixed in mineral oil) at the end of the growing season and re-sprouting woody stems were removed the subsequent years via manual clipping, when needed.

Table 2. Vegetation suppression treatments

Treatment Code[a]	N plots[b]	Growing Season[c] 1	2	3	4	5	6	7	8	9	10	Description
NoTrt	9	-	-	-	-	-	-	-	-	-	-	no vegetation suppression
Herb4	9	h	h	h	h	-	-	-	-	-	-	non-woody suppression
Woody10	3	w	w	w	w	w	w	w	w	w	w	woody suppression
Both4	3	hw	hw	hw	hw	w	w	w	w	w	w	four years complete suppression followed by woody only
BroadOp	9	-	hw	-	-	-	-	-	-	-	-	operational broadcast, complete suppression at year 2 followed by no vegetation suppression

[a] Letters indicate suppression treatment applied: h – non-woody suppression, w – woody suppression, "-" no treatment

[b] Replicate numbers are uneven between treatments because each replicate of initial deciduous tree regeneration densities were pooled.

[c] Growing season refers to the number of growing seasons for planted white pine. Growing season 1 was 2001 for Clearcut-ON site and 2002 for both the two Shelterwood sites.

2.3. Stand characteristics and tree performance indicator

The residual overstory stand characteristics at the two shelterwood sites were quantified by measuring diameter at breast height (DBH; 1.3 m) of all live trees within each of the original 14 treatments in the White Pine Competition Study (n = 72). At Shelterwood-ON a 1.5 m buffer zone around each plot was included (i.e. 19.5 m x 19.5 m) whereas at Shelterwood-NB measurements were limited to the 18 m x 18 m treatment plots. A subsample of tree heights were measured in order to develop a height-to-diameter relationship using the Chapman-Richard's function (Richards, 1959) to predict missing heights. Gross total tree volumes were estimated using standard volume equations described in (Honer et al., 1983) (equation 14) and scaled to the hectare level by the total plot area sampled. Canopy closure was estimated from stand level basal area using a relationship developed by (Parker, 2014).

Within each treatment plot (18m x 18m) consisted of 9 rows of 9 planted white pine and an inner 10 m x 10 m area, coinciding with the 5 rows of 5 planted trees was established as the measurement plot. Basal stem diameter and the percentage of trees meeting or exceeding an age-10 target height of 2.5 m height were used as practical indicators of planted pine performance (Pitt el al. 2016). This was determined through measures of total height and basal stem diameter measured 5 cm above the ground line of the surviving planted trees and was measured at the end of the tenth growing season.

2.4. Vegetation Survey

Non-crop vegetation was assessed from five 2 m x 2 m subplots randomly located within each treatment plot during July or early August of growing seasons one, three, six and ten. Percent cover of all non-crop vascular species was visually estimated to the nearest (1%). Identification was done to the species level following the nomenclature of the Database of Vascular Plants of Canada (VASCAN), with the exception of 13 genera (*Actaea*, *Alnus*, *Amelanchier*, *Aster, Hieracium, Lactuca, Pyrola, Ribes, Salix, Solidago, Vaccinium, Viburnum, Viola*) which were identified at the genus level. Grass percent cover was pooled at the family level. Cover of natural conifer regeneration was not assessed for individual species, rather percent cover was called for all naturally regenerated conifers within the subplot. To make data comparable, percent cover was determined by multiplying the proportion (%) of stems per plot for each conifer species by the total percent cover for all conifer species within the plot. We did not included cover values of planted white pine in our analysis of plant community response as we were interested in the response of naturally regenerating species to competition suppression treatments.

2.5. Species Traits

Plant traits were obtained from the TOPIC database (Aubin et al. 2012) and from field observations. Selected traits included characteristics related to dispersal, germination and survival (Table 3). We use the term trait in its broader sense, and include morphological, physiological or phenological features of the plant, as well as performance traits related to individual fitness (Violle et al., 2007).

2.6. Analysis

Analyses were designed to find similarities in plant community response to treatments across the three study sites. We based the analyses on four distinct matrices: (1) matrix *L* (plots by species) containing percent cover data, (2) matrix *Q* (traits by species) containing mixed species traits data, (3) matrix *R* was comprised of an ordinal variables representing competition supression treatment (NoTrt, Herb4, Woody10, Both4, BroadOp), and (4) a matrix containing pine performance (plots by performance indicator). Figure 1 summarizes the various datasets and analytical steps used to answer the four questions addressed by our study. All statistical analyses were performed with R version 3.2.2.

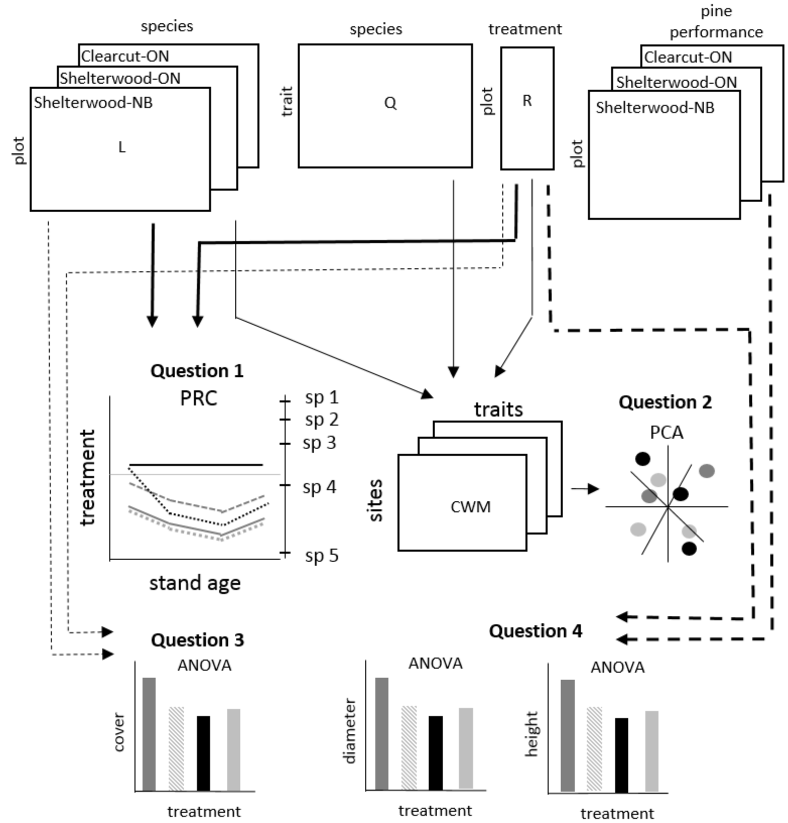


Fig. 1. The datasets used in analytical steps required to answer four study questions.PRC stands for principal response curves; CWM stands for Community weighted mean. Questions include: 1) How do these treatments alter community development trajectories over time? 2) How do communities respond to these treatments? 3) What is

the response on target species (competitive species and interior forest species) 4) What is the effect of these treatments on tree growth and performance?

Table 3 Traits included in analysis

Trait	Code	Data	Description
Raunkiaer Lifeform	RA	0, absence 1, presence	mg, mega or meso-phanerophyte (height ≥ 8m); mc, micro or nano-phanerophyte (25 cm to 8 m); ch, chamaephyte, herb or shrub with buds between 0.1 and 25 cm above ground); h, hemicryptophyte, herb with bud at the ground surface; hf, hemicryptophyte fern, fern with bud at the ground surface g, geophyte, herb with underground bud; gf, geophyte fern, fern with underground bud t, therophyte, annual
Height (cm)	HT	3.8 - 4850	The shortest distance between the upper boundary of the main photosynthetic tissues on a plant and the ground level
Foliage Architecture	SFO	0, absence 1, presence	*Phanerophytes* m, mono-stem; mu, multi-stem; *Other life forms without stems;* r, rosette and semi-rosette; e erect leaves; *Other life forms with stems;* el, erect leafy stem; de, decombent or prostrate stem; h, horizontal stem bend in an arch or umbel-shaped stem
Lateral Extension	LE		*Phanerophytes*
		0	pl, limited
		1	pcc, compact
		3	pci, intermediate
		4	pce, extensive
			Other life forms
		0	l, limited
		1	cc, compact
		2	ce, extensive
Seed Weight (seeds/kg)*	SDWT	1200 - 100000000	Seeds and spores are defined as generative units of reproduction
Seed Production	SPRO	0	f, few(< 20 seeds ind $^{-1}$ yr $^{-1}$)
		0.5	s, semi-abundant (20 to 1000 seeds ind $^{-1}$ yr $^{-1}$)
		1	a, abundant (>1000 seeds ind $^{-1}$ yr $^{-1}$)
Dispersal Distance	DD	0.2	short, gravity and ant dispersed
		0.5	short wind, dispersed but lack seed structures that facilitate long distance dispersal
		0.7	intermediate, dispersed by animals through internal or external transport
		1	long wind, seeds dispersed by birds or specialized seed structures that facilitates long dispersal
Shade Tolerance	LI	0	s, shade tolerant
		0.5	m, mid-tolerant
		1	i, shade intolerant

** reciprocal transformation applied to assist in visual interpretation of results*

2.6.1. How do treatments converge across time?

To explore the effect of treatment on species composition over time, we used the principal response curve method (PRC) (Van den Brink and Ter Braak, 1999) on the L and R matrix. PRCs are a special case of redundancy analysis (rda) that use a multivariate approach based on constrained ordination techniques to test the interaction between time and a given treatment ($\alpha = 0.05$), relative to the change between a given treatment and an *a priori* determined control. Clearcut was analyzed separately from shelterwood sites (Shelterwood-ON and Shelterwood-NB) because preliminary analyses found the two shelterwood sites responded similarly to treatments whereas the clearcut site responded differently (Appendix S1). Prior to analysis, a Hellinger transformation was performed on the L matrix to control for absent species (Legendre and Gallagher, 2001) using the "decostand" function of the "vegan" package (Oksanen et al., 2008). We considered treatment and year as factors. The *a priori* control for this analysis were the no vegetation suppression treatments (NoTrt), although not a true control, this was the most appropriate to compare changes in community composition after the application of different suppression treatments. Significance for the overall PRC was assessed using Monte-Carlo permutations (n=999). To test if there were significant differences between the five experimental treatments in the 10th year, the PRC includes a Tukey's post-hoc comparison to its site scores, which were evaluated against a multivariate t-distribution using the multcomp package (Hothorn et al., 2008). To avoid the bias introduced by multiple comparisons between treatments, a Bonferroni correction was then applied when significant effects were detected.

2.6.2. What is the impact of competition suppression on functional community composition over the long-term (i.e. 10 years)?

To investigate how species traits related to vegetation suppression treatments in year 10, we first generated a Community-Weighted Mean (CWM) matrix using matrix *L* and matrix *Q*. The CWM matrix was generated by calculating the mean of the trait value present in the community weighted by its abundance (percent cover) for each species (Garnier et al., 2004). To describe community trait assemblage in response to suppression treatments, we performed a Principal Component Analysis (PCA) on the CWM matrix, allowing us to relate patterns of co-occurring traits to prescribed treatments. Seed weight and stem height were both skewed in distribution and were log-transformed to obtain a normal distribution (Májeková et al., 2016).

2.6.3. What is the response on target species?

We tested differences in mean percent cover of competitive between vegetation suppression treatments at each of the three study sites separately using a one-way ANOVAs with permutations (n=999) with a post-hoc pairwise t-test to test for differences between treatments (Borcard et al., 20011). Ten species in this dataset were identified in the literature as competitive species (*Acer rubrum*, *Acer spicatum*, *Betula papyrifera*, *Corylus cornuta*, *Epilobium angustifolium*, *Kalmia angustifolia*, *Populus tremuloides*, *Prunus pensylvanica*, *Pteridium aquilinum*, and taxa from the *Rubus* genus, and Grass) (Jobidon, 1995; Young and Peffer, 2010). Because two treatments, Woody10 and Both4, suppressed woody species every year for the full 10 years of the experiment (Table 2), these treatments were only included in the analysis of herbaceous species (*Pteridium aquilinum, Epilobium angustifolium).*

Ten interior forest species, that are known to be typical if interior habitats, and that are shade tolerant, less nutrient demanding, and are known to be more sensitive disturbance frequent intense disturbances (Avon et al., 2010) were identified in our dataset. These species included *Clintonia borealis*, *Coptis trifolia*, *Cornus canadensis*, *Dryopteris* sp., *Maianthemum canadense*, *Mitchella repens*, *Polygaloides paucifolia*, *Rubus pubescens*, *Lysimachia borealis and Trillium* *undulatum*. Because of low cover values of these species we used an average percent cover for all ten interior forest species to tested differences between vegetation suppression treatments using a one-way ANOVAs with permutations (n=999) with a post-hoc pairwise t-test to test for differences between treatments (Borcard et al., 20011).

2.6.4. What is the effect of vegetation suppression on tree growth and performance in the long-term (ie. 10 years)?

To assess the effects of vegetation suppression treatments on white pine performance indicators, we tested differences between treatments in terms of stem diameter 5 cm above ground line, and percentage of trees reaching year 10 targets of 2.5m (P250), and percent cover at each of the three study sites separately. This was tested using a one-way ANOVAs with permutations (n=999) (Borcard et al., 20011) with a post-hoc pairwise Bonferroni comparison to test for differences between treatments.

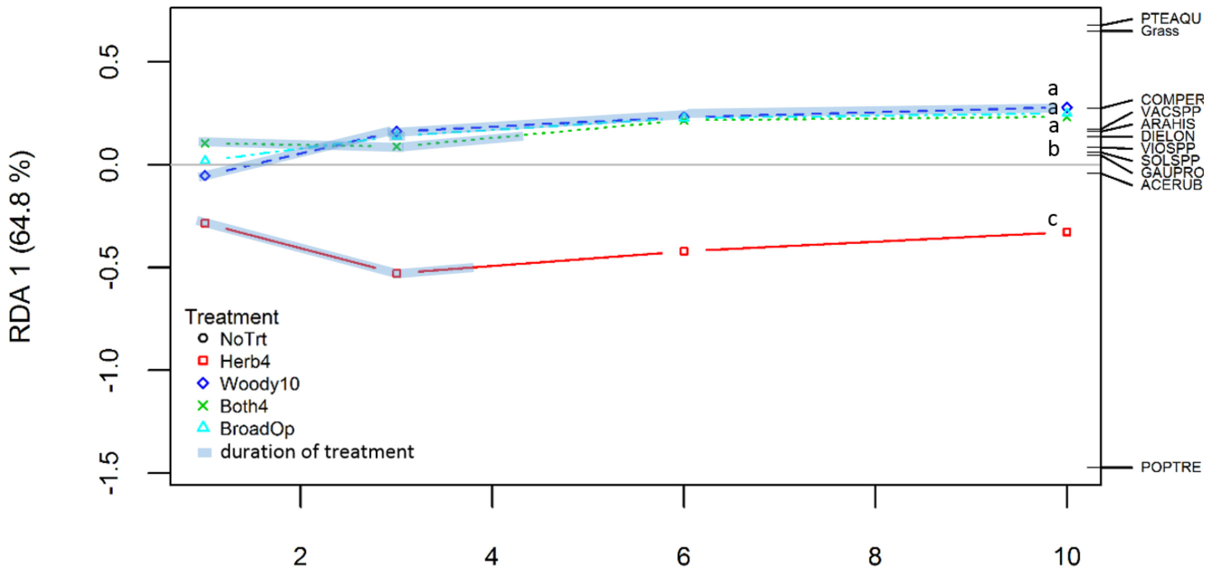
3. Results

3.1 How do treatments converge across time?

Clearcut - ON

A total of 19 woody species and 24 non-woody species were identified in the Clearcut site. Principal response curves for that site revealed that all vegetation suppression treatments led to changes in species composition over the duration of the monitoring period of 10 years when compared to treatments that received no vegetation suppression treatment (NoTrt) (RDA axis 1 = 64.8%, F= 217.41, $p = 0.01$) (Fig. 2a). Time explained 11% percent of the variance and the treatment × time interaction explained 67% of the variance. Changes in species composition in response to treatment application were most pronounced between years 1 and 3 of the study. After this community composition appear to remain relatively stable until year 10 (Fig. 2a). Post-hoc comparison of curves with Bonferroni correction revealed significant differences between treatments in terms of composition with the exception of Woody10, BroadOp, Both4 (Fig. 2a, Appendix S2). In year 10 all treatments lead to significantly altered plant communities (still visible at year ten, $\rho < 0.001$) in comparison to treatments that received no vegetation suppression (NoTrt). Positive scores for *Pteridium aquilinium*, Grass, and *Comptonia peregrina* indicate that these species benefited from conditions generated by Woody10, BroadOp and Both4 vegetation suppression treatments (Fig. 2a). *Populus tremuloides* became more prevalent in treatments where only non-woody species (Herb4) were suppressed (Fig. 2a).

a) Clearcut-ON



b) Shelterwood sites

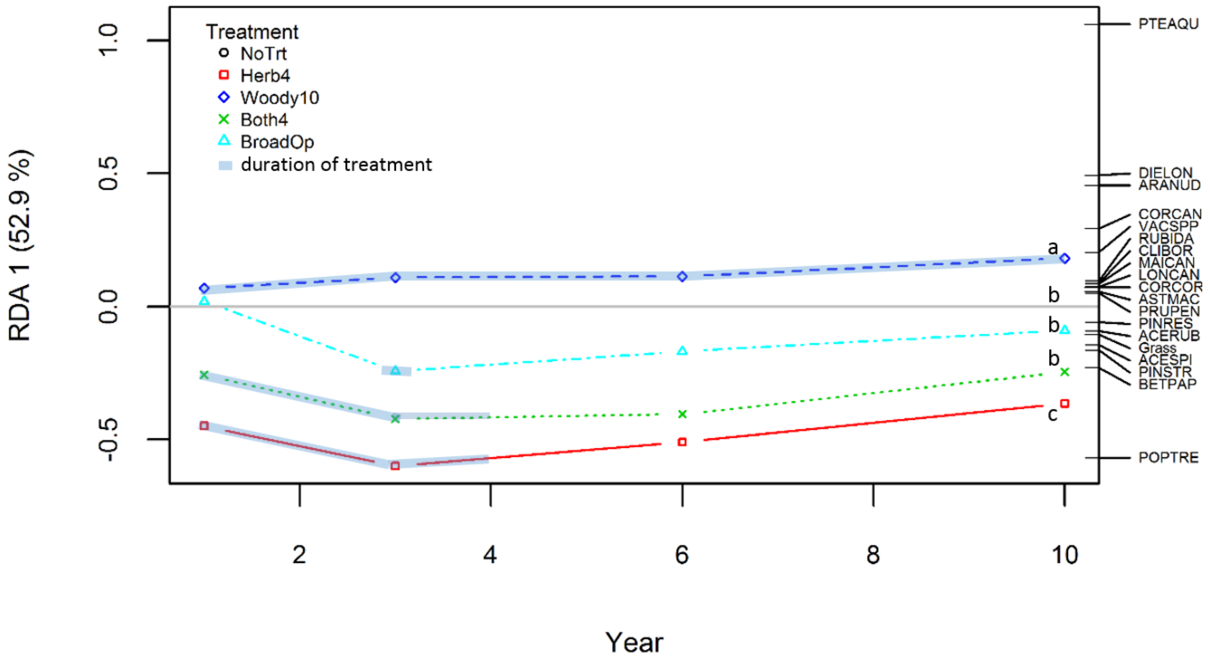


Fig. 2ab. Principal response curves (PRC) for the first axis of the RDA testing the effect of treatment × time on species composition, representing the dominant temporal trajectory in a planted *Pinus strobus* stand, a) Clearcut-ON b) Shelterwood sites. See Table 2 for treatment descriptions. Response curves for each treatment are presented, with the zero line representing the reference condition (NoTrt). PRCs with the same lowercase letter were not significantly different at year 10 of the experiment following vegetation suppression treatments. Significance based on Tukey post-hoc test, with Bonferroni correction. Species scores of the most dominant species are displayed along the right-side vertical axis (species scores > = |0.05|). Scores indicate how strongly each species is correlated with the temporal patterns displayed by the curves, and thus illustrating the main drivers of the temporal trajectories. Positive score = positive correlation, 0 = no correlation, negative score = negative correlation.

Shelterwood sites

In the two shelterwood sites, 32 woody species and 41 non-woody species were identified across the two sites. At those sites, all vegetation suppression treatments appear to cause changes in species composition (Table 5, RDA axis 1 = 52.9%, F=76.96, p = 0.001) relative to NoTrt. Time explained 11% percent of the variance and the treatment × time interaction explained 33 % of the variance. Changes in species composition were the most pronounced between years 1 and 3 of the study (Fig. 2b). Although sites appear to converge towards the NoTrt treatment with time, at year 10 a Tukey's post-hoc comparison of curves with Bonferroni correction detected differences between some of the treatments in terms of species composition (Fig. 2b, Appendix S2). Treatments that suppressed only non-woody vegetation (Herb4), and woody vegetation (Woody10) were both found to differ significantly from the treatments where vegetation was not suppressed (NoTrt), operational broadcast treatment (BroadOp) and treatments where both woody and non-woody vegetation was suppressed (Both4). Due to differences in species composition the treatments that suppressed non-woody species (Herb4), and woody vegetation (Woody10) were found to differ significantly from one another (Fig. 2b, Appendix S2). Species scores on the right axis indicate how strongly species correlate temporally with the treatments (Fig. 2b). Positive scores for *Pteridium aquilinium*, *Diervilla lonicera*, and *Aralia nudicaulis* indicate that these species benefited from conditions generated by treatments where only woody plants were suppressed (Woody 10) (Fig. 2b). *Populus tremuloides* became more frequent throughout the experiment when compared to the no suppression treatment (NoTrt) and were associated with conditions created by treatments where only non-woody vegetation was suppressed (Herb4) (Fig. 2b).

3.2. What is the impact of competition suppression on functional community composition?

Clearcut-ON

The two PCA axes of the CWM show trait distribution in relation to their relative importance for each treatment (Clearcut site: Fig 3a; Shelterwood sites: Fig 3b,). For the Clearcut-ON site, the first two axes explained 87.2 % of the total variation (Fig. 3a.). There is a clear separation in NoTrt, Woody10 treatments on the left and Herb4, Both4 and BroadOp treatments on the right. On the left side of the axis, NoTrt treatments showed a high prevalence of tall, shade intolerant species, abundant seed producers with small seeds and long dispersal ability which was driven by prevalence of *Populus tremuloides*. Woody10 was associated with species capable of spreading extensively though vegetative means, species with erect stems and geophyte ferns which are traits associated with *Pteridium aquilinum*. Multi-stemmed micro-phanerophytes were also prevalent in these treatments. Both4 and BroadOp treatment were associated with large seed size, shade tolerant species, short height, low seed production and low dispersal ability. Herb4 treatments tended to be associated with mono-stemmed meso-phanerophytes.

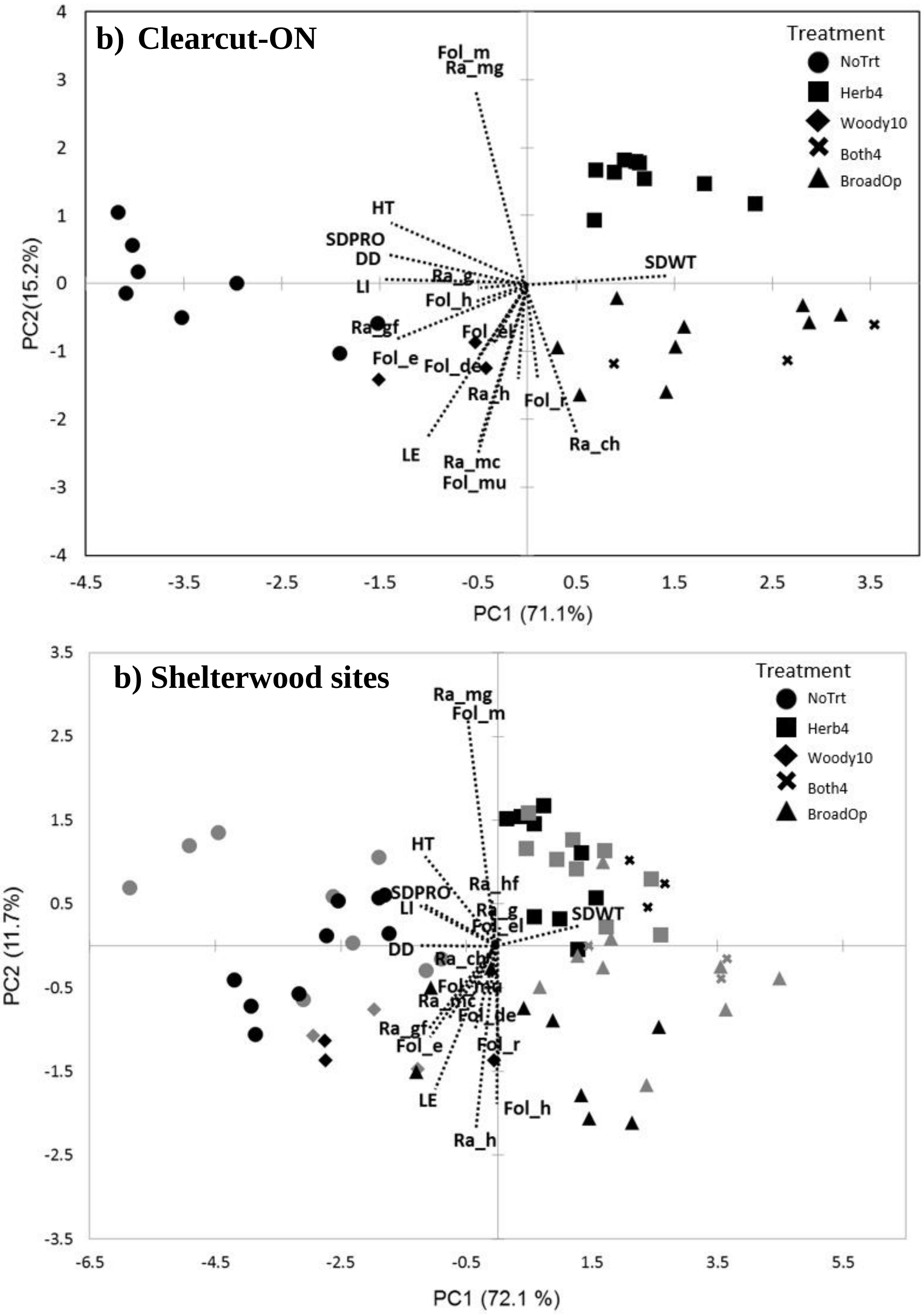


Fig. 3ab. Patterns in trait occurrences for the a) Clearcut-ON site and b) Shelterwood sites at year 10 of the investigation. See Table 3 for treatment code description and Table 4 for trait code description. First two axes of the PCA performed on the CWM trait × site matrix. Shelterwood sites in Ontario (Grey), and New Brunswick (Black).

Shetlerwood sites

The two axes explained 86% of the total variation for the two shelterwood sites (Fig.3b). Similar to the Clearcut-ON, the first axis shows a clear separation in NoTrt and Woody10 treatments on the left and Both4, Herb4 and BroadOp treatments on the right. Traits of species associated with NoTrt included tall shade intolerant abundant seed producers, with small seeds and good dispersal ability. Woody10 tended to be associated with species capable of spreading extensively though vegetative means, species with erect stems and geophyte ferns. These treatments were also associated with multi-stemmed micro-phanerophytes. Traits associated with Both4 and BroadOp included shade tolerant species that produce few larger seeds with low dispersal ability. Herb4 treatments were associated with mono-stemed meso-phanerophytes.

3.3. What is the response on target species

In general we found that tall woody competitive species had greater cover in plots that received no vegetation suppression treatments (NoTrt) and treatments that suppressed only non-woody vegetation (Herb4) (Table 4, Appendix S2). *Pteridium aquilinum* cover was also greater in plots with no vegetation suppression treatments (NoTrt) and where woody suppression treatment was applied (Woody10) (Table 4). Interior forest herbs were only found to have significantly greater cover in the Shelterwood Ontario site in treatments that reflected operational standards (BroadOp) (Table 4, Appendix S3).

Table 4. Sites with Treatments found to have significantly* greater mean percent cover of competitive species and interior forest species 10 years following initiation of vegetation suppression treatments. Sites are ^{1}Clearcut-ON; ^{2}Shelterwood-ON; ^{3}Shelterwood-NB. See Table 3 for treatment description and Tables S2 and S3 of appendix for more detailed results

Species **Competitive Species**	**Treatment**
Woody	
Acer rubrum	NoTrt^{23}; Herb4^{23}
Acer spicatum	--
Betula papyrifera	NoTrt^{3};Herb4^{3}
Corylus cornuta	--
Epilobium angustifolium	--
Kalmia angustifolia	NoTrt^{1}
Populus tremuloides	NoTrt^{12}; Herb4^{12}
Prunus pensylvanica	--
Non-Woody	
Pteridium aquilinum	NoTrt^{123}; Woody10^{123}
Rubus allegheniensis	--
Rubus idaeus	--
Grass	--
Interior Forest Species	BroadOp^{2}

*Tested each site separately using a using one-way ANOVAs with permutations (n=999) with a post-hoc pairwise t-test to test for differences between treatments with Bonferoni correction.

*Woody10 treatments were only included when testing herbaceous competitive species

3.4. What is the effect of vegetation suppression on the ten year tree growth and performance?

To determine the effect of vegetation suppression treatments on planted white pine growth and performance we performed a one-way ANOVAs with permutations (n=999) for each of the three study sites separately. Vegetation suppression treatments were found to have a significant effect on basal stem diameter measured 5 cm above the ground line at all three of the sites (*p* = 0.001; Table 5 or Appendix S4). Lowest stem diameters in year 10 of the study were consistently found in plots that did not receive vegetation suppression treatments, (NoTrt, mean diameter 27 mm). Individually, non-woody suppression (Herb 4), woody suppression (Woody 10); and the operational (BroadOp) treatments provided a similar growth response with mean diameters of 40, 44, 56 mm respectively. The stem diameter response was nearly doubled when both woody and non-woody vegetation were suppressed (Both4; Table 5 or Appendix S4).

Vegetation suppression treatments were also found to have a significant effect on percentage of trees reaching year 10 height targets of 2.5m (P250) at each of the three sites (*p* = 0.001) (Table 5 or Appendix S4). Only the shelterwood sites were capable of exceeding year 10 height targets of 50%. Treatments that did not suppress vegetation had the poorest performance, with the lowest values across the three sites (NoTrt, 5 to 21%). Similar to stem diameter, at the shelterwood sites, intermediate results were achieved when either non-woody species (Herb4, 45%) or woody species were suppressed (Woody10, 48%), and the highest P250 rates were achieved when both woody and non-woody vegetation suppression treatment were applied, with mean P250 of 70% and 85% for BroadOp and Both4 respectively (Table 5 or Appendix S4). The clearcut site overall had the lowest P250 (averages <36%).

The percent cover of planted pine at each of the three sites was also found to be significantly affected by vegetation suppression treatments (*p* = 0.001) (Table 5 or Appendix S4). Treatments that did not suppress vegetation had the lowest cover values across the three sites (NoTrt, 12 to 13%). Similar to stem diameter and P250, at the shelterwood sites, intermediate cover results were achieved when either non-woody species (Herb4) or woody species were suppressed (Woody10), and the greatest cover was found when both woody and non-woody vegetation suppression treatment was applied, with mean cover of approximately 32% and 54% on average for BroadOp and Both4 respectively (Table 5 or Appendix S4).

Table 5. Sites that had treatments with significantly[1] greater performance indicators of white pine growth 10 years following initiation of competition suppression treatments. See Table 2 for treatment description and Table S4 of appendix for more detailed results.

Performance Indicator		
Stem diameter (mm) 5cm AGL[3]	**Sites**	**Treatments with ↑ Diameter**
	Clearcut-ON	Both4 (82mm); BroadOp (56 mm); Herb4 (52mm)
	Shelterwood-ON	Both4 (72 mm); BroadOp (54mm); Woody10 (43mm)
	Shelterwood-NB	Both4 (74 mm); BroadOp (60 mm); Herb4 (50 mm)
Percentage of Trees reaching 10 year target	**Sites**	**Treatments with ↑ Percentage of Trees > 2.5 m**
	Clearcut-ON	Herb4(36%); Both4 (21%)
	Shelterwood-ON	Both4 (97%); BroadOp(70%); Woody10(59%)
	Shelterwood-NB	Both4 (73%); BroadOp (70%); Herb4 (59%); Woody10 (36%)
Percent Cover	**Sites**	**Treatments with ↑ Pw Cover**
	Clearcut-ON	Both4 (56%); Herb4 (42%)
	Shelterwood-ON	Both4 (54%); BroadOp(37%); Woody10(24%)
	Shelterwood-NB	Both4 (53%); BroadOp (38%); Herb4 (34%)

[1] Values are averaged from surviving planted white pine (up to 25 trees) in each of *n* treatment plots at each site. df =4.

4. Discussion

In this study, we were able to quantify both white pine performance and understory response to a range of vegetation suppression treatments that varied in duration and intensity with the aim of finding a balance between dependable tree growth and sustainable forest management. By comparing the response at three sites, we were able to describe general patterns and identify treatments beneficial for tree performance and with minimal effect on understory species composition and functional assemblage, a central goal of current silviculture.

An ideal treatment would release white pine from competition and conserve enough canopy cover to reduce weevil damage. This would favour rapid white pine growth while allowing the understory community to recover quickly enough to occupy available niches and converge toward the attributes of a mature forest. In comparison to the range of experimental treatments observed in this investigation, the BroadOp treatment, which emulates current practices, was the most effective in these regards (Table 4 and 5). White pine performance in the BroadOp treatment at the shelterwood sites was almost as successful as more intense treatments (Both4). Using a single broadcast application of herbicide targeting both woody and non-woody vegetation has become a popular silvicultural practice because application using aerial sprayers is cost effective over large areas. Although some studies looking at conifer performance have found stand volume to be positively related to duration of vegetation suppression treatment (Hoepting et al., 2011; Wagner and Robinson, 2006), there is as yet no consensus on this question. Other studies looking at five year effects of vegetation suppression treatments on jack pine growth had results similar to ours. In this study, aerial spraying once after planting had comparable growth and performance in white pine to jack pine growing in treatments where competition was removed annually for three years (Pitt et al., 2000).

The BroadOp treatment was also associated with shade tolerant understory communities comprised of species with low dispersal ability and only produce a few large seeds (Fig. 3ab). These traits are strongly associated with later successional forests (Dölle et al., 2008; Knapp et al., 2016; Messier et al., 2009). Several studies have also reported that a single application of herbicide at initial stand development has only minor short-term effects on plant communities due to adaptation of boreal species to disturbance-prone environments (Miller and Miller, 2004). Seed banks in the soil and crown, effective seed dispersal, resprouting ability, and abundance and locality of survivors all contribute to the resilience of boreal forest species that can rebound quickly provided no other compounding disturbance follows. (Seidl et al., 2014).

Treatments that suppressed either woody (Woody10) or non-woody vegetation (Herb4) appeared to cause major shifts in community composition due to the prevalence of competitive plants forming stable communities at the end of our 10 year study (Fig. 2ab). *Pteridium aquilinium* tended to dominate Woody10 plots at all three sites, whereas Herb4 plots tended to be dominated by competitive trees and tall shrubs, primarily *Populus tremuloides* (Fig. 2ab). Other studies have also shown that suppression of non-woody species increases abundance and cover of competitive woody species, while the suppression of woody species alone typically favours the release of competitive herbaceous species (de la Cretaz and Kelty, 1999; Miller et al., 2003). Disturbances such as logging and vegetation suppression increase community colonization potential by freeing up space and increasing resource availability of light and soil moisture for opportunistic species left on site (Balandier et al., 2006; Davis et al., 2000). The release of these remaining competitive species, either woody or non-woody, means that positive effects on crop trees can be only temporary (Balandier et al., 2006). These competitive species can then become a management problem themselves, reducing not only crop tree growth but also limiting successional development of the overall plant community. In the worst case scenario, these changes in community composition can cascade into changes in ecological services and functions that these species drive (Milligan et al., 2016; Mori et al., 2017).

The release and dominance of *Pteridium aquilinium* in Woody10 treatments and of *Populus tremuloides* in Herb4 treatments are of particular concern because both of these species can remain locally dominant for several decades (Balandier et al., 2006; Douterlungne et al., 2013). Success of *Pteridium aquilinium* as a competitor is attributed to its extensive rhizomes containing considerable carbon stores, nutrients and dormant buds. Rapid growth allows this species to develop dense understory layers quickly which compete aggressively for light with other forest herbs. *Pteridium aquilinium* also causes the accumulation of a thick litter layer containing potentially allelopathic compounds which can limit establishment of other species (Milligan et al., 2016).

By comparison, competitive overstory tree species are considered one of longest persisting competitive groups (Balandier et al., 2006). Traits that make *Populus tremuloides* a strong competitor include its ability to colonize sites quickly through its abundant and wind dispersed seeds and its aggressive resprouting ability from extensive underground root systems. Rapid juvenile growth allows *Populus tremuloides* to overtake neighbouring trees quickly following establishment (Landhäusser and Lieffers, 1998; Lavertu et al., 1994). The strong competitive ability of *Pteridium aquilinium* and *Populus tremuloides* and their ability to dominate communities for long periods of time suggest that beyond the challenge they pose at stand initiation, they can create longer term issues that affect future rotations.

We observed some differences in community response to treatment depending on whether the site had been harvested following the clearcut or shelterwood system. Treatments at the Clearcut-ON site were dominated by competitive, open habitat species, including *Pteridium aquilinum*, Grass, *Comptonia peregrina* and *Populus tremuloides* (Fig 2a, Appendix S2). Clearcuts are known to be colonized quickly by fast growing, early successional species because they can more efficiently utilize newly available resources (Parker et al., 2009; Thompson and Pitt, 2003). In contrast, we observed more rapid convergence of plant community composition at shelterwood sites in vegetation-suppressed treatments, towards similar composition of untreated ones. Strong competition combined with increased occurrence of weevil attack suggests that a clearcut harvesting method may be unsuitable to the effective regeneration of white pine unless additional measures are taken to mitigate effects of weevil and competition (Pitt et al., 2016; Parker et al., 2010, 2009) .

Findings from our study suggest a balance can be struck between sustainable fiber production and conserving biodiversity within managed forests. For eastern white pine, management practices that provide early release from competition and conserve enough canopy cover will be necessary to favour seedling survival and growth while allowing the understory community to recover. Treatments that suppressed only woody or non-woody vegetation caused major shifts in community composition due to the persistence of competitive species that formed stable and dominate layers. Comparing the magnitude of change in understory community to gains in crop tree performance will be useful in identifying vegetation suppression strategies that manage competitive vegetation, minimize the effects on biodiversity, and enhance crop tree performance.

Next Steps for component 1 assessing long-term changes in understory using a legacy dataset (2018-2019)

- Submit draft manuscript for peer review (March 2018)
- Disseminate results including scientific publications, CWFC technology transfer document (Fibre Facts), presentations in conferences, workshops and meetings

Component 2: Assessing impacts on understory structure using field measurements

Site Description

Two ongoing shelterwood white pine study sites were chosen. The site located in the Petawawa Research Forest near Chalk River, Ontario, was established in 1994 by Darwin Burgess *et al.* and the site located in Britt, Ontario was established in 1997 by Andree Morneault. The two sites share common initial vegetation management treatments across a range of site conditions (e.g. soil depth, moisture, and topography). Stand history and characteristics as well as floristic measurements at those sites are summarized below. For more details on the establishment history and stand characteristics at the Petawawa Research Forest, site see Burgess et al. (2002) and Burgess and Wetzel (2000). For more detailed summaries of study sites in Britt, see Morneault et al. (2010) and Parker et al. (2013).

Petawawa study site

The Petawawa study site is located within the Petawawa Research Forest (45^{o} 57’N, 77^{o}34’W), which is part of the Great Lakes – St. Lawrence forest region of central Ontario, Canada and is classified as ecosite type ES11.2 (white pine-red pine, fresh to moist) based on the Central Ontario Forest Ecosystem Classification System (Chambers et al. 1997). The site is comprised of three 110-year-old natural white and red pine stands of fire origin. The soils are classified as either podzols, or brunisols. Soil texture is predominantly fine sand, but ranges from coarse sand to very fine sand and loamy sand. Depth ranged from 0.5 m to >1.2 m in depth. This area has a mean annual, January, and July temperatures (1981-2010 averages) of 5.6 °C, -11.8 °C, and 20.3°C, respectively, with 387.4 mm of precipitation during May to September (Environment Canada, 2018). Before harvest, the canopy was comprised mainly of white pine (*Pinus strobus*) and red pine (*Pinus resinosa*) with minor components of white spruce (*Picea glauca*) and trembling aspen (*Populus tremuloides*) (Burgess et al., 2002). The pre-harvest subcanopy was made up of balsam fir (*Abies balsamea*), white spruce (*Picea glauca*), sugar maple (*Acer saccharum*) and poplar (*Populus sp*.), beaked hazel (*Corylus cornuta*), mountain maple (*Acer spicatum*), wild raisin (*Viburnum cassinoides*), serviceberry (*Amelanchier* spp.), green alder (*Alnus viridis* subsp. *crispa*) and fly honeysuckle (*Lonicera canadensis*) (Burgess and Wetzel, 2000). The experimental design was comprised of a randomized complete block, split-split plot design with four replicates. Blocks were stratified using plot measurements of white pine basal area. The main plot factor consisted of canopy thinning treatment and subplot factors were site preparation treatments (Table 2) (Burgess and Wetzel, 2000). Stand history is presented in Table 1.

Canopy thinning and site preparation treatment

Winter logging operations took place in January to March 1994 by crews using chain saws and Timberjack 230 and 350 cable skidders. The crown thinning took place during the regeneration cut phase of the uniform shelterwood system. The three canopy thinning treatments include (T0) uncut controls, (T1) thinning leaving a one-crown width opening between residual trees, and (T2) thinning leaving a two-crown width opening between residual trees. The uncut control treatment had an 80% canopy cover, the one-crown width treatment consisted of 37% canopy cover, and the two-crown width treatment had 16% canopy cover (Burgess and Wetzel, 2000).

Site preparation treatments were applied to randomly selected sub-plots that measured 0.25 ha, and were separated by a 5 m wide buffer. The site preparation treatments are presented in Table 1. In in August 1994, blade scarification (S) treatments were completed using a John Deere 350 bulldozer with a mounted six-way blade. Chemical site preparation (H) consisted of herbicide application of Vision® (*n* -phosphonomethyl) that was applied one year later in mid-September 1995 using back-pack sprayers at a rate of 1.5 kg ha^{-1} of active ingredient (Burgess and Wetzel, 2000).

Planting

In May and June of 1995, half of each sub-plot was planted with two-year-old container-grown white pine seedlings at a 2 m spacing. For destructive sampling, an additional 50 seedlings planted at a 1 m × 1 m spacing. The remaining sub-plot area was left to regenerate naturally (Burgess and Wetzel, 2000). We only examined planted plots in our study.

Britt study site

The Britt study site is located near Britt, Ontario (45°56'N, 80°30'W) situated within the Great Lakes – St. Lawrence forest region and is classified as Ecosite 11.2 (white pine-red pine, fresh to moist) based on the Central Ontario Forest Ecosystem Classification System (Chambers et al. 1997). This site was comprised of 90- to 100-year-old stands dominated by white pine that are presumed to be of fire origin (Parker et al. 2013). Soils of the nearby Parry Sound District are characterized as podzols (Hoffman et al., 1962). Soils can vary greatly over short distances (from 0 to 60 cm in depth) but are commonly less than 30 cm deep. Soils are classified as well-drained medium sandy loam (Parker et al. 2013, Morneault et al., 2010). This area has a mean annual, January, and July temperatures (1981 – 2010 average) of 4.7 °C, -12.0°C, and 19.4 °C, respectively, and of 445.3 mm precipitation during May to September (Environment Canada, 2018). Before harvest, canopies were dominated by white pine (*Pinus strobus*) with minor components of red pine (*Pinus resinosa*), trembling aspen (*Populus tremuloides*), and white spruce (*Picea glauca*). Understory vegetation was dominated by balsam fir (*Abies balsamea*), red maple (*Acer rubrum*), trembling aspen, low sweet blueberry (*Vaccinium angustifolium*), beaked hazel (*Corylus cornuta*), bracken fern (*Pteridium aquilinum*), and wild raisin (*Viburnum cassinoides*). These sites were reported to have very little advanced reproduction of white pine (Morneault et al., 2010). Stand history is presented in Table 1. The experimental design was comprised of a randomised complete block design, with five treatments replicated in three blocks (Table 2). Blocking was separated by approximately 10 km and was based on site characteristics related to differences in total pre-harvest basal area of pine and moisture regime (Morneault et al., 2010).

Canopy thinning and site preparation treatment

Unlike Petawawa, the canopy of all treated stands were thinned to 50% crown cover. Based on light measurements taken following canopy thinning, Britt sites are likely most similar to the one crown width treatment at the Petawawa site (mean light levels 45% and 50% for Britt and Petawawa respectively). Between 1995 to 1997, trees greater than 10 cm at breast height (DBH) were removed by experienced operators who manually felled and skidded tree-length stems. Prior to thinning of the canopy, plots were marked to retain 50% crown closure in dominant and co-dominant trees, following Ontario Ministry of Natural Resources and Forestry tree marking guidelines (OMNR 1998).

Treatment plots were applied randomly, measured 100 m x 50 m surrounded by a 30 m wide buffer. The site preparation treatments are presented in Table 1. In early fall of 1996 and 1997, blade scarification (S) was treatments were completed using a D4 bulldozer with a mounted six-way blade. Chemical site preparation consisted of broadcast sprayed (H) application of Vision® herbicide at 2.1 kg a.e. ha^{-1} that was applied in late summer of 1997 using a mist blower mounted on a rubber-tired skidder. The herbicide was applied one year after the mechanical treatment on the combined mechanical scarification and chemical treatment plots (Morneault et al., 2010).

Planting

In May of 1998, each treatment plot was planted with one-year-old container-grown white pine seedlings at a 2.7 by 2.7 m spacing. After planting, 40 seedlings were selected from each treatment plot and tagged for repeated growth and survival measurements (Morneault et al., 2010).

Tending and removal cuts

In 2006, tending was accomplished through a combination of thinning from below in May to remove mid-canopy trees (balsam fir, red maple, white birch), basal bark treatment in August to remove tall deciduous competition (poplar, maple, pin cherry, and birch), and backpack foliar treatments in August to remove beaked hazel (within 1 m spacing or taller than white pine regeneration). Prior to the first removal cut, plots were marked to retain 40% crown closure in dominant and codominant trees, following Ontario Ministry of Natural Resources and Forestry tree marking guidelines (OMNR 1998). Marked trees greater than 10 cm diameter at breast height (DBH) were removed by experienced operators manually in 2009 who felled and tree-length skidded the marked trees (Morneault et al., 2010)

Table 1. Stand history

Petawawa, Ontario	
1994	Crown thinning, scarification (S,SH)[1]
1995	Chemical treatment; Planted white pine seedlings
Britt, Ontario	
1995	Crown thinning (SH)
1996	Crown thinning (S,H); scarification (SH)
1997	Crown thinning (C); scarification (S); chemical site preparation (H, SH)
1998	Planted white pine seedlings
2006	Tending
2009	First Removal cut (C, S,H,SH)

[1] NC: no cut, no site preparation; C: thinning, no site preparation; S: thinning, scarification; H: thinning, chemical site preparation; SH: thinning, scarification and chemical site preparation. The crown thinning was the regeneration cut phase of the uniform shelterwood system.

Table 2. Crown Thinning and Treatments

Site	N	Crown thinning[1]	Treatment
Petawawa, Ontario	12	T0,T1,T2	C: thinning, no site preparation
	12	T0,T1,T2	S: thinning, scarification
	12	T0,T1,T2	H: thinning, chemical site preparation
	12	T0,T1,T2	SH: thinning, scarification and chemical site preparation
Britt, Ontario	5	T1	NC: no cut, no preparation
	5	T1	C: thinning, no site preparation
	5	T1	S: thinning, scarification
	5	T1	H: thinning, chemical site preparation
	5	T1	SH: thinning, scarification and chemical site preparation

[1] crown thinning is T0: no thinning; T1: one-crown spacing; T2: two crown spacing. Treatments are NC: no cut, no site preparation; C: no site preparation; S: scarification; H: chemical site preparation; SH: scarification and chemical site preparation.

Measurements

Structural understory response

Stratified floristic composition was determined though field measurements made in July of 2016 for research plots at the Petawawa site and in July of 2017 for research plots at the Britt site. This was achieved using a linear vegetation survey method following Aubin et al. (2000). In each study plot, 60 circular points (15 cm radius) were systematically sampled along four 22.5 m transects. Vascular species (including all woody species with DBH < 5 cm) were recorded. To describe vertical structure, vegetation was sampled in 50 cm vertical layers, from the soil surface to the top of the vegetation (0 – 50cm height, 50-100 cm, 100-150 cm, 150-200 cm and >200 cm). A trait-based approach following analytical steps suggested by Aubin et al. (2008) was used to ascertain the mechanisms behind recalcitrant layer formation while maintaining comparability among studies. Plant trait data were provided by the TOPIC database (Aubin 2012; http://www.nrcan.gc.ca/forests/research-centres/glfc/topic/20303) and by field measurements (see Table 2).

Preliminary Results

The aim of these analyses is to provide an assessment of long-term vegetation response to a set of silvicultural treatments. As a preliminary analysis, we grouped species according to light requirement and competitive ability. These *a priori* emergent groups provide an indication of understory response to the different treatments in terms of functional and structural assemblage.

Legend


Fern
Competitive fern
Shade intolerant grass
Shade tolerant grass
Competitive grass
Shade intolerant herb
Shade tolerant herb
Competitive herb
Non−vascular plant
White Pine
Shade intolerant shrub
Shade intolerant competitive shrub
Shade tolerant shrub
Shade tolerant competitive shrub
Shade intolerant tree
Shade intolerant competitive tree
Shade tolerant tree
Shade tolerant competitive tree

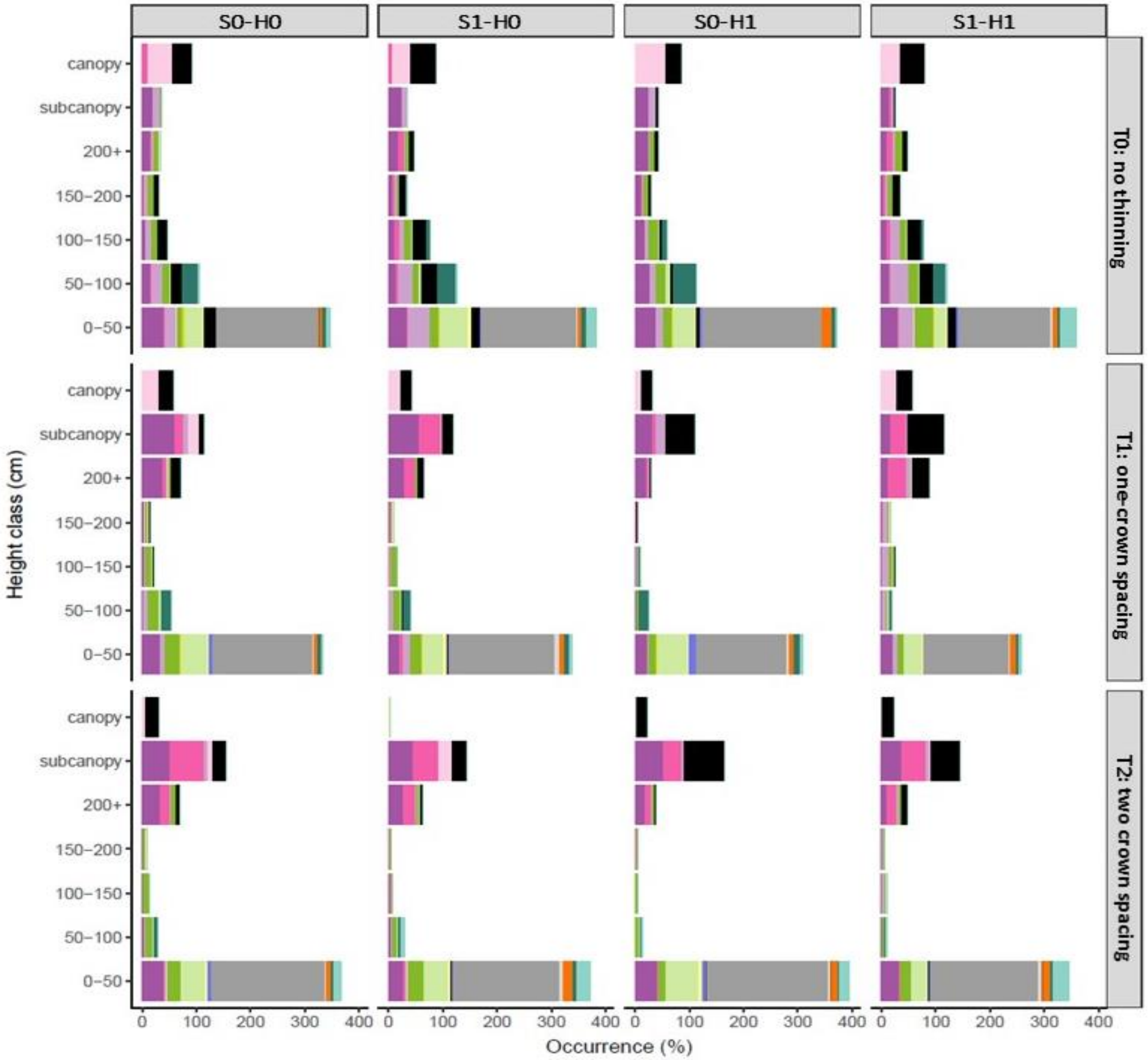


Figure 1: Cumulative relative occurrence of plant species by emergent groups at the Petawawa site

Canopy thinning appears to have had a strong influence on understory vegetation, both in terms of abundance of the different emergent groups and in terms of vertical structure (Fig. 1). Across all treatments, plots with no canopy thinning tend to have more shade intolerant species and more competitive ferns. Plots with greater canopy spacing had fewer competitive species at lower strata levels (up to 200 cm height) and had more shade tolerant species. This is likely related to the positive growth response of white pine and tree species like aspen and maple to canopy thinning, which is reflected by more trees of these species in higher strata (200+ cm and subcanopy). There are also differences in understory community response to treatments. In our study, non-vascular plants only occurred in treatments that were not blade scarified. This agrees with other studies that found lower non-vascular diversity with increasing disturbance severity, including herbicide application and blading (Haeussler 2002; Bell et al. 2004). Plots that were not mechanically scarified and did not receive herbicide application tended to have the most competitive shrubs and trees across all strata and had less white pine in higher strata.

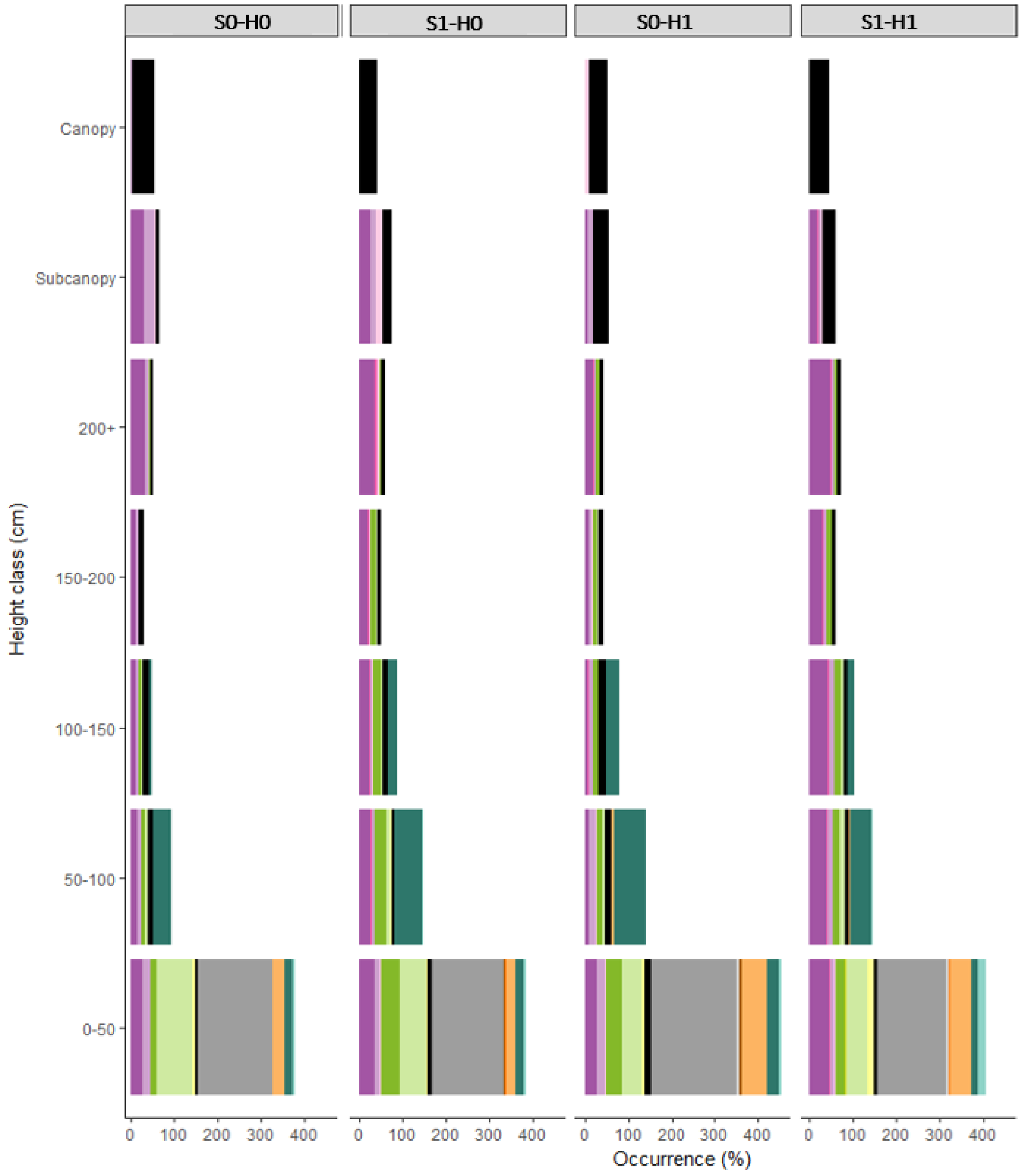


Figure 2: Cumulated relative occurrence of plant species by emergent groups at the Britt site.

Unlike the research site in Petawawa, plots in Britt only had one level of canopy thinning and are considered most similar to one crown spacing at the Petawawa site. At this site, we see differences in understory community response to treatments in terms of both community composition and strata; however, differences between treatments are not as clear as seen in Petawawa (Fig. 2). More white pine occurred in the subcanopy in treatments that received herbicide application. Shade tolerant ferns also occurred in greater frequencies in treatments that were blade scarified and treated with herbicide. Plots that did not receive any site preparation had lowest frequency of white pine in higher strata.

Next Steps for Assessing impacts on forest structure using field measurements (2018-2019)

- Complete leaf trait laboratory measurements collected from Britt Site
- Finalising the compilation of plant traits
- Perform analysis of vegetation response to silvicultural treatment
- Analyze and disseminate results including scientific publications, CWFC technology transfer document (Fibre Facts), presentations in conferences, workshops and meetings
- Present recommendation to the CIF seminar series
- Final SERG-I report and presentation to SERG-I workshop

Sources of funding 2017-2018

Funding source	Amount
Canadian Forest Service	12,000$
SERG-I OMNRF SERG-I FPL	8,000$ 7,600$

Acknowledgements

Financial and in-kind support for the associated data collection and analyses presented in this report, we thank the following people and organizations.
For the legacy dataset, Laura Boisvert-Marsh performed data structuring and assisted with data analysis. Marianne Bachand provided technical advice on statistical analysis. For field measurements exploring forest structure, Peter Arbour and the staff at Petawawa Research Forest assisted with logistical support in the field, providing lab space and equipment. Craig Zimmerman, from Canadian Forest Service, and Sam Sommer, from Petawawa Research Forest, performed fieldwork. Kevin Good, from Canadian Forest Service, assisted with logistics and field work. Financial support and in-kind donations in 2016 and 2017 were provided by Canadian Forest Service (CWFC and GLFC), and the Ontario Ministry of Natural Resources and Forestry (SERG-I). We also acknowledge collaborating partners Nipissing Forest Resource Management Inc., J.D. Irving Limited, Westwind Forest Stewardship Inc., Ontario Ministry of Natural Resources and Forestry, and the Petawawa Research Forest (Canadian Forest Service).

Prior to 2016, generous support for study site installation and data collection activities were provided by the following organizations.

White Pine Competition Study (Ontario and New Brunswick): Canadian Forest Service – Canadian Wood Fibre Centre, Ontario Ministry of Natural Resources and Forestry, Canadian Ecology Centre – Forestry Research Partnership (Tembec, Canadian Forest Service, Ontario Ministry of Natural Resources), Living Legacy Trust, Canada Foundation for Innovation, Enhanced Forest Productivity Science Program - Forestry Futures Trust Ontario, Ontario Innovation Trust Fund, Monsanto Canada Inc., Dow AgroSciences Canada Inc., Domtar Wood Products Inc., Nipissing Forest Resource Management Inc., J.D. Irving Limited, Forest Protection Limited, Spray Efficacy Research Group International (SERG-I), ULERN (Upper Lakes Environmental Research Network), and the U.S. Forest Service.

Petawawa White Pine Shelterwood and Site Preparation Trial: Canadian Forest Service, Ontario Ministry of Natural Resources and Forestry, Algonquin Forest Authority, Northern Ontario Development Agreement (NODA), Canadian Ecology Centre – Forestry Research Partnership (Tembec, Canadian Forest Service, Ontario Ministry of Natural Resources).

Britt White Pine Shelterwood and Site Preparation Trial: Ontario Ministry of Natural Resources and Forestry, Westwind Forest Sterwardship Inc. , Canadian Ecology Centre - Forestry Research Partnership (Tembec, Canadian Forest Service, Ontario Ministry of Natural Resources), Canadian Forest Service, Roy's Lumber.

SERG-i Project FPL-786 Dynamics and management of rising spruce budworm outbreaks

Interim Report February 2018.

Régnière J, Delisle J, Martel V, Seehausen ML, Boulanger Y, Béchard A, Labrecque A, Trudeau S, St-Amant R.

Laurentian Forestry Centre, Quebec City, QC.

Abstract.

In 2017, work on the dynamics and management of rising spruce budworm outbreaks progressed on four fronts.

1. Monitoring of the endemic SBW populations of Armagh and Epaule.

1.1 SBW populations were monitored by foliage sampling (no SBW larvae were recovered on 200 host branch tips), pheromone and light traps. Moth populations were higher in 2017 than in 2016, but remained < 100 males per pheromone trap, well below the range expected of epidemic populations (Fig. 1a). No clear mass immigration events were recorded in 2015, 2016 or 2017 in Armagh or Epaule. Total catch in pheromone traps in 2015 and 2017, along a transect west from Rimouski to Quebec City, was closely related to distance from Rimouski (near the outbreak front), an indication that even in the absence of mass immigration, there is a spatial gradient in moth abundance suggesting of spread by movement of moths (Fig. 1b).

1.2 The impact of natural enemies was again measured on implanted insects using the sentinel and cohort methods. Overall parasitism rates have remained very high in both locations, which explains why those populations have not been rising. Among sentinel larvae, parasitism by the ichneumonid *Tranosema rostrale* has continued its slow decline initiated in 2011. However, this decline has been compensated by a rise in the contribution of the ectoparasitic eulophid *Elachertus cacoeciae* to total mortality (Fig. 2). In Epaule, mortality from those two wasps has remained very high and has fluctuated without clear trend in recent years (Fig. 2). It is interesting to note the strong negative correlation in attack rates of *T. rostrale* and *E. cacoeciae* at the yearly level (Fig. 3), a feature that had already been clearly observed at the daily level.

2. Modeling of the seasonal ecology of the parasitic wasp *Tranosema rostrale*. The seasonal biology model of *T. rostrale* (Seehausen, PhD thesis, 2017, University of Toronto), was linked to the seasonal biology model of the spruce budworm and that of a likely alternate host, the obliquebanded leafroller (*Choristoneura rosaceana*) developed from literature data. The interaction between the parasitoid and its two hosts was mediated through Holling's disk equation relating parasitoid attack rates to host density. This hybrid model allows us to investigate the consequences of synchrony between adult

parasitoids and host larvae on the overall fitness of the parasitoid in response to weather across the landscape (Fig. 4). This work is in progress.

3. Results of three large-scale aerial applications of pheromone for mating disruption (2008, 2013, 2014) were compiled and analysed, and a manuscript is in advanced stages of preparation. While pheromone applications had a clear effect on male captures in pheromone traps and on the success of caged females at capturing males or getting mated m (Fig. 5), no effect on egg densities or apparent fecundity (E/M ratios) was found (Fig. 6). Thus, pheromone applications failed to reduce SBW populations.

4. Flight modeling, now fully incorporated in the BioSIM/11 software, and Doppler radar image processing were further advanced 2017. The flight model was improved by adding wing-frequency modifications representing the energy required for horizontal moth displacement and through that flight altitude in the wind field. Tools for the incorporation of high-resolution 3D weather data in BioSIM/11, and for overlaying animated flight model output on radar images were developed. The team, composed of Canadian and US researchers or computer scientists, is now developing analytical tools aimed at calibrating and validating the flight model based on radar images and trap networks. Among the most interesting products that the SBW flight model can now deliver are annual egg deposition maps, compiling the cumulative egg deposition of immigrating moths during a season (Fig. 7).

Résumé.

En 2017, la recherche dans le cadre du projet sur la dynamique et la gestion des populations de la TBE en début d'épidémie a progressé sur quatre fronts.

1. Suivi des populations endémiques d'Armagh et Épaule.

1.1 Les niveaux de populations de la TBE ont été mesurés par échantillonnage du feuillage (aucune larve n'a été trouvée sur plus de 200 bouts de branches), et à l'aide de pièges à phéromones ou lumineux. Les populations de papillons ont augmenté de 2016 à 2017, mais sont demeurées sous les 100 mâles par piège à phéromones, bien en deçà du niveau attendu d'une population épidémique (Fig. 1a). Aucun événement clair d'immigration massive n'a été enregistré en 2015, 2016 ou 2017 à Armagh ou Épaule. La capture totale saisonnière en 2015 et 2017, le long d'une ligne de piégeage entre Rimouski et Québec, était corrélée étroitement avec la distance de Rimouski (près du front épidémique), une indication que même en l'absence de migration massive il y a un gradient d'abondance des papillons suggérant l'expansion par mouvement des papillons (Fig. 1b).

1.2 L'impact des ennemis naturels a de nouveau été mesuré sur des insectes implantés par les méthodes de sentinelles et de cohortes. Les taux de parasitisme global sont demeurés très élevés, ce qui explique pourquoi ces populations n'ont montré aucune tendance à l'augmentation. Parmi les sentinelles, le parasitisme dû à l'ichneumone *Tranosema rostrale* a poursuivi son lent déclin depuis 2011. Par contre ce déclin a été compensé par une augmentation de la contribution à la mortalité totale par l'eulophide ectoparasitique *Elachertus cacoeciae* (Fig. 2). À Épaule, la mortalité due à ces deux guêpes est demeurée très élevée et a fluctué sans tendance claire au cours des dernières années (Fig. 2). Il est intéressant de noter la forte corrélation négative entre les taux d'attaque de *T. rostrale* et *E. cacoeciae* au niveau annuel (Fig. 3), une caractéristique qui avait déjà été notée au niveau quotidien.

2. Modélisation de l’écologie saisonnière de la guêpe parasitique *Tranosema rostrale*. Le modèle de biologie saisonnière de *T. rostrale* (Seehausen, thèse de PhD, 2017, Université de Toronto), a été couplé au modèle saisonnier de la TBE et celui d’un hôte alterne probable, la tordeuse à bandes obliques (TBO, *Choristoneura rosaceana*) mis au point à partir de données provenant de la littérature. L’interaction entre le parasitoïde et ses deux hôtes a été approximée par l’équation des disques de Holling qui met en relation les taux d’attaque et la densité des hôtes. Ce modèle hybride nous permet d’explorer les conséquences de la synchronie entre adultes du parasitoïde et les larves des ses hôtes sur la performance globale (fitness) du parasitoïde en réponse au climat à l’échelle du paysage (Fig. 4). Ces travaux se poursuivent.

3. Les résultats de trois applications à grande échelle de phéromones pour la confusion sexuelle de la TBE (2008, 2013, 2014) ont été compilés et analysés, et un manuscrit est en préparation avancée. Bien que les applications aient eu un effet clair sur les taux de capture de papillons dans les pièges à phéromones et sur le succès de femelles en cages à capturer des mâles et à s’accoupler (Fig. 5), aucun effet sur la densité des œufs ou la fécondité apparente (rapport œufs/papillon) n’a été décelé (Fig. 6). Donc, l’application de phéromones a échoué à réduire les populations de TBE.

4. La modélisation du vol de la TBE, désormais incorporée dans le logiciel BioSIM/11, et le traitement d’images de radar Doppler, ont été avancés en 2017. Le modèle de vol a été amélioré en introduisant un facteur de fréquence alaire représentant l’énergie requise pour le déplacement horizontal des papillons et par son truchement l’altitude de croisière de ceux-ci dans le champ de circulation de l’air. Des outils pour l’incorporation de données météo tridimensionnelles à haute résolution dans BioSIM/11 et pour la superposition des extrants du modèle de migration sur des images radar ont été mis au point. L’équipe, composée de chercheurs et informaticiens canadiens et américains, est maintenant à mettre au point des outils analytiques qui permettront de calibrer et valider le modèle de vol à partir d’images radar et de réseaux de pièges. Parmi les produits les plus prometteurs que peut désormais livrer le modèle de vol, figurent des cartes de déposition totales annuelles des œufs par les migrants (Fig. 7).

Financial

Much of this project (Sections 1 to 3) was funded through the EIS/SBW project to the amount of $73,500 (ACOA, FPL and Newfoundland Department of Natural Resources). Section 4 (the moth flight modeling and radar work) was funded outside of SERG through CANUSA funds (project FS-NRS-13) to the tune of $60,000 USD. CSF O&M totaled $24,500 ($9,000 to each Johanne Delisle and Véronique Martel, and $6,500 to Jacques Régnière). All external funds (from SERG/I and CANUSA) were handled through a contract between FPL and SOPFIM, and we express our gratitude to both organisations, especially to SOPFIM, for making this collaboration possible.

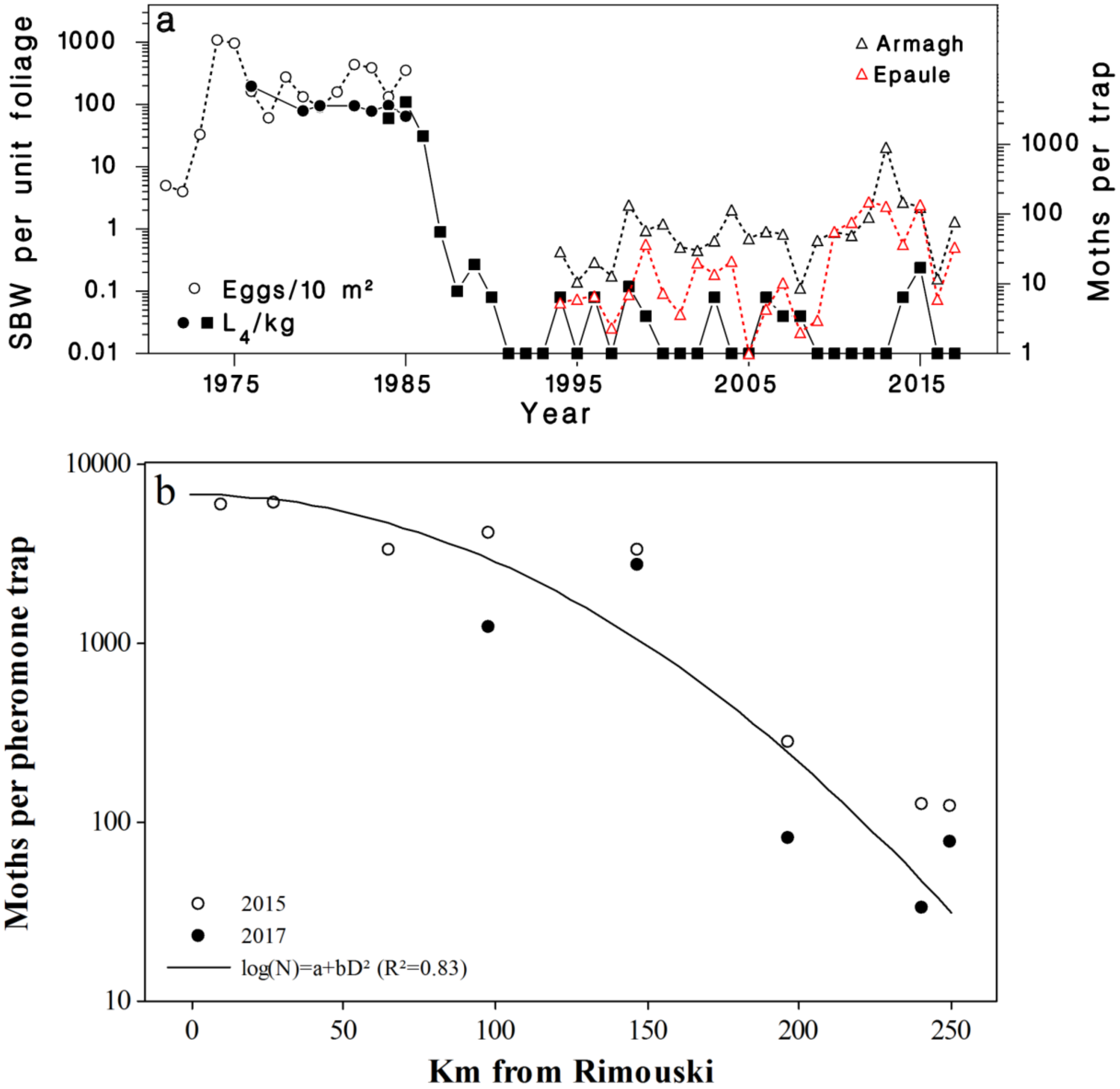


Figure 1. (a) History of spruce budworm population density fluctuations in Armagh and Epaule, 1971-2017 (○: eggs/10m²; ●: larvae/kg; Δ: males per pheromone trap, black in Armagh, red in Epaule). (b) Relationship between total seasonal catch of male SBW moths in pheromone traps and distance from the main outbreak front (Rimouski) in 2015 and 2017 (Armagh and Epaule are at 249 and 240 km from Rimouski, respectively).

Figure 1. (a) Historique des fluctuations de populations de la tordeuse des bourgeons de l'épinette à Armagh et Épaule de 1971 à 2017 (○: oeufs/10m²; ●: larves/kg; Δ: mâles par pièges à phéromones, noir à Armagh, rouge à Épaule). (b) Relation entre la capture saisonnière totale de papillons mâles de la TBE dans des pièges à phéromones et la distance du front épidémique (Rimouski) en 2015 et 2017 (Armagh et Épaule sont à 249 et 240 km de Rimouski, respectivement).

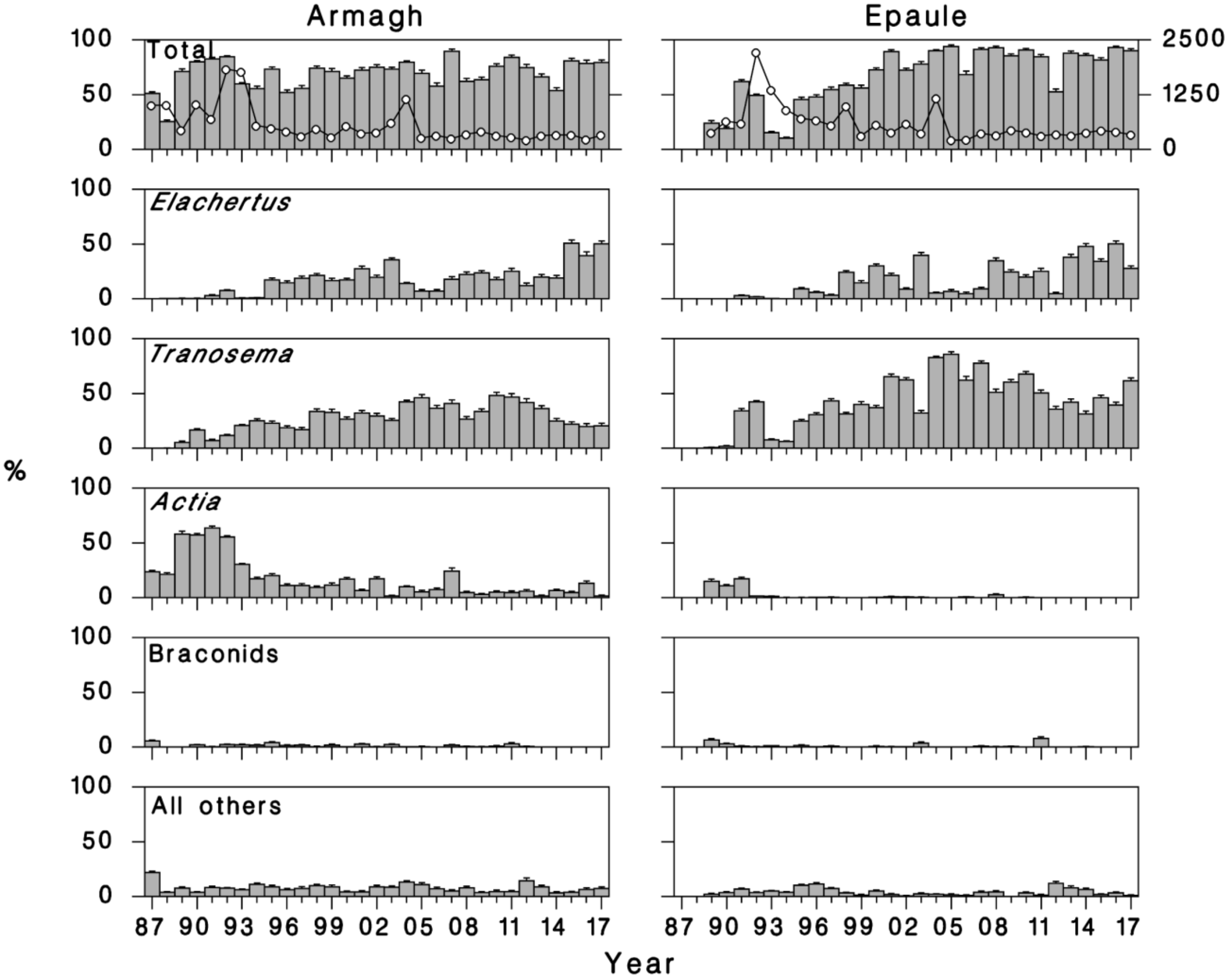


Figure 2. Annual fluctuations of parasitism rates among the implanted SBW sentinel larvae since the collapse of the last outbreak in Armagh and Epaule (1987 to 2017).

Figure 2. Fluctuations annuelles des taux de parasitisme parmi les larves de TBE implantées (sentinelles) depuis le déclin de la dernière épidémie à Armagh et Épaule (1987 à 2017).

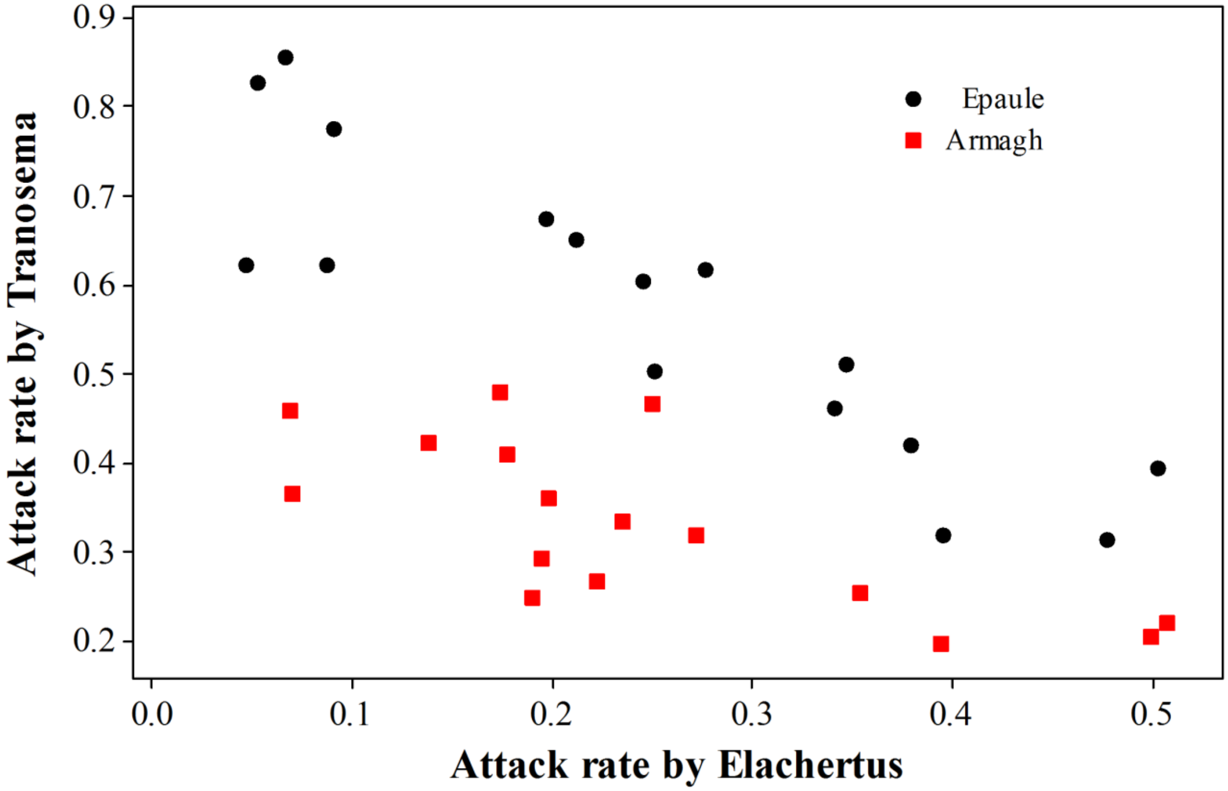


Figure 3. Negative correlation between overall attack rates by *T. rostrale* and *E. cacoeciae* in Armagh and Epaule between 2001 and 2017. This feature has been clearly observed at the daily level.

Figure 3. Corrélation négative des taux d'attaque par *T. rostrale* et *E. cacoeciae* à Armagh et Épaule entre 2001 et 2017. Cette caractéristique a été clairement observée au niveau quotidien.

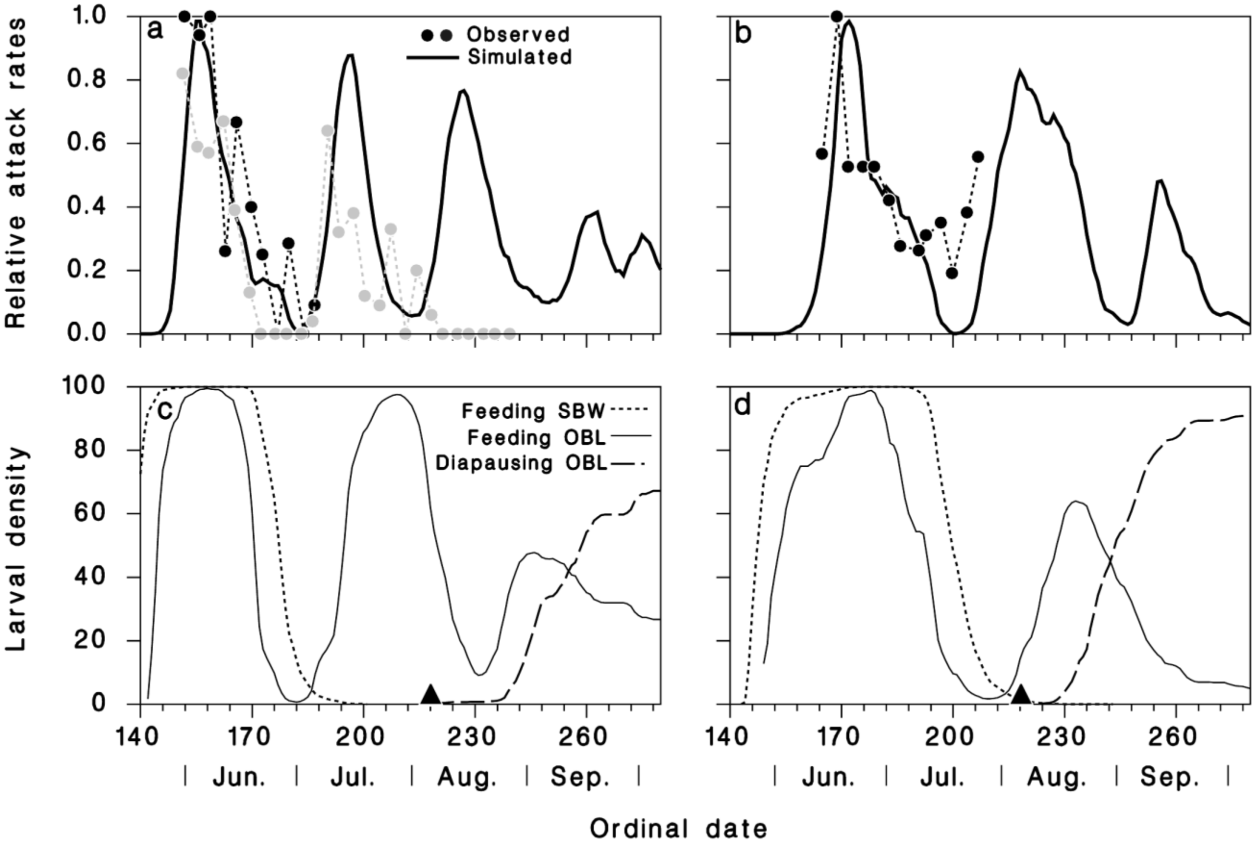


Figure 4. *T. rostrale* seasonal biology model output. Comparison of observed (●, ●) and simulated (——) seasonal oviposition trends by the parasitoid in 2016 in (a) Armagh and (b) Epaule. Corresponding predicted seasonal abundance of host larvae from the SBW and OBL models in (c) Armagh and (d) Epaule. ▲: Date at which day length < 14.5 h (OBL diapause induction).

Figure 4. Extrants du modèle de biologie saisonnière de *T. rostrale*. Comparaison des patrons saisonniers d’oviposition par le parasitoïde en 2016 à (a) Armagh et (b) Épaule. Patrons correspondants prédits pour la fréquence des larves hôtes de TBE et TBO à (c) Armagh et (d) Épaule. ▲: Date à partir de laquelle la longueur du jour < 14.5 h (induction de la diapause de la TBO).

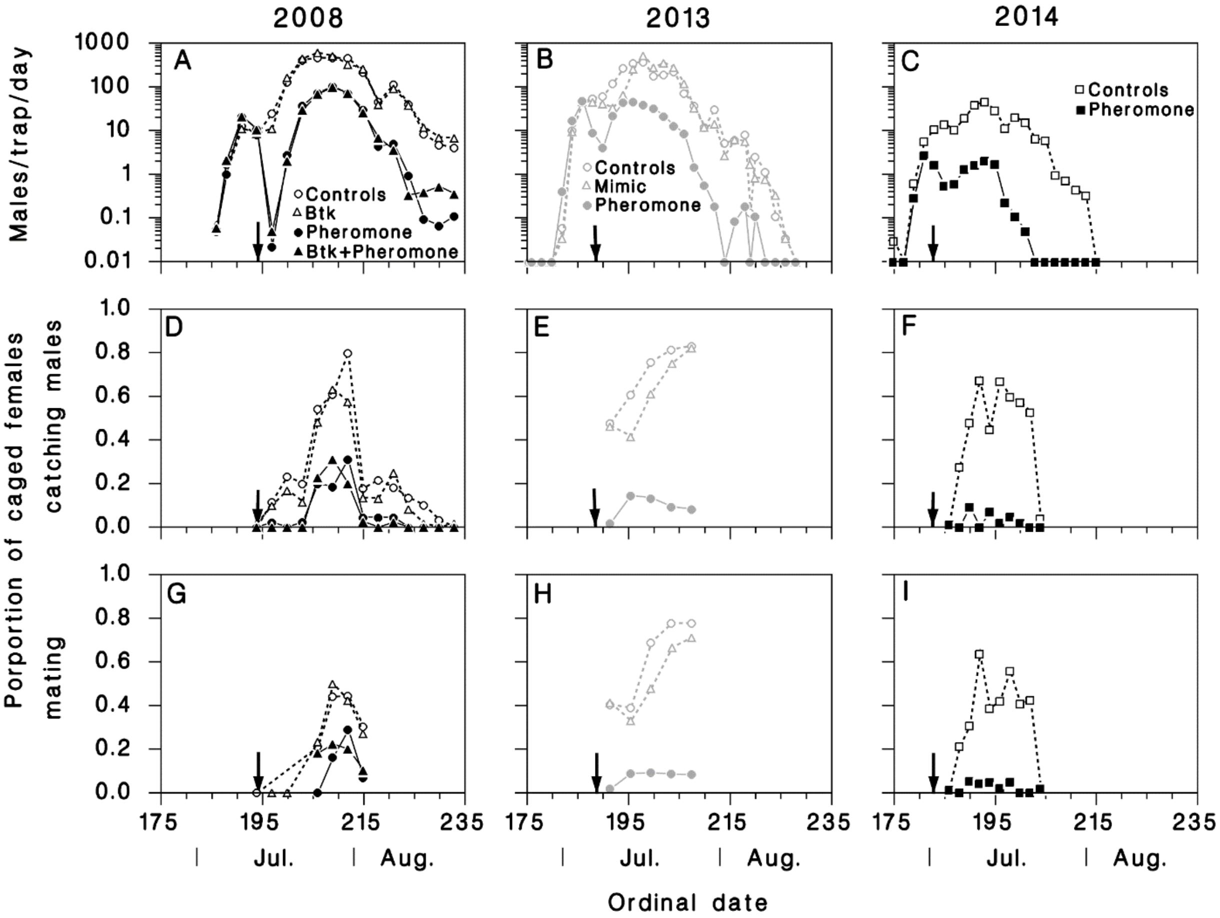


Figure 5. Daily capture rates of male spruce budworm moths in pheromone traps, success of caged virgin females at capturing males and getting mated in (A, D, G) 2008 (○, controls; Δ, Btk ; ●, Pheromone; ▲, Btk + pheromone), (B, E, H) 2013 (○, controls; △, Mimic ; ●, Pheromone) and (C, F, I) 2014 (□, controls; ■, Pheromone). Arrows indicate the date of pheromone treatment.

Figure 5. Taux de capture quotidien de males de la tordeuse des bourgeons de l'épinette dans des pièges à phéromones, succès de femelles vierges en cage à capturer des mâles et à s'accoupler (A, D, G) en 2008 (○, témoins; Δ, Btk ; ●, Phéromones; ▲, Btk + phéromones), (B, E, H) en 2013 (○, témoins; △, Mimic ; ●, Phéromones) et (C, F, I) en 2014 (□, témoins; ■, Phéromones). Les flèches indiquent le moment de l'application des phéromones.

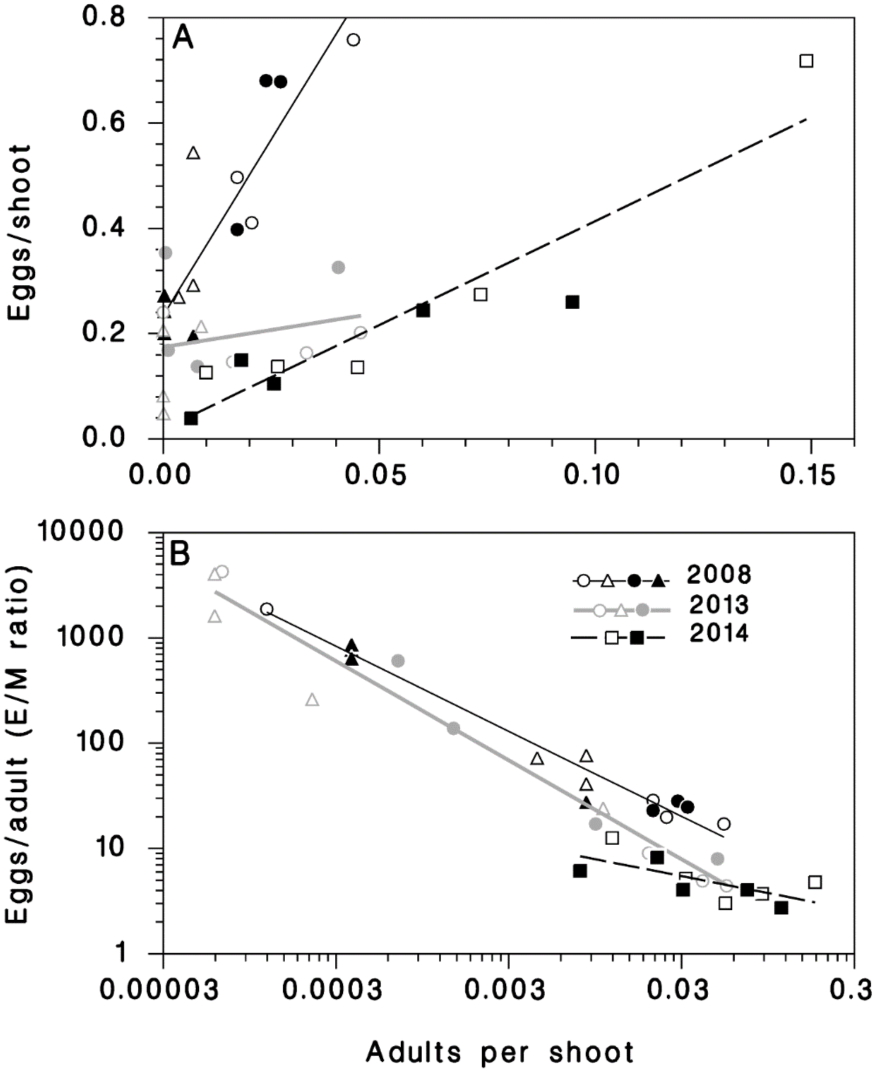


Figure 6. Relationship between adult density, (A) egg density (lines: eq. 7) and (B) apparent fecundity (eggs/adult)) in 2008 (○, controls; ∆, Btk ; ●, Pheromone; ▲, Btk + pheromone; ——, regression), 2013 (○ controls; ∆, Mimic ; ●, Pheromone; ▬▬, regression) and 2014 (□, controls; ■, Pheromone; — — —, regression).

Figure 6. Relation entre la densité des populations adultes, (A) la densité des œufs et (B) la fécondité apparente (œufs/adulte) en 2008 (○, témoins; ∆, Btk ; ●, Phéromones; ▲, Btk + phéromones; ——, régression), en 2013 (○ témoins; ∆, Mimic ; ●, Phéromones; ▬▬, régression) et 2014 (□, témoins; ■, Phéromones; — — —, régression).

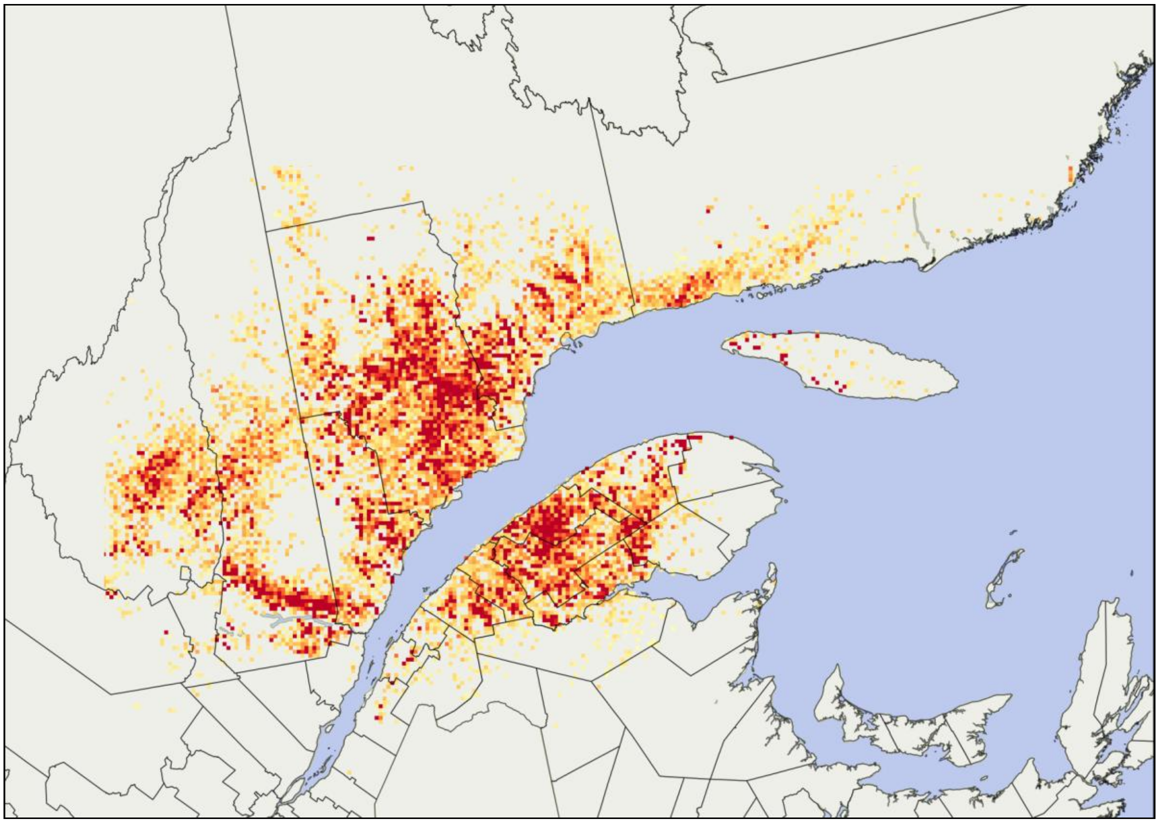


Figure 7. Example of map output from the SBW moth migration model under BioSIM/11. Density of eggs deposited over migration events of 2017. Color intensity is proportional to the density of deposited eggs.

Figure 7. Exemple d’extrant cartographique du modèle de migration des papillons de la TBE dans BioSIM/11. Densité des œufs déposés lors des événements migratoires de 2016. L’intensité de la couleur est proportionnelle à la densité des œufs déposés.

Landscape level impacts of EIS on SBW, other herbivores and associated natural enemies (ACOA RD100 2.2.2)

Principal Investigators
V. Martel, R. Johns

Collaborators:
E. Eveleigh (CFS-AFC), K. McCann (University of Guelph), D. Pureswaran (CFS-LFC), Z. Sylvain (CFS-AFC), A. Morrison (FPL), B. Morin (CFS-AFC), E. Owens (FPL), C. Hébert (CFS-LFC)

Contacts:
V. Martel
Telephone Number: (418) 640-2625
Address: CFS-LFC, 1055 du PEPS, PO Box 10380, Stn Ste-Foy, Québec, QC, G1V 4C7

R. Johns
Telephone Number: (506) 452-3785
Address: CFS-AFC, 1350 Regent St., Fredericton, NB, E3B 5P7

Abstract

We report on results from the final year of our research project aimed at addressing three key questions underlying the Early Intervention Strategy: (1) Does treatment cause enough additive mortality (i.e., mortality in addition to what would otherwise occur naturally) to cause populations to decline? (2) Does mass moth migration offset the efficacy of treatments? (3) Do treatments cause unintended effects on non-target caterpillars and natural enemies. In 2017, preliminary results suggest that treatments caused ~20% additive mortality across the range of densities studied. Moreover, intergenerational population growth rates (based on L2 larval collections in 2016 and 2017), indicated that treatments cause mild to significant budworm declines, whereas in untreated sites budworm densities generally increased. Our results also indicated that treating sites relatively early (~L4.5) yielded higher efficacy than treating late (~L6). Validation of molecular approaches to identify parasitoids in frozen larvae remains underway.

Titre

Impacts au niveau du paysage de la SIH sur la TBE, les autres herbivores et les ennemis naturels associés (APECA RD100 2.2.2)

Résumé

Ce rapport présente les résultats de la dernière année de notre projet de recherche visant à répondre à trois grandes questions dans le développement du cadre de travail de la Stratégie d’intervention hâtive : (i) Les traitements causent-il suffisamment de mortalité additionnelle (c.-à-d., de la mortalité en plus de ce qui se produirait naturellement) pour causer le déclin des populations? (ii) La migration de masse des papillons empêche-t-elle l’efficacité des traitement? (iii) Les traitements amènent-ils des effets non voulus sur les chenilles non-ciblées et sur les ennemis naturels?. En 2017, les résultats préliminaires suggèrent que les traitements ont causé ~20% de mortalité additionnelle dans la gamme des densités testées. De plus, les taux de croissance intergénérationnelles des populations (basés sur les collections de L2 en 2016 et 2017) indiquent que les traitements ont causé un déclin des tordeuses de faible à significatif, alors que les densités de tordeuses des sites non-traités ont généralement augmentés. Nos résultats indiquent que traiter les sites relativement tôt (~L4.5) amène une plus grande efficacité que de traiter les sites tard (~L6). La validation de la détection et de l'identification des parasitoïdes par biologie moléculaire dans les larves congelées est toujours en cours.

Introduction

Spruce budworm outbreaks have been managed historically through a 'Foliage protection' approach whereby insecticides are used to preserve foliage in severely defoliated forests before substantial mortality or growth loss occur. While foliage protection may offer a short-term reprieve to budworm damage, it is mainly a stopgap measure and does not aim to suppress the overall rise or spread of outbreaks. Recent advances in our understanding of budworm population dynamics (e.g., Régnière et al. 2013) have prompted efforts to develop a complementary approach to managing budworm, the so-called Early Intervention Strategy (EIS). Briefly, the EIS entails controlling relatively low-density populations along the leading edge of outbreaks as a way of containing outbreak spread. In general, the EIS program shares many characteristics with area-wide containment programs often used to contain invasive species, such as the "Slow the Spread" program for gypsy moth being used in the United States. Many practical and theoretical questions underlie the development of a pest containment program. How do we monitor and decide when and where to treat hot spots? What insecticide products should we use and when should they be applied? Does treatment cause enough additive mortality (i.e., mortality in addition to what would otherwise occur naturally) to cause populations to decline? Does mass moth migration offset the efficacy of treatments? In addition, given that natural enemies (parasitoids in particular) are a major source of this natural budworm control, there are also concerns around the potential non-target impacts of treating low density populations. Most of the key parasitoids thought to control budworm are generalists that attack other herbivores when SBW densities are low and these may be adversely affected if a low-density population is treated (Huber et al. 1996; Eveleigh et al. 2007). In particular, an unwanted impact of the treatments on the general community could have an adverse effect on the SBW in years following treatment if parasitoid populations are negatively affected, either directly by insecticide-induced mortality, or indirectly through alternate/alternative hosts mortality.

In this report, we discuss our results from 2017 addressing the above questions. As analyses are still underway, the presented results and conclusions are preliminary. Results for the first three years of this project won't be discussed here and may be found in previous reports (Martel et al. 2015-2017 SERG Reports).

Objectives

(1) Assess the efficacy of insecticide application over large areas on relatively low-density populations of SBW and determine the ability of moths to reinvade large areas that have been treated with insecticides.

(2) Validate the Bar coding ‘chip’ (see Project A2.2) that will provide rapid and precise identification of spruce budworm natural enemies.
(3) Determine the impact of EIS on parasitoid abundance and impact within the local herbivore community.

Methodology

Objective 1: Treatment efficacy and migration effects

In 2017, we carried out population surveys in 62 total sites spanning northern NB through Cape Breton, NS (Fig. 1). The bulk of sites were clustered inside (21 sites) and outside (28 sites) of the spray block north of Mirimichi, NB, and these will be the focus of this report (very few budworm were found south of Mirimichi). Treatments in 2017 consisted of single applications of either tebufenozide or *Bacillus thuringiensis kurstaki* (Btk) (Fig. 1). Due to a spell of inclement weather in the middle of the treatment period, we also ended up with a *de facto* experiment allowing us to examine the efficacy of relatively ‘early’ (~L4.5) vs. ‘late’ (~L6) treatments. For further details on the timing of application for tebufenozide and Btk application, see Amirault et al. (2018 SERG Report).

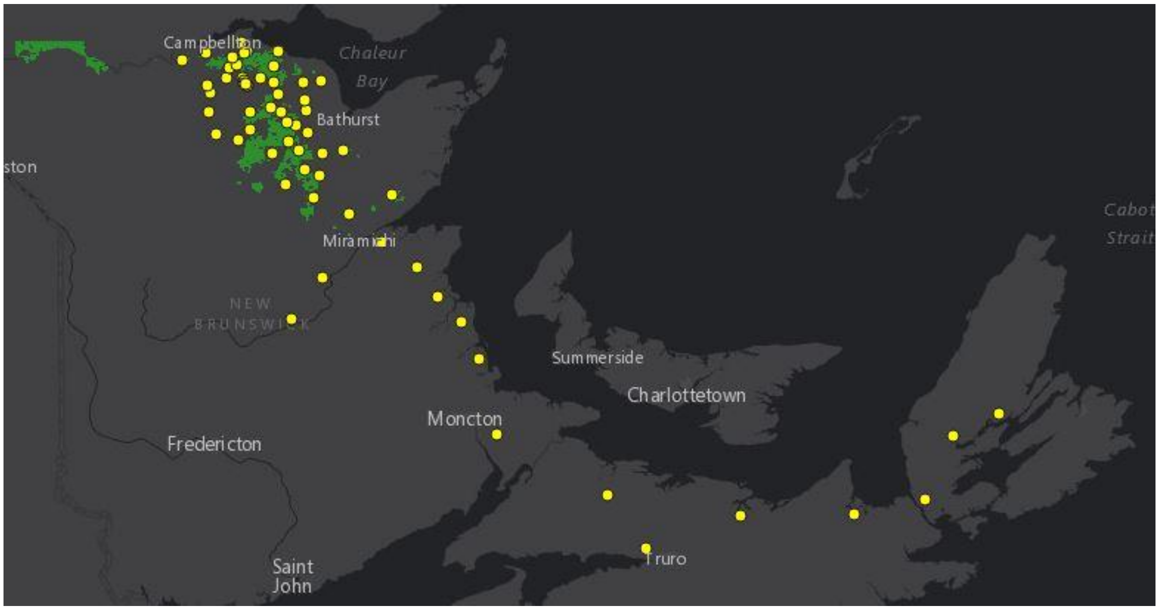


Figure 1. Circles represent study sites used in 2017 to assess spruce budworm population responses inside and outside areas treated with single applications of either Btk or tebufenozide. The shaded areas in northern NB represent the areas treated with the insecticides.

To determine the effects of insecticide treatments on budworm survival, we collected 15-30 branches (45 cm in length) from the mid crown of trees along transects beginning from the edge of the plot and running roughly 100-200 m into each site. We sampled branches at ~L4.5 (prior to treatments) and again at peak L6, early pupation, late pupation, and peak egg lay. Branches were processed in the laboratory in Fredericton and all larval insects (including non-budworm) were identified and assessed for whether they were live or dead. Any collected pupae were placed in petri dishes and reared through to adult or parasitoid emergence. When collecting branches during the egg stage, we also counted empty pupal cases and identified whether they showed signs of predation, parasitism, or adult emergence. Egg masses were assessed for egg number and categorized as alive, sterile, parasitized, or hatched. All living and dead insects were placed individually in centrifuge tubes and placed in a freezer at -18^{o}C for eventual parasitoid barcoding (see Objective 3). Assessments of seasonal treatment efficacy were based on comparisons of survival from L4.5 to adult ('adult' being derived from the number of 'live' pupae/pupal cases on branches from our final branch collection).

Intergenerational population growth rates, which would reflect both seasonal survival rates and effects of migration/egg lay, were estimated from L2 branch collections carried out in the fall of 2016 and 2017. We used the same protocol described above to collect L2 branches in each site, although the branches were 75 cm long rather than 45 cm.

Objectives 2 & 3: Non-target effects of treatments on natural enemies

All frozen samples collected from branches in the previous section (both budworm and non-budworm) are still awaiting soon processing for parasitoids using the DNA barcoding approach being developed by Smith et al. (2015-2018 SERG Reports).

Results and Discussion

Objective 1: Treatment Efficacy and migration effects

Our *de facto* experiment testing how the timing of treatments influences treatment efficacy suggested that survival was lower when treatments were applied 'early' and less effective when applied 'late' when compared with untreated sites (Fig. 2). It's worth noting that the timing of the late treatment occurred very near to pupation, though there were mainly larvae (mostly L6) found in the collection prior to the 'late' treatment. This trend will require further study with better replication to tease out effects for the different insecticides used.

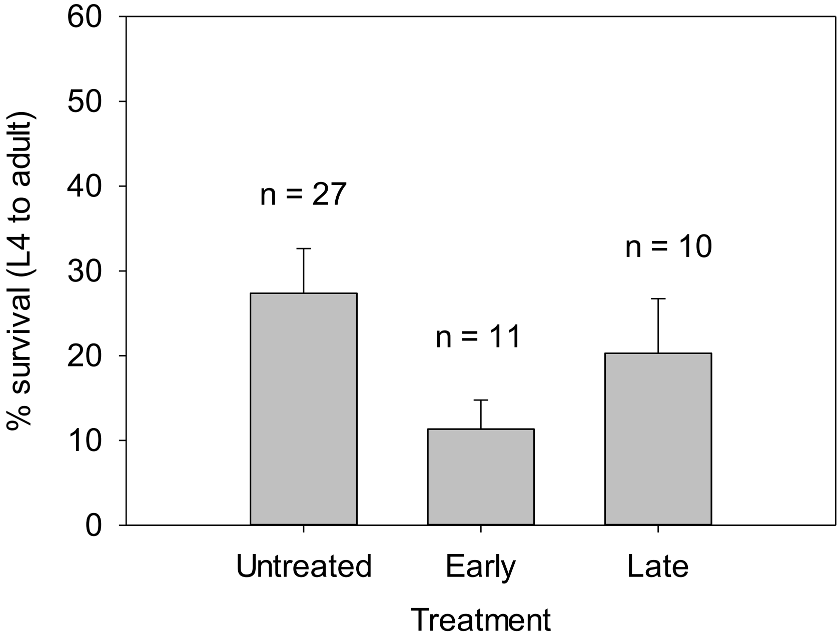


Figure 2. Effects of treatment timing (early vs. late) on spruce budworm survival from L4 to adult compared with untreated sites.

Overall, survival across the range of densities that occurred in the field was ~20% lower in treated vs. untreated areas, suggesting that treatments were providing mortality over and above that occurring naturally (i.e., additive mortality) (Fig. 3a). The magnitude of effects appeared to increase marginally (at least for lower budworm density sites) when we considered only the data collected from the 'early' treated sites (Fig. 3b). It is also notable that treatments were effective in sites that had densities exceeding the (tentative) Allee threshold of ~7-8 larvae per branch.

Intergenerational population growth rates, based on comparisons of L2 densities between years, were stable or increased in ~77% of untreated sites (i.e., budworm populations stayed the same or increased from 2016 to 2017) (Fig. 4a), but declined in ~67% of treated sites (i.e., budworm populations declined from 2016 to 2017) (Fig. 4a). However, if we removed all of the 'late' treated sites, then all the remaining sites that were treated 'early' had negative growth rates (Fig. 4b). Overall, this suggests that the effects of insecticide treatments were not substantially (or at least completely) offset by moth immigration and egg lay and, again, that the strongest effects of treatments may be yielded from treating relatively earlier rather than later in larval development.

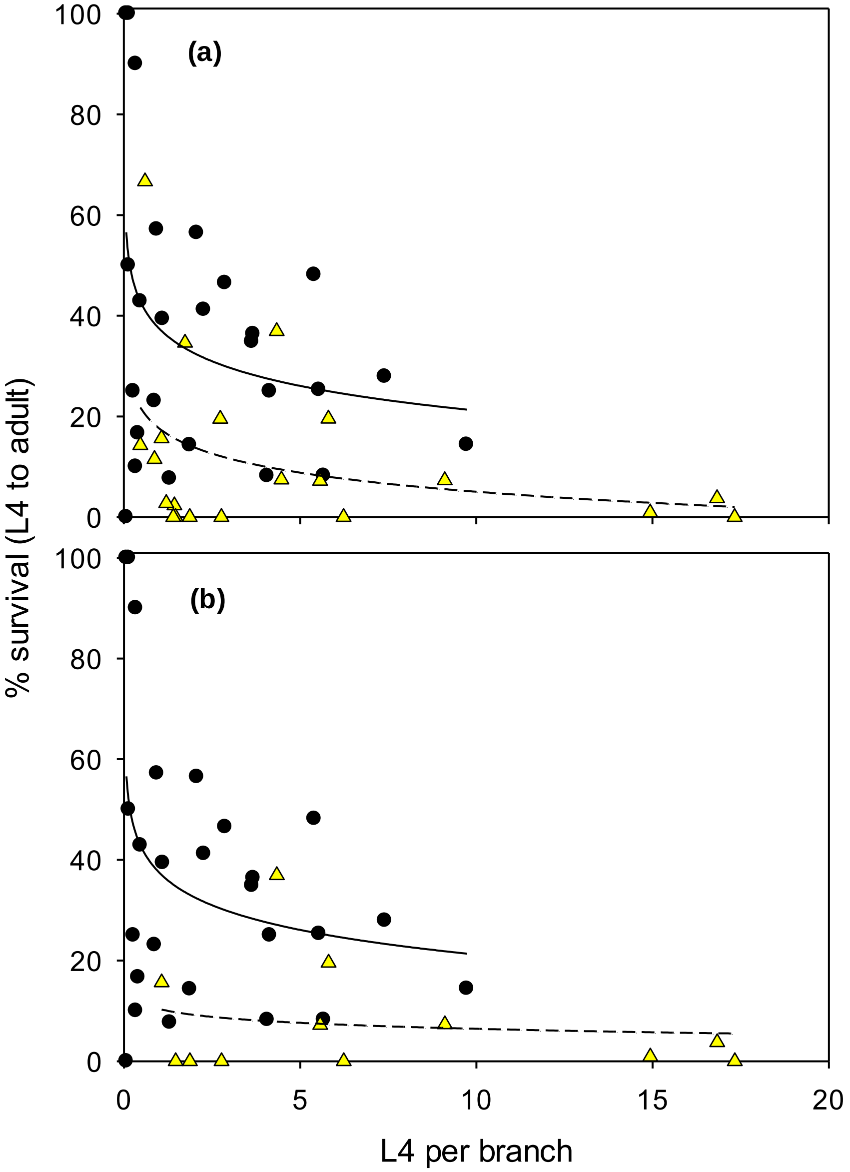


Figure 3. The effect of insecticide treatment in 2017 on spruce budworm survival from L4 to adult and across a range of population densities. Sites treated with tebufenozide vs. Btk are not distinguished. (a) Including both ‘early’ and ‘late’ treated sites, (b) Only including sites treated ‘early’.

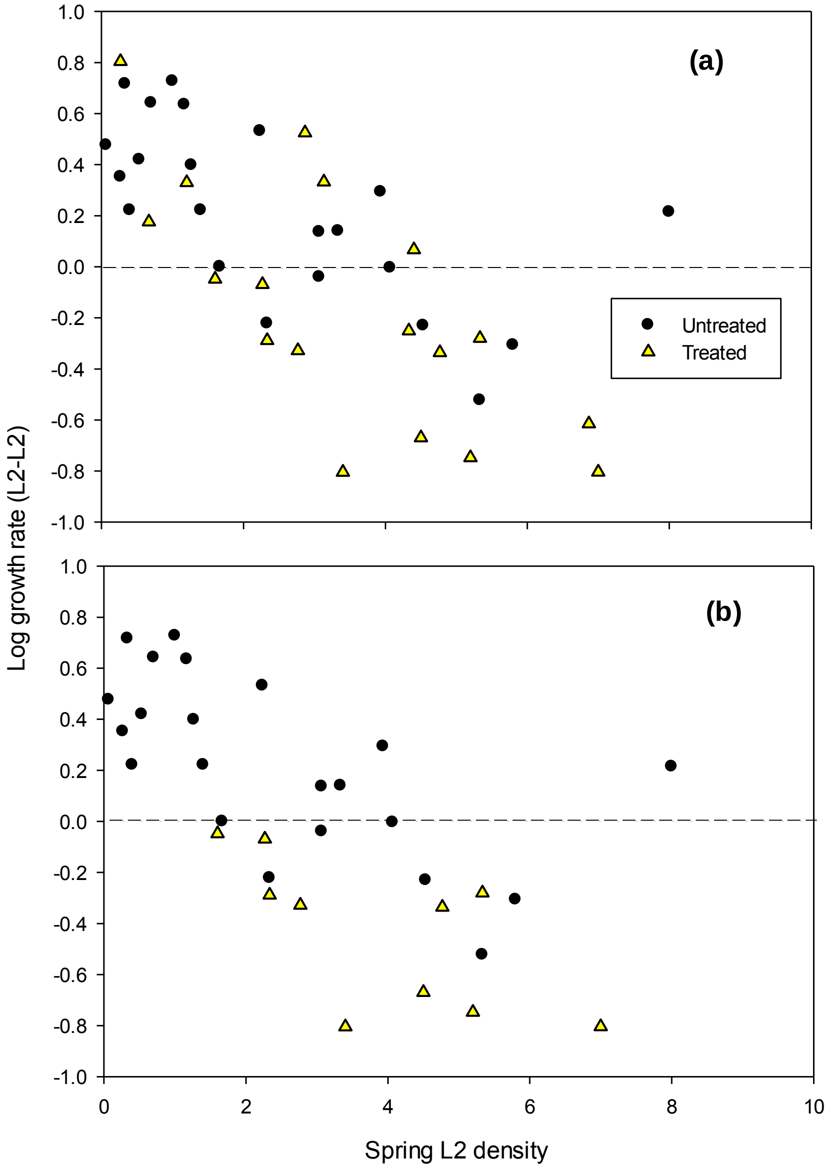


Figure 4. Average budworm densities at different life stages throughout the season from three sites that had been treated in 2015 with a double application of Btk and again in 2016 with a single application of Btk.

Objectives 2 & 3: Non-target effects

Sixteen parasitoid species (5 Diptera and 11 Hymenoptera) among the most important ones have been collected and sequenced. The primers to identify if a budworm larva is parasitized or not, and if it is, if it's by a Diptera (fly) or an Hymenoptera (wasp) have been identified and tested on these 16 species. All of these species react to the identified primers. The next step (starting imminently) will be to test these primers on field-collected SBW larvae (from Lower-St-Lawrence and from North Shore in Quebec) for which we know the parasitism rates for the different species (by rearing the same number of SBW larvae for these sites), as a validation step for the technique. Once this validation step has been completed we will begin processing the multiple years of frozen larvae (budworm and non-target) to assess parasitism.

Tentative Conclusions

Our results to date show some promise for depressing low-density populations in the context of the EIS, though it remains to be seen whether these effects can be sustained or maintained as the outbreak in Quebec continues to encroach on the NB border. In 2017 at least, there were no substantial mass migration events of the kind we saw in 2016, which likely contributed to the effectiveness of treatments this year. Results of our efficacy trials also appear to be consistent with broader L2 trends collected by DERD this year, which suggested only a modest increase in NB budworm densities from the previous year. We suspect that some of this modest growth may have come from sites that were not picked up in L2 treatments and which were therefore untreated. While results to date are encouraging, there remains work to be done to refine both budworm monitoring and treatment efficacy. Moreover, once the barcoding approach is completed and applied to the four years of frozen samples we have, we are likely to accrue additional insights regarding how treatments affect non-target caterpillars and the natural enemy community.

Acknowledgements

We thank the ACOA Atlantic Innovation Fund, Forest Protection Limited, forest industry in New Brunswick, the New Brunswick Department of Natural Resources, SERG-International, and the Canadian Forest Service for providing financial and in-kind support for this work. We are also grateful for in-kind support from various collaborators private woodlot owners, including Acadia Timber and JD Irving for providing us with access to their land and property for use during this study. Numerous people also provided support in the lab and the field, including A. Morrison, E. Owens, Z. Fitch, H. Blaquiere, Z. Berry, K. Mossler, C. Forbes, T. Morin, M. Labrecque, F. Douville, S. Trudeau, P. Huron, M. Leblanc. Maps for NB sites were provided by I. Demerchant, S. Heartz, and J. Gullison.

References

Eveleigh, E.S., McCann, K.S., McCarthy, P.c., Pollock, S.J., Lucarotti, C.J., Morin, B., McDougall, G.A., Strongman, D.B., Huber, J.T., Umbanhowar, J. & Faria L.D.B.(2007) Fluctuations in density of an outbreak species drive diversity cascades in food webs. PNAS 104: 16976-16981.

Régnière, J., Delisle, J., Pureswaran, D.S. & Trudel, R. (2013) Mate-finding allee effect in spruce budworm population dynamics. Entomologia Experimentalis et Applicata 146: 112-122.

Innovative DNA-based diagnostic for SBW, its natural enemies and other conifer-feeding species

M. Alex Smith[1], *Eldon Eveleigh*[2]

[1]Department of Integrative Biology - University of Guelph, ON
[2]Canadian Forest Service – Atlantic Forestry Center, Fredericton, NB

Abstract

In an original approach for DNA-base identification of insect predators of spruce budworm (SBW) *Choristoneura fumiferana* in Quebec and New Brunswick, we developed a TaqMan qPCR assay to detect and identify parasitoids in SBW larvae. The strategy proposed is largely inspired by the paper by Stewart et al. PlosOne 2016, and consists in using a qPCR approach requiring specific primers designed to enable discrimination among species. In the first step of the assay, we answer the questions: (i) Is this SBW parasitized? And (ii), if so, is the parasitoid a Diptera or a Hymenoptera? In subsequent steps, the assay will provide more information on the family or the actual species detected.

The 28S rRNA sequence share greater similarity among insect orders than the COI barcode while allowing discrimination between Diptera and Hymenoptera. Thus, we successfully designed a first set of specific primers in the 28S rRNA, which allowed DNA quantification of parasitoid DNA and *Hymenoptera* and *Diptera* specific TaqMan assay. Comparative sequences analysis for the different parasitoid species will help us design new specific primer strategies for subsequent steps.

Résumé

Dans une approche originale, nous avons développé une méthode d'essai qPCR TaqMan pour identifier les parasitoïdes impliqués dans le parasitisme de la tordeuse de bourgeons d'épinette (TBE) *Choristoneura fumiferana*, au Québec et au Nouveau Brunswick. La stratégie proposée largement inspirée de celle décrite dans l'article de Stewart et al. PlosOne 2016 consiste à utiliser des amorces de qPCR spécifiques pour permettre la distinction entre les espèces. Dans une première étape de l'essai, nous répondons à la question: (i) Est-ce que la TBE est parasitée ? et (ii) si oui, le parasitoïde est-il un diptère ou un hyménoptère? Dans des étapes suivantes, l'essai fournira des informations complémentaires sur la famille ou l'espèce particulière détectée.

La séquence de l'ARNr 28S partage une meilleure homologie entre les ordres des espèces ciblées, que la séquence du COI, permettant ainsi de distinguer les diptères des hyménoptères. Nous avons produit un premier ensemble d'amorces dans le 28S qui permet de quantifier l'ADN des parasitoïdes et de discriminer diptère et hyménoptère dans un essai TaqMan spécifique. L'analyse comparative des séquences COI et 28S des différentes espèces parasitoïdes permettra de développer d'autres amorces spécifiques pour les étapes subséquentes.

Early Intervention Strategies to Suppress a Spruce Budworm Outbreak: Aerial Application of Pesticides Including Pheromones

Peter Amirault, Luke Amos-Binks and Andrew Morrison
Forest Protection Limited, Lincoln, NB, E3B 7E6, Canada

ABSTRACT

This was the final season for the four-year project. Once again, the area treated for the control of spruce budworm in Northern New Brunswick increased. This was consistent with projections in the original project proposal. The authors have attempted to give the readers a vision of the complexities involved in planning and executing a modern aerial insect control project throughout a considerable area. In meeting this objective various aspects of the planning, obtaining authorizations, abiding by permit requirements, and the extent of resources required are outlined. Many organizations and individuals played a role in meeting the project timelines documented and their contributions are noted in the report.

In 2017, larvicide applications targeted late-instar spruce budworm larvae from three bases of operation (Charlo, Boston Brook and Miramichi). Some 147 230 hectares received a single application of Bacillus thuringiensis or Tebufenozide products. These applications were conducted during a span of 18 days in the latter half of June. There were no pheromone applications during 2017.

RÉSUMÉ

Il s’agissait de la dernière saison d’un projet de quatre ans. Encore une fois, on a agrandi la zone traitée contre la tordeuse des bourgeons de l’épinette dans le nord du Nouveau-Brunswick, ce qui concorde avec les projections de la proposition de projet originale. Les auteurs ont tenté de donner aux lecteurs une idée des complexités qu’impliquent la planification et l’exécution d’un projet aérien de lutte antiparasitaire moderne sur une grande superficie. Pour ce faire, les auteurs décrivent divers aspects touchant la planification, l’obtention des autorisations, le respect des exigences des permis et l’ampleur des ressources nécessaires. Un grand nombre d’organismes et de personnes ont joué un rôle dans le respect de l’échéancier établi du projet, et leur apport est souligné dans le rapport.

En 2017, des applications de larvicides lancées à partir trois bases opérationnelles (Charlo, Boston Brook et Miramichi) ont ciblé des larves du dernier stade de la tordeuse des bourgeons de l’épinette. Environ 147 230 hectares ont reçu une seule application de *Bacillus thuringiensis* ou de tébufénozide. L’application des produits s’est déroulée sur une période de 18 jours durant la deuxième moitié de juin. Aucune application de phéromones n’a eu lieu en 2017.

INTRODUCTION

The aerial applications described within are in support of the Early Intervention Strategies to Suppress a Spruce Budworm Outbreak (EIS-SBW) Project. The Research Project is a partnership between Forest Protection Limited (FPL), the Canadian Forestry Service – Atlantic and Laurentian Forestry Centres, the University of New Brunswick, the New Brunswick Department of Energy and Resource Development (NBDERD), Maritime Forest Research Limited, and major Atlantic region forest products companies.

The goal of the applications are to test the theory that an impending spruce budworm outbreak can be prevented or at least minimized by targeted applications to rising populations. There are also several companion research studies, some of which also depend on the applications. For example, the consequences of pesticide treatments on natural enemies of the spruce budworm was examined. The applications followed unprecedented surveys of spruce budworm population levels by many of the project partners. These surveys were the basis for decision making as to where populations in New Brunswick are on the rise and where control efforts should be targeted. The 2017 project applications in Northern New Brunswick were conducted from three bases of operation. Forest Protection Limited treated 79 088 ha with Bacillus thuringenisis (Btk) – Foray 76B from Charlo. JD Irving, Limited (JDI) was contracted to treat an area of 22 399 ha with tebufenozide (Limit 240) from Boston Brook. Forest Protection Limited treated an additional 45 743 ha with either Limit 240 or Mimic 240LV (also tebufenozide) from Miramichi. Pheromone applications were discontinued in 2017.

A permit to allow the larvicide applications was obtained from the New Brunswick Department of Environment and Local Government. A unique aspect of the 2017 Project was that applications from the Charlo location were not confined to New Brunswick. FPL entered into an agreement with SOPFIM to share project resources and treat a portion of SOPFIM's Quebec SBW project from the Charlo site (roughly 12 500 ha, largely double applications). The applications done in Quebec were not part of the Early Intervention Project.

The information in this report has been assembled in fulfillment of the SERG-I requirements placed on projects receiving SERG-I funding. The authors would like to thank SERG-I members for their support of this project.

PRE-PROJECT

In order to conduct operations of this complexity a large amount of information was exchanged between the parties involved. Company staff met regularly to discuss needs of both the EIS-SBW and SOPFIM Projects. There were information exchanges and joint training with SOPFIM staff to plan operations in both provinces. FPL and J.D. Irving, Limited staff met to discuss concurrent operations from Boston Brook and other bases.

In addition to the Aerial Forestry Permit from the Department of Environment and Local Government, previously mentioned, certain other authorizations were required. Forest Protection Limited was granted permission to conduct applications in Protected Natural Areas and a Watershed Exemption for Btk in certain areas. Finally, the authority to conduct the Project under the Crown Lands and Forests Act was granted from the Minister of Energy and Resource Development.

Pre-Project – Preparation of Final Treatment Area

Representatives of NBDERD provided eligible treatment areas in the form of feature services that were published to ArcGIS Online. The eligible treatment areas were defined using a process that weighed spruce budworm larval population estimates and adjacent forest condition to identify likely hotspots that should be treated. The larval population estimates had been obtained by extensively sampling spruce budworm populations throughout potential treatment areas. The process development is a separate component of the project under the Integrative Modelling and DSS/Economic Assessment Component.

It was FPL’s responsibility to produce the final treatment areas (subdivided by block) that met any permit, aerial management system, reporting, planning, and operational requirements as well as private land opt outs and product allocation commitments. These tasks were performed using GIS processes that have been developed in support of this project. Once all the requirements were met the final data products were produced and distributed.

Pre-Project – Charlo

The Charlo location was the first to be activated due to the fact that Quebec applications commenced before the EIS applications. The tarmac area available had been expanded from 2016 (Figure 1). Product storage capacity and pumping stations were also increased from the previous season. These improvements were required to complete applications in a timely manner given increases in treatment area.

Figure 1. Aerial view, Charlo base, 2017.

The first staff arrived on site to commence set-up on May 8 and by May 18 the pit setup was ready. A rented office trailer, a standby trailer, and washroom facilities along with an FPL-owned travel trailer (for security staff) were added to the site. FPL staff also had access to a Charlo Airport hangar for equipment/vehicle storage. The first deliveries of Foray 76B occurred on May 22 and continued periodically as the project progressed. The first application aircraft arrived on May 28. FPL pilots and aircraft had previously undergone training and calibration flights at Miramichi. Provision for aircraft fuel was handled through the Charlo Airport. The main pre-project briefing was held on May 29 using the SOPFIM guidelines plus a few additional topics specific to the EIS-SBW.

Pre-Project – Boston Brook

As a result of a contractual arrangement between Forest Protection Limited and J.D. Irving, Limited the later was given the responsibility of treating areas in Northwestern New Brunswick designated to receive Limit 240. Sufficient quantities of Limit 240 to complete applications were shipped directly to the site.

In anticipation of Boston Brook and Charlo airstrips plus any number of Quebec Airstrips operating simultaneously, staff from FPL, JDI, and SOPFIM established inter-base communication procedures regarding application priorities. This was necessary to prevent airspace conflicts between application aircraft.

Pre-Project – Miramichi

The preparations at Miramichi were fairly straight forward as FPL has a permanent presence at the site. Assembly of temporary mixing/loading facilities commenced on May 17 and was completed in early June. Aircraft commenced arriving on-site on June 11 and the pre-project briefing was June 13.

Pre-Project – Block Opening

The task of timing the applications was done by Canadian Forestry Service and FPL Research staff. According to information provided by Emily Owens, the initial process involved using BIOSIM to predict when Spruce Budworm were in specific developmental stages. After bud burst crews began sampling tree and insect-development in the treatment areas (JDI staff assisted in the Northwest) on a weekly basis.

The optimal timing for all larvicide applications was determined to be larval instar 4.5. This was partially based on delaying commencement as late as possible to encourage the beneficial effects of natural enemies of spruce budworm. An additional input into this process was an analysis to estimate the amount of time needed to complete applications (prior to pupation) done by Andrew Morrison.

There was some speculation that given the geographical extent and differences in elevation of areas involved in 2016 that there would be some variation in block opening dates. This did not turn out to be the case as Spruce Budworm development was consistent throughout the research area. The number of larval samples collected, in support of project timing, was expanded in 2017 in a further effort to refine the process. Based on the data provided, standby for applications commenced on June 12.

Pre-Project – Access Control

In 2015, EIS-SBW applications were conducted on a relatively limited scale. To meet public access control requirements outlined in the Pesticide Use Permit essentially all points of entry to treatment areas were manned during applications. The expansion of the treatment area in Northeast NB in 2016 made this logistically impossible to repeat. In order to overcome this situation and meet permit requirements, FPL staff discussed the situation with representatives of the Department of Environment and Local Government. It was suggested that an access control plan be developed and submitted for review. A plan was developed for the Northeast areas by Pierre Mezzetta (AVCell) and JP Astorino (FPL) and submitted to the Department of Environment and Local Government and subsequently approved. A variation of the plan incorporating the expanded (2017) area was once again approved.

The access control plan in the Northeast was implemented by DERD, AVCell, and Fornebu staff. Meetings were held during which the plan and communication protocols were explained. Daily communication with the access control staff occurred from each base. The individuals involved in the access control exercise also posted the individual blocks and provided project information to members of the public.

In the Northwest access control and posting was handled by Acadian Timber staff in consultation with JDI staff.

PROJECT

Project – Boston Brook

Areas scheduled to be treated with Limit 240 from Boston Brook (Figure 2) involved significant ferry distances from base. In all cases areas received one application at 1.5 L/ha. Applications from Boston Brook commenced in the morning of June 15 and continued until the assigned blocks were complete on June 25.

Further details of the applications from Boston Brook were summarized in Session Summaries, Aircraft Loading Summaries, and Pilot Session Spray Summaries and retained as a permanent record. In order to complete the Project, Forest Patrol Limited positioned four Ayres Turbo Thrush Aircraft plus an observation aircraft on site. The final area treated with Limit 240 from Boston Brook was 22 399 ha.

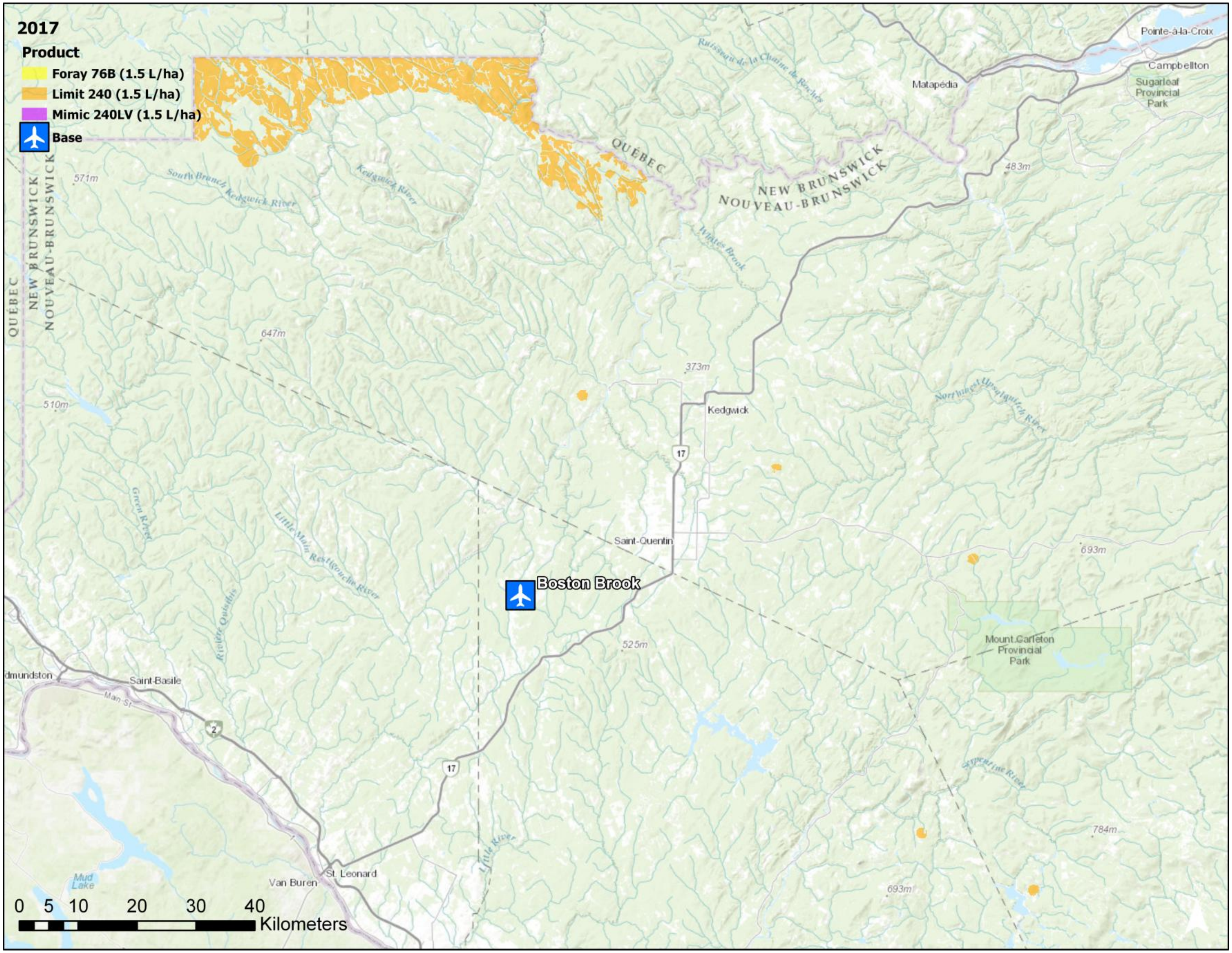


Figure 2. Areas in northwest New Brunswick receiving product applications during 2017 EIS-SBW Project

Project – Charlo

The extent of the integration of operations with SOPFIM included the sharing of aircraft resources, using two SOPFIM Aerial Observers, the joint purchase of Foray 76B, and many components of the site set-up. This was all covered by a contractual arrangement.

The EIS-SBW applications from Charlo (Figure 3) were completed in eleven sessions between June 13 and June 30. This was the most active airstrip on the project and at its peak the site featured twelve application aircraft. The entire supply of Btk on site was used. Applications in Quebec were completed on the same day as the EIS-SBW applications and a site tear down ensued.

Extensive details regarding the progression of applications from Charlo were recorded on Session Summary and Application Observer Reports. In addition, a Rapport Météo was prepared by Aerial Supervisors for each application flight. The details of each Session Summary are retained in electronic files on permanent storage by FPL's IS department.

From Charlo 79 088 ha were treated with Foray 76B in New Brunswick. All areas received one application at 1.5 l/ha.

Project – Miramichi

The EIS-SBW applications from Miramichi were completed in ten sessions between June 15 and June 30. There were four Air Tractor 802 and one Cessna 188 application aircraft assigned to the base for the duration of the project.

As with the Charlo base extensive details regarding the applications were kept as a permanent record. The duration of the applications from Miramichi and Charlo were longer than optimal due to weather delays.

Some 8 729 ha were treated with Limit 240 and 34 240 ha were treated with Mimic 240LV from Miramichi (Figure 4). An area of 2 774 ha originally assigned to Miramichi was treated with Limit 240 from Boston Brook.

COMMUNICATIONS

The bases were equipped with a number of communications options (cell phones, FM radios, and internet for e-mail and text). During applications bases kept in touch with Aerial Supervisors and Access Control Personnel via FM Radio. Communications between Aerial Supervisors and Application Pilots occurred via VHF radio.

Base managers meet daily by conference call to discuss issues and formulate plans going forward. As necessary the same individuals communicated with bases in Quebec to ensure aircraft separation during applications. Daily plans were disseminated to Access Control personnel.

Gerry Cormier distributed project updates to a wider audience through e-mail. He also contributed to the Communications Committee.

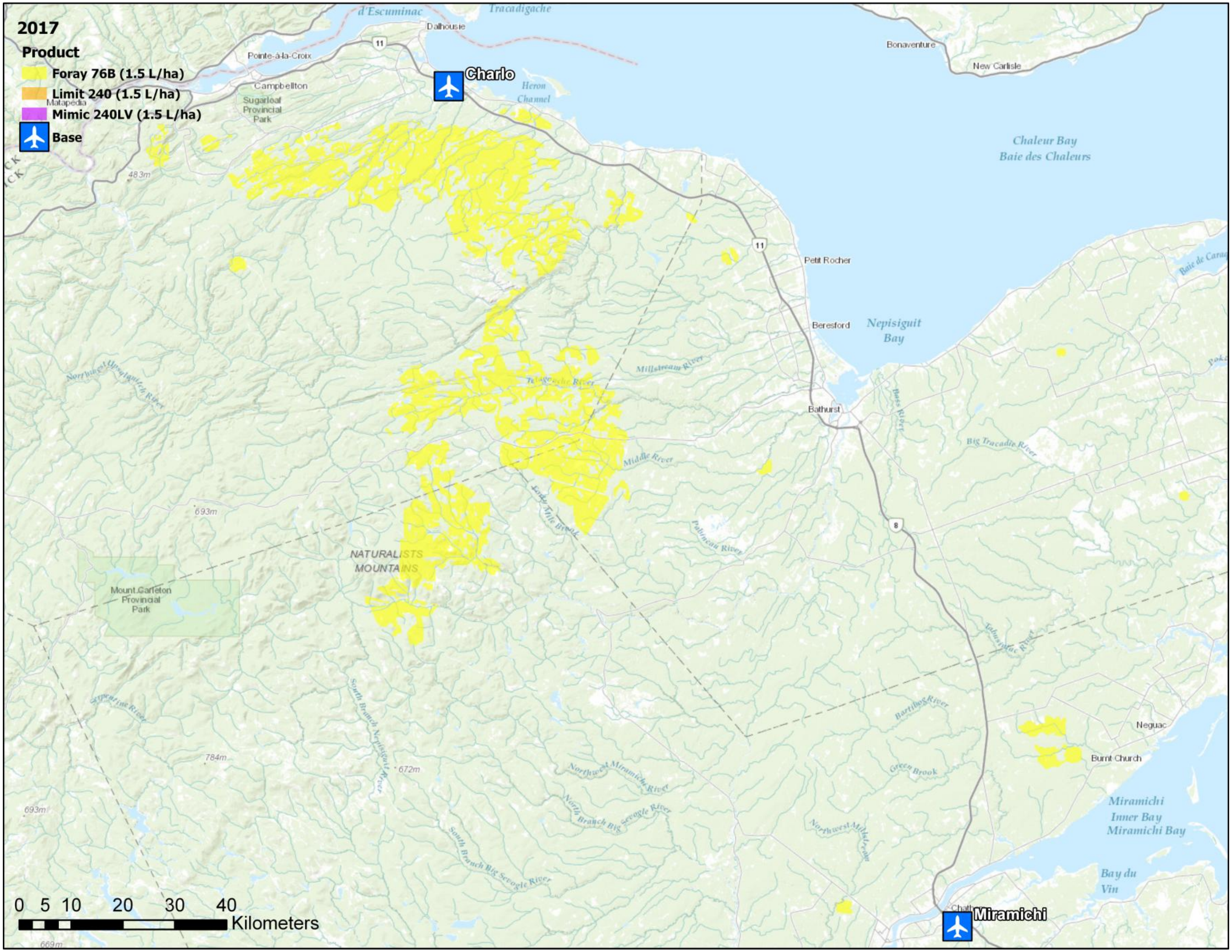


Figure 3. Areas in northeast New Brunswick receiving product applications from Charlo during 2017 EIS-SBW Project

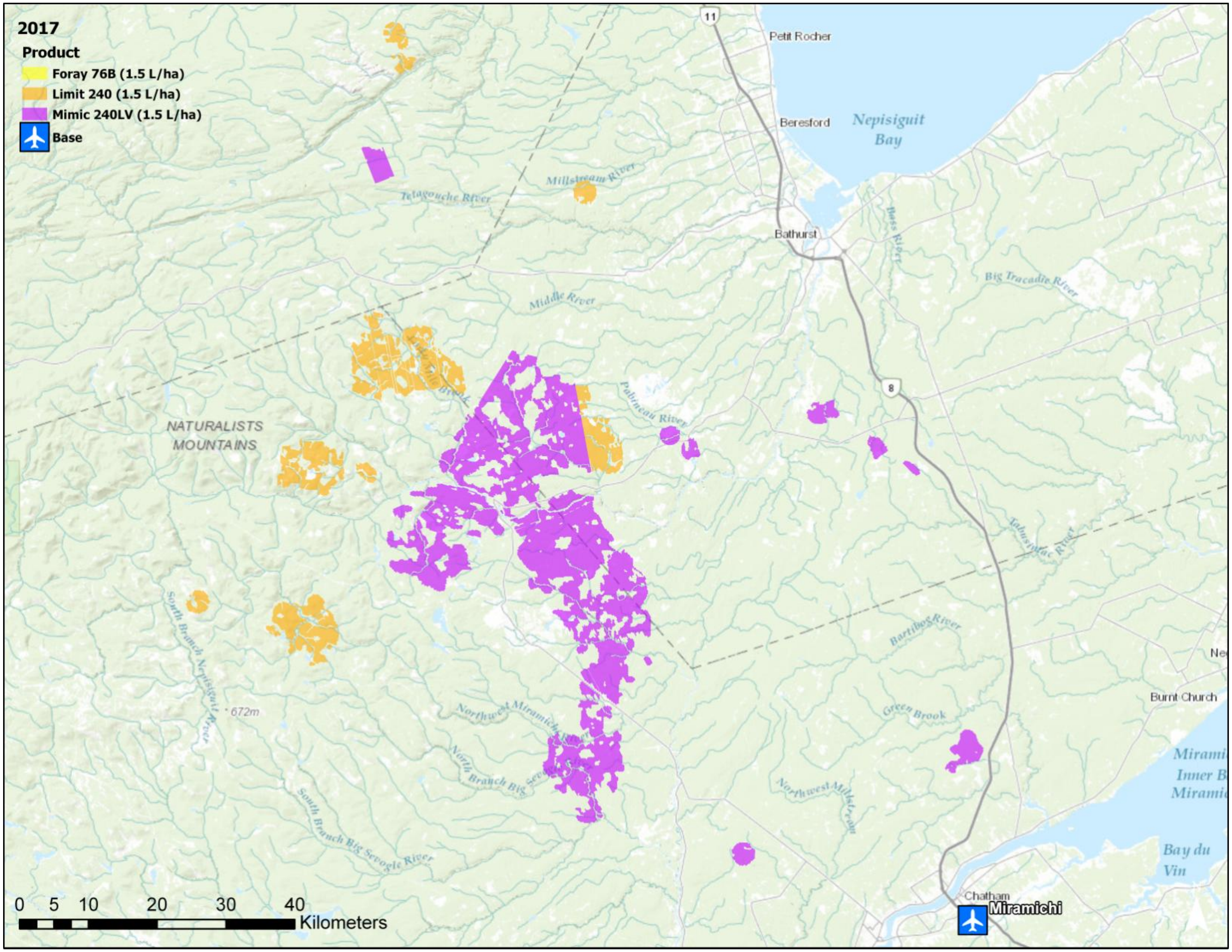


Figure 4. Areas in northeast New Brunswick receiving product applications from Miramich during 2017 EIS-SBW Project

FOUR YEAR – APPLICATION SUMMARY

The 2017 season was the fourth and final year for aerial applications of pesticides under the original EIS-SBW project proposal. Table 1 contains a summary of the year-by-year areas treated by product.

Year	Area By Product (ha)			
	Foray 76B	Mimic 240LV	Limit 240	Disrupt BioFlake
2014	169	4 472	0	490
2015	12 093	3 263	0	300
2016	41 889	18 119	1 600	1 000
2017	79 088	34 240	33 902	0
Total	133 239	60 094	35 502	1 790

Table 1. Area Treated By Product During the EIS-SBW Project (2014-2017)

FUNDING

Funding breakdown of Year 4 of 4 for Early Intervention Strategies to Suppress a Spruce Budworm Project (projected to March 31, 2018).

NB-DERD	$2,038,109
ACOA	$3,559,928
INDUSTRY	$ 418,752
CANADIAN WOOD FIBRE CENTRE (NRC)	$ 500,000
	$6,516,789

Epicentre formation and dispersal behavior of adult spruce budworm in eastern Canada: Implications for the rise and spread of outbreaks and Early Intervention Strategy

(ACOA RD100 2.2.5)

Project co-leads. *Deepa Pureswaran (LFC), Rob Johns (AFC)*

Telephone Number (contact person): D. Pureswaran. (418) 648-7532
Address: CFS-LFC. 1055, rue du PEPS, Quebec City, QC

Team members. *Stephane Bourassa (LFC), Louis De Grandpré (LFC), Drew Carleton (FPL), Eric Moise (AFC), Zach Sylvain (AFC)*

Collaborators. *Dan Kneeshaw (UQAM), Patrick James (Université de Montréal), Yan Boulanger (LFC)*

Abstract. This project addresses the role of moth dispersal in the context of early intervention to combat spruce budworm. Although the dispersal of spruce budworm moths has been the subject of extensive study and discussion for more than 50 years, many questions remain unanswered regarding the role of dispersal in driving the rise and spread of outbreaks. Results provide evidence of long-distance dispersal of spruce budworm moths from Quebec to southern New Brunswick. Evidence also indicates, however, that occurrence of dispersal events do not necessarily translate into increased population growth in areas inundated by moths. Stands that are potential sources of moths are characterised by high cumulative defoliation, position in the landscape and phenological match in moth flight with sinks. Source sites emit moths with low body weight and large wing area. Processing remains underway to assess regional differences in genetic markers, which together should help to identify the original sources of captured moths. Analyses of radar imagery can measure large-scale spatial patterns of moth movement on clear nights. We also have significant regional expansion of Citizen Science moth sampling to include all of the eastern provinces and Maine, USA.

Titre. Développement des épicentres et le comportement d'immigration de la tordeuse des bourgeons d'épinette dans l'est du Canada. Les conséquences pour l'augmentation et la dispersion des épidémies et l'intervention hâtive (ACOA RD100 2.2.5).

Résumé en français. Ce projet évalue le rôle de la migration des papillons dans le contexte d'intervention hâtive pour combattre la tordeuse des bourgeons d'épinette. La dispersion de la tordeuse est le sujet d'étude pendant plus que 50 ans, mais il nous reste encore plusieurs questions à répondre par rapport au rôle du vol long distance dans l'augmentation est dispersion des épidémies. Les résultats de l'étude fournis l'évidence qu'il y a des dispersions des papillons du Quebec au sud du Nouveau Brunswick. Il y a l'évidence aussi que l'immigration des papillons nécessite pas l'augmentation de la population de la tordeuse. Les placettes sources des papillons sont caractérisées par un haut niveau de défoliation sévère, leurs positions dans le paysage, et les périodes de vol des papillons qui rapprochent les puits. Les placettes source produisent des papillons avec

un poids bas et une surface d'ailes plus haut. Nous sommes en train d'analyser des données avec des marqueurs génétiques afin d'identifier les populations sources des immigrants. Nous analysons aussi des images radar afin de mesurer le moment et l'endroit de ce phénomène. Dans les prochaines années, il y aura des échantillonnages aussi dans toutes les provinces dans l'est du Canada et dans le Maine aux États-Unis.

Introduction. The benefit of the "Early Intervention Strategy" (EIS) of spruce budworm, vis à vis conventional suppression, depends on the role that moth dispersal plays in outbreak maturation. There is recent evidence (Régnière et al. 2013) that a mate-finding Allee effect is a factor in natural suppression of low populations. Emigration of male moths from epicentres could release the low-populations from this natural control. There is evidence (Greenbank et al. 1980) that immigration of gravid females to areas of low population increases population levels beyond a threshold where natural control is effectual. In either case, critical information is still missing for the effective implementation of the EIS.

Most previous empirical work on SBW dispersal was carried out when regional population densities were extremely high (e.g., Greenbank et al. 1980). Because of this, many questions remain unanswered regarding the role dispersal plays in escalating or spreading outbreaks when populations are low or as populations are rising. For example, what are the pre-determinants (e.g., forest conditions, population levels, defoliation levels) of epicentre formation leading to significant moth (male and female) dispersal? What is the effect on outbreak maturation of moth (male and female) immigration to areas of low population? How can we quantify the probability that a given site will produce migrating adults that can spread to other regions (i.e., a risk index)? To what extent does moth dispersal from epicentres contribute to population increases in surrounding low-density regions? Can we distinguish confidently between resident and migrant populations? Can we explain or predict instances where apparent moth mass influx into low-density areas do or do not contribute to population rise? Our project seeks to provide answers to these questions.

Objectives

1) Develop an emigration risk index based on stand and population parameters (from ground surveys and satellite imagery) in relation to moth immigration that can be used to identify potential epicentre candidates for EIS.
2) Develop tools to differentiate between resident and migrant moths based on neutral genetic markers (SNPs) and/or various phenotypic traits.
3) Study the dispersal of male and female SBW moths during the rise and spread of outbreaks from apparent epicentres (e.g., QC) into low-density areas (e.g., NB).

Methodology. To determine how biophysical context, location, and SBW characteristics influence whether resident SBW will become a significant source of dispersing adults (i.e., an epicentre), in 2014 we selected a total of 10 sites on the north shore of the St. Lawrence in QC and 12 sites from northern to southern NB. Selected sites were composed primarily of pure and mixed conifer stands (balsam fir and or spruce). In each plot, we setup one light trap, one pheromone trap, and one malaise trap. There was a minimum distance of 50 m between traps to ensure that they do not interfere with one another. Hobo temperature/humidity data loggers were also set up in each site to monitor local weather conditions during the study period. During the period of adult flight for SBW, traps were checked every two to three days and the contents of the traps emptied. Collected adults are currently being processed for phenotypic traits (e.g., phoretic mites, egg load, moth weight, body size, relative abdominal weight, relative wing muscle weight, wing load, wingspan, wing shape, lipid contents) and through DNA analysis (based on genetic markers) to determine whether captured moths are migrants or residents. After adult flight ended in each plot, we sampled 75 cm branch tips from the mid-crown of 9 trees per plot (3 trees near each trap type) to be processed in the laboratory for overwintering L2. Information on apparent dispersal provided by this study is being compared to weather patterns in the region using our data loggers and radar imagery.

Citizen Science Program

Our moth-monitoring network expanded significantly in 2015 through the introduction of a Citizen Science program (i.e., Budworm Tracker), which harnesses the efforts of volunteers throughout eastern Canada. Volunteers were sent pheromone traps and asked to hang them in their woodlot or back yard and had a choice to collect trap contents once, twice, or three times per week (with three times per week being the ideal). A subset of ~40 light traps was also deployed to citizens in New Brunswick to collect potential migrant female moths throughout the province. Volunteers had the option of recording the data manually or accessing the website on their computer or through their smart phone (using a QR code) to upload their data to our website (http://budwormtracker.ca/) for real-time updates of results. All data was later counted in the lab to verify reported numbers. The target for 2015 was the deployment of 300 traps. These traps were distributed among Ontario, Quebec, New Brunswick, Prince Edward Island, Nova Scotia, Newfoundland, and Maine with provincial or state co-ordinators overseeing the deployment and collection of traps. In late-September, the moths were returned to their respective co-ordinators for counting and processing.

Results to date and Discussion.

1. We have characterised source populations in terms of defoliation severity. We found that defoliation is related to larval density ($r = 0.33$; $P < 0.0001$) and larval density is in turn is related to the presence of buds on trees that are experiencing defoliation ($r = 0.24$; $P = 0.0008$). However, bud production declined during severe defoliation, but recovered in 2015 when local populations crashed and defoliation was lower (Figure 1).

2. Moth characteristics in source populations. Stand defoliation had a positive relationship on moth emergence ($r = 0.16$; $P < 0.0001$). There was an effect of stand composition and flight time on moth phenotype. Moths were lightest ($F_{2,1364} = 4.94$; $P = 0.007$) with largest wing area ($F_{2,1364}=16.7$; $P < 0.0001$) (Fig. 2) at high balsam fir composition where cumulative defoliation was highest in 2013 and 2014, suggesting that these sites would serve as source populations relatively early in the outbreak cycle, because moths that are lighter have a greater propensity to engage in long distance

dispersal (Blais 1953). The trend is reversed after trees recovered from defoliation in 2016 and balsam fir was consumed again by the larvae.

Figure 1. Number of buds per branch during seven years of severe defoliation in Quebec's source stands.

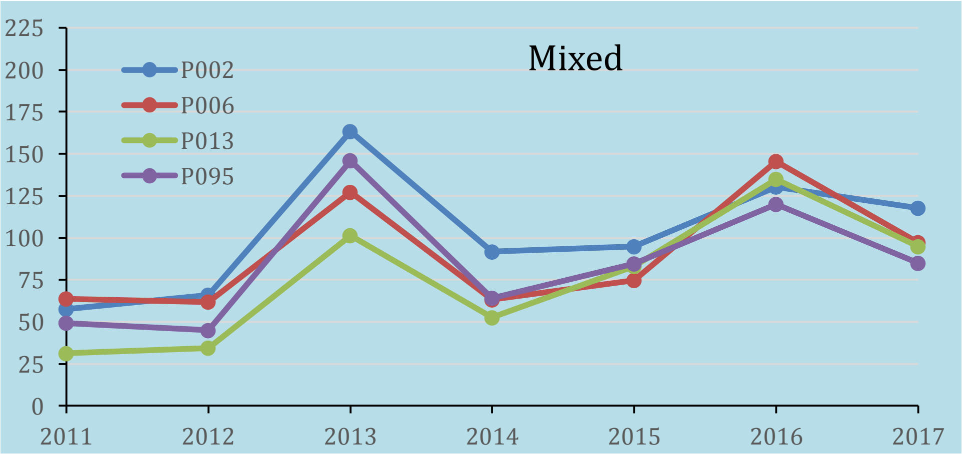
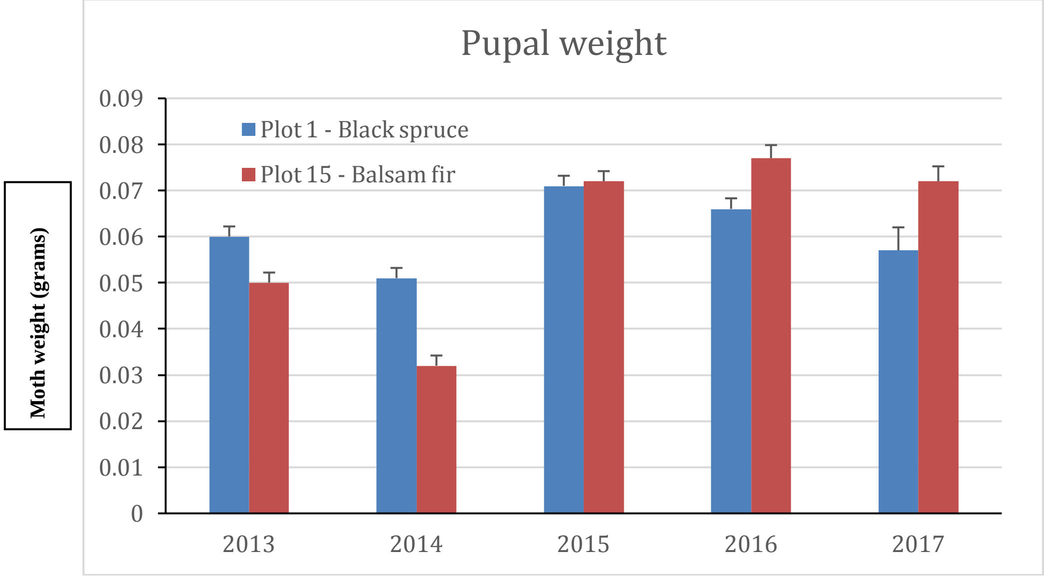


Figure 2. Moth weight in grams in a black spruce and a balsam fir dominated site. Note decline in weight from 2013 to 2014 following severe defoliation and population crash. Moth weight rebounded in 2015 when defoliated trees recovered and produced buds.



Citizen Science Program

Of the 456 traps deployed throughout eastern Canada in 2016 (Fig. 3), we received data for 87% of traps with a total of over 16, 000 moths collected and processed (Table 1). Most participants have kept their traps and agreed to participate again in 2017, though the results from 2017 are still being processed. Processing of collected moths to validate the results collected b

Figure 3. A citizen checking her pheromone trap, which hangs from a lower crown branch of a balsam fir tree. Note the unique QR code sticker that allows citizens to access the website via phone App to check-in with results.

Table 1. Budworm Tracker participation and return rate data from 2015 to 2017 (to date).

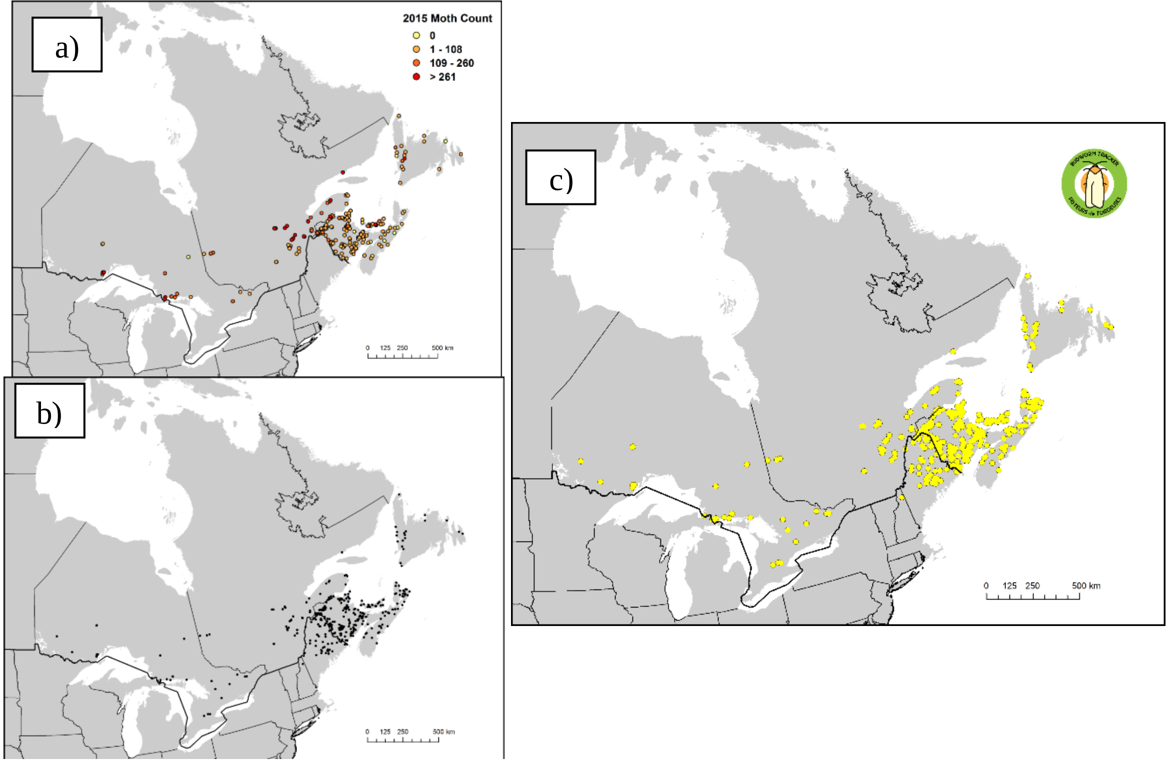
	2015	**2016**	**2017**
# traps sent to volunteers	284	405	413
# volunteers that returned data	259	352	279 as of December, 2017…. more on their way (we did reminder phone calls to BT's to return traps in November)
Return rate	90%	87%	TBD by April
Total days of data collected	3,328	5.328	TBD by April
Total # moths collected	~28,000	~16,000	TBD by April

A subset of moths from both 2015 and 2016 are currently being processed for genetic SNP markers and further information on the potential origin of moths (and the extent to which we can detect differences) is expected before the upcoming summer.

Engaging volunteers to assist with pheromone trapping offers numerous additional advantages over the traditional pheromone trapping approaches. First and foremost, the BT program is simple for volunteers to carry out and cost-effective owing in large part to the significant reduction in travel for setting up traps. Moreover, since participants tend to set up traps in locations near their home, they can be checked at more frequent intervals throughout the flight season. The use of a species-specific sex pheromone essentially eliminates issues of misidentification, which is a common issue in plant or animal Citizen Science programs that require volunteers to recognize different species (Dickinson et al. 2010). The program could be scaled up with little additional costs and, if adopted at a regional scale, can help to harmonize methodologies used among different jurisdictions seeking to use this technique to aid in management.

Citizen Science offers a compelling solution to the issue of collecting detailed data for outbreaking insects that span broad geographical areas, potentially providing relatively inexpensive population estimates for management and ecology. Perhaps equally significant to the scientific data collected are the ancillary benefits to public education – public engagement in scientific research can draw much needed attention to critical environmental issues, and in the case of outbreaking insects offers an entry point to discussions around how and when (and even if) we should manage outbreaks. In this sense, any management program addressing issues with outbreaking insects may find benefits in incorporating Citizen Science into their broader communication and outreach strategy.

Figure 4. (a) Total moth abundance estimates from 285 pheromone traps in eastern Canada collected by citizens in 2015. (b) Pheromone trap distribution from the ca. 405 traps sent out in 2016, and (c) 413 traps distributed in 2017.



Conclusion. Most of the samples from our study have been processed and are currently being analysed. Preliminary conclusions are as follows: There is evidence of long-distance dispersal of spruce budworm moths from Quebec to southern New Brunswick. Occurrence of dispersal events do not necessarily translate into increased population growth in areas inundated by moths. Stands that are potential sources of moths are characterised by high cumulative defoliation, position in the landscape and phenological match in moth flight with sinks. Source sites emit moths with low body weight and large wing area. Processing remains underway to assess regional differences in genetic markers, which together should help to identify the original sources of captured moths.

References

Blais 1953. Effects of the destruction of the current year’s foliage of balsam fir on the fecundity and habits of flight of the spruce budworm. *The Canadian Entomologist*. 85: 446-448.

Greenbank et al. 1980. Spruce budworm (Lepidoptera: Tortricidae) moth flight and dispersal: New understandings from canopy observations, radar and aircraft. *Memoirs of the Entomological Society of America*. 112: 1-49.

Régnière et al. 2013. Mate-finding Allee effect in eastern spruce budworm population dynamics. *Entomologia Experimentalis et Applicata*. 146: 112-122.

Acknowledgements

We thank the ACOA Atlantic Innovation Fund, Forest Protection Limited, forest industry in New Brunswick, the New Brunswick Department of Natural Resources, SERG-International, and the Canadian Forest Service for providing financial and in-kind support for this work. We are also grateful for in-kind support from various collaborators the Fundy National Park, Mt. Carleton Provincial park, and various private woodlot owners, PNA of NB, Acadia Timber, and JD Irving for providing us with access to their land and property for use during this study. Numerous people also provided support in the field, including A. Morrison, E. Owens, Z. Fitch, H. Blaquiere, Z. Berry, K. Mossler, C. Forbes, T. Morin, and summer research assistants in NB and QC. Maps for NB sites were provided by I. Demerchant, S. Heartz, and J. Gullison.

Identification of Male Hairpencil Pheromone Components in *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae): Chemistry and function.

P.J. Silk[1], E.S. Eveleigh[1], L.E. Roscoe[1], M. Brophy[2]

[1] Natural Resources Canada, Canadian Forest Service - Atlantic Forestry Centre, PO Box 4000, Fredericton, NB, E3B 5P7, Canada; Phone: 506-451-6084;

[2] Forest Protection Limited, c/o Natural Resources Canada, Canadian Forest Service - Atlantic Forestry Centre, P.O. Box 4000, 1350 Regent Street, Fredericton, New Brunswick, E3B 5P7 Canada

Abstract

Pheromones are essential mediators of numerous insect behaviors and processes. In Lepidoptera, male pheromones fulfill essential roles in the mating sequence, such as location of the male, induction of acquiescence in the female, and advertisement of male quality. These compounds can also act upon competing males, either through disrupting their location of females interacting with a primary male, or by indicating the status of previously mated females. Recently, the importance of male pheromones in *Choristoneura fumiferana* (Clemens) (Lep.: Tortricidae) to mating success was confirmed; however, both their specific role and the behaviorally important components were unknown. Electroantennogram detection identified two compounds within in the blend, nonanal (9:Ald) and decanal (10:Ald) that elicited strong dose-dependent antennal responses in the antenna of both males and females. Observations from bioassays showed that females marked with nonanal mated significantly less than females marked with either a solvent or decanal. Additionally, mass spectrographic analyses showed that spermatophores removed from mated females contained nonanal. Proportions of males wing fanning and locating a pheromone source containing nonanal were significantly less than pheromone alone and with other male pheromone components. Interestingly, 9:Ald was also found in quantities on adult females that were similar to those on males. Our findings suggest that nonanal is used by primary males to both disrupt female location by secondary males, and to 'mark' females during mating so that the possibility of extra copulations with other males is reduced.

Resumé

Les phéromones sont des médiateurs chimiques qui jouent un rôle essentiel dans le comportement et les processus de nombreux insectes. Chez les Lépidoptères, les phéromones mâles ont des rôles essentiels dans la séquence de l'accouplement, notamment pour la localisation du mâle, l'induction de l'acceptation chez la femelle et l'étalage des qualités du mâle. De plus, ces composés peuvent avoir une incidence sur les mâles concurrents, en perturbant la localisation des femelles interagissant avec un premier mâle ou en agissant comme indicateur de l'état accouplé des femelles. Récemment, l'importance des phéromones mâles pour le succès de l'accouplement a été confirmée chez le *Choristoneura fumiferana* (Clemens) (Lep.: Tortricidae); cependant, le rôle précis des phéromones et leurs composantes importantes sur le plan comportemental étaient inconnus. Un électroantennogramme a permis

de déterminer que deux composés du mélange, le nonanal (9:Ald) et le décanal (10:Ald), induisent de fortes réactions antennaires liées à la dose chez les mâles et les femelles. Dans le cadre d'épreuves biologiques, il a été observé que le taux d'accouplement était considérablement moins élevé chez les femelles marquées au nonanal que chez les femelles marquées par un solvant ou marquées au décanal. De plus, des analyses spectrographiques de masse ont révélé que les spermatophores prélevés chez les femelles accouplées contenaient du nonanal. Les proportions de mâles battant des ailes et localisant la source de phéromone étaient considérablement inférieures dans le cas où cette source contenait du nonanal que dans le cas où elle contenait uniquement la phéromone ou contenait d'autres composantes de la phéromone mâle. Fait intéressant, le 9:Ald a été détecté chez les femelles adultes en quantités semblables à celles observées chez les mâles. Nos résultats donnent à penser que le nonanal est principalement utilisé par les mâles pour perturber le repérage des femelles par les mâles secondaires et pour « marquer » les femelles durant l'accouplement, de façon à réduire les possibilités de copulation avec d'autres mâles.

3. **Project Details**

A. **Background and Rationale**

Choristoneura fumiferana Clemens. (Lepidoptera: Tortricidae) is an indigenous defoliator of spruce (Pinaceae, *Pinus* spp.) and balsam fir (*Abies balsamea* L.) in Eastern North America (Hennigar et al. 2008, MacLean 1984). Larvae primarily feed on current year shoots, with repeated feeding eventually leading to tree death. Population cycles of this species oscillate approximately every 35 years in New Brunswick (Royama 1984), with high number of *C. fumiferana* causing major tree mortality over widespread areas (Royama et al. 2005). Due to the severe ecological and economic consequences associated with *C. fumiferana* outbreaks (Hennigar et al. 2007, Chang et al. 2012), significant research has been committed to several mitigation and detection strategies. One area that has been studied in detail is the pheromone ecology of *C. fumiferana*. It has been determined that adult females release a blend of 95/5 (*E*)- and (*Z*)-11 tetradecenal (E11-14:Ald and Z11-14:Ald) that acts as a long-range sex pheromone for conspecific males (Sanders and Weatherson 1976, Silk et al. 1980). Another compound, (*Z*)-11-hexadecenal (Z11-16:Ald), was thought to be another potential minor component based on its presence in the female sex pheromone gland (Silk and Kuenen 1986). Recent research confirmed that Z11-16:Ald and another compound, (*Z*)-5-tricosane, synergized with the primary sex pheromone components to stimulate upwind flight (Silk et al. 2017). Additionally, (*Z,Z,Z*)-3,6,9-tricosatriene (ZZZ3,6,9-23:H) present in the female cuticle released male copulatory attempts when in close proximity to the female. While significant insight into the female *C. fumiferana* pheromone system has been generated, little information exists about the existence of male pheromones.

Male pheromones can fulfill several important roles within the courtship sequence, including: advertisement of fitness (Iyengar et al. 2001); short-range female chemotaxis (Baker et al. 1981a, Zagatti et al. 1987); and, induction of female acceptance for mating (Lundgren and Bergström 1976, Phelan et al. 1986, Birch et al. 1989). Male pheromones can also be perceived by other males, and in some cases may be disseminated by a primary male to maximize his own fitness by preventing secondary males from locating his female mate. An example of this strategy is chemical mate guarding, where a male will deposit detectable compounds either onto or within a female via his spermatophore that will inhibit or reduce her receptiveness to other males (Alcock 1994, Malouines 2016). Primary males may also disseminate pheromones to disrupt the mating sequences of downwind secondary males. Behaviors such male wing fanning are often essential for inducing female reception to mating a number of species (Bijpost et al. 1985, Hendrikse 1986, Collins and Cardé 1989, Kamimura and Tatsuki 1993, Knight and Flexner 2007, Cremer and Greenfield 2010). Courtship sequences are most often stereotypical, and any disruption to the sequence of events will result in unsuccessful matings (Birch et al. 1989, Sanders and Lucuik 1992, Curkovic et al. 2009). Such mate protection strategies are often essential for maximizing male fitness in species where females are polyandrous and where last sperm precedence exists (Thornhill and Alcock 1983, Alcock 1994)

Wing fanning is a prominent behavior in the mating sequence of *C. fumiferana* (Sanders and Lucuik 1992). Males will fan their wings upon detection of the female pheromone and only stop this behavior upon successful mating (Palaniswamy et al. 1979). Mate guarding in *C. fumiferana* has been suggested to be an important component of ensuring male fitness, however whether or not male pheromones are used in this role is unknown (Kipp et al. 1990). Recently, the presence and importance of male pheromones in the mating sequences was confirmed (Roscoe et al. 2016). As part of the investigations into the specific role of *C. fumiferana* male pheromones, we wished to identify behaviorally active components of these pheromones, and identify if these components elicited behaviors consistent with mate guarding behaviors. Preliminary results determined that two major components of the male hair pencil blend, nonanal (9:Ald) and decanal (10:Ald), could be pheromonally active in *C. fumiferana* (Roscoe et al., unpublished). We now report their role as anti-aphrodisiac pheromones in the mating sequence of *C. fumiferana*.

B. Methods:

Electroantennogram Analysis (EAG): Extracts and synthetics were tested for antennal stimulation in *C. fumiferana* using an EAG system and Pasteur pipette “puff stimuli”. To assemble an antennal preparation, the antennae of an adult *C. fumiferana* was removed with a razor blade in the region of the scape. The distal segment of the antennae was also removed to ensure a positive contact of the antennae with the probe. The antennae was affixed to a gold-plated EAG fork-style holder (Syntech, Kirchzarten, Germany) with each end attached to the holder using Spectra©-60 electrode gel (Fairfield NJ, USA). The holder with antennae was then inserted into an EAG probe (Syntech, Kirchzarten, Germany) connected to an IDAC-2 amplifier (Syntech, Kirchzarten, Germany). The amplifier was connected to a computer and the test recording was analysed using a software program (GC-EAD 2011 v1.2.3, Syntech). During the test, the antennal preparation was inserted into a glass tube through which a constant humidified stream of air flowed over the preparation at approximately ~40 ml/min. To test the response of the antenna to a stimulus, 3 µl of a treatment compound was applied to a 0.5 x 3.5 cm filter paper cartridge using a microsyringe. The test volatiles were: 1). Pure hexane (Sigma Aldrich, 95%); 2). nonanal (9:Ald) (Sigma Aldrich); and 3). decanal (10:Ald) (Sigma Aldrich). After the solvent had evaporated from the cartridge, it was then inserted into the pipette. A rubber bulb was then attached to the pipette and the distal tip of the pipette was inserted through a small hole in the glass tube in which the antennal preparation was placed. The volatile was dispensed into the air stream from the pipette, with each puff being 1 ml of air emitted in approximately 0.2 s. For each treatment compound, the antennae was exposed to three puffs separated by refractory window of 30 seconds. Between treatment compounds, a 1-minute refractory window was provided to allow the previous treatment compound to completely disperse from the airstream. Treatment compounds were presented to each preparation in a random sequence. The EAG response (mV) for a replicate was calculated as the mean of the responses generated by the three puffs. Concentrations of 0.01, 0.1, and 1.0 mg/ml were tested, with twelve individuals tested at each concentration.

Mate Marking Bioassay: Mating pairs consisting of one 3-day-old adult male SBW and one 12-18-hour-old female SBW were observed in small mating arenas. Mating arenas consisted of a Petri dish bottom (diameter = 9.0 cm) holding a filter paper circle (Whatman No. 2, Buckinghamshire, UK). Insects were caged onto the Petri dish with a 7.5 cm tall plastic mesh cone. To apply a treatment compound to a female, the insect was first anesthetized with CO_2 after which 3 µl of a treatment compound was added using a micropipette. Treatment compounds were: 1). pure hexane; 2). nonanal; 3). decanal. The female was then placed in the mating arena followed by one adult male. Mating pairs were held together for 3 hours with observations on the mating status of the pair conducted every 30 minutes using a red LED headlamp. During each observation, the mating status of the pair, determined as whether or not they were *in copula* at the time of observation, was recorded. Bioassays commenced two hours after scotophase and were conducted in a darkened room at 22-25^{o}C and 60% relative humidity. Concentrations of treatments was 1 mg/ml. Insects were only used once.

Wing Fanning Bioassay: To determine if wing fanning of males stimulated by the primary components of the female sex pheromone was affected by the presence of male pheromone components, males were caged downwind from a humidified air stream and exposed to one of three odor treatments. The treatments included: 1). 3 µl 95/5 E/Z11-14:Ald (1 mg/ml) + 3 µl hexane; 2). 3 µl 95/5 E/Z11-14:Ald (1 mg/ml) + 3 µl nonanal (1 mg/ml); and 3). 3 µl 95/5 E/Z11-14:Ald (1 mg/ml) + 3 µl decanal (1 mg/ml). The cage consisted of a 4.5 x 4.5 x 4.5 cm steel mesh cage on in which a single 3-day-old adult male was placed. The cage containing the adult male was placed onto a filter paper circle (Whatman No. 2, Buckinghamshire, UK) approximately 10 cm on the exhaust end of a humidified air stream flowing at 1.3 ml/s. The air stream was emitted from a plastic Teflon tube attached to a 5 cm glass tube approximately 1.5 cm in diameter. A microsyringe was used to apply the treatment compounds to a 0.5 cm x 3.5 cm paper cartridge that was then placed in the open end of the glass tube using clean forceps. Insects were exposed to odors for three minutes with the occurrence of wing fanning and duration (s) recorded. The filter paper floor of each cage was changed for every new insect. The cage was rinsed with 95% ethanol between treatments, and the glass tube was cleaned with 95% ethanol and hot soapy water every three replicates. Males were used only once and were 3 days-old at the time of the experiment.

Flight Tunnel Bioassay: The wind-tunnel (90 x 90 x 240 cm) was fabricated as described by Kuenen and Rowe (2006). Briefly, the floor consisted of factory-painted white medium-density fiberboard which was patterned using green vinyl adhesive disks of varying diameter, arranged in a random pattern. The sides and top were covered with 6 mm-thick Lexan® panels. At the upwind end of the tunnel, the ducting was reduced to 63 x 63 cm and a filter box added for a single 5 cm thick charcoal filter (Filterfast.com, product #FFOKGM). A tube (64 cm diameter, 92 cm long) housed the 61-cm fan blade and a Dayton 0.25HP 90VDC variable speed motor (Dayton, OH; #4Z248). An array of eight incandescent light fixtures was fixed above the tunnel and controlled via a Lutron

(Coopersburg, PA) dimmer. Tunnel air velocity and temperature were recorded using a heated wire anemometer/thermometer (VWR #21800-024), luminosity with a lux meter (VWR #21800-014), and relative humidity with an analog household meter (Accu-Temp #99047).

All wind-tunnel tests were conducted at 20–25 °C and 40–60% RH under a red incandescent light at 2 lux. Test extracts and solutions were loaded either onto pre-extracted red rubber septa (Wheaton, NJ, USA) and the source placed 35.6 cm from the floor and 35.6 cm from the upwind end of the tunnel. The test extracts were: 1). 3 µg 95/5 E/Z11-14:Ald (1 mg/ml); and 2). 3 µg 95/5 E/Z11-14:Ald + 1 µg nonanal (1 mg/ml). After a brief acclimatization period (20–30 min), males (2– to 4-d-old) were released at the downwind end of the tunnel from small mesh cages (3.2 cm x 3.2 cm) placed 168 cm from the septum. Male moths were allowed to leave the cage and attempt to locate the odour source. Insects were observed for a period of up to 10 minutes.

C. Results:

EAG Analysis: The mean absolute EAG response (±S.E.) for males was significantly greater to both nonanal and decanal than that for hexane at all concentrations (Figure 1, Table 1). There was no significant difference in mean EAG responses to nonanal and decanal any concentration. There was a significant effect of dose with antennal responses to both nonanal and decanal increasing significantly as concentration increased.

Table 1: Mean (±S.E.) electroantennogram responses (mV) of 3-day old adult male *C. fumiferana* to volatile compounds at two different doses.

	Volatile concentration (mg/ml)			
Volatile	0.01	0.1	1.0	*P* (Student's *t*-test)
Hexane	0.228 ± 0.047 mV	0.319 ± 0.031 mV	0.409 ± 0.056 mV	0.1777
Nonanal	0.452 ± 0.05 mV	0.959 ± 0.138 mV	2.056 ± 0.128 mV	< 0.001
Decanal	0.411 ± 0.09 mV	0.948 ± 0.098 mV	2.196 ± 0.216 mV	< 0.001
P (ANOVA)		< 0.001	< 0.001	

Mating Bioassays: While the proportion of mating pairs *in copula* was not significantly different where females had been labelled with hexane or decanal (19/29; 65.5% and 25/40; 62.5% respectively), or were unlabelled (46/71; 64.8%), the proportion or pairs *in copula* where the female had been marked with nonanal was significantly lower (23/71; 32.4%; X^2 = 19.3386, *P* =

0.0002). The proportion *in copula* of these unmanipulated mating pairs not significantly different from proportions where the female had been marked with either hexane or decanal.

Wing Fanning: There was a significant effect of treatment combination on the occurrence of male wing fanning. When exposed to 95/5 *E*/*Z*11-14:Ald + nonanal, the proportion of males wing fanning was significantly less than the proportion of males wing fanning while exposed to either 95/5 *E*/*Z*11-14:Ald-only and 95/5 *E*/*Z*11-14:Ald + decanal. There was no significant difference in proportions between 95/5 *E*/*Z*11-14:Ald-only and 95/5 *E*/*Z*11-14:Ald + decanal. Mean amount of time spent wing fanning was not significantly different across all three combinations (Table 3).

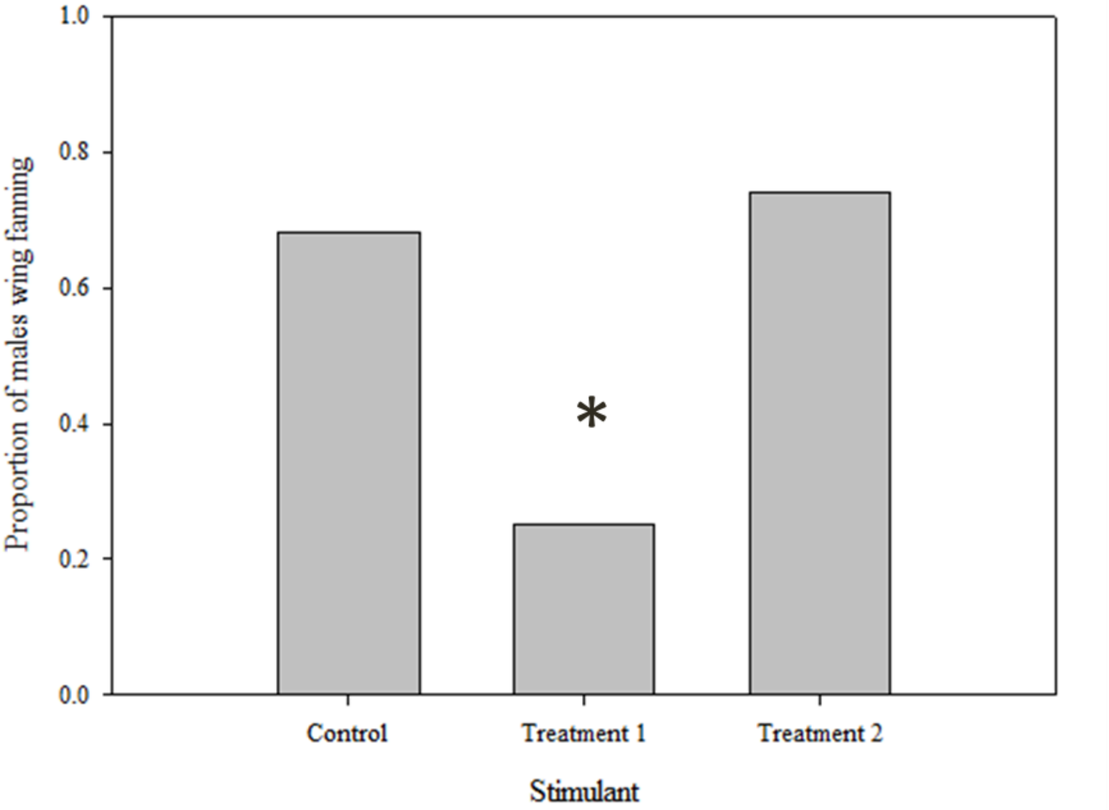


Figure 1: Proportions of adult male *C. fumiferana* wing fanning when exposed to different odour treatments. Odour source are: treatment 1 = 3µL 95/5 E11-14:Ald/Z11-14:Ald (1 mg/ml) + 3 µL (1mg/ml) nonanal; treatment 2 = 3µL 95/5 E11-14:Ald/Z11-14:Ald (1 mg/ml) + 3 µL (1mg/ml) decanal. Control = 6 µL of hexane (99%). '*' represents a significant difference in proportions of males wing fanning (X^2= 6.378, df = 2, *P* = 0.041)

Wind tunnel bioassay: There were significant differences in the proportions of males wing fanning and displaying their hair pencils near the source. When septa had been treated with 95/5 E/Z11-14:Ald + nonanal, the proportion of males both fanning at the septa and displaying their hair pencils was significantly lower than when the septa contained 95/5 E/Z11-14:Ald-only. While proportions of males demonstrating these two behaviors were affected, the mean durations for all behaviors were not significantly affected by treatment blends (Table 2).

Table 2: Proportions of adult male *C. fumiferana* undertaking behaviors in wind tunnel in response to different pheromone sources. [1]Blend 1 = 3 µg 95:5 E:Z 11-14 ald (1 mg/ml); Blend 2 = 3 µg 95:5 E:Z 11-14 ald (1 mg/ml) + 1 µg nonanal (1 mg/ml).

Blend[1]	**n**	**% wing fanned at cage**	**% flew in scent plume**	**% wing fanned at source**	**% displayed hair pencils**
1	46	54.36	39.1	39.1	37
2	45	37.8	22.2	17.8	8.9
χ2		*P* = 0.112	*P* = 0.079	*P* = 0.023	*P* = 0.0011

D. Discussion

Both decanal and nonanal were antennally-active components of the male pheromone blend, however, only nonanal elicited behavioral responses. Nonanal is found in several plants, including apple (Casado et al. 2006), rice (Ramachandran et al. 1990), and peach (Xiang et al. 2017). In addition to *C. fumiferana,* nonanal has been observed as a male pheromone component of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) (Leyrer and Monroe 1973), and *Lycaeides argyrognomon* Bgstr. (Lepidoptera: Lycaenidae) (Lundgren and Bergström 1976). Rather than acting as an anti-aphrodisiac, the pheromone blends of these species initiate female attraction and receptivity to mating. Schmidt and Monroe (1976) demonstrated through radiolabelling of fatty acid precursors that nonanal biosynthesis in *G. molesta* utilized several precursors. They concluded that the most important precursor was oleic acid, likely due to the high amounts of this molecule in the larval diet (Yendol 1970). Oleic acid is component of the waxes in balsam fir needles, an important larval food source for *C. fumiferana* (Beri and Lemon 1970). The biosynthetic pathway for nonanal in *C. fumiferana* may be similar to that of *G. molesta*. Future studies involving radiolabelling of oleic and other fatty acid precursors are required to confirm this.

The lower proportions of males wing fanning when exposed to nonanal suggests that males emit compounds to disrupt the courtship sequences of secondary males. This strategy has been observed in other species. In *Adoxophyes orana* (F.v.R.) (Lepidoptera: Totricidae), males exhibit both significantly less wing fanning and incorrect courtship sequence behaviors when exposed to pheromones produced by upwind sexually excited males (Bijpost et al. 1985). Similar results were observed in *Acrolepiopsis assectella* (Zell.) (Lepidoptera: Acrolepiidae) (Lecomte et al. 1998) and *Yponomeuta padellus* (L.) (Lepidoptera: Yponomeutidae) (Hendrikse 1986), where both mate searching and wing fanning were reduced in males exposed to primary male pheromone components. The disruption of wing fanning in secondary males is an important mate guarding technique, as wing fanning is a vital behavior for inducing female receptiveness. Males of both *Grapholita molesta* (Busck) (Lepidoptera: Totricidae) and *Achoria grisella* (Fabricius) (Lepidoptera: Pyralidae) disseminate attractive pheromones towards females using wing fanning (Baker et al. 1981b, Greenfield and Coffelt 1983), while male *Ephestia cautella* (Walk.), *Ephestia kuehniella* Z. and *Plodia interpunctella* (Hb.) (Lepidoptera: Pyralidae) generate acoustical cues that stimulate courtship behavior in female conspecifics (Trematerra and Pavan 1995). Wing fanning also assists searching males in locating female pheromones plumes by directing and increasing air flow rates over their own antenna, a scenario observed in male *Bombyx mori* L. (Loudon and Koehl 2000). For these and other species, a disruption by a primary on the wing fanning of secondary males outside of the courtship sequence may have a negative effect on one or more processes related to female location. The results of Roscoe et al. (2016) suggest that one or more compounds in the male hair pencil blend induce female mating, either through arresting her behaviors or by inducing a state of receptivity. If male wing fanning is required to project this receptive compound onto a prospective female, then any disruption to this behavior could reduce its effect and possibly prevent successful mating. Evidence of this possibility was shown by Sanders (1975), who observed lower proportions of mating in males with clipped wings versus males with unmanipulated wings. Such a disruption to wing fanning could be an important strategy used by primary males to prevent interference in their attempted courtship by other searching males.

We observed that the number of mating pairs formed where females marked with nonanal was significantly less than mating pairs containing decanal-marked and unmarked females. This suggests the existence of chemical mate guarding in *C. fumiferana*. Mate guarding is an important strategy in for males in species where the female is polyandrous and where sperm precedence occurs (Thornhill and Alcock 1983). Both of these conditions exist in *C. fumiferana*: females will mate more than once (Outram 1967, 1971), and, depending on timing after the previous mating, sperm precedence will occur (Retnakaran 1974). Physical mate guarding, whereby males remain in physical contact with the female in some cases for over 10 hours, has been described in *C. fumiferana* (Kipp et al. 1990). While such a strategy increases the likelihood of the female using their genetic material when fertilizing her eggs, the male is sacrificing potential matings with additional females that could achieve the same result (Alcock 1994). Chemical mate guarding involves the male depositing compounds that can protect his genetic investment while allowing him the flexibility to continue mating with other females (Malouines 2016). Several variations of this strategy exist, including the deposition of a physical mating plug (Matsumoto and Suzuki 1992), and compounds in addition to the spermatophore that induce pheromonostasis (Kingan et al. 1995). Another strategy is the deposition of compounds onto or into the female that make her unattractive to other males. For example, *Pieris napi* L. (Lepidoptera: Pieridae) males will deposit methyl-salicylate via the spermatophore to females during copulation. Applications of this compound to unmated virgin females resulted in males consistently rejecting them in favor of unmarked virgins (Andersson et al.

2000). A similar response to females marked with a male anti-aphorodisiac was observed in *Heliothis virescens* (Fabricius), where virgin females marked with either male pheromone extract hexadecyl acetate mated significantly less than unmarked females (Hosseini et al. 2016). These reductions in mating success of females artificially marked with male pheromone components are very similar to our observations of *C. fumiferana* females marked with nonanal, and suggest that a similar mechanism is occurring.

Previous understanding of the courtship sequence of *C. fumiferana* indicates that male upwind flight is initiated by reception of a female-produced Type I pheromone blend of 95:5 (*E*)- and (*Z*)-11 tetradecenal (Silk and Kuenen 1986). Following landing, the male begins searching for the female while wing fanning. Recent evidence suggests that the following close-range male behaviors are mediated by Type II female pheromones that include (*Z*)-11-hexadecenal, (*Z*)5-tricosene and (*Z,Z,Z*)-3,6,9-tricosatriene (Silk et al. 2017). Upon locating contacting the female, the male curls his abdomen towards the abdomen of the female while displaying his hair pencils and attempts to copulate with the female (Sanders and Lucuik 1992). We now propose that the mating sequence of *C. fumiferana* includes the role of anti-aphrodisiac pheromones released by a primary male that act upon downwind secondary males. While our results demonstrate the effects of nonanal on secondary males, the results of Roscoe et al. (2016) indicate that additional components in the male pheromone blend act upon the female during male courtship. Here, the removal of male pheromones from the hair pencils or antennectomization of females resulted in lower mating success in mating pairs versus in pairs where the pheromone was still present or where females were unmanipulated. In other species, male pheromones are necessary to initiate mating postures and/or arrest movements in females so that mating can occur (Birch 1970, Pliske 1975, Phelan and Baker 1986). It is likely that similar actions are initiated in *C. fumiferana* females when exposed to male pheromones. Identification of these active components are currently underway. Additionally, very little is known about the male pheromone blends of other *Choristoneura* spp. It is possible, given the similarities in female pheromone blends of species in this genus (Silk and Eveleigh 2016), that similarities in the male pheromone blends may also exist. Furthermore, the disruption of male courtship behaviors demonstrated here suggests the possibility of the application of male pheromones in mate disruption for field populations. Though most attempts at mate disruption involve utilization of synthetic female pheromones (Vickers et al. 1985, Lawson et al. 1996, Il'ichev et al. 2004), including *C. fumiferana* (Silk and Eveleigh 2016), the use of male pheromones has not been studied to the same extent. Future experiments that integrate male pheromones into existing mate disruption protocols could be useful in improving protocols for management of *C. fumiferana* in Atlantic Canada.

E. Acknowledgements:

We wish to acknowledge the following supporters/funders: Canadian Forest Service, Forest Protection Limited, ACOA/AIF. Total funding = $36 000 (AIF/ACOA)

F. References:

Alcock. **1994**. Postinsemination associations between males and females in insects: the mate-guarding hypothesis. Annu. Rev. Entomol. 39: 1–21.

Andersson, J., A.-K. Borg-Karlson, and C. Wiklund. **2000**. Sexual cooperation and conflict in butterflies: a male-transferred anti-aphrodisiac reduces harassment of recently mated females. Proc. R. Soc. London B. 267: 1271–1275.

Baker, T. C., W. Meyer, and W. L. Roelofs. **1981a**. Sex pheromone dosage and blend specificity of response by oriental fruit moth males. Entomol. Exp. Appl. 30: 269–279.

Baker, T. C., R. Nishida, and W. L. Roelofs. **1981b**. Close-range attraction of female oriental fruit moths to herbal scent of male hairpencils. Science 241: 1359–1361.

Beri, R. M., and H. W. Lemon. **1970**. Chemical examination of the wax from needles of black spruce *(Picea mariana)* and balsam fir *(Abies balsamea)*. Can. J. Chem. 48: 67–69.

Bijpost, S. C. A., G. Thomas, and J. P. Kruijt. **1985**. Olfactory interactions between sexaully active males in *Adoxophyes orana* (F.v.R.) (Lepidoptera: Totricidae). Behaviour. 95: 121–137.

Birch, M. C. 1970. Pre-courtship use of abdominal brushes by the nocturnal moth, *Phlogophora meticulosa* (L.) (Lepidoptera: Noctuidae). Anim. Behav. 18: 310–316.

Birch, M. C., D. Lucas, and P. R. White. **1989**. The courtship behavior of the cabbage moth, *Mamestra brassicae* (Lepidoptera: Noctuidae), and the role of male hair-pencils. J. Insect Behav. 2: 227–239.

Casado, D., C. Gemeno, J. Avilla, and M. Riba. **2006**. Day-night and phenological variation of apple tree volatiles and electroantennogram responses in *Cydia pomonella* (Lepidoptera: Tortricidae). Environ. Entomol. 35: 258–267.

Chang, W.-Y., V. A. Lantz, C. R. Hennigar, and D. A. MacLean. **2012**. Economic impacts of forest pests: a case study of spruce budworm outbreaks and control in New Brunswick, Canada. Can. J. For. Res. 42: 490–505.

Collins, R. D., and R. T. Cardé. **1989**. Wing fanning as a measure of pheromone response in the male pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). J. Chem. Ecol. 15: 2635–2645.

Cremer, S., and M. D. Greenfield. **2010**. Partitioning the components of sexual selection: Attractiveness and agonistic behaviour in male wax moths, *Achroia grisella* (Lepidoptera: Pyralidae). Ethology. 104: 1–9.

Curkovic, T., J. F. Brunner, and P. J. Landolt. **2009**. Courtship behavior in *Choristoneura rosaceana* and *Pandemis pyrusana* (Lepidoptera: Tortricidae). Ann. Entomol. Soc. Am. 99: 617–624.

Greenfield, M. D., and J. A. Coffelt. **1983**. Reproductive behaviour of the lesser waxmoth, *Achoria grisella* (Pyralidae: Gelleriinae): Signalling, pair formation, male interactions, and mate guarding. Behaviour. 84: 287–315.

Happ, G. M. 1969. Multiple sex pheromones of the mealworm beetle, *Tenebrio molitor*. Nature. 222: 180–181.

Hendrikse, A. 1986. The courtship behavior of *Yponomeuta padellus*. Entomol. Exp. Appl. 42: 45–55.

Hennigar, C. R., D. A. MacLean, K. B. Porter, and D. T. Quiring. **2007**. Optimized harvest planning under alternative foliage-protection scenarios to reduce volume losses to spruce budworm. Can. J. For. Res. 37: 1755–1769.

Hennigar, C. R., D. A. MacLean, D. T. Quiring, and J. A. Kershaw. 2008. Differences in spruce budworm defoliation among balsam fir and white, red, and black spruce. For. Sci. 54: 158–166.

Hosseini, S. A., M. van Wijk, G. Ke, S. H. Goldansaz, C. Schal, and A. T. Groot. 2016. Experimental evidence for chemical mate guarding in a moth. Sci. Rep. 6: 38567.

Il'ichev, A. L., D. G. Williams, and A. D. Milner. 2004. Mating disruption barriers in pome fruit for improved control of oriental fruit moth *Grapholita molesta* Busck (Lep., Tortricidae) in stone fruit under mating disruption. J. Appl. Entomol. 128: 126–132.

Iyengar, V., C. Rossini, and T. Eisner. 2001. Precopulatory assessmen of male quality in an arctiid moth (*Utethesia ornatrix*): hydroxydanaidal is the only criterior of choice. Behav. Ecol. Sociobiol. 49: 283–288.

Kamimura, M., and S. Tatsuki. 1993. Diel rhythms of calling behavior and pheromone production of oriental tobacco budworm moth, *Helicoverpa assulta* (Lepidoptera: Noctuidae). J. Chem. Ecol. 19: 2953–2963.

Kingan, T. G., W. M. Bodnar, A. K. Raina, J. Shabanowitz, and D. F. Hunt. 1995. The loss of female sex pheromone after mating in the corn earworm *Helicoverpa zea*: Identification of a male pheromonostatic peptide. Proc. Natl. Acad. Sci. 92: 5082–5086.

Kipp, L. R., R. Ellison, and W. D. Seabrook. 1990. Copulatroy mate guarding in spruce budworm. J. Insect Behav. 3: 121–131.

Knight, A. L., and L. Flexner. 2007. Disruption of mating in codling moth (Lepidoptera: Tortricidae) by chlorantranilipole, an anthranilic diamide insecticide. Pest Manag. Sci. 63: 180–189.

Kuenen, L. P. S., and H. C. Rowe. 2006. Cowpea weevil flights to a point source of female sex pheromone: analyses of flight tracks at three wind speeds. Physiol. Entomol. 31: 103–109.

Lawson, D. S., W. H. Reissig, A. M. Agnello, J. P. Nyrop, and W. L. Roelofs. 1996. Interference with the mate-finding communication system of the obliquebanded leafroller (Lepidoptera: Tortricidae) using synthetic sex pheromones. Environ. Entomol. 25: 895–905.

Lecomte, C., E. Thibault, D. Pierre, and J. Auger. 1998. Transfer, perception, and activity of male pheromone of *Acrolepiosis assectella* with special reference to conspecific male sexual inhibition. J. Chem. Ecol. 24: 655–671.

Leyrer, R. L., and R. E. Monroe. 1973. Isolation and identification of the scent of the moth, *Galleria mellonella*, and a revaluation of its sex pheromone. J. Insect Physiol. 19: 2267–2271.

Loudon, C., and M. A. Koehl. 2000. Sniffing by a silkworm moth: wing fanning enhances air penetration through and pheromone interception by antennae. J. Exp. Biol. 203: 2977–2990.

Lundgren, L., and G. Bergström. 1976. Wing scents and scent-released phases in the courtship behavior ofLycaeides argyrognomon (Lepidoptera: Lycaenidae). J. Chem. Ecol. 1: 399–412.

MacLean, D. A. 1984. Effects of spruce budworm outbreaks on the productivity and stability of balsam fir forests. For. Chron. 60: 273–279.

Malouines, C. 2016. Counter-perfume: using pheromones to prevent female remating. Biol. Rev. 92: 1570–1581.

Matsumoto, K., and N. Suzuki. 1992. Effectiveness of the mating plug in *Atrophaneura alcinous* (Lepidoptera: Papillionidae). Behav. Ecol. Sociobiol. 30: 157–163.

Outram, I. 1967. Polyandry in spruce budworm. Can. Dep. For. Bi-monthly Res. Notes. 24: 6–7.

Outram, I. 1971. Aspects of mating in the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Totricidae). Can. Entomol. 103: 1121–1128.

Palaniswamy, P., W. D. Seabrook, and R. J. Ross. 1979. Precopulatory behavior of males and perception of a potential male pheromone in spruce budworm, *Choristoneura fumiferana*. Ann. Entomol. Soc. Am. 72: 544–551.

Phelan, P. L., and T. C. Baker. 1986. Male-size-related courtship success and intersexual selection in the tobacco moth, *Ephestia elutella*. Experientia. 42: 1291–1293.

Phelan, P. L., P. J. Silk, C. J. Northcott, S. H. Tan, and T. C. Baker. 1986. Chemical identification and behavioral characterization of male wing pheromone of *Ephestia elutella* (Pyralidae). J. Chem. Ecol. 12: 135–146.

Pliske, T. E. 1975. Courtship behavior and use of chemical communication by males of certain species of ithomiine butterflies (Nymphalidae: Lepidoptera). Ann. Entomol. Soc. Am. 68: 935–942.

Ramachandran, R., Z. R. Khan, P. Caballero, and B. O. Juliano. 1990. Olfactory sensitivity of two sympatric species of rice leaf folders (Lepidoptera: Pyralidae) to plant volatiles. J. Chem. Ecol. 16: 2647–2666.

Retnakaran, A. 1974. The mechanism of sperm precidence in the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Can. Entomol. 106: 1189–1194.

Royama, T. 1984. Population dynamics of the spruce budworm *Choristoneura fumiferana*. Ecol. Monogr. 54: 429–462.

Royama, T., W. E. MacKinnon, E. G. Kettela, N. E. Carter, and L. K. Hartling. 2005. Analaysis of spruce budworm outbreak cycles in New Brunswick, Canada, since 1952. Ecology. 86: 1212–1224.

Sanders, C. J. 1975. Factors affecting adult emergence and mating behavior of the eastern spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Can. Entomol. 107: 967–977.

Sanders, C. J., and G. S. M. Lucuik. 1992. Mating behavior of spruce budworm moths, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. Entomol. 124: 273–286.

Sanders, C. J., and J. Weatherson. **1976**. Sex pheromone of the eastern spruce budworm (Lepidoptera: Totricidae): optimum blend of *Trans*- and *cis*-11-tetradecenal. Can. Entomol. 108: 1285–1290.

Schmidt, S. P., and R. E. Monroe. 1976. Biosynthesis of the waxmoth sex attractants. Insect Biochem. 6: 377–380.

Silk, P. J., and E. S. Eveleigh. 2016. Pheromone communication, behavior, and ecology in the North Ameican *Choristoneura* genus, pp. 265–277. *In* Pheromone Commun. Moths Evol. Behav. Appl.

Silk, P. J., E. S. Eveleigh, L. E. Roscoe, K. Burgess, S. Weatherby, G. Leclair, P. Mayo, and M. Brophy. 2017. Unsaturated cuticular hydrocarbons enhance responses to sex pheromone in spruce budworm, *Choristoneura fumiferana*. J. Chem. Ecol. 43: 753–762.

Silk, P. J., and L. P. S. Kuenen. **1986**. Spruce budworm (*Choristoneura fumiferana*) pheromone chemistry and behavioral responses to pheromone components and analogs. J. Chem. Ecol. 12: 367–383.

Silk, P. J., S. H. Tan, C. J. Wiesner, R. J. Ross, and G. C. Lonergan. 1980. Sex pheromone chemistry of the eastern spruce budworm, *Choristoneura fumiferana*. Environ. Entomol. 9: 640–644.

Thornhill, R., and J. Alcock. **1983**. The Evolution of Insect Mating Systems. Harvard University

Press, Cambridge.

Trematerra, P., and G. Pavan. **1995**. Ultrasound production in the courtship behaviour of *Ephestia cautella* (Walk.), *E. kuehniella* Z., and *Plodia interpunctella* (Hb.) (Lepidoptera: Pyralidae). J. Stored Prod. Res. 31: 43–48.

Vickers, R. A., G. H. L. Rothschild, and E. L. Jones. 1985. Control of the oriental fruit moth, *Cydia molesta* (Busek) (Lepidoptera: Tortricidae), at a district level by mating disruption with synthetic female pheromone. Bull. Entomol. Res. 75: 625–634.

Xiang, H.-M., R.-Y. Ma, H.-L. Diao, X.-W. Li, X.-J. He, and Y.-F. Guo. **2017**. Peach-specific aldehyde nonanal attracts female Oriental fruit moths, *Grapholita molesta* (Lepidoptera: Tortricidae). J. Asia. Pac. Entomol. 20: 1419–1424.

Yendol, W. G. 1970. Fatty acid composition of *Galleria* larvae, hemolymph, and diet (Lepidoptera: Galleriidae). Ann. Entomol. Soc. Am. 63: 339–341.

Zagatti, P., G. Kunesch, F. Ramiandrasoa, C. Malosse, D. R. Hall, R. Lester, and B. F. Nesbitt. **1987**. Sex pheromones of rice moth, *Corcya cephalonica* Stainton I. Identification of male pheromone. J. Chem. Ecol. 13: 1561–1573.

Evaluating endophyte application strategies

Greg Adams[1,4], *David Miller*[2], *Pamela Nicks*[3] *Andrew McCartney*[3]

[1] J.D. Irving, Limited, 181 Aiton Road, Sussex East, NB, E4G 2V5
[2] Ottawa-Carleton Institute of Chemistry, Carleton University, Ottawa, ON, K1S 5B6
[3] Maritime Innovation Limited, 181 Aiton Road, Sussex East, NB, E4G 2V5
[4] Corresponding author: adams.greg@jdirving.com; phone: 506-432-2844

Abstract

Our research over the past several years has focused on endophytes, fungi that result in symptomless infections in the needles of conifer trees that improve fitness. Over the past three decades we have identified multiple populations of endophytes that produce secondary metabolites that reduce the growth of pests, namely spruce budworm (*Choristoneura fumiferana*), white pine blister rust (*Cronartium ribicola*) and *Dothistroma septosporum*. We have further developed applications whereby cultures of endophytic fungi are applied to nursery seedling stock to improve tree tolerance to these insect and disease pests. Long-term field studies have demonstrated the persistence and spread of endophytes in inoculated trees and their presence causes reduced budworm survival and defoliation.

Needle endophytes isolated from white pine were shown to produce potently antifungal compounds including the known antifungals pyrenophorol and griseofulvin. In infected needles, these compounds are present in concentrations shown to be toxic to white pine blister rust and related rust fungi in *in vitro* assays. Field experiments to test the efficacy of these selected white pine endophytes are underway. Research during the past few years of the SERG-I project has focused on conducting research on endophyte application strategies to evaluate the inoculation efficiency of seedlings after treatment with endophyte mixtures.

At present, endophyte cultures are applied to seedlings as individual strains but mixtures of diverse species may be desirable, both from the standpoint of diversity and also to address seeding genotype X fungal strain interactions which may be present. This might increase the percentage of seedlings inoculated with true endophytes. However, little is known about how individual strains interact in the context of other, potentially antagonistic, fungal species. To better understand these interactions, we treated 1000 red spruce, white spruce and white pine seedlings with endophyte mixtures. Samples were collected and are currently being analyzed by qPCR methods to determine the percent inoculation of seedlings and the identity of the endophyte strain(s). Early results indicate that one strain appears to dominate over other strains in the white spruce and white pine treatments and two strains co-dominate in the red spruce treatments. There also appears to be no difference between mixture and serial application strategies. The results will be used to support decisions regarding the future deployment of endophytes in large scale applications.

Résumé

Depuis quelques années, notre recherche porte sur une catégorie de champignons endophytes qui habitent à l’intérieur des aiguilles de conifères, formant des associations mutualistes avec leurs hôtes. Nous avons identifié parmi ceux-ci des souches de champignons endophytes qui produisent des métabolites secondaires se comportant de manière antagoniste avec certains ravageurs forestiers, dont la tordeuse des bourgeons de l’épinette (*Choristoneura fumiferana*), la rouille vésiculeuse du pin blanc (*Cronartium ribicola*) et *Dothistroma septosporum*. Nous avons perfectionné des applications pour ajouter des champignons endophytes à des semis de pépinière afin d’améliorer la tolérance des arbres à ces maladies et insectes nuisibles. Des études de longue durée sur le terrain ont montré la persistance et la propagation des endophytes dans les arbres inoculés et aussi que la présence de ces endophytes réduit la survie de la tordeuse et la défoliation. De plus, les expériences en laboratoire utilisant des extraits antifongiques prélevés sur le pin blanc ont montré que ceux-ci étaient toxiques pour le champignon de la rouille vésiculeuse du pin blanc et d’autres champignons qui causent la rouille dans des essais in vitro. Des expériences sur le terrain sont en cours pour vérifier l’efficacité de ces endophytes du pin blanc. La recherche menée au cours des dernières années dans le cadre du projet SERG-I a consisté à faire des travaux de recherche sur les stratégies qui utilisent l’application d’endophytes pour évaluer l’efficience de l’inoculation des semis suivant leur traitement avec des mélanges d’endophytes. À l’heure actuelle, on applique sur les semis des souches individuelles de cultures endophytes, mais il est peut-être souhaitable d’appliquer des mélanges de plusieurs espèces, aussi bien du point de vue de la diversité que pour prendre en compte la possibilité d’interactions de souches fongiques de génotype X des semis (augmentant ainsi le pourcentage de semis inoculés avec des endophytes utiles). Toutefois, on en connaît très peu sur l’interaction des souches individuelles en présence d’autres espèces fongiques potentiellement antagoniques. Pour mieux comprendre ces interactions, nous avons traité avec des mélanges d’endophytes 1000 semis d’épinette rouge, d’épinette blanche et de pin blanc. Des échantillons ont été prélevés, et leur analyse est en cours au moyen de méthodes de PCR quantitative pour déterminer le pourcentage d’inoculation des semis et identifier les souches d’endophytes. Les premiers résultats montrent qu’une souche semble dominer sur les autres dans le traitement de l’épinette blanche et du pin blanc et deux souches co-dominent pour l’épinette rouge. De plus, il ne semble y avoir aucune différence, peu importe le mélange ou les stratégies d’application en série. Les résultats de l’analyse serviront à appuyer les décisions concernant le déploiement futur d’endophytes dans des applications à grande échelle.

1. Background

Plants commonly form symbiotic mutual associations with microbiological organisms and these associations are mutualistic: plants provide microbes with protection and photosynthate in exchange for increased tolerance of the host to biotic and abiotic stresses. Certain mutualistic associations between plants and microbes are well-characterized and Some are in commerce. An important example is endophyte positive cool season fescues for lawns and golf courses. These are more tolerant to insects damage and have improved drought tolerance (Clay, 1988; Kuldau and Bacon, 2008). Foliar endophytes of conifers are another class of mutualisms receiving more attention in recent years and also demonstrate potential to improve tolerance of certain forest insect and disease pests.

Our research over the past several years has identified and characterized specific endophyte strains of spruces from Acadian forests that produce mixtures of toxic, anti-insectan and antifungal compounds (Calhoun *et al*., 1992; McMullin *et al*., 2015; McMullin *et al*., 2016; McMullin *et al*., 2017; Sumarah and Miller, 2009; Sumarah *et al*., 2010; Sumarah *et al*., 2011; Richardson *et al*., 2014; Richardson *et al*., 2015; Tanney *et al*., 2016). These compounds have been shown to reduce the performance of spruce budworm (*Choristoneura fumiferana*) in laboratory, nursery and field trials (Miller *et al*., 2002; Miller *et al*., 2008; Sumarah *et al*., 2005). This provided some of the first evidence in trees showing beneficial endophytes improve tolerance to biotic stresses.

Further research efforts have focused on isolating and characterizing endophytic fungi from pine needles that produce antifungal toxins and testing them for their effectiveness to improve tree tolerance to white pine blister rust (*Cronartium ribicola*) and other fungal diseases. Several endophyte strains isolated from white pine produce compounds known to have activity have been identified that demonstrate broad antifungal activity in laboratory assays (Sumarah *et al*., 2011; Richardson *et al*., 2014; Richardson *et al*., 2015; Sumarah *et al*., 2015). Some of these are present in concentrations in needles shown to be toxic to white pine blister rust and related rust fungi (McMullin *et al*., 2018).

The beneficial effect of toxin-producing endophytes is persistent, systemic and transmissible. Foliar endophytes of conifers are transmitted horizontally i.e., young seedlings become inoculated by exposure to older, infected trees. This is different than the grass endophytes which are transmitted by seed or vertical transmission (Kuldau and Bacon, 2008). Toxin-producing endophytes that were artificially inoculated into trees were detected in foliage throughout those same trees 3-4 years later (Sumarah *et al.*, 2008). An extensive analysis of young, nursery grown seedlings showed they were relatively endophyte free. However, when these young seedlings were planted beneath older, endophyte-infected trees, the young seedlings obtained the endophyte over time (Miller *et al.*, 2009). It appears that young seedlings obtain endophytes through exposure from cast needles of infected trees, suggesting that the natural mechanism of endophyte transmission in conifers is limited and confined to a short physiological and temporal window. For some endophytes, persistence of the fungus and its principal toxin have been shown to extend for a decade or more so far (Frasz *et al.*, 2014; McMullin *et al.*, 2018).

Our research demonstrates there is a clear benefit to producing conifer seedlings that harbor beneficial endophytes and a significant effort has been made to identify many unique strains. A diversity of endophytes applied to young nursery-grown seedlings allows for greater seedling inoculation rates because mixtures offer a higher probability of more favourable endophyte/seedling genotype combinations and would also increase the diversity of endophyte species in forest stands.

The goal of this project builds upon on-going research with the specific objective to evaluate the inoculation efficiency of nursery-grown seedlings after treatment with endophyte mixtures.

2. Objective

Evaluate the inoculation efficiency of nursery-grown seedlings after treatment with endophyte mixtures

The purpose of this study was to evaluate the inoculation efficiency of seedlings when applied as mixtures. Through our on-going efforts to commercialize endophyte-enhanced seedling technology we continually seek improved methods to deploy endophytes operationally. Endophyte cultures are applied to seedlings as individual strains; however, ideally, they would be applied as mixtures of diverse species. This would allow for potentially greater seedling inoculation rates because mixtures offer a higher probability of more favourable endophyte/seedling genotype combinations. It would also allow for a greater diversity of endophytes, and thereby toxins, within forest stands once seedlings are planted out. Very little is known, however, about how individual strains interact in the context of other, potentially antagonistic, fungal species.

In 2015, 1000 seedlings each of red spruce, white spruce and white pine were treated with endophyte mixtures. The red spruce seedlings were treated with endophyte strains 06-255A, 06-073C and 08-018A; the white spruce seedlings were treated with endophyte strains 22E1, 11I1 and 05-037A; the white pine seedlings were treated with endophyte strains T6-4B-2, SG-15 and 1747-1. The seedling lots were divided into three treatment groups: treatment 1 received no endophytes and served as the control group; for treatment 2 the endophytes were mixed together and applied at one time; for treatment 3 the endophytes were applied through serial application. All seedlings were grown in the nursery under standard growing conditions.

3. Preliminary Results

In 2016, a subset of 100 seedlings from each of the three treatments were sampled and are currently being analyzed using qPCR methods to determine the percent seedling infection and the identity of the endophyte strain(s). When present in the mixture it appears that one strain appears to dominate over other strains in the white spruce and white pine treatments and two strains co-dominate in the red spruce treatments. There is very little known regarding the infectiveness of each strain. Inoculum viability likely varies between the individual cultures. From these data, it appears for white spruce and white pine endophytes that whichever strain becomes established first will dominate. This preliminary assessment does not support a difference between serial and mixture application treatments. A subset of seedlings (30 seedlings/species/treatment) will be carried forward for future evaluation in the field. The results of this analysis will be used to support decisions regarding the future deployment of endophytes in large scale applications. At this point, it appears that portions of crops of a given species will need to be treated with different individual strains of endophytes to maintain a broad diversity of strains in seedling crops.

References

Bingham, R.T. 1983. Blister rust resistant western white pine for the Inland Empire: the story of the first 25 years of the research and development program. General Technical Report INT-146. USDA Forest Service, Intermountain Forest and Range Experiment Station; Ogden, Utah. 45 p.

Calhoun, L.A., Findlay, J.A., Miller, J.D., Whitney, N.J. 1992. Metabolites toxic to spruce budworm from balsam fir needle endophytes. Mycol. Res. 96: 281-286.

Clay, K., 1988. Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69:10-16.

Frasz SL, Walker AK, Nsiama TK, Adams GA, Miller JD (2014) Distribution of the foliar fungal endophyte *Phialocephala scopiformis* and its toxin in the crown of a mature white spruce tree as revealed by chemical and qPCR analyses. Can J Forest Res 44:1138-1143.

Kuldau G, Bacon C (2008) Clavicipitaceous endophytes: their ability to enhance resistance of grasses to multiple stresses. Biological Control 46:57-71.

Mahalovich, M.F. 2010. U.S.A. Inland northwest western white pine breeding and restoration program: history, current and future directions. In M. Cleary (ed.). Proc. 3rd Western White Pine Management Conference, June 17–18, 2008, Vernon, BC. pp. 50–74. B.C. Ministry of Forests and Range, Kamloops, BC.

McMullin, D.R., Green, B.D., Prince, N.A., Tanney, J.B., Miller, J.D. 2017. Natural products of Picea endophytes from the Acadian forest. Journal of Natural Products 80: 1475-1483.

McMullin DR, Green BD, Prince NC, Tanney JB, Miller JD (2017) Natural products of *Picea* endophytes from the Acadian forest. J. Natural Products 80:1475-1483.

McMullin DR, Nguyen HDT, Daly GJ, Menard BS, Miller JD (2018) Detection of foliar endophytes and their metabolites in *Picea* and *Pinus* seedling needles. Fungal Ecology 31 https://doi.org/10.1016/j.funeco.2017.09.003

Miller, J.D., Cherid, H., Sumarah, M.W., Adams, G.W. 2009. Horizontal transmission of the Picea glauca foliar endophyte *Phialocephala scopiformis* CBS 120377. Fungal Ecol. 2: 98-101.

Miller, J.D., Mackenzie, S., Foto, M., Adams, G.W., Findlay, J.A. 2002. Needles of white spruce inoculated with rugulosin-producing endophytes contain rugulosin reducing spruce budworm growth rate. Mycol. Res. 106: 471-479.

Miller, J.D., Sumarah, M.W., Adams, G.W. 2008. Effect of a rugulosin-producing endophyte in *Picea glauca* on *Choristoneura fumiferana*. J. Chem. Ecol. 34: 362-368.

Moreau, G., Quiring, D. T., Eveleigh, E. S., and Bauce. 2003. Advantages of a balanced diet: feeding on several foliar age classes increases the fitness of an insect herbivore. Oecologia 135: 391-399.

Muller, J. J. (2002). Environmental variables associated with field performance of blister rust resistant F2 western white pine in the Inland Empire. University of Idaho, Moscow Master's Thesis.

Quiring, D.T. and M.L. McKinnon. 1999. Why does early-season herbivory affect subsequent budburst? Ecology 80: 1724-1735.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical computing. Vienna, Austria. URL http://www.R-project.org/.

Rack K, Butin H. 1973. A quick method for the production of *Dothistroma pini* spores in culture. Eur J Forest Pathol 3: 201-209.

Richardson, SN, Walker AK, Nsiama TK, McFarlane J, Sumarah MW, Ibrahim A, Miller JD (2014) Griseofulvin-producing *Xylaria* endophytes of *Pinus strobus* and *Vaccinium angustifolium*: evidence for a conifer-understory species endophyte ecology. Fungal Ecology 11: 107–113.

Richardson SN, Nsiama TK, Walker AK, McMullin DR, Miller JD (2015) Antimicrobial dihydrobenzofurans and xanthenes from a foliar endophyte of *Pinus strobus*. Phytochemistry 117:436–443.

Schwandt, J.W., Kearns, S.J., Miller, D.L., & Ferguson, B.A. 2013. Impacts of white pine blister rust in 22 plantations of western white pine in Northern Idaho: 1995-2011. FHP Report No. 13-08. USDA Forest Service, Northern Region, State and Private Forestry, Forest Health Protection; Missoula, Montana. 23 p.

Sumarah, M.W., Adams, G.W., Berghout, J., Slack, G.J., Wilson, A.M., Miller, J.D. 2008. Spread and persistence of rugulosin-producing endophyte in *Picea glauca* seedlings. Mycol. Res. 112: 731-736.

Sumarah, M.W., Kesting, J.R., Sorensen, D., Miller, J.D. 2011. Anti-fungal metabolites from fungal endophytes of *Pinus strobus*. Phytochemistry. 72: 1833-1837.

Sumarah, M.W., Miller, J.D., Adams, G.W. 2005. Measurement of a rugulosin-producing endophyte in white spruce seedlings. Mycologia. 97: 770-776.

Sumarah, M.W., Miller, J.D. 2009. Anti-insect secondary metabolites from fungal endophytes of conifer trees. Nat. Prod. Commun. 4: 1497-1504.

Sumarah, M.W., Puniani, E., Sorensen, D., Blackwell, B.A., Miller, J.D. 2010. Secondary metabolites from anti-insect extracts of endophytic fungi isolated from *Picea rubens*. Phytochemistry. 71: 760-765.

Sumarah, M.W., Walker, A.K., Seifert, K.A., Todorov, A., & Miller, J.D. 2015. Screening of Fungal Endophytes Isolated from Eastern White Pine Needles. In: Pirttila, A.M., Frank, A.C. (eds) The Formation, Structure and Activity of Phytochemicals. Recent Advances in Phytochemistry 45, Springer 195-206.

Tanney JB, McMullin DR, Green BD, Miller JD, Seifert KA (2016) Production of antifungal and antiinsectan metabolites by the *Picea* endophyte *Diaporthe maritima sp. nov*. Fungal Biology 120: 448-1457

Zuur, A.F., Ieno, E.,N., Walker, N.J., Saveliev, A.A., and G..M. Smith. 2009. *Mixed effects models and extensions in ecology with R.* Springer, 574pp. U.S.A.

Early intervention strategies against spruce budworm: modeling and DSS analyses in 2017

David A. MacLean[1], Jeremy Gullison[2], Chris Hennigar[1,2], Bo Zhang[1],
and Drew Carleton[2]
[1] Faculty of Forestry and Environmental Management, University of New Brunswick
[2] NB Dept. of Energy and Resource Development, Fredericton, NB

Study 2.2.8 of the Early Intervention Strategies (EIS) against spruce budworm (SBW) project includes modelling and DSS/economic analyses, determining the mechanism of hardwood effects in reducing defoliation, and developing methods to remotely sense defoliation. PhD student Bo Zhang completed analysis of 5 years of SBW defoliation in 27 plots representing a gradient of hardwoods in balsam fir-hardwood stands near Amqui, Quebec. Average defoliation severity in softwood stands and percent hardwood content were the two most important predictor variables in predicting balsam fir defoliation caused by SBW, with 38% and 15% increase in mean square error, respectively. Excluding average defoliation severity in softwood stands as a predictor variable decreased the accuracy of prediction of fir defoliation. Methods to optimize operational blocking in insecticide spray programs, linked to the Spruce Budworm Decision Support System (DSS), were refined and used to prioritize the planned 2018 SBW EIS spray program. Study of remote sensing technology to assess SBW defoliation were continued by MScF student Shawn Donovan, including testing Hyperion satellite imagery and ground-based hemi-spherical canopy photographs to measure changes in leaf-area index and canopy gap fraction with defoliation. Study of economic analyses of EIS against SBW was continued by MSc student Eric Liu.

L'étude 2.2.8 du projet de stratégies d'intervention précoce contre la tordeuse des bourgeons de l'épinette (TBE) comprend la modélisation et des analyses de SSD/économiques pour déterminer le mécanisme des effets des feuillus dans la réduction de la défoliation et mettre au point des méthodes permettant de détecter la défoliation à distance. L'étudiant au doctorat Bo Zhang a analysé cinq années de données sur la défoliation par la TBE dans 27 parcelles représentant un gradient de feuillus dans des peuplements de sapins baumiers-feuillus près d'Amqui, au Québec. La gravité moyenne de la défoliation dans les peuplements de résineux et le contenu de feuillus en pourcentage ont été les deux plus importantes variables de prédiction de la défoliation du sapin baumier causée par la TBE, avec des hausses respectives de 38 % et de 15 % de l'erreur quadratique moyenne. Le fait d'exclure la gravité moyenne de la défoliation dans les peuplements de résineux comme variable de prédiction a réduit la précision de la prédiction de défoliation chez le sapin. Les méthodes pour optimiser les parcelles opérationnelles des programmes de pulvérisation d'insecticides avec un système de soutien à la décision (SSD) de la TBE ont été raffinées puis ont servi à établir les priorités du programme de pulvérisation pour la stratégie d'intervention précoce contre la TBE de 2018. Shawn Donovan, étudiant à la maîtrise en sciences de l'environnement, poursuit l'étude de technologies de détection à distance pour évaluer la défoliation par la TBE, y compris l'utilisation d'images satellitaires d'Hyperion et de photographies hémisphéricales du couvert forestier à partir du sol pour mesurer les changements dans l'indice de surface foliaire et la fraction de trous du couvert forestier présentant une défoliation. Eric Liu, étudiant à la maîtrise, a poursuivi l'étude des analyses économiques des stratégies d'intervention précoce contre la TBE.

Eastern North America is undergoing another spruce budworm (SBW) outbreak, and we are testing an early intervention strategy (EIS) to suppress SBW populations in New Brunswick and prevent widespread damage. Government and Industry are actively involved in a 4-year project. Strategies include short-term (applied control measures designed to suppress populations), longer-term (understanding effects of natural enemies and factors affecting outbreak initiation, and inoculating seedlings with endophytic fungi that have insect suppressing qualities), and improving decision support capabilities to facilitate planning. More information about the 4-year New Brunswick EIS-SBW project is available at www.healthyforestpartnership.ca

The SBW DSS is a computer system including stand and forest-level models and a GIS that projects effects of SBW defoliation, and SBW management/ protection strategies, on tree and stand growth, timber supply, economic indicators, and forest carbon (MacLean et al. 2001; Hennigar et al. 2007, 2011, 2013). The most recent version of the SBW DSS is the Accuair Forest Protection Optimization System (*ForPRO*) (McLeod et al. 2011). Ecological SBW population data is being integrated into computer-based simulation models to develop an optimized SBW management DSS, Accuair *ForPRO II*. The new optimized DSS will help reduce social and regulatory barriers to the adoption of new pest-control products. The livelihoods of many forest-resource based communities across Canada depend upon successful suppression of SBW populations. The DSS analyses are being conducted in collaboration with end-users. The DSS is currently the only tool available to permit users to integrate forest harvest planning, protection using pesticides, and salvage, within a spatial optimization framework, to reduce losses to SBW (Hennigar et al. 2007).

Objectives (for activities in 2017):

1. Evaluate the mechanism of observed hardwood effects in reducing SBW defoliation on balsam fir and spruce (whether increased natural enemies and/or small larvae dispersal losses) and incorporate into the SBW DSS.
2. Refine optimum operational blocking heuristics for use in planning annual insecticide spray programs, and use for planning the SBW EIS 2018 blocking in New Brunswick.
3. Conduct timber supply analyses of EIS and reactive SBW outbreak scenarios on timber supply from Crown land in NB and determine their economic outcomes.

Overall project objectives and timeline from 2014-2018 are summarized in Table 1.

Table 1. Summary of planned studies and timeline for completion of activities of EIS-SBW Study 2.2.8 Integrative modeling and DSS/economic analyses. (Q = Quarter of year)

ACTIVITY DESCRIPTION	COMPLETION DATE
1. SBW population-derived defoliation scenarios tested in *ForPRO* II	Q4 2017
2. Population-based pesticide efficacy models	Q1 2016
3. Field studies of mechanism of hardwood protective effect	Q3 2017
4. Hardwood protective effect defoliation scenarios tested in *ForPRO* II	Q1 2018
5. EIS control strategies using operational blocking developed using heuristic algorithms	Q1 2017
6. Economic outcomes of EIS and reactive SBW control scenarios	Q1 2018
7. A satellite-based model for rapid monitoring of current defoliation	Q2 2016
8. Tests of CASI-1500 and SASI-600 hyperspectral imagery to quantify current defoliation	Q2 2016

Results

1. Mechanism of hardwoods reducing SBW defoliation and incorporation into DSS

In 2014, UNB PhD student Bo Zhang established 27 plots near Amqui, QC and is studying effects of hardwood content on SBW dispersal, natural enemies, and defoliation. Three replicate mature (age >40 years) balsam fir-hardwood stands were selected within each of three percent basal area of hardwood classes: 0-25% (termed softwood), 40-65% (mixedwood), and 75-95% (hardwood). Methods and early results were described in detail in Zhang and MacLean (2015).

Strong trends of decreasing defoliation with increasing hardwood content occurred each year from 2012 to 2016, during the initiation and building phase of the outbreak, across the gradient of softwood, mixedwood, and hardwood plots (Fig. 1). Balsam fir defoliation in the sample plots was significantly related to hardwood content (% basal area) in each of the 5 years (explaining 25-70% of the variability).

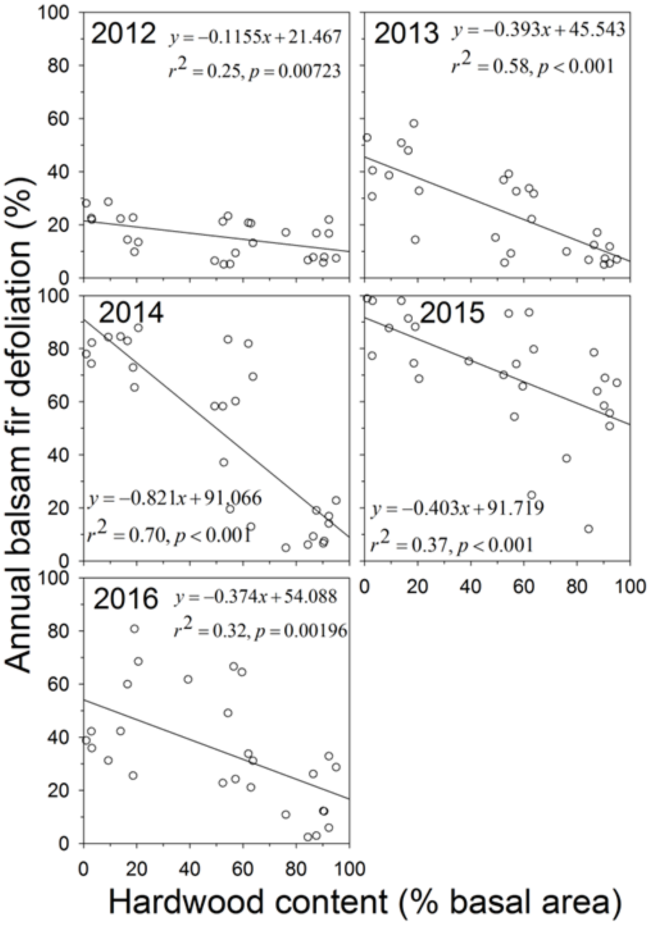


Fig.1. Regression analyses of relationships between balsam fir defoliation and hardwood basal area percentage in each stand from 2012 to 2016.

In a manuscript in preparation (Zhang et al., unpublished), results from the Amqui plots were compared with similar defoliation/hardwood relationships from Su et al. (1996), collected in northern NB from 1989-1993, during the declining phase of the outbreak. The relationships between balsam fir defoliation and hardwood content were stronger during the declining phase (1989-1993, from Su et al. 1996) (r^2 0.57-0.80) than during the building phase (2012-2016) (r^2 0.25-0.70). For both declining and building phases, the relationships were highest under severe defoliation and lowest under light defoliation. Average defoliation severity in softwood stands and percent hardwood content were the two most important predictor variables in predicting balsam fir defoliation caused by SBW, with 38% and 15% increase in mean square error, respectively. Excluding average defoliation severity in softwood stands as a predictor variable decreased the accuracy of prediction of fir defoliation. Our results did not support the previous assumption that hardwood content caused the same proportional reduction of defoliation regardless of whether SBW populations, and thus defoliation, were high, moderate, or low. Accurate prediction of effects of hardwood content on defoliation requires data about average defoliation level in softwood stands, as an indicator of overall outbreak severity that year.

2. Overstory composition and seedling height influence understory regeneration defoliation

The same set of plots used by Bo Zhang (above), were also used by UNB MScF student Zhuoyi Nie to evaluate effects of overstory defoliation and other factors on defoliation of understory balsam fir regeneration. Results were published in Nie et al. (2018). Plots represented three classes of hardwood content (0–25%, 40–65%, and 75–95%) across a gradient of fir-hardwood stands, with 27 plots sampled near Amqui, Quebec in an early stage spruce budworm outbreak (3 years of defoliation), and 9 plots sampled in three stands in the North Shore of Quebec in a later stage budworm outbreak (7 years of defoliation). Linear mixed-effects models with repeated measures (years) were used to analyze differences in defoliation of fir regeneration as a function of hardwood content, six height classes, and three years (2013, 2014, 2015). In the Amqui plots, defoliation of fir regeneration was significantly related to all factors and interaction terms except for hardwood content, while in the North Shore plots, defoliation was significantly related to all factors and the hardwood content x height class and hardwood content x year interaction terms (Nie et al. 2018). Defoliation of balsam fir regeneration was 85% higher in softwood than in hardwood stands in 2013 and 2014 in the North Shore plots, when the budworm outbreak was severe. Defoliation was at least 10% higher on regeneration taller than 30 cm than on smaller regeneration in the Amqui plots in 2015 and over 15% higher in the North Shore plots (Nie et al. 2018). In general, balsam fir regeneration in softwood stands had higher levels of defoliation than in hardwood stands when defoliation was severe, and regeneration taller than 30 cm had higher defoliation than smaller regeneration.

3. Needle longevity of balsam fir is increased by defoliation by spruce budworm.

The plots in Amqui and Causapscal, QC were used by UNB MEM student Olivia Doran in a study of whether defoliation increases longevity of remaining needles on balsam fir, published in Doran et al. (2017). Conifers experiencing environmental change affecting photosynthetic capacity have been observed to compensate by adjusting foliage longevity and increasing retention of old foliage. Defoliation by SBW is one such change, therefore, it was hypothesized that conifers may increase foliage life spans to compensate for losses of photosynthetic capacity to defoliation. Understanding foliage longevity is a key component of predicting foliage complement, which is the main driver of effects of defoliation on forest growth and productivity. Defoliation and needle loss were assessed on 16 age classes of foliage on mid-crown branches sampled from 134 mature balsam fir trees near Amqui and Causapscal, Quebec, and related to needle age and cumulative defoliation (summed annual defoliation from 2012-2016). A general linear mixed model fitted to the needle survivorship data accounted for 68% of the total deviance (Doran et al. 2017). The model interaction term of cumulative defoliation with foliage age indicated that for foliage ages less than about 9 years, increased cumulative defoliation resulted in lower needle survival, possibly because of backfeeding of spruce budworm on 5+ year-old foliage, while in the older age classes, needle survival increased with increasing cumulative defoliation. For the oldest, 11 to 16 year old foliage age classes, 10-16% more foliage per age class was retained under severe defoliation than under light defoliation. As a result, median needle age increased from 9.5 years to 10.4 years as cumulative defoliation increased from 0% to 500% (Doran et al. 2017). Mean needle longevity of 9.5 years for 50-year-old balsam fir with low defoliation observed in this study was considerably higher than the mean of 5.5 years previously observed for 22- to 27-year-old fir in Cape Breton, Nova Scotia. Such differences in needle longevity and retention would cause considerable differences in predictions of tree growth using foliage-based stand growth models.

4. Develop an optimum operational spray blocking tool

The Accuair *ForPRO* DSS (McLeod et al. 2011) allows integration between forest management planning and optimization models and underlying tree impact information. Advantages of *ForPRO* include optimization of treatment schedules to reduce losses and area sprayed, more accurate determination of impacts on harvest levels, and integration of salvage activities with protection. The goal of the *ForPRO* II model is to minimize the cost and effort required to achieve a given control objective, and to judiciously using Btk and tebufenozide biological control and application technologies to increase the social acceptability of these technologies.

An operational insecticide block planning tool was developed by Dr. Chris Hennigar using heuristic algorithms to implement EIS control strategies. This is being used by FPL and NB ERD (Jeremy Gullison) in designing the 2018 EIS spray trials. The blocking tool (Fig. 2) uses 1 ha (100m x 100m) cells, and combines an interpolated SBW L2 sample raster and a % spruce- fir layer, along with tests of alternative desired flight directions, to produce optimal spray blocks.

The blocking tool process and initial results for 2018 are shown in Fig. 3. For 2018, the L2 spatial interpolation method algorithm was changed to be a combination of four interpolation methods. Emphasis in the blocking algorithm was put on the SBW population data: interpolated log L2 (scaled 1-100) + % spruce-fir (scaled 1-10) (Fig. 3A). Known L2 sample points with moderate SBW population levels (≥ 7 L2/branch) were set to high priority, to include them in blocks, and treated for 1000m surrounding the actual sample point (Fig. 3B). In the surrounding area, we assumed that SBW population levels would be higher in areas with high spruce-fir, given a specific interpolated SBW population level; this was the reason for including %spruce-fir as an additive factor. Areas with <20% SPBF were excluded. Habitation and other operational setbacks were included in all scenarios (Fig. 3C). The New Brunswick policy permits landowners to opt out of any planned provincial spray program, if desired, and all landowners are notified. However, given that EIS is area-wide SBW population management, we attempt to treat all high SBW area regardless of ownership. The resulting blocking tool solution (Fig. 3D) is then refined by FPL analysts. Advantages of use of the blocking tool are 1) it is an objective, optimal solution, and 2) it can be rerun in hours to incorporate any desired alternative changes (e.g. differing budgets and program sizes, differing inclusion of low SPBF and high hardwood areas, riparian buffers, etc.).

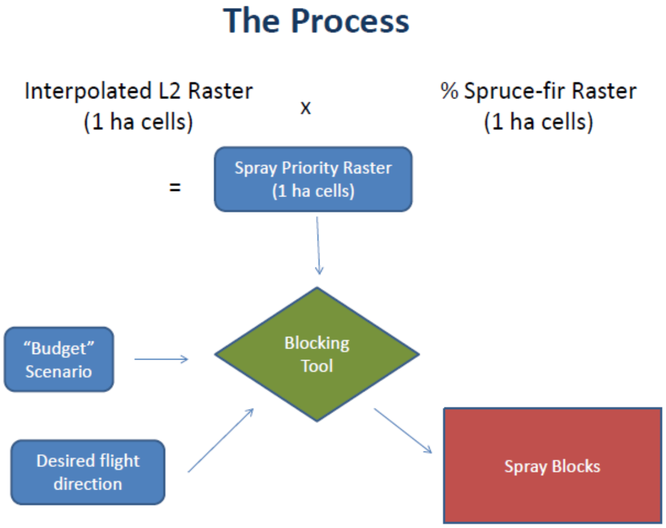


Fig. 2. The blocking tool process developed by Dr. Chris Hennigar and tested by Jeremy Gullison and Chris Hennigar

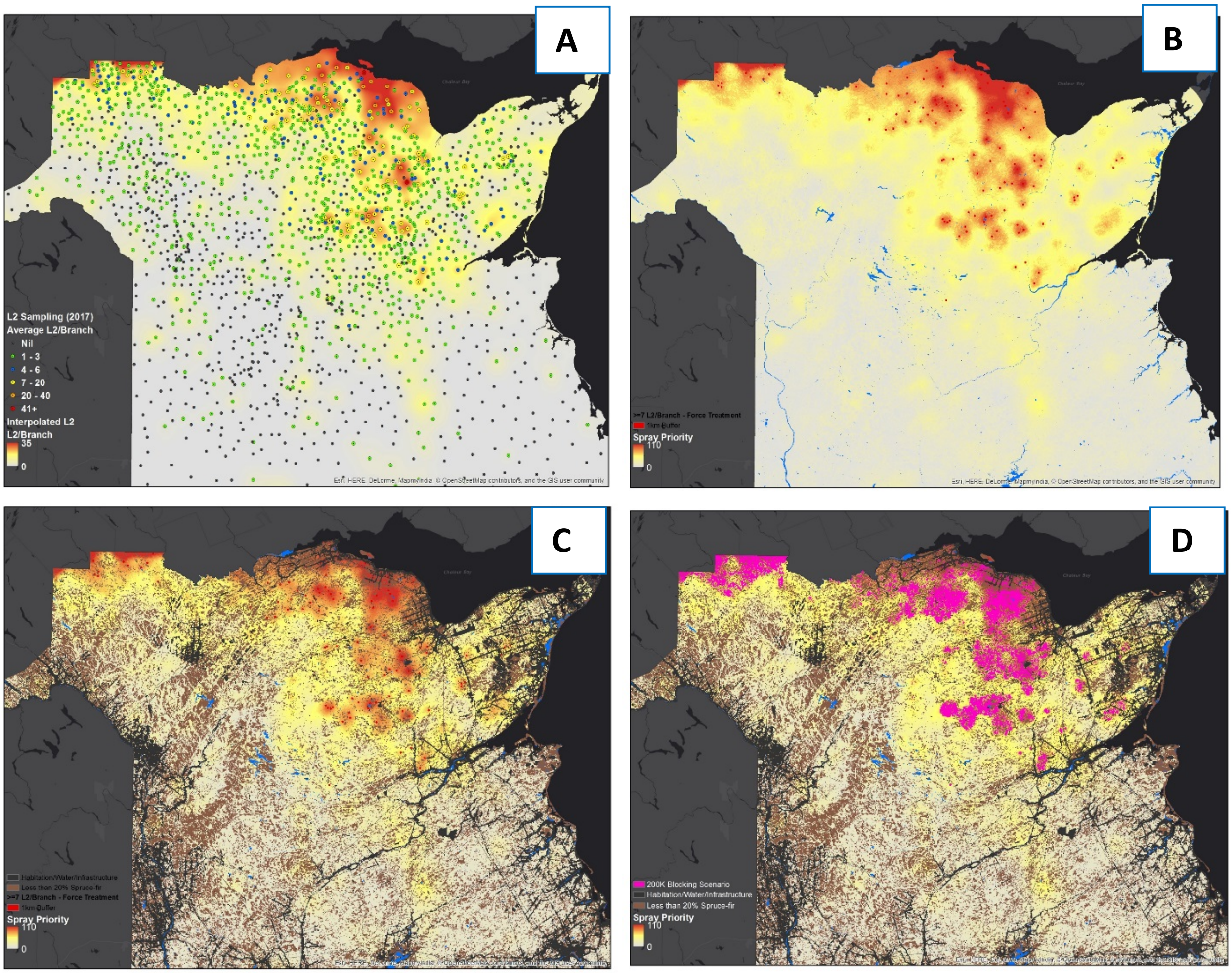


Fig. 3. Results of a test of the blocking tool in planning the 2018 New Brunswick EIS spray program. A. Interpolated SBW Spray priority layer = log L2 (scaled 1-100) + %SPBF (scaled 1-10). B. Spray priority layer with moderate sample points (≥7 L2/branch) given high priority. C. Operational exclusions (brown). D. Proposed treated area (pink) based on a 200,000ha budget.

Based on both project budget and tests of alternative program sizes from 150,000ha to 300,000ha, the tentative recommended 2018 EIS spray program will be 200,000ha to 225,000ha (Fig. 3D), depending upon available budget.

5. Timber supply and economic analyses of EIS and reactive protection scenarios

Dr. Chris Hennigar and other staff from NB ERD have conducted timber supply analyses for all Crown licenses in NB, projecting effects of EIS, moderate, and severe SBW outbreak scenarios and differing levels of protection (5-40% of area) on timber supply for 80 years. UNB MScF student Eric Liu is then taking the timber supply results and determining direct and indirect effects on the NB economy. Numerous model runs and revisions of the data and methods have been completed, and initial results produced, but they are under revision currently and not quite ready for presentation. Initial results (Fig. 4) indicated that moderate and severe SBW outbreak scenarios, with no protection, would result in up to 30% and 50% maximum reduction in 5-year timber supply in NB, occurring 20 to 30 years following the beginning of the SBW outbreak. Final results should be available by spring 2018.

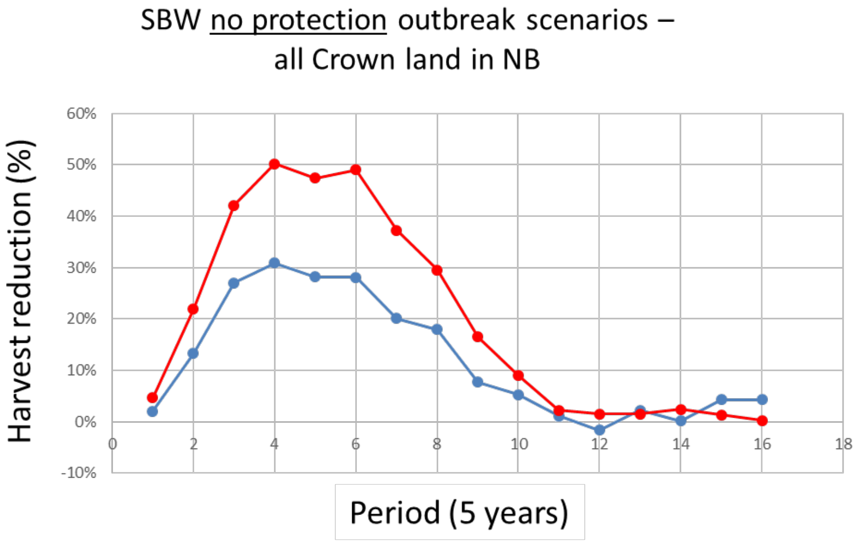


Fig. 4. Projected harvest reductions for Crown land in NB under moderate and severe SBW outbreak scenarios (MacLean et al. 2001) with no insecticide protection.

Conclusions

Following 4 years of EIS treatments of low but increasing SBW populations, L2 populations across northern NB are considerably lower than adjacent SBW populations across the border in QC. Populations in blocks treated with both Btk and tebufenozide are consistently reduced and generally do not require treatment in the subsequent year. Increases in SBW populations in northern NB, based on intensive L2 sampling, are continuing to increase but at a relatively slow pace (so far). These successful results, to date, have resulted in the Healthy Forest Partnership EIS Tea submitting proposals to the federal and Atlantic Canada provincial governments for funding to continue the EIS SBW project in northern NB.

Acknowledgements

This research was supported by the ACOA Atlantic Innovation Fund, Forest Protection Limited, forest industry in New Brunswick, the New Brunswick Department of Natural Resources, and SERG-International. It forms Study 2.2.8 of the project *Early Intervention Strategies to Suppress a Spruce Budworm Outbreak.*

Literature Cited

Doran, O., D.A. MacLean, and J.A. Kershaw Jr. 2017. Needle longevity of balsam fir is increased by defoliation by spruce budworm. Trees 31(6): 1933-1944.

Hennigar, C.R., MacLean, D.A., Porter, K.B., and Quiring, D.T. 2007. Optimized harvest planning under alternative foliage-protection scenarios to reduce volume losses to spruce budworm. Can. J. For. Res. 37: 1755-1769.

Hennigar, C.R., Wilson, J.S., MacLean, D.A., and Wagner, R.G. 2011. Applying a spruce budworm decision support system to Maine: projecting spruce-fir volume impacts under alternative management and outbreak scenarios. J. Forestry 109: 332-342.

Hennigar, C.R., Erdle, T.A., Gullison, J.J., and MacLean, D.A. 2013. Reexamining wood supply in light of future spruce budworm outbreaks: a case study in New Brunswick. For. Chron. 89: 42-53.

Huang, Z. 2015. Remote sensing of spruce budworm defoliation in Québec, Canada using EO-1 Hyperion data. MSc Engineering Thesis, University of New Brunswick, Fredericton, NB. 152p.

MacLean, D.A., Erdle, T.A., MacKinnon, W.E., Porter, K.B., Beaton, K.P., Cormier, G., Morehouse, S., and Budd, M. 2001. The Spruce Budworm Decision Support System: forest protection planning to sustain long-term wood supplies. Can. J. For. Res. 31: 1742-1757.

McLeod, I.M., Lucarotti, C.J., Hennigar, C.R., MacLean, D.A., Holloway, A.G.L., Cormier, G.A., and Davies, D.C. 2012. Advances in aerial application technologies and decision support for integrated pest management. In: Integrated Pest Management and Pest Control, S. Soloneski and M.L. Larramendy, Eds. InTech Open Access Publisher, Rijeka, Croatia. ISBN 978-953-307-926-4. Pp. 651-668.

Nie, Z., MacLean, D.A., and Taylor, A.R. 2018. Forest overstory composition and seedling height influence defoliation of understory regeneration by spruce budworm. For. Ecol. Manage. 409: 353-360.

Su, Q., Needham, T.D., MacLean, D.A., 1996. The influence of hardwood content on balsam fir defoliation by spruce budworm. Can. J. For. Res. 26: 1620-1628.

Zhang, B., and MacLean, D.A. 2015. Determining the mechanism of impact of hardwood content on spruce budworm defoliation of balsam fir. Pp. 140-147. In: Proc. SERG International 2015 Workshop. Feb. 3-5, 2015, Sault Ste. Marie, ON.

Validation of a spruce budworm phenology model across environmental and genetic gradients: applications for budworm control and climate change predictions

Interim Report

Jean-Noël Candau[1]

2018-01-19

Project #: SERG-International 2016/06-2016-114

Collaborators: Ashlyn Wardlaw[1], Kerry Perrault[1], Amanda Roe[1], Emilie Vaillancourt[1], John Dedes[1], Kala Studens[1], and Chris MacQuarrie[1]

[1] Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste Marie, P6A 2E5, Ontario.

Abstract

This report presents the results of an ongoing investigation of the variability of spruce budworm development rates over its geographic distribution. The first phase of the study consisted in rearing laboratory colonies from the IPQL in SSM at 7 constant temperatures (i.e. 5-35ºC). This allowed us to develop and test rearing protocols. It also provided an opportunity to assess the phenology of laboratory colonies that have been kept in isolation from wild populations for many generations against Biosim's predictions. Our results indicate that the model fits very well the phenology of the laboratory colonies over the 15-25ºC range. For lower and higher temperatures (5-10ºC and 30-35ºC, respectively), the model tends to overestimate development rates. In 2017, we successfully completed the second phase of the project that consisted in establishing colonies from wild budworm populations sampled in 5 locations across its geographic distribution, from Inuvik to Northern Quebec. A trend analysis of budworm simulated phenology at these 5 locations for the period 1998-2017 reveals a trend towards a faster overall larval development (L2-L6) statistically significant in Inuvik (NWT) and almost significant in High Level (AB). The samples collected in these locations were reared through to the next generation to provide enough individuals in each colony to run 7 temperature experiments. Preliminary results for the Ontario colony reared at 30ºC and 35ºC are similar to what was observed with the IPQL colonies, i.e. the current phenology model tend to overestimate the development rate at these temperatures. Other temperature treatments for Ontario and Quebec are ongoing while the other colonies have not yet completed their obligatory diapause.

Résumé

Ce rapport présente les résultats de travaux de recherche en cours sur la variabilité du taux de développement de la tordeuse des bourgeons de l'épinette à travers son aire de distribution. La première phase des cette étude consistait à élever des colonies du Service de Production d'Insectes de SSM à 7 températures constantes (i.e. 5-35ºC). Ceci nous a permis de développer et tester les protocoles d'élevages qui seraont utilisés par la suite. Cela nous a aussi donné la possibilité de comparer la phénologie de colonies isolées des populations sauvages depuis de nombreuses générations avec les prédictions du modèle Biosim. Nos résultats indiquent que le modèle est bien ajusté à la phénologie des populations de laboratoire pour des températures comprises entre 15-25ºC. Pour les températures plus basses ou plus hautes (5-10ºC et 30-35ºC, respectivement), le modèle surestime le taux de dévelopement. En 2017, nous avons complété avec succés la deuxième phase du projet qui consistait à établir des colonies à partir de populations sauvages échantillonnées dans 5 endroits répartis à travers l'aire de distribution de la tordeuse, depuis Inuvik jusqu'au nord du Québec. Une analyse de tendance des estimations de phénologie de la tordeuse pour les endroits échantillonnés durant la période 1998-2017 révèle une tendance vers un développement larvaire total (L2-L6) plus rapide statistiquement significatif pour Inuvik (NWT) et pratiquement significatif pour High Level (AB). Les échantillons prélevés dans les 5 endroits ont été élevées jusqu'à la génération suivante de façon a assurer assez d'individus dans chaque colonie pour réaliser 7 traitements a différentes températures. Les résultats préliminaires obtenus sur la colonie de l'Ontario à 30ºC et 35 ºC sont similaires aux résultats obtenus avec les colonies du Service de Production d'Insectes, i.e. le modèle de dévelopement larvaire de la tordeuse surestime le taux de dévelopement à ces températures. Le reste des traitements pour la colonie de l'Ontario et les traitements pour la colonie du Québec sont en cours. Les autres colonies sont encore en diapause.

Introduction

The ability to accurately predict spruce budworm phenology is important in many aspects of the management of this insect. In control programs, the efficacy of all current spruce budworm IPM strategies hinges on the ability to target the right development stage. In predicting population dynamics, a reliable model of adult emergence phenology is a key component in modeling landscape-scale dispersal, a factor that is increasingly recognized as being critical in budworm large-scale dynamics. In longer-term predictions, an accurate knowledge of budworm phenology, intrinsically and in relation with its hosts' phenology, is of particular importance in our ability to predict the potential impacts of climate change on the distribution of this insect. The most accurate model currently available to predict the phenology of spruce budworm over large areas is BIOSIM's Spruce Budworm Biology model (Régnière et al. 2014). This model has been tested against field observations in several occasions. In most cases, it was able to predict budworm phenology with exceptional accuracy. However it is important to note that the data used so far to develop and validate this model, have come from a fairly limited geographic range (i.e., roughly between 45º-50ºN and 70º-90ºW). A comparison with the current distribution of spruce budworm (between 43º-68ºN and 55º-133ºW) questions ultimately the limits of applicability of the model. This project investigates the variability of spruce budworm development rates for each larval stage across the geographic distribution of the insect. Particular attention is given in detecting clinal variation in development rates related to environmental and/or genetic gradients. Comparing the observed rates to Régnière's model for a wide distribution of locations will help better define the geographic limits of applicability of the model.

Development rate of laboratory colonies at different temperatures

The first step in this project consisted in rearing laboratory colonies at different constant temperatures. The main objective of this exercise was to test if the relationships between temperature and development rates in these colonies, which had been reared for dozens of generations at constant temperature regimes, had "drifted" from the observations made 30 years prior. It also provided the opportunity to develop and test specific rearing protocols, test the rearing equipment and train staff. To our knowledge, the mass rearing of spruce budworm at different temperatures through its entire life cycle had not been carried since the mid-1980's, so most of the expertise in this type of experiment has to be rebuilt.

Material and Methods

Laboratory colonies were obtained from the Insect Production and Quarantine Lab (IPQL) facility at the Great Lakes Forest Centre (Sault Ste Marie). Each temperature experiment started with a large number of overwintering L2s that were kept at 25±3°C, 55±10%RH and 16L:8D, regardlesss of the experimental temperature treatment, until emergence from diapause. From the emerged L2s, 250 larvae were sampled, placed in individual cups with artificial diet (provided by IPS), and labeled for the experiment. All 250 cups were then placed in a growth chamber (Conviron) at a set experimental temperature. We tested 7 temperature treatments: 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C, all ±1°C with 70±20%RH and 14L:10D. The temperature in each chamber was recorded every minute with a data logger (Hobo). Out of the 250 larvae in each chamber, 200 were monitored daily for molting, the remaining 50 was left untouched until pupation and used as backup for mating and genetic analyses. Daily monitoring consisted in checking the status (live/dead) and larval stage of each larva. When the development reached pupal stage, the sex of each individual was assessed by counting the number of abdominal rings. Adults were mated shortly after emergence. After harvesting, The eggs were hatched at 20°C regardless of the temperature treatment. First stage larvae were reared at the same temperature to pre-diapause L2s. The pre-diapause L2s were then placed at 4°C for 24 weeks for diapause. The rearing protocol was repeated for the second generation, the temperature treatment of each F1 was identical to the treatment for its parent population.

Extreme temperatures near development thresholds (high and low) have detrimental effects on larval development and result in high rates of mortality. Development rates at these temperatures have to be estimated using temperature transfers (Régnière et al. 2012). This technique consists in rearing the larval stage at the extreme temperature for a period of time long enough to measure developement but not too long to induce high levels of mortality, then transfer to a more favorable temperature to complete the stage development, then repeat for the next larval stage. Temperature transfers were applied to the 5°C, 10°C, 30°C, and 35°C temperature treatments. All transfers were made to 20°C to complete stage developments.

Observed development rates were compared to Régnière's phenology model by applying the set of equations published in Régnière et al. 2012 to the temperatures recorded in each chamber.

Results

We will present here the results for 5ºC-35ºC for the first generation. The experiments for the second generation are still on-going at the time of this report. Simulated and observed development rates are represented here as average instar (Appendix A) and larval stage development times (Appendix B).

At 5ºC (Fig. A1), the simulated development is consistently faster than observed (R^2=0.69). The differences are particularly pronounced at the L2 and L4 stages (Fig. B1). Observed days to moult at the L4 stage are 15 days longer than simulated. This difference in development time can be partly due to the fact that Biosim will simulate an immediate increase in the development rate when the larvae are transfered to 20ºC whereas there is likely a delayed response due to physiological constraints. This difference is compounded by the fact that Biosim slightly overestimates the development rate at 20ºC (see below). Considering the importance of the response of early stages of development to temperatures in the overall timing of the post-diapause phenology and the likelihood that early stages are exposed to low temperatures, it is critical to improve the development model for early stages at low temperatures. The model improves in simulating the overall development at 10ºC (R^2=0.82, Fig. A2). The development rates of the L2, L4 and L5 stages at this temperature are overestimated (Fig. B2). At 15ºC, the fit of the overall development (Fig. A3) is excellent (R^2=0.98). The development rate of the L2 stage is overestimated by the model. The model underestimates the development rates of later stages (L5 and L6). The model is able to fit very well the development at 20ºC (R^2=0.97) with only a slight overestimation after L3 (Fig. A4). At 25ºC (Fig. A5), the model simulates overall development very well (R^2=0.99). L2 and L4 development rates are slightly overestimated while the rate of later instars are underestimated, particularly the female L6 stage. The performance of the model are not as good at higher temperatures. At 30ºC and 35ºC (Fig. A6 and A7, respectively), the model does not perform as well (R^2=0.68 R^2=0.81, respectively). It overestimates the development rate of the L2 stage at 30ºC and 35ºC and underestimates de rate at later stages (Fig. B3 and B4).

Discussion

In summary, the current phenology model performs very well in predicting the larval development of IPQL laboratory colonies for temperatures ranging from 15-25ºC. This suggests that the phenology of the current colonies is not noticeably different from the phenology of the colonies used to parametrized de model over 3 decades ago. The model does not perform as well at extreme temperatures, particularly 5ºC and 30ºC. The temperature transfers required at these temperatures (see Methods above) are simulated as instanteneous shifts from one development rate to the other which is very unlikely to reflect what happens physiologically. Indeed, there is likely a period of time necessary for the physiology of the larva to adjust to the new temperature. This could in part explain why the model almost systematically overestimates the developemnt rate at these temperatures. It also points to the challenges of using estimates of development rates measured at constant temperatures to model development at variable temperatures.

Development rates across environmental and genetic gradients

To assess the variability of budworm development rates across its geographical range requires sampling different locations and establishing laboratory colonies from each sample. To our knowledge, no new colonies of spruce budworm have been established from wild populations since the development of the IPQL colonies over 30 years ago. The task can be challenging because of the absence of published protocols and potential high levels of mortality and low fecundity due to microsporidia and viruses. In an effort to minimize the risk of disease cross-contamination that would lead to colony collapse, we reared the larvae individually and mated adults in separate pairs (vs a large number of males and females in the same container). Infection loads were checked regularly along the process so infected individuals could be discarded. It took 3 FTEs from March to Oct. 2017 to complete the rearing of 5 populations from flushing diapausing L2s to the next generation of L2s in diapause. We will describe below the protocol we used and the results of the first temperature treatments for the Ontario population.

Sampling and rearing methods for wild populations

The objective of the sampling was to cover the maximum range of budworm geographical distribution. At the same time, samples had to be collected from areas that were currently or recently at outbreak population levels to maximize the chances of having enough larvae to start colonies. We believe that the 5 populations we sampled (Fig. 4) were the maximum that could be done considering the current population levels across the range. If outbreaks were to develop in other areas, it would be interesting to add sampling locations between Alberta and Ontario, in the southern edge of the distribution, and in NewFoundland.

Our sampling protocol followed Morris (1955): 3 branches, 75-100 cm long each, were sampled from the crown of approximately 30 trees in each location except for the Quebec population that was sampled in 18 locations along a transect from Manic-5 to Fermont. The branches were separated by source tree and stored in canvas or plastic bags for storage and shipping. The samples were collected from June 2016 to May 2017 (Table 1).

Table 1: Summary of sampling locations

Prov/T	Date	Location	Lat	Long	Host	Collector credit
NWT	02-05-2017	Inuvik	67.5	-133.7	Sw	Martin Callaghan (Forest Management Division, Gvt of NWT)
AB	05-04-2017	High Level	58.6	-118.2	Sw	R. Hermanutz (Alberta Agriculture and Forestry)
ON	01-03-2017	Timmins	48.6	-82.1	Fb, Sb	G. Brand (CFS-GLFC)
QC	19-09-2016	Manic-5	50.7	-68.7	Fb, Sb	L. deGrandpré and his crew (CFS-LFC)
NB1	28-06-2016	Campbelton	47.8	-66.4	Fb	R. Johns and his crew (CFS-AFC)
NB2	07-03-2017	Balmoral	47.8	-66.7	Fb	R. Johns and his crew (CFS-AFC)

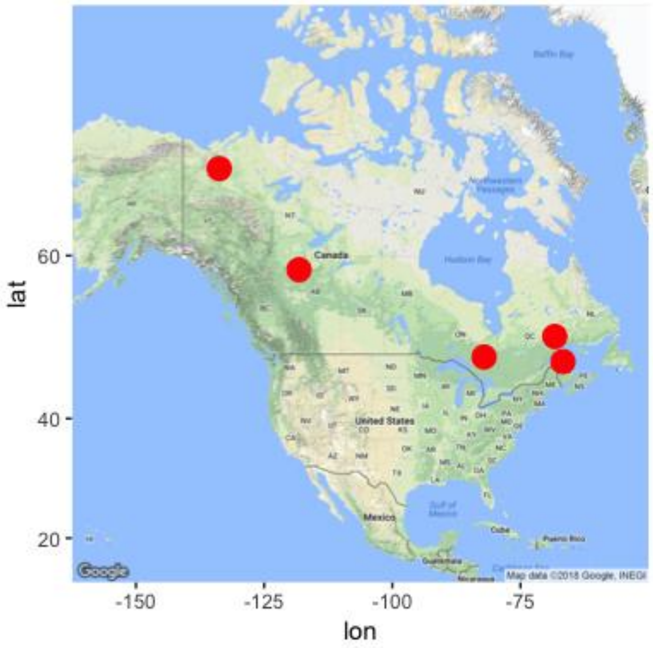


Figure 4: Location of wild populations sampled in 2017

Flushing Methods

If the samples were collected in the spring, flushing was carried out immediately after receiving the branches. Branches that were collected in fall or winter during diapause were stored at 4°C, 70% RH in the dark until the following spring. Traditionally, there are 2 methods of flushing overwintering larvae from branches (Sanders, 1980). The “paper cone”" method consists in wrapping branch samples cut in small bundles in a cone-shaped paper suspended over a wash basin filled with water. Emerging L2s are collected while they are crawling at the surface of the paper or floating at the surface of the water after they dropped from the branches. The “enclosed box” method consists in placing the branches in pyramid-shaped opaque boxes fitted with transparent vials at the top that are pointed towards a bank of lights. Phototactic larvae are collected from the vials. The 2 methods were compared on the Ontario and Quebec samples. The enclosed box method was much less successful than the paper cone method. There were several issues with the enclosed box method that may have limited its success. First, mold developed in the boxes that were set up with water soaked paper towel around the base of the branches. The high level of mold in the boxes may have caused mortality or limited upward movement of the larvae. Open glass vials containing water-soaked gauze were a second option to allow some moisture in the box without directly touching the branches. This seemed to have little effect on emergence versus completely dry boxes, but did limit mold growth. Third, many of the boxes had noticeable levels of mortality inside the bottom of the box. If this method is attempted, attention needs to be paid to humidity levels. It is also recommended that branch segments placed inside the box stay as intact as possible, and that boxes are not overstuffed, allowing the best circumstances for larvae to move upwards. In contrast to the enclosed box method, the branches in the paper cone method are fully visible during emergence, allowing more larvae to be spotted and collected, rather than relying on them to maneuver to a collection area on their own. Additionally, moisture levels can more easily be controlled with the branches exposed. One issue with this method is high levels of needle loss. Water basins will need to be cleaned almost daily with a straining device after collection of larvae from the water surface. Sometimes, the amount of needles in the basin hindered collection because they obscured the water's surface. Additionally, water had to be changed weekly in order to prevent mold growth on stray needles floating in the water. A final issue with both methods was the collection of non-target species. When the larvae are in the L2 stage, they are hard to identify by species. Larvae were often not identified as a non-target species until several instars later, when they had already been placed on diet. At that time, they were discarded. The number of larvae flushed from the samples ranged from 4466 in NB to 648 in NWT (Table 2).

Rearing Methods

Once larvae were collected, they were individually placed on McMorran artificial spruce budworm diet in ¾ oz cups with a cardboard lid. Since the populations were wild and contained possible pathogens, the diet contained Fumidil-B at 4000 ppm (van Frankenhuyzen et al. 2004). They were labelled by population and collection date, and placed into a 20° C walk-in chamber. Diet was changed every 14 days. For each population, a subsample of 10-12% of L4 or L5 stage larvae was tested for pathogen load. The results of these tests (Table 2) dictated the level of isolation needed for mating the adults in each population. Populations with low or no presence of virus or fungal infection were placed into several group matings as well as several single matings. If pathogen levels were medium to high, only single matings (one to two males and one to two females per mating group) were used. After single matings were completed, every adult female used for mating was sent to the quality control laboratory for pathogen testing. If fungi or more than one type of virus was found, the eggs produced by that mating were discarded. This procedure is necessary because of the potential vertical transfer of microsporidia (van Frankenhuyzen et al. 2007). Microsporidia infection rates were high in AB, while NPV and EPV were high in ON and QC (Table. 2).

Table 2: Infection rates in wild samples collected in 2016-17

Population	Larva flushed	% Microsporidia	% NPV	% EPV
AB	2370	36.5	8.7	0.0
NWT	648	1.2	9.5	0.0
NB	4466	6.8	15.0	1.6
ON	1084	10.8	41.2	5.9
QC	765	0.0	33.9	32.1

An analysis of 20 years of simulated spruce budworm phenology across its distribution

While our rearing experiments of wild colonies are starting to produce empirical data to measure the variability of spruce budworm phenology across its natural range, we can already establish a baseline by simulating budworm phenology for different locations using historical weather data and the current phenology model. We are presenting here an analysis of the simulation of budworm's phenology for the past 20 years in locations across its distribution range. We downloaded Environment Canada's daily temperature data for the past 20 years for 5 weather stations close to the wild populations sampling locations, i.e. Inuvik (NWT), High Level (AB), Timmins (ON), Baie Comeau (QC), and Frederiction (NB). We used Sen's (1968) non-parametric method to test for linear trends in the time-series, and Pettit's (1979) test to identify change points (i.e. abrupt changes in the time-series). For the period 1998-2017, annual mean daily temperatures for each location (Fig.1) show a significant warming in Inuvik (+0.11ºC/year, p<0.001). The graphs seemed to indicate a slight cooling in Eastern Canada but no significant trend was detected. The change point analysis detected a probable change in Inuvik in 2007 with an increased warming rate (+0.17ºC/year, p<0.001 for 2007-2015). Change points were detected in the other locations but they did not reveal significant trends.

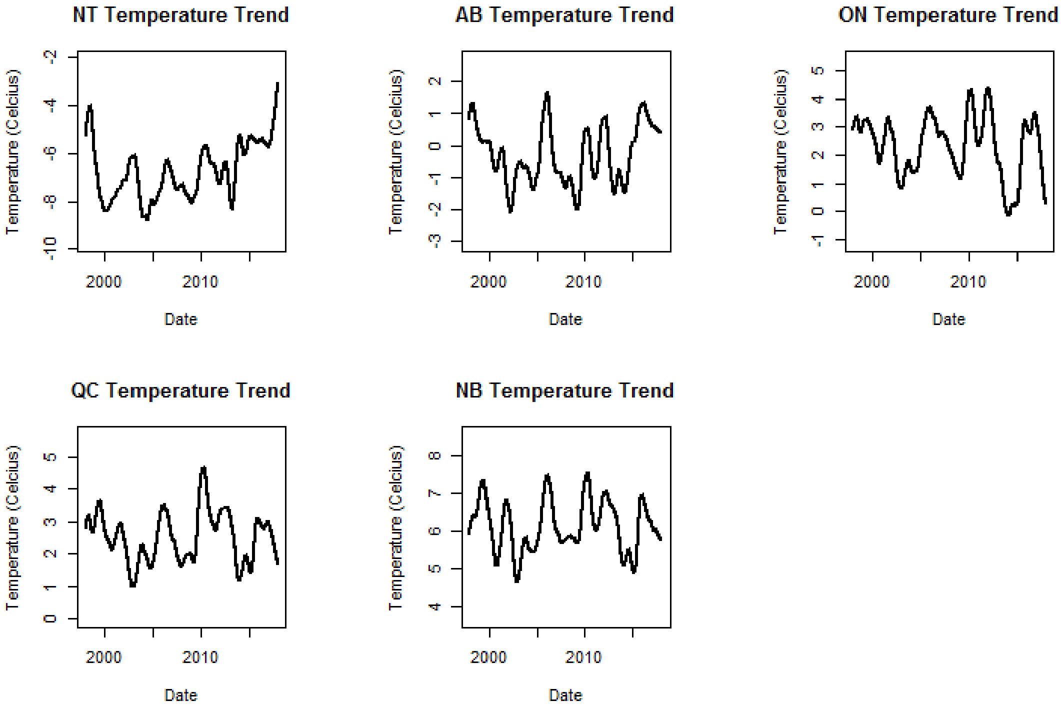


Figure 1: Trends in mean daily temperature between 1998-2017 for 5 weather stations close to the sampling locations: Inuvik (NT), High Level (AB), Timmins (ON), Baie Comeau (QC), and Fredericton (NB)

Annual spruce budworm phenology was simulated for each location, using daily temperature records and Biosim. Development times at each location (Fig.2) show a clear segregation of the number of days at the overwintering L2 (L2o) stage with longer duration in NT, followed by QC, AB, ON, and NB. On average, the duration at the L2o stage is 38 days longer in Inuvik than in Fredericton. It is noticeable that, at the L3 stage the ranking among locations is almost reversed: the duration in that stage is shorter in the NWT followed by AB, ON, QC and NB.

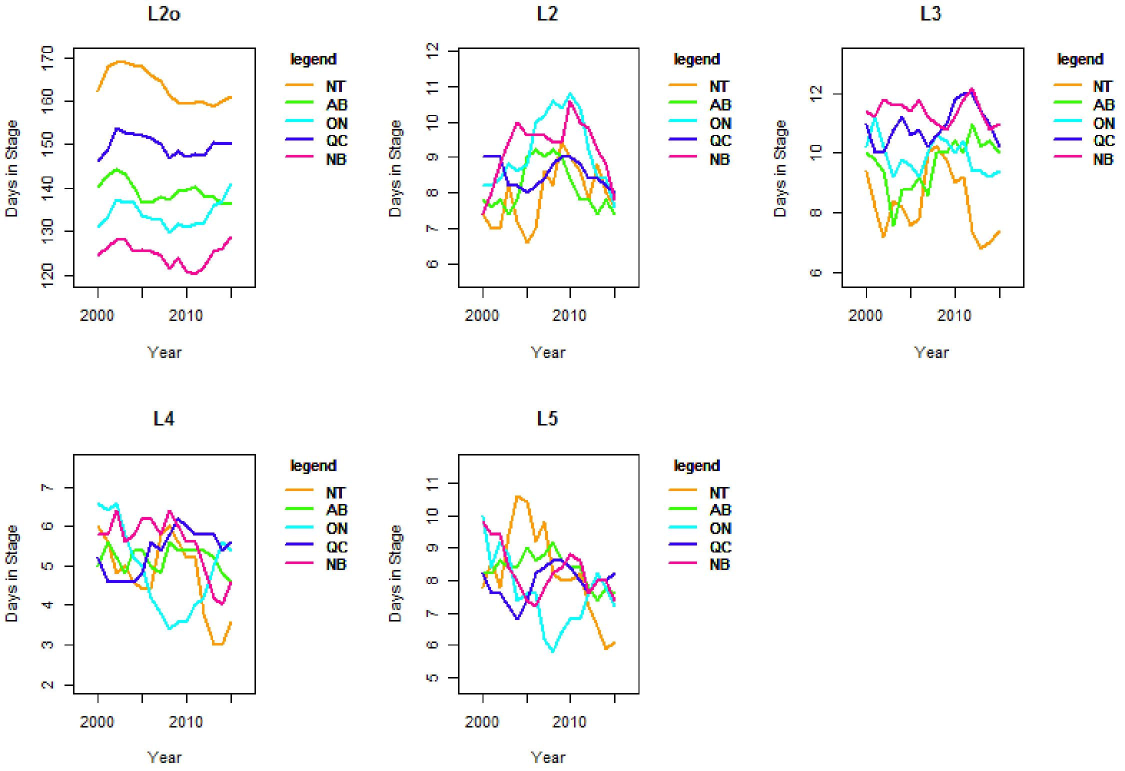


Figure 2: Simulated development times at each larval stage for 5 weather stations close to the sampling locations

Trend analyses reveal significant trends for different larval stages in various locations. In the NWT, the time in stage decreased significantly for L2o (-0.7d/yr), L4 (-0.2d/yr), L5 (-0.2d/yr) and L6 (-0.4d/yr) since 1999. In AB, stage duration decreases at the L2o and L6 stages (-0.4d/yr and -0.1d/yr respectively), but it increases at the L3 stage (+0.1d/yr). In ON, the only significant trend is a small increase in the duration of the L6 stage (+0.1d/yr). In QC, the L4 stage shows a small increase (+0.1d/yr). In NB, the duration of the L6 stage shows a significant positive trend (+0.2d/yr).

When the duration of development is summed across stages from L2o to pupa (Fig. 3), the total duration decreases significantly in the NWT (-0.7d/year, $p<0.05$) and almost significantly in AB (-0.6d/yr, $p=0.06$).

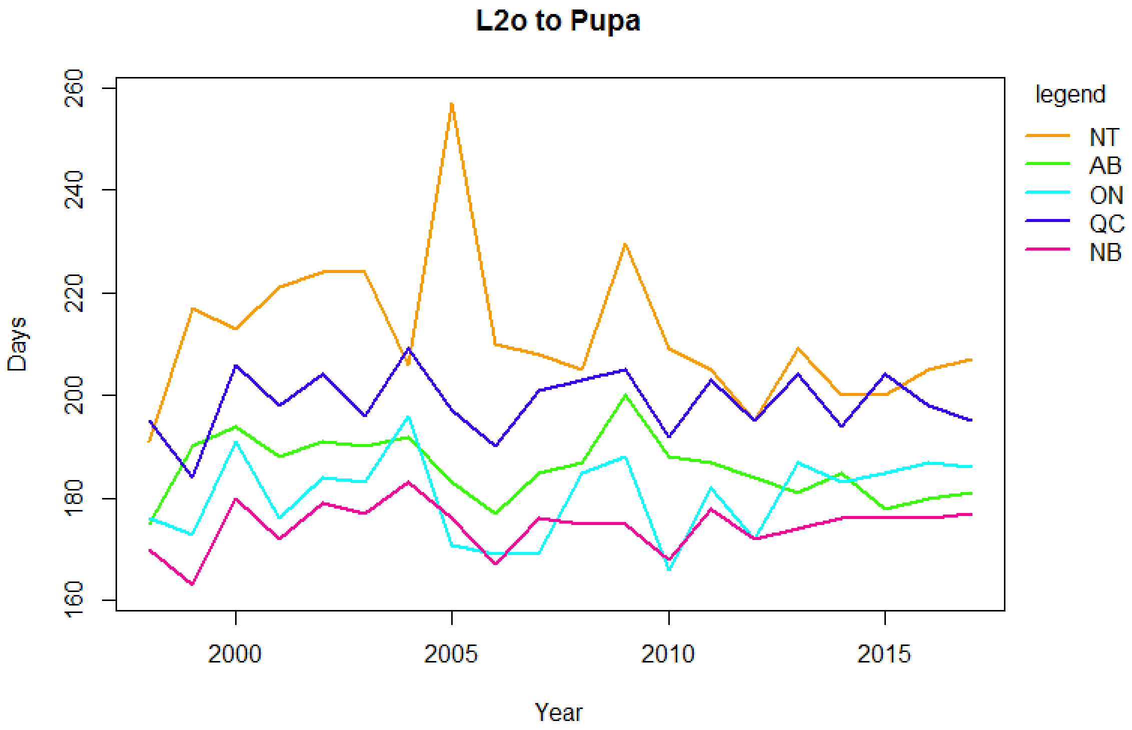


Figure 3: Simulated development time to reach pupal stage close to the sampling locations of the wild populations

Development rates of the Ontario colony at 30ºC and 35ºC

At the time of this report, we have completed the rearing the Ontario colony at 30ºC and 35ºC. The rest of the temperature treatments (i.e., 5, 10, 15, 20, 25ºC) are ongoing. We also started to rear the Quebec colony. The other colonies are still in diapause. The expected start of the temperature treatments for these is between the end of January (NB) and the beginning of April (NWT). The experimental protocol used for rearing the wild colonies is identical to the protocol used for the IPQL laboratory colonies.

At 30ºC, the development of the Ontario colony appears slower than Biosim predictions (Fig. 4). This is similar to what was observed with the IPQL colony. The observed development at the L2 stage (Fig. 5) is slower than simulated and slightly slower than the development of the IPQL colony. This is partly compensated by a faster development of the Ontario and IPQL colonies at the L5 stage compared to the model predictions. The model predicts well the development of L3 (better than it predicts IPQL colonies) and L6 stages.

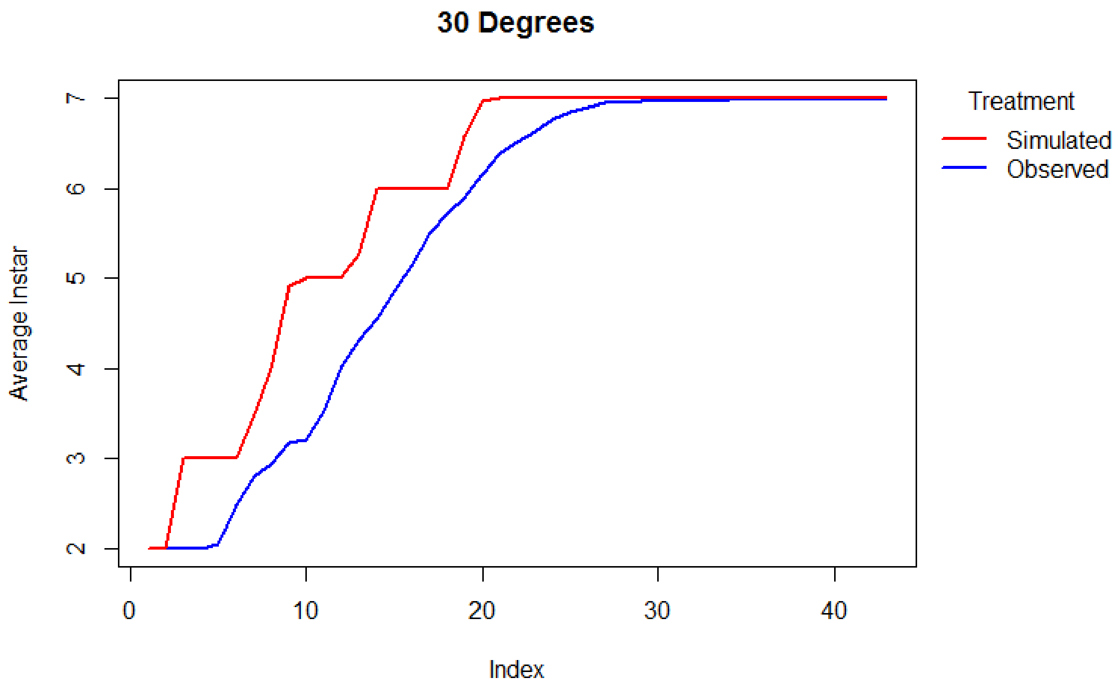


Figure 4: Average instar development of the Ontario colony compared to Biosim simulation at 30ºC

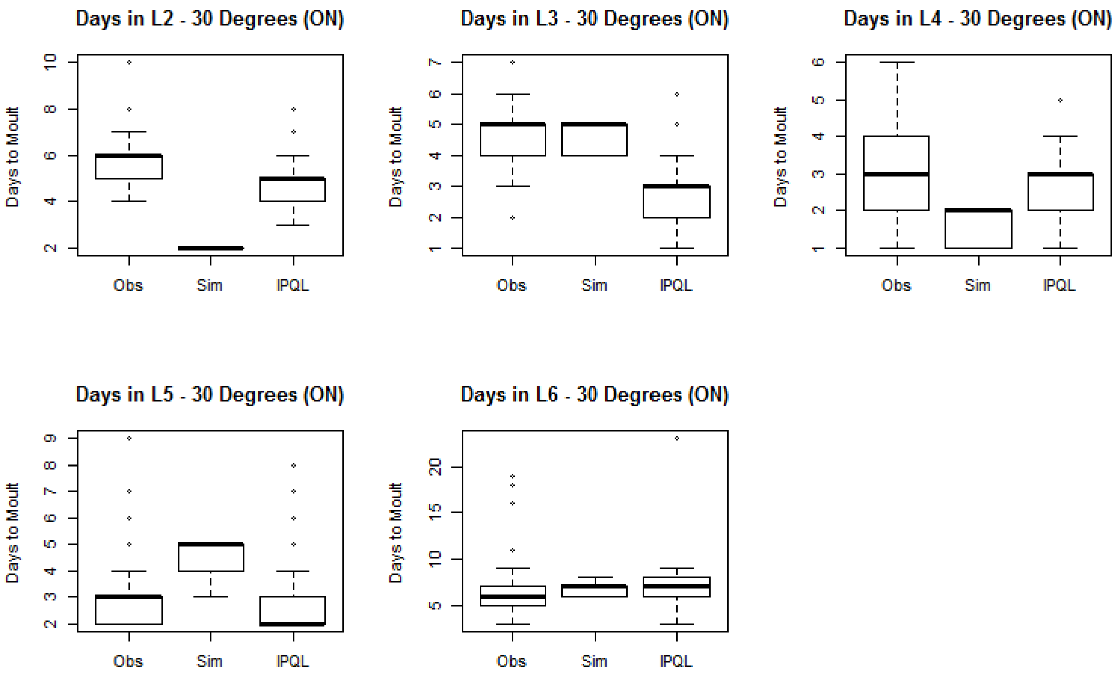


Figure 5: Instar development of the Ontario colony compared to Biosim simulation and IPQL colony at 30ºC

At 35ºC, the observed development is again slower than simulated (Fig. 6). The development of the Ontario colony is slower than predicted by the model at the L2 and L4 stages, and to a lesser degree at the L6 stage (Fig. 7). The model agrees with the observations at the L3 and L5 stages.

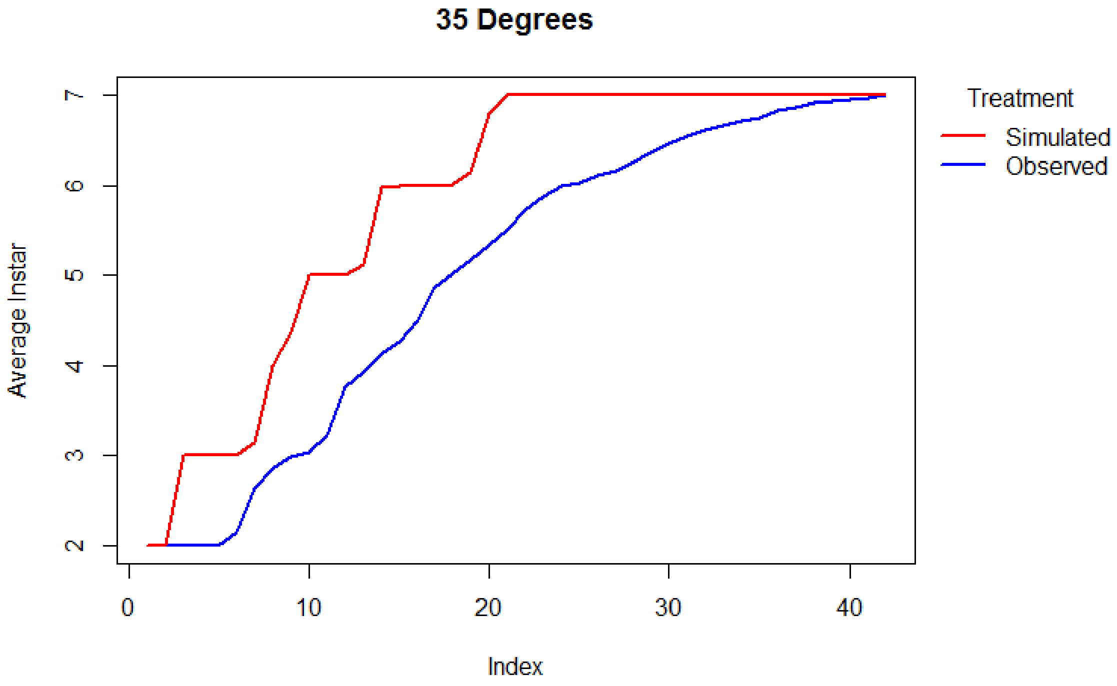


Figure 6: Average instar development of the Ontario colony compared to Biosim simulation at 35ºC

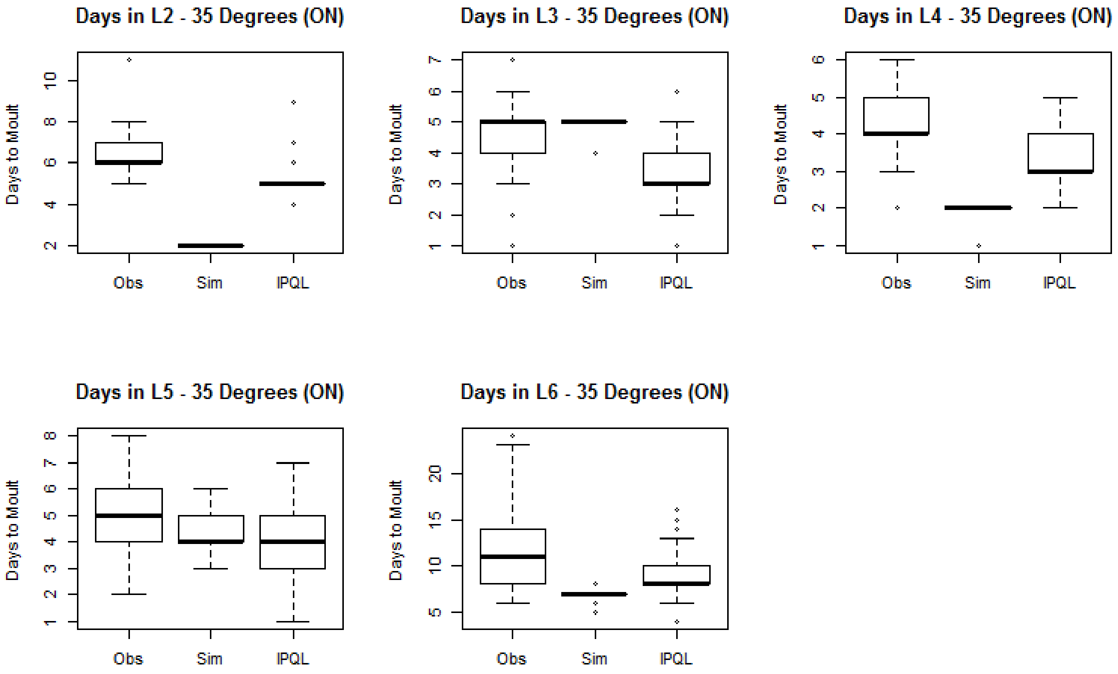


Figure 7: Instar development of the Ontario colony compared to Biosim simulation and IPQL colony at 35ºC

In summary, the preliminary results for the Ontario colony suggest that the model overestimates the development rate at 30ºC and 35ºC, a result that is consistent with what was observed on the IPQL colony. However, it is important to point that: (1) the temperature transfers that are required to rear budworm at these temperatures are probably not well addressed in the model as pointed earlier, (2) differences between observed and simulated development rates of early instars as observed here have likely little impact on the usability of the model because early instars are very unlikely to encounter these temperatures.

Aknowledgements

This work was carried-out with financial support from SERG-international members (NSDNR, SOPFIM, AB-AAF, SK MOE, OMNRF) and in-kind support from AB-AAF and NWT government. The Great Lakes Forestry Centre of the Canadian Forest Service contributed significant financial and in-kind support. We thank Stephane Bourassa (CFS-LFC), Gord Brand (CFS-GLFC), Roger Brett (CFS-NoFC), Martin Callaghan (NWT Environment and Natural Resource), Louis De Grandpré (CFS-LFC), Ryan Hermanutz (AB Agriculture and Forestry), Rob Johns (CFS-AFC), Greg Pohl (CFS-NoFC), and Joris Wiersinga (FPL) for their help in sampling branches for this experiment. Without them, this large-scale sampling would not have been possible.

References

Cooke, B.J.; Régnière, J. 1999. Predictability and measurability of Bacillus thuringiensis efficacy against spruce budworm (Lepidoptera: Tortricidae). Environmental Entomology 28(4): 711-721.

Morris, R.F. 1955. The development of sampling techniques for forest insect defoliators, with particular reference to the spruce budworm. Canadian Journal of Zoology, 1955, 33:225-294

Régnière, J.; Saint-Amant, R.; Béchard. 2014. BioSIM 10 – User's manual. A. Nat. Resour. Can., Can. For. Serv., Laurentian For. Cent., Québec (Quebec). Inf. Rep. LAU-X-137E

Régnière, J.; St-Amant, R.; Duval, P. 2012. Predicting insect distributions under climate change from physiological responses: spruce budworm as an example. Biol. Invasions 14:1571-1586.

Régnière, J. 1987. Temperature-dependent development of eggs and larvae of Choristoneura fumiferana (Clem.) (Lepidoptera: Tortricide) and simulation of its seasonal history. The Canadian Entomologist 119: 717-728.

van Frankenhuyzen, K.; Ebling, P.M.; McCron, B.; Ladd, T.R.; Gauthier, D.; Vossbrinck, C. 2004. Occurrence of Cytosporogenes sp. (Protozoa, Microsporidia) in a multi-species insect production facility and its elimination from a colony of the eastern spruce budworm, Choristoneura fumiferana (Clem.) (Lepidoptera: Tortricidae). Journal of Invertebrate Pathology 87: 16-28.

Volney, W.J.A.; Cerezke, H.F. 1992. The phenology of white spruce and the spruce budworm in northern Alberta. Canadian Journal of Forest Research 22(2): 198-205.

Weber, J.D.; Volney, W.J.A.; Spence, J.R. 1999. Intrinsic development rate of spruce budworm (Lepidoptera: Tortricidae) across a gradient of latitude. Environmental Entomology 28 (2): 224-232.

Appendix A: Simulated vs observed development times of the IPQL colony at 7 constant temperatures - Overall development

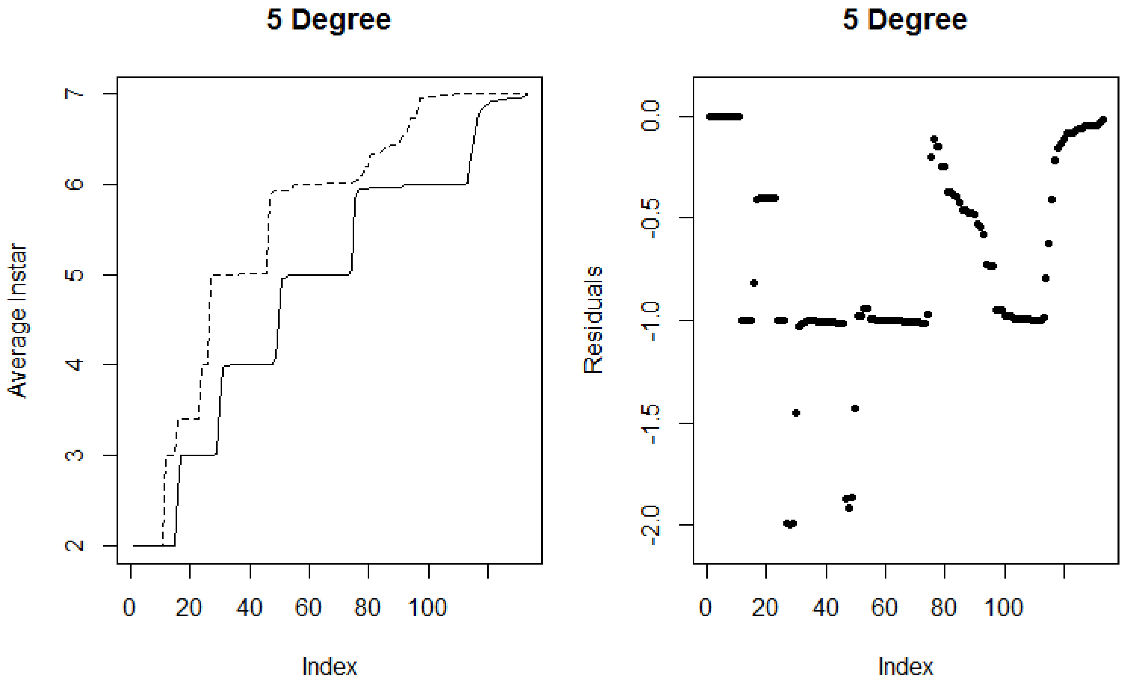


Figure A1: Comparison between simulated (dotted line) and observed (plain) development times at 5ºC for IPQL colony generation F0

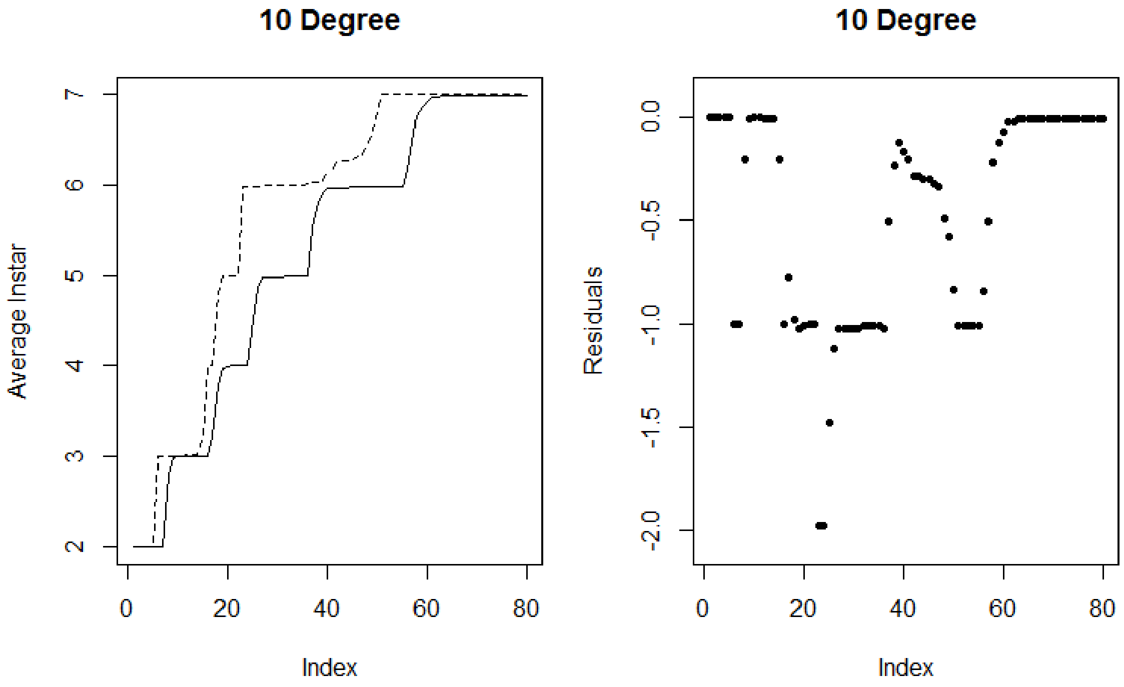


Figure A2: Comparison between simulated (dotted line) and observed (plain) development times at 10ºC for IPQL colony generation F0

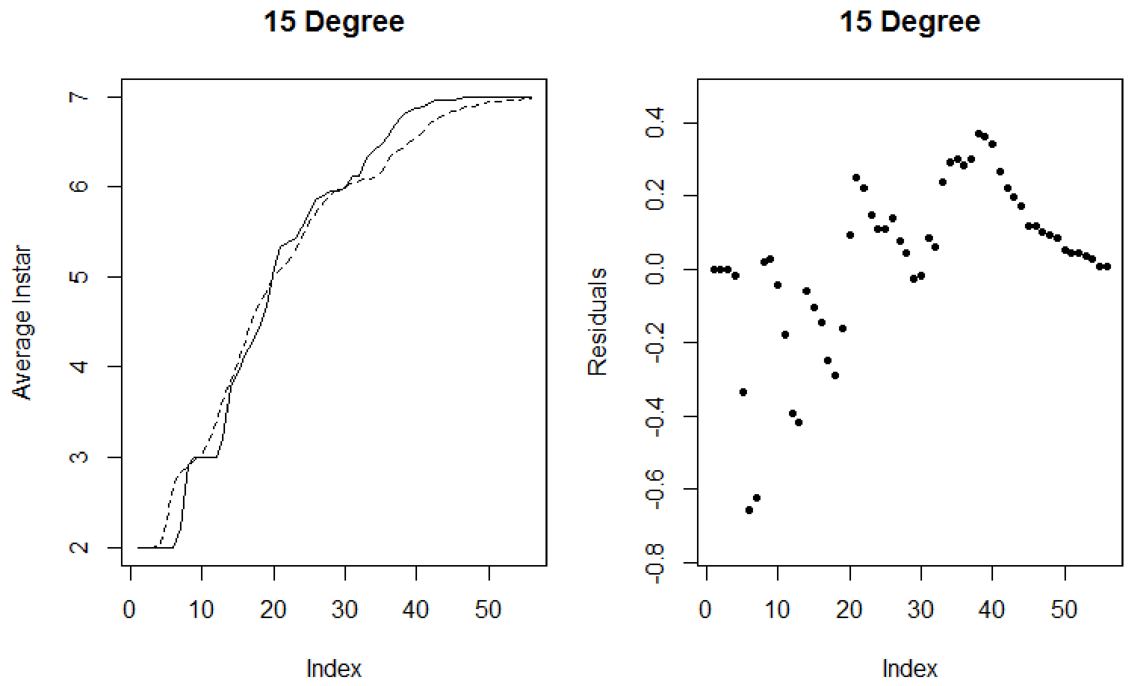


Figure A3: Comparison between simulated (dotted line) and observed (plain) development times at 15ºC for IPQL colony generation F0

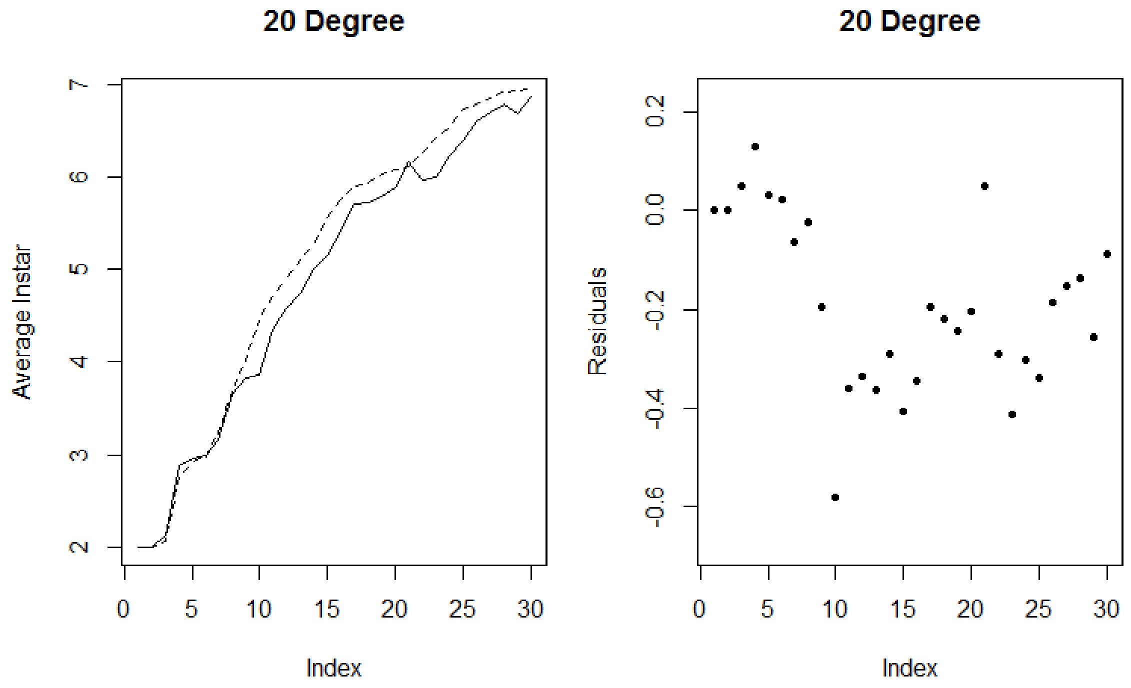
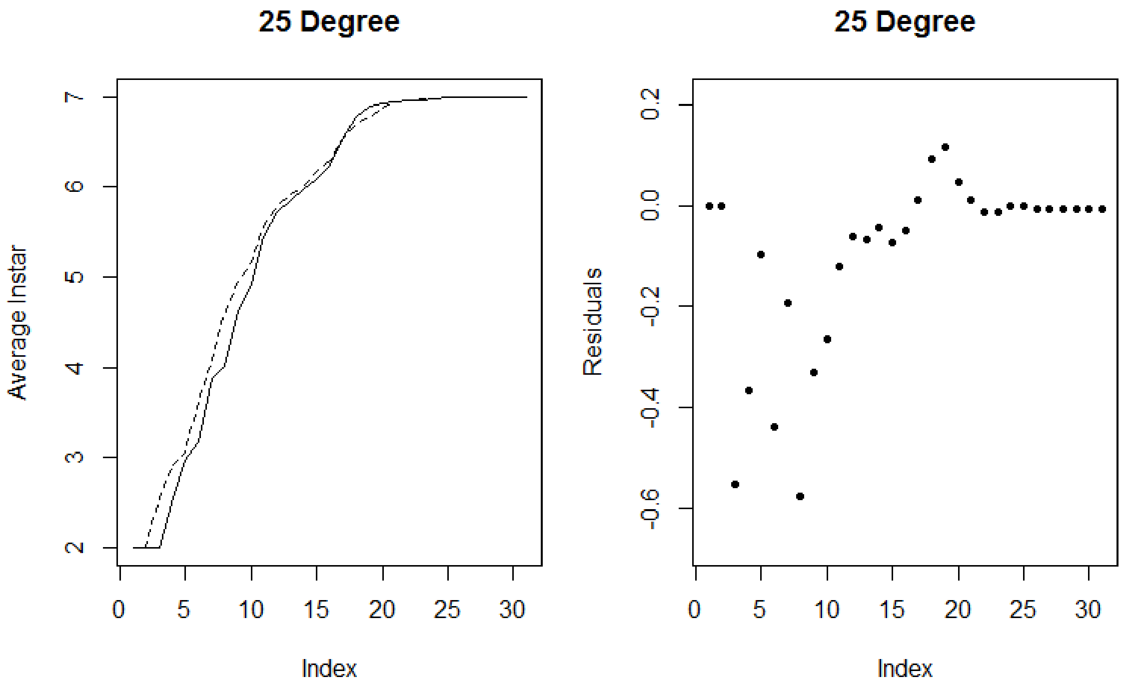


Figure A4: Comparison between simulated (dotted line) and observed (plain) development times at 20ºC for IPQL colony generation F0



FigA5: Comparison between simulated and observed development rates at 25ºC for IPQL stock generation F0

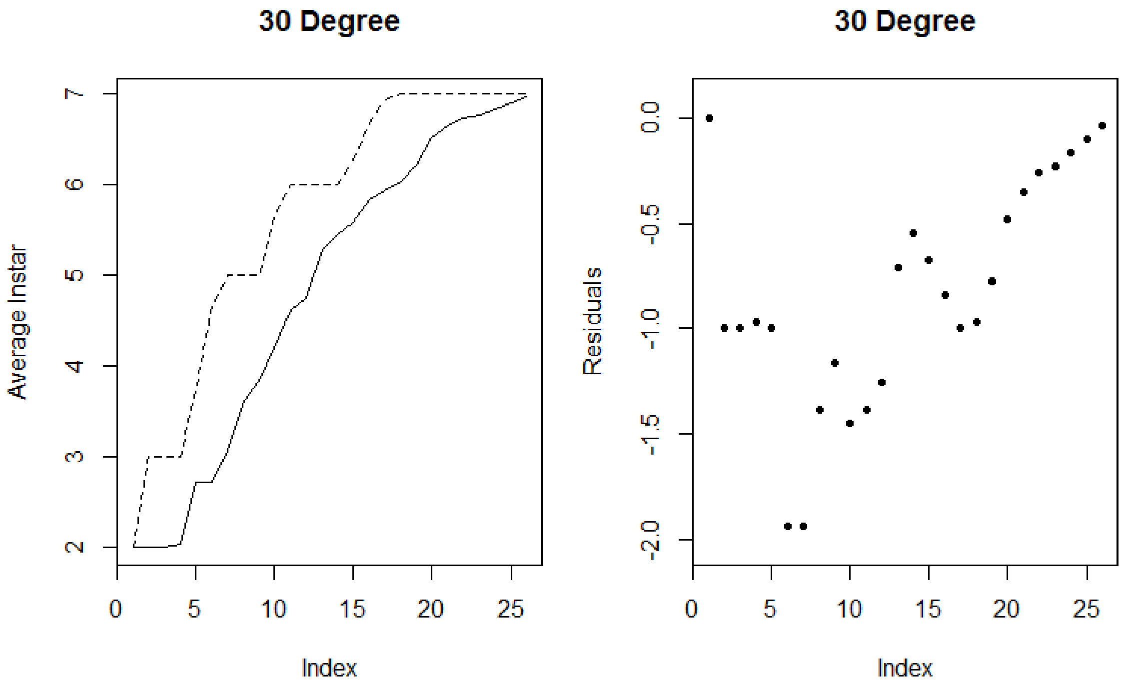


Figure A6: Comparison between simulated (dotted line) and observed (plain) development times at 30ºC for IPQL colony generation F0

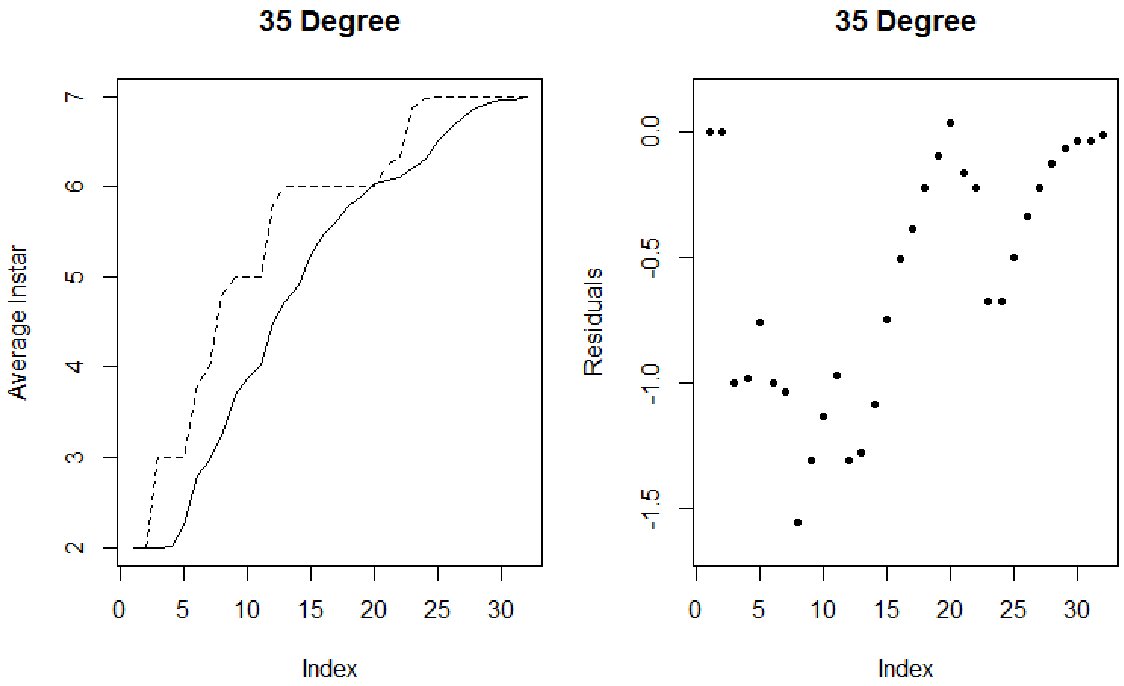
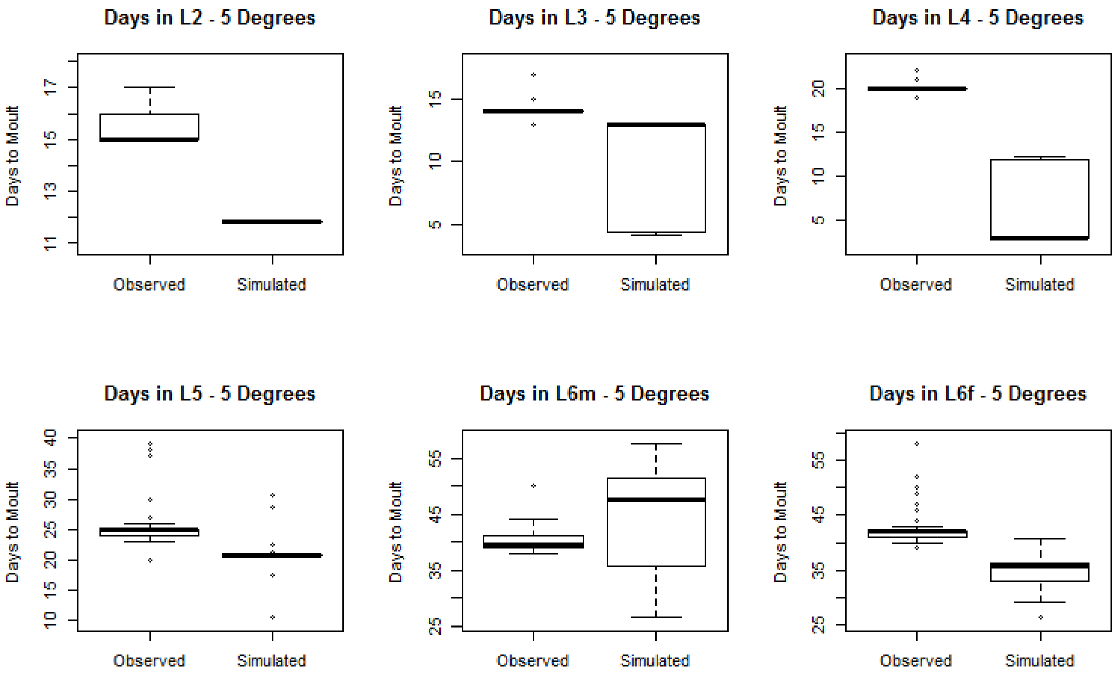


Figure A7: Comparison between simulated (dotted line) and observed (plain) development times at 35ºC for IPQL colony generation F0

Appendix B: Simulated vs observed development times of the IPQL colony at 7 constant temperatures - Larval stage development



Days in L2 - 5 Degrees

Days in L3 - 5 Degrees

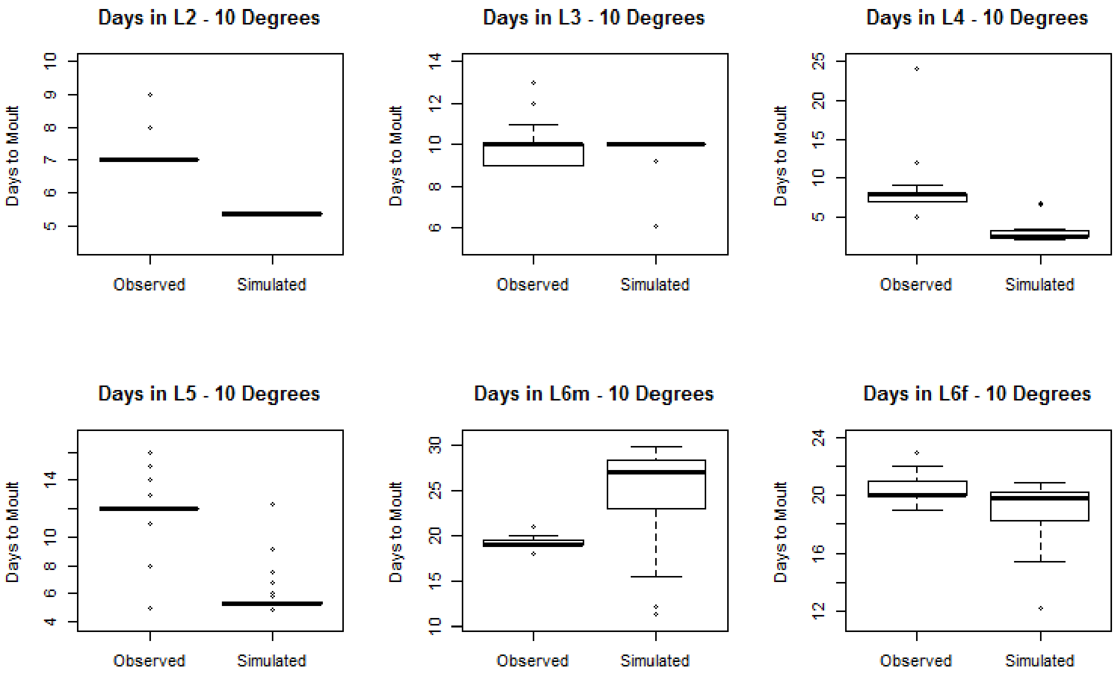
Days in L4 - 5 Degrees

Days in L5 - 5 Degrees

Days in L6m - 5 Degrees

Days in L6f - 5 Degrees

Figure B1: Comparison between simulated (dotted line) and observed (plain) development times at 5ºC for IPQL colony generation F0



Days in L2 - 10 Degrees

Days in L3 - 10 Degrees

Days in L4 - 10 Degrees

Days in L5 - 10 Degrees

Days in L6m - 10 Degrees

Days in L6f - 10 Degrees

Figure B2: Comparison between simulated and observed development times at 10ºC for IPQL colony generation F0

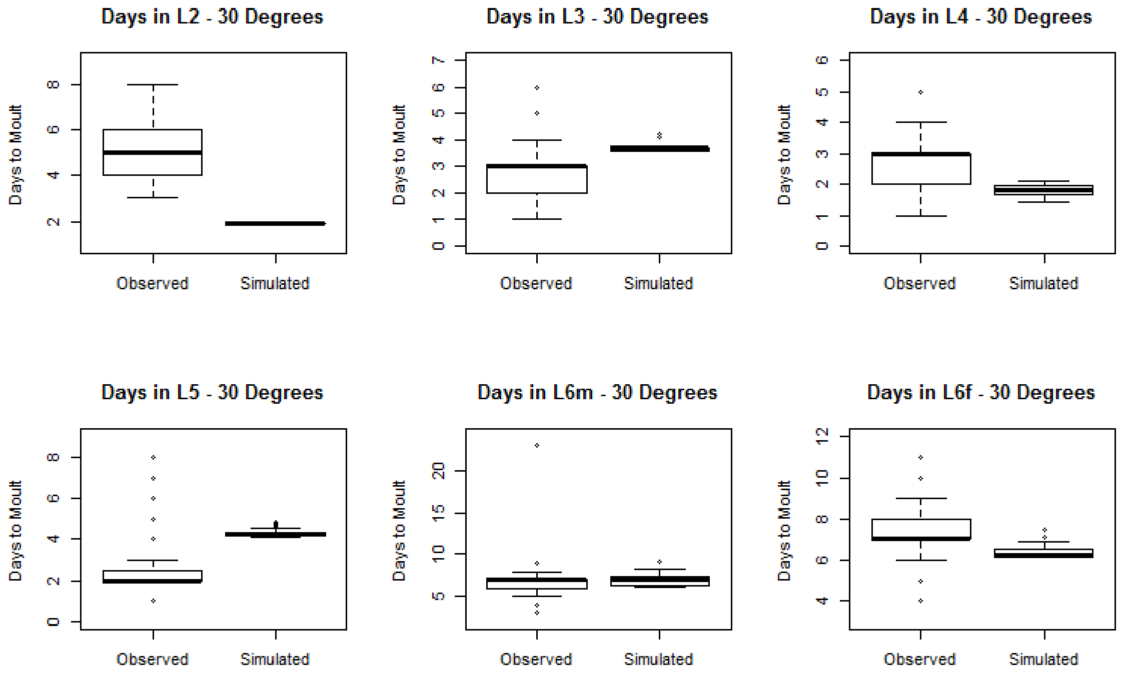


Figure B3: Comparison between simulated and observed development times at 30ºC for IPQL colony generation F0

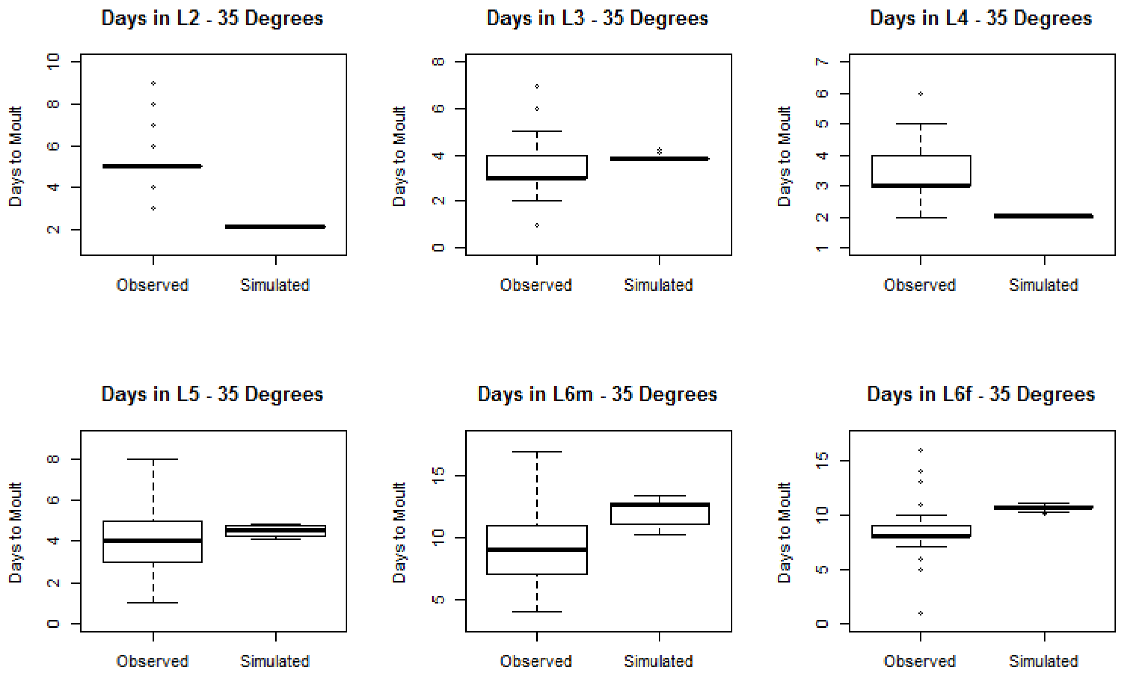


Figure B4: Comparison between simulated and observed development times at 35ºC for IPQL colony generation F0

Understanding overwintering survival as a component of pest management strategies for spruce budworm

Co-Principal Investigators and Agency (alphabetical order):

Dr. Joe Bowden [1]
NRCan-CFS Research Scientist, Forest Entomology, joseph.bowden@canada.ca

Dr. Eric Moise (contact person) [1]
NRCan-CFS Research Scientist, Forest Entomology, eric.moise@canada.ca, T: 709-637-4921

Dr. Michael Stastny [2]
NRCan-CFS Research Scientist, Insect Forest Ecology, michael.stastny@canada.ca

[1] Atlantic Forestry Centre – Corner Brook
26 University Drive, Corner Brook, Newfoundland and Labrador, A2H 6J3
[2] Atlantic Forestry Centre – Fredericton

Collaborators:
Lauren Stead, UNB/NRCan-CFS (AFC), lauren.stead@unb.ca
Dr. Rob Johns, NRCan-CFS (AFC), rob.johns@canada.ca
Dr. Véronique Martel, NRCan-CFS (LFC), veronique.martel@canada.ca

Funding:

Funding Estimates (x $1000) **(Current Funding)**

Item	2017-18	2018-19	2019-20
Lab/Field Supplies	$7	$10	$10
Field work (mileage, per diems)	$12	$15	$15
Student/Casual labour	$22	$25	$25
Totals	**$41**	**$50**	**$50**

Proposed Funding Sources	2017-18	2018-19	2019-20
CFS PROMIS	$21	$25	$25
SERG request	$20	$25	$25
Total	**$41**	**$50**	**$50**

Funding request from SERG Partners: $25K/year.

Abstract: The Early Intervention Strategy aims to mitigate the current spruce budworm outbreak by treating rising population "hotspots" to limit spread via moth migration. Identifying these hotspots relies on fall forecasts of second instar (L2) spruce budworm densities, a process that assumes overwintering mortality is equal across all sample sites. However, this ignores the potential for biotic and abiotic influences. This project aims to quantify how overwintering mortality varies with tree-level differences in the quality of overwintering sites (e.g. host species, crown position, substrate for L2 hibernacula), as well as stand (composition, age) and landscape-level factors (elevation, latitude). Initial results for tree-level effects suggests that both overwintering mortality and parasitism are greater in balsam fir than in black spruce. To further quantify the role of stand- and landscape-level factors, environmental gradients have been established in New Brunswick (elevation, latitude) and Newfoundland (elevation), with initial results anticipated for Spring 2018. Ultimately, this data will help 1) determine if sampling logistics affect the accuracy of L2 monitoring due to intra-tree variation in the strength of different mortality factors, and 2) identify stand conditions that compromise larval survival, thereby delineating low-risk sites for SBW outbreaks, and leading to a reduction in the size or number of necessary treatment blocks.

Résumé : La stratégie d'intervention précoce vise à atténuer l'infestation en cours de la tordeuse des bourgeons de l'épinette en traitant les « points chauds », où les populations sont en croissance, dans le but de limiter la propagation via la migration des papillons. Le repérage de ces points chauds dépend des prévisions automnales des densités de stade larvaire L2 de la tordeuse des bourgeons de l'épinette, processus qui suppose une mortalité hivernale égale dans tous les sites d'échantillonnage. Or, ce processus ne tient pas compte d'éventuelles influences biotiques et abiotiques. Ce projet examine la mesure dans laquelle la mortalité hivernale varie selon les différences au niveau des arbres dans la qualité des zones d'hivernage (p. ex. : espèces hôtes, position de la couronne, substrat pour l'hibernacle de L2) et des peuplements (composition, âge), ainsi que les facteurs au niveau du paysage (élévation, latitude). Les résultats initiaux concernant les effets au niveau des arbres semblent indiquer qu'aussi bien la mortalité hivernale que le parasitisme sont plus élevés chez le sapin baumier que chez l'épinette noire. Pour quantifier plus en détail le rôle des facteurs au niveau du peuplement et du paysage, des gradients environnementaux ont été établis pour le Nouveau-Brunswick (élévation, latitude) et Terre-Neuve (élévation); les premiers résultats sont attendus au printemps 2018. Au bout du compte, ces données contribueront : 1) à déterminer si la logistique de l'échantillonnage a des effets sur l'exactitude du relevé L2 dus à la variation de la force de différents facteurs de mortalité d'un arbre à l'autre, et 2) à déterminer les conditions d'un peuplement qui compromettent la survie des larves, ce qui permettrait de délimiter les zones à faible risque de connaître une infestation de TBE et de réduire la taille et le nombre de parcelles qu'il est nécessaire de traiter.

Introduction: Spruce budworm (*Choristoneura fumiferana* Clem.) poses ecological and economic threats to the spruce-fir forests of northeastern North America. As the current outbreak continues to rise in Quebec and approach Atlantic Canada, there have been efforts to contain the spread through managing so-called 'hot spots' at the leading edge of the outbreak (i.e., Early Intervention Strategy). Early detection of hot spots has relied primarily on fall collections of larvae (L2) prior to their overwintering. These L2 density estimates have been the basis of locating and contouring hot spots for subsequent experimental treatments with insecticides, and evaluating their success (e.g., comparisons of annual density estimates inside and outside of treated areas).

Inherent to L2 monitoring is the assumption that overwintering survival of SBW is equal across all sampled sites. However, larval mortality can vary greatly (15-48%)[1], driven by abiotic (e.g., temperature) and biotic (e.g., energy stores, predation/parasitism) factors. These factors vary 1) within trees[2], 2) across environmental gradients (i.e., elevation/latitude)[2] and 3) with stand structure (composition and age)[3], and may help explain discrepancies between L2 surveys and subsequent densities and damage. Therefore, investigation of how tree-level, stand-level and landscape-level variables influence L2 overwintering period (timing and duration)[4] and survival is necessary to improve the predictive value of L2 density estimates and optimize spray treatments.

Our study is comprised of two components; 1) Quantify intra-tree drivers of overwintering survival (i.e. host species, substrate, crown position), and 2) Employ environmental gradients (i.e., elevation and latitude) in combination with stand-level characteristics (i.e., composition and age structure) to evaluate predictors of overwintering survival to identify stand conditions that create an increased risk of higher budworm density and defoliation. Because aerial spray programs for SBW in eastern Canada rely heavily on fall L2 collections to determine treatment blocks, our study aims to 1) determine if sampling logistics affect the accuracy of L2 monitoring due to intra-tree variation in the strength of different mortality factors and 2) incorporate landscape and stand-level results into models used for integrated pest management. In conjunction with other variables used in decision-making (e.g. stand commercial value, number of consecutive years with detectable populations), this research will help identify stand conditions that compromise larval survival, thereby delineating low-risk sites for SBW outbreaks, and leading to a reduction in the size or number of necessary treatment blocks.

Methodology

1) *Quantifying tree-level drivers of spruce budworm overwintering mortality*

In Spring 2017, we selected trees from a mixed balsam fir – black spruce forest stand outside of Charlo, New Brunswick at the leading edge of the spruce budworm outbreak. One week prior to L2 emergence we felled 10 healthy trees of each species (dbh 8-12 cm). We divided trees into the upper and middle crown levels, from which four different 45 cm sections of tree were collected; upper-crown branch, middle-crown branch, upper-crown bolt, and middle-crown bolt (Fig. 1). We further divided the upper and middle branches into branch classes “base” and “tip”. In the rearing lab, we wrapped each tree section in wet paper towel and hung them under a light to promote budworm emergence. Collected individuals were fed on McMorran diet until pupation, and we recorded whether a budworm moth or parasitoid eclosed. For individuals that emerged but did not survive to adulthood, we recorded mortality as “unknown”. Three days after emergence ended, we processed tree sections in a sodium hydroxide wash and collected budworm were recorded as having died over winter.

Overwintering mortality was calculated as:

Total number of budworm collected from wash / Total number of collected budworm

Percent parasitism was calculated as: Total number of parasitoids / Total number of parasitoids + moths

2) *Quantifying stand- and landscape-level drivers of spruce budworm overwintering mortality*

We have established a manipulative experiment over an elevation gradient in western Newfoundland comprised of four stand types (Young Spruce, Young Fir, Mature Spruce, Mature Fir) repeated at four different altitudes (~400 m range). Sections of cheesecloth each containing 30 sentinel pre-diapause L2 budworm larvae were placed in organza bags and affixed to the mid-bole of six trees in each stand. A similar experiment has been established along an elevation gradient (~350 m) in central New Brunswick, with 3-4 trees of each host species chosen at random in mixed spruce-fir stands within each elevation belt. Sentinel larvae in hibernacula were translocated on cut branches from an intermediate-density (mean 17.3 L2 per 75cm branch) population near Campbellton, NB, and one branch of each host species was affixed within the lower crown of the selected trees in a paired design. In parallel, five 60-100 km long transects along a phenological gradient (coast-to-inland) in northeastern NB have been selected for a non-manipulative approach that relies on sampling natural SBW densities across four phenological zones and a combination of host tree attributes (species, age).To quantify overwintering mortality, budworm will be collected in early spring prior to emergence, and larvae will be reared out in the laboratory. The observed variation in overwintering mortality will be tested against climatic and stand-level factors measured at each site.

Preliminary Results

Initial results for tree-level effects reveal that overwintering mortality was higher on balsam fir than black spruce (Fig. 2), suggesting that biotic and/or abiotic mortality drivers may differ between tree species. Parasitism, for instance, appears to be greater on balsam fir than on black spruce (Fig. 3). Despite reduced survival on balsam fir, both spruce budworm performance (e.g. development) and feeding damage are greater on this species[5,6], thus suggesting a potential tradeoff in host suitability.

Figure Captions

Figure 1. Example of sampling units that were taken from the tree: a) 45 cm from the tip and base of the branch from the upper and lower crowns; b) 45 cm bolt from the upper and middle thirds of the tree bole.

Figure 2. Percent overwintering mortality for budworm collected from a) boles b) branch tips, and c) branch bases. Overwintering mortality was calculated as the number of budworm extracted in the sodium hydroxide wash divided by the total number of budworm collected. Bars represent means ±SE.

Figure 3. Percent parasitism for budworm collected from a) boles, b) branch tips, and c) branch bases. Bars represent means ±SE.

Figure 1

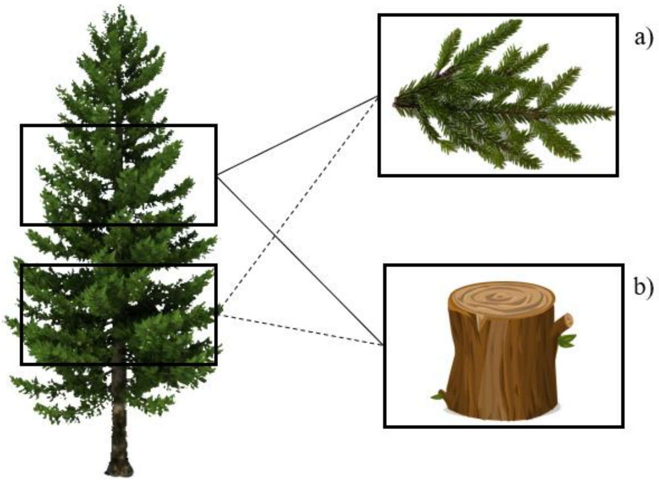
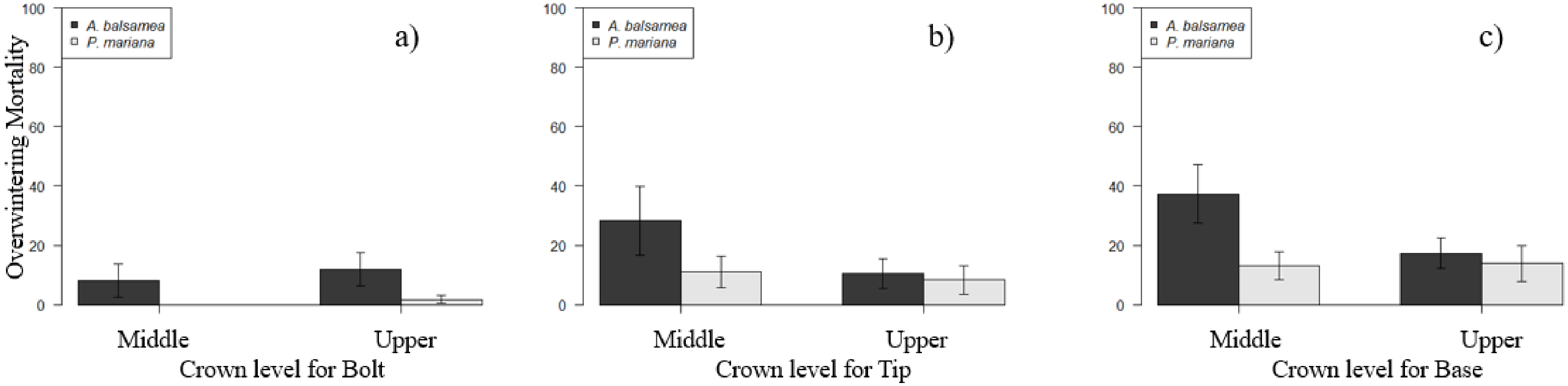
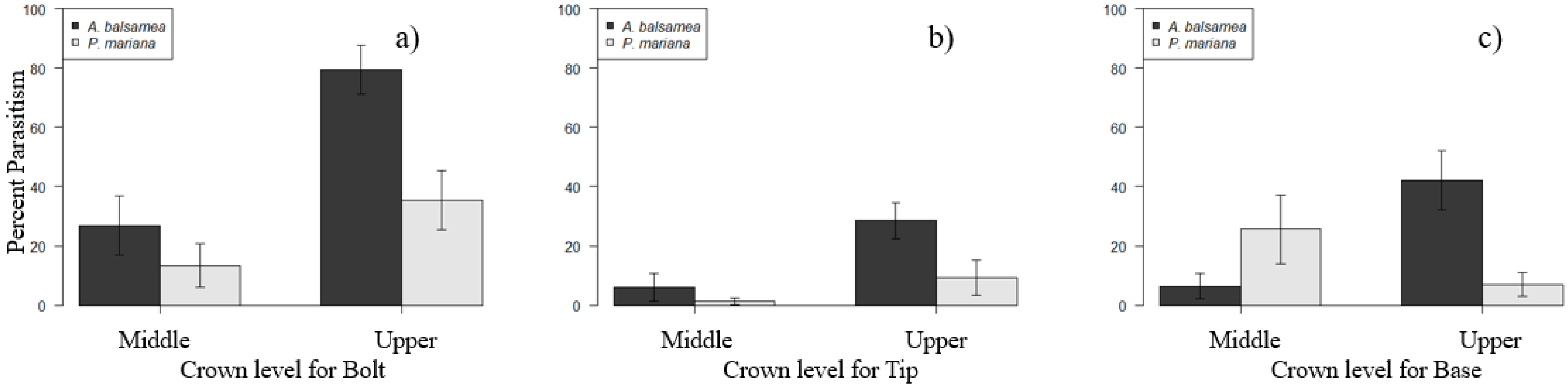


Figure 2



a)
b)
c)
■ A. balsamea
□ P. mariana
Overwintering Mortality
100
80
60
40
20
0
Middle
Upper
Crown level for Bolt
Crown level for Tip
Crown level for Base

Figure 3


Percent Parasitism
0
20
40
60
80
100
■ A. balsamea
□ P. mariana
a)
Middle
Upper
Crown level for Bolt
0
20
40
60
80
100
■ A. balsamea
□ P. mariana
b)
Middle
Upper
Crown level for Tip
0
20
40
60
80
100
■ A. balsamea
□ P. mariana
c)
Middle
Upper
Crown level for Base

References:

1- Régnière J & Duval P (1998) *Can. Ent.* 130:13–26, **2-** Nealis VG & Régnière J (2016) *Ecol. Ent.* 41:633–641; **3-**Bouchard M & Auger I (2014) *Land.Ecol.* 29:111-126; **4-** Han E-N & Bauce E (1998) *Ecol. Ent.* 23:160–167; **5-**Hennigar CR et al (2008) *For. Sci.* 54:158-166; **6-** Blais JR (1957) *For. Chron.* 33:364-372

Acknowledgements

We thank SERG-International, the Canadian Forest Service, and the Newfoundland Department of Fisheries and Land Resources for providing financial and in-kind support for this work. We are also grateful for the support from our field technicians including H. Spicer, K. Casey, J. Pinksen, M. Ungurain, A. Scott, J. Fowlow, R. Graves, T. Doncaster, and K. Burgess. Maps for Newfoundland sites were provided by O. van Lier, J. Luther and G. Strickland.

INTERIM PROJECT REPORT January 19, 2018

Title: Using landscape-level forest management to reduce the intensity of spruce budworm outbreaks

Investigators and affiliations

Dan Kneeshaw (principal investigator). Université du Québec à Montréal. 514-987-3000 ext. 4480. kneeshaw.daniel@uqam.ca
Louis De Grandpré. CFS-LFC.418-648-5846. louis.degrandpre@nrcan.gc.ca.
Deepa Pureswaran. CFS-LFC. 418-648-7532. dpureswa@nrcan.gc.ca.
Dave MacLean, UNB, macleand@unb.ca
Brian Sturtevant, USDA Forest Service, bsturtevant@fs.fed.us
Laura Kenefic, USDA Forest service, lkenefic@fs.fed.us
Patrick James, Université de Montréal, patrick.ma.james@umontreal.ca
Steven Kembel, UQAM, kembel.steven_w@uqam.ca

Collaborators

Mathieu Bouchard, MFFP-QC; Loic D'Orangeville UQAM, Louis-Etienne Robert, UQAM and Malcolm Itter (Michigan State University) and Andria Dawson (Mount Royal University

Contributing SERG Members:

NSDNR, SOPFIM, AB-AAF, OMNRF

EXECUTIVE SUMMARY

Recent studies have suggested that large-scale effects of forest management on landscape structure have influenced the severity and duration of insect outbreaks as well as future composition. However, the risk of mortality i.e. the severity of an outbreak will also be influenced by other global changes particularly climate warming. The objectives of this project were to evaluate landscape-level effects of forest management on spruce budworm (SBW) outbreak severity and duration for balsam fir and black spruce, to evaluate whether forest composition responds similarly to SBW outbreaks and to logging and to how climate change may be impacting mortality risk through interactions between moisture stress and defoliation. We also evaluated biotic effects of microbial communities on SBW health with the goal of pursuing this at the landscape scale next year. Our results show that SBW and harvesting lead to different outcomes in terms of composition. We further show that large blocks of spruce are little vulnerable to SBW and could be a good strategy to reduce forest vulnerability but that although they are not vulnerable to SBW they are to forest operations. Climate change,risk in terms of negative feedbacks between moisture stress and defoliation were only weakly observed and unless excarbated may not be a major issue. Similarly, lab experiments do not yet lend much support for the role of microbe communities on SBW health These results suggest that forest management may have unintended consequences on forest vulnerability to SBW outbreaks, that a concerted effort will be required to reduce fir and increase spruce during forest management. Climate change over the last 30-40 years is not a compounding factor across much of the SBW range although this should be verified for future scenarios. Thus we may be our own worst enemy due to large landscape level changes in forest contiguity and composition.

RÉSUMÉ EXÉCUTIF

Des recherches récentes suggèrent que les effets à grande échelle de l’aménagement forestier sur la structure du paysage ont influencé la sévérité et la durée des épidémies d’insectes ainsi que la composition forestière future. Cependant le risque de mortalité est aussi influencé par d’autres changements globaux dont principalement le réchauffement climatique. Les objectifs de ce projet étaient d’évaluer, à l’échelle du paysage, les effets de l’aménagement forestier sur la sévérité et la durée des épidémies de la tordeuse des bourgeons de l’épinette (TBE) pour le sapin baumier et l’épinette noire, d’évaluer si la composition forestière répond similairement aux épidémies de la TBE et des coupes forestières; et d’évaluer les pertes de croissance causées par la TBE est excaerbée par les stresses hydriques. Nos résultats montrent que la composition forestière est différente après épidémie et après coupe forestière. De plus, nous montrons que les massifs d’épinette sont peu vulnérables à la TBE, et donc de maintenir ou d’augmenter leur proportion peut être une bonne stratégie de lutte contre la TBE bien qu’ils soient davantage prisés par les forestiers. Le changement climatique, en termes de secheresses n’ont pas empiré les pertes de croissance due aux épidémies d’insectes (livrée de forêt ou tordeuse des bourgeons d’épinette). Ces résultats suggèrent que l’aménagement forestier pourrait avoir des conséquences indésirables sur la vulnérabilité des forêts face aux épidémies de TBE et qu’un effort accru sera nécessaire pour réduire le sapin et augmenter l’épinette durant les activités d’aménagement forestier. Les secheresses des derniers 30-40 ans n’augmentent pas les pertes de croissance suite aux épidémies d’instectes. Les résultats préliminaires, en laboratoire, ne montre pas non plus un effet des microbes sur la santé de la TBE, il faut donc valider ces résultats avec les études sur le terrain. Nous devrons faire attention à nos interventions forestières à grande échelle car celles-ci semblent être une plus grande source de risque que les changements climatiques.

PROJECT OBJECTIVES:

Global : Evaluate the influence of climate induced stress, landscape level forest management and microbes on risk of SBW outbreak damage. Specifically,

- Assess whether SBW outbreaks and forest management lead to the same outcomes in terms of forest response
- Determine differences in SBW outbreak patterns overtime as the proportion of landscapes under management regimes changes
- Evaluate the interaction between effects of climate change stress and defoliation on tree growth loss.
- Evaluate the effect of microbes on SBW growth and survival
- Identify risk factors to use in designing pest management strategies.

BACKGROUND:

Humans have an omnipresent influence on forested landscapes which can lead to significant and unintentional effects on ecosystem processes such as disturbances (Sanderson et al. 2002). Recent studies have suggested that changes to landscape structure, including forest composition and configuration, have influenced insect outbreak characteristics. For example, fragmentation of forest landscapes has been identified as increasing the severity of forest tent caterpillar outbreaks **(**Roland 1993) and large landscapes with different forest management strategies have been shown to influence the duration, frequency and severity of spruce budworm outbreaks in a forested region where host species occur but are not dominant (Robert et al. 2012)). Thus the impact of forest management legacies on a severe SBW outbreak in forests where fir is a more dominant species still needs to be elucidated. It has also been suggested that large contiguous blocks of the most vulnerable host to the SBW (balsam fir) would lead to more severe outbreaks (Baskerville 1975). Recently, we showed that at the stand level that black spruce growing in stands mixed with fir were more vulnerable in terms of defoliation and growth reductions that in stands dominated by spruce (Bognounou et al. 2017) At the landscape level this still needs to be demonstrated. Divergent landscape configurations within a region or across jurisdictions offer the possibility to evaluate the effect of different forest management regimes on spruce budworm outbreak disturbance characteristics. This comparison can be made of volume losses in landscapes undergoing different intensities of forest management and also using dendrochronological records of growth ring reductions through time as landscape structure changes. We hypothesized that outbreak duration and severity would increase as the area clearcut in a landscape increased, and that this effect would be strongest in fir dominated ecozones

As recently shown (Pureswaran et al. 2015), these factors will also change as climate warms as both host species and the insect will change ranges. As climate warms soil moisture is expected to decrease throughout much of boreal Canada. Drought stress can predispose trees to attack by herbivores (Kolb et al. 2016). Insects as ectotherms may also respond directly to warmer temperatures and thus (warmer and drier climates) drought conditions could also lead to increased outbreak severity. Several physiological feedbacks in tree responses to drought and insect defoliation have been proposed to explain a negative interaction between drought and insect defoliation. First, insect defoliation may reduce tree growth and xylem formation making trees more susceptible to future drought (Anderegg and Callaway, 2012). Second, limited available carbon and water in drought-stressed trees may reduce metabolic defenses against defoliating insects (McDowell et al., 2008, 2011). As drought events and insect outbreaks are expected to increase with climate warming an evaluation of mortality risk needs thus not only to consider the influence of forest harvesting but also that of climate change. It has been suggested that high temperature and low precipitation coincide with initiation of SBW outbreaks in the Green River (Morris 1963). Should we be more concerned about greater impacts of defoliation during dry years.

Biotic relationships with other organisms found on trees could also play a role in differential defoliation and survival. For example, endophytic fungi on spruce needles have been shown to reduce budworm growth and survival. Spruce budworm ingest a large quantity of bacteria and fungi in the leaves that they eat, leading to differences in microbes present in the budworm gut. These microbes are likely to influence budworm health, and could help explain the large differences in vigour amongst larvae, and potentially also why some trees fare better than others in defoliated zones. Preliminary results from lab experiments show that there are differences in vigour between SBW fed on leaves with and without microbial organisms present, and SBW fed on spruce versus fir foliage. Plant-associated microbial communities change between host species and environmental gradients. If we can identify how landscape configurations interact with the bacteria and fungi that lead to reduced budworm vigour, then these microorganisms could potentially be used as another biological agent to reduce budworm damage.

In the following part of the report we will present methods and results for each of the three main research areas: landscape effects on outbreak severity, the effect of climate change as represented by moisture limitation in interaction with defoliation severity on tree growth and finally the influence of tree microbial communities on insect health.

Effect of forest management on spruce budworm outbreak characteristics: Methods

In a previous study (Robert et al. 2012) we evaluated the effect of landscape level patterns caused by harvesting on spruce budworm outbreak characteristics. This study investigated response in three landscapes in the Border Lakes area on the Ontario-Minnesota border: 1) a wilderness area with no influence of industrial forest harvesting; 2) a landscape dominated by small scale (approx 10 Ha clear cuts) and 3) a landscape dominated by larger 100Ha clearcuts. In other words, an unfragmented (by anthropogenic disturbance) landscape, a finely fragmented and a coarsely fragmented landscape. We used the outbreak characteristics and performed a non-parametric co-variance function to analyse spatial pattern in outbreak characteristics in the different landscapes.

Results

Results showed that the coarsely fragmented landscape did not have a pattern different than the average pattern for the entire Border Lakes area (Figure 1). The finely fragmented landscape significantly reduced the spatial patterns below that observed in the overall landscape for most tested distances. In the unfragmented wilderness the pattern was far more strongly correlated with synchronised outbreak characteristics at very large scales (Figure 1). Despite the large and generally overlapping confidence intervals, inferences are consistent with earlier non-spatial analyses suggesting differences in outbreak responses between the zones. As previously demonstrated (Robert et al. 2012) a fine grain of landscape fragmentation (10Ha cutblocks) tends to desynchronise outbreaks and create more local patterns. In non-fragmented landscapes the outbreak patterns are more synchronised over much larger areas than in the other zones. These patterns suggest that forest management by breaking up forests into smaller parcels could reduce large-scale, high amplitude effects of outbreaks. Outbreaks will still occur but effects will be more heterogenous, with severity and synchrony of effects dampened. It could be argued that less synchronous outbreaks could permit interventions to be spread out in-time and thus permit more wood fibre to be protected for harvesting.

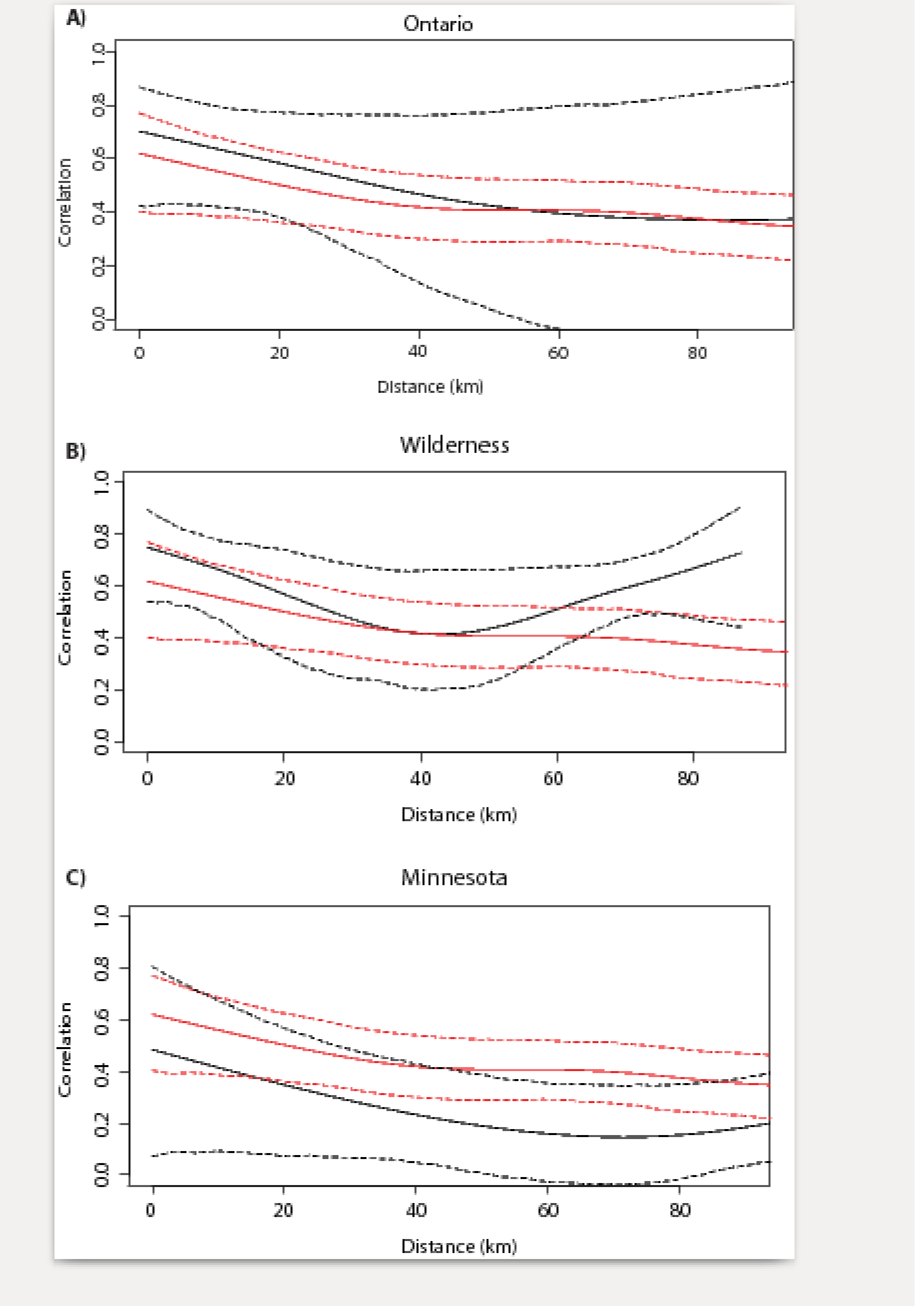


Fig. 1 Spatial non-parametric covariance functions (SNCF) of spruce budworm outbreak characteristics in landscapes with different spatial configurations. The Ontario landscape is characterised by large cut-blocks (approx. 100 Ha) and is coarsely fragmented, the wilderness landscape is natural and has not undergone fragmentation by harvesting, the Minnesota landscape has undergone fine fragmentation by harvesting (approx. 10 Ha cutblocks). The redlines represent the overall SNCF for the entire Border Lakes area (dotted lines are 95% confidence intervals) and the black lines represent the SNCF for each landscape type.

Methods

To compare the relative impact of harvesting and of spruce budworm outbreak on forest composition across the budworm-susceptible forest of Quebec, we used the SIFORT geospatial database. We refined the analysis presented last year to focus on the white birch balsam fir climatic subdomain where we evaluated 227 941 tesserae. We then determined for each dominant composition the proportion of tesserae affected by either harvesting (with or without spruce budworm disturbance) or by the spruce budworm. Two disturbance intensities were used: partial disturbance (25 to 75% basal area removed; partial cutting/salvage logging and light/moderate SBW-caused mortality) and total disturbance (≥75% basal area removed; clearcutting and severe SBW-caused mortality). For each disturbance intensity (partial disturbance and total disturbance), we used the Gallagher index (LSq) to assess whether the proportion of tesserae that were harvested is proportional to the proportion of tesserae where spruce budworm disturbance occurs (Gallagher 1991, Gallagher and Mitchell 2005).

$$\mathrm{LSq} = \sqrt{\frac{1}{2}\sum_{i=1}^{n}(V_i - S_i)^2}$$

Where V is the proportion of forests a given stand type *i* in a landscape affected by the SBW, S is the proportion of forests of a given stand type that were harvested and *n* is the number of stand types. The index weighs the deviations between managed and unmanaged landscapes such that the larger the differences between the observed disturbances (harvesting and SBW outbreak), the larger the Gallagher index. We used this index to compare the two severities of natural disturbance with the two severities of harvest disturbance, i.e. partial (salvage) harvesting with light/moderate SBW-caused mortality and clearcuts with severe outbreak mortality.

We also assessed the occurrence of forest management and spruce budworm disturbance in stands of different size and composition. Tesserae having the same dominant cover and that were direct neighbours i.e. that shared at least one border (before or early in the outbreak 1964-1975) were grouped. We then summed these neighbouring tesserae to determine patch size of a given cover type (balsam fir or spruce). We grouped stands into patch sizes of less than 100 ha, from 101-500 ha, from 501 to 1000 ha, and greater than 1000 ha. Then, we determined the proportion of harvesting (clearcutting) and spruce budworm disturbance (spruce budworm-caused mortality) in each patch and used this to determine the proportion of disturbance per patch size class.

To assess long-term changes on SBW severity and duration due to forest alteration across landscapes (i.e. the proportion of a landscape that has been harvested) we used a dendrochronological analysis of thousands of balsam fir, white spruce and black spruce tree cores collected by the MFFP in their temporary and permanent sample plots. Spruce cores provide a good proxy of effects as they record defoliation effects on growth while the tree usually survives. The decrease in ring widths and the proportion of trees affected is a good proxy for outbreak severity but it does not directly measure severity (i.e. tree mortality).

RESULTS / DISCUSSION

Disturbance by harvesting is disproportional to the disturbance by SBW for the two intensities of disturbance (Gallagher index greater than zero) (Table 1). This disproportionality was expected to decrease with the larger sampling effort conducted this year but instead was found to increase. It almost tripled for partial disturbances and although the increase was less for total disturbances from the previous calculations it still remained of greater overall magnitude.

For partial disturbance, although fir stands were more often affected by SBW disturbance and partial harvesting (salvaging) than spruce stands, there was a greater targeting by salvaging of spruce stands than fir stands: 62% of all lightly to moderately and 75% of severely SBW-affected stands were fir stands. On the other hand, spruce stands were more targeted by harvesting than their proportion in the landscape. The Gallagher index directly evaluates where the proportions of harvested vs SBW affected stands are proportional to their representativity ? in the landscape. The disproportionality between clearcutting and severe SBW outbreak was high for both partial and severe disturbances (31 and 49 respectively where the numbers above one indicate disproportionality and greater than ten indicates a high disproportionality.

Table 1: Area proportion per disturbance type for stands initially dominated by host species of the spruce budworm (fir and spruce). Disturbances are separated by their severity, partial disturbance (partial salvage logging and light/moderate outbreak in the table and text) and total disturbance (called clearcut and severe outbreak in the table and text). For a given composition, the proportion of disturbed host stands for each type of disturbance is shown. The Gallagher index is a measure of disproportionality. When the value of the index is zero, the variables are completely proportional as the index increases there is a difference between harvesting and SBW.

	Proportion per Stand type (%)				Gallagher index
	Fir	**Fir-spruce**	**Spruce-fir**	**Spruce**	
Pre-disturbance	36	1	4	59	
Partial disturbance					
Partial (salvage) cut	34	1	3	63	
Light/moderate SBW outbreak	62	2	5	30	30.72
Total disturbance					
Clearcut	26	1	4	69	
Severe SBW outbreak	75	1	3	20	48.81

SBW vs Harvesting and fir\spruce patch size

In balsam fir dominated stands the proportion of affected cells was much higher for SBW than for cutting during the last outbreak period (1970s and 1980s). There is also a pattern that SBW had a stronger affect when the balsam fir stands were larger in size (over 40% of cells affected when blocks of fir exceeded 1000ha vs 30% when blocks of fir were less than 100Ha (Figure 2).
Black spruce is a much less vulnerable species and it is little affected (less than 10% of stands disturbed) by the SBW. However, even though spruce is less affected than fir by SBW, small blocks are about twice as disturbed as large blocks by SBW. This underlines the importance of maintaining large blocks of spruce or ensuring that large blocks of spruce are regenerated following harvesting (Ste-Marie et al. 2014, Bognounou et al. 2017). Future work should also consider natural succession which tends to favour fir, and within block variability in stand composition, and gradients in composition at multiple scales

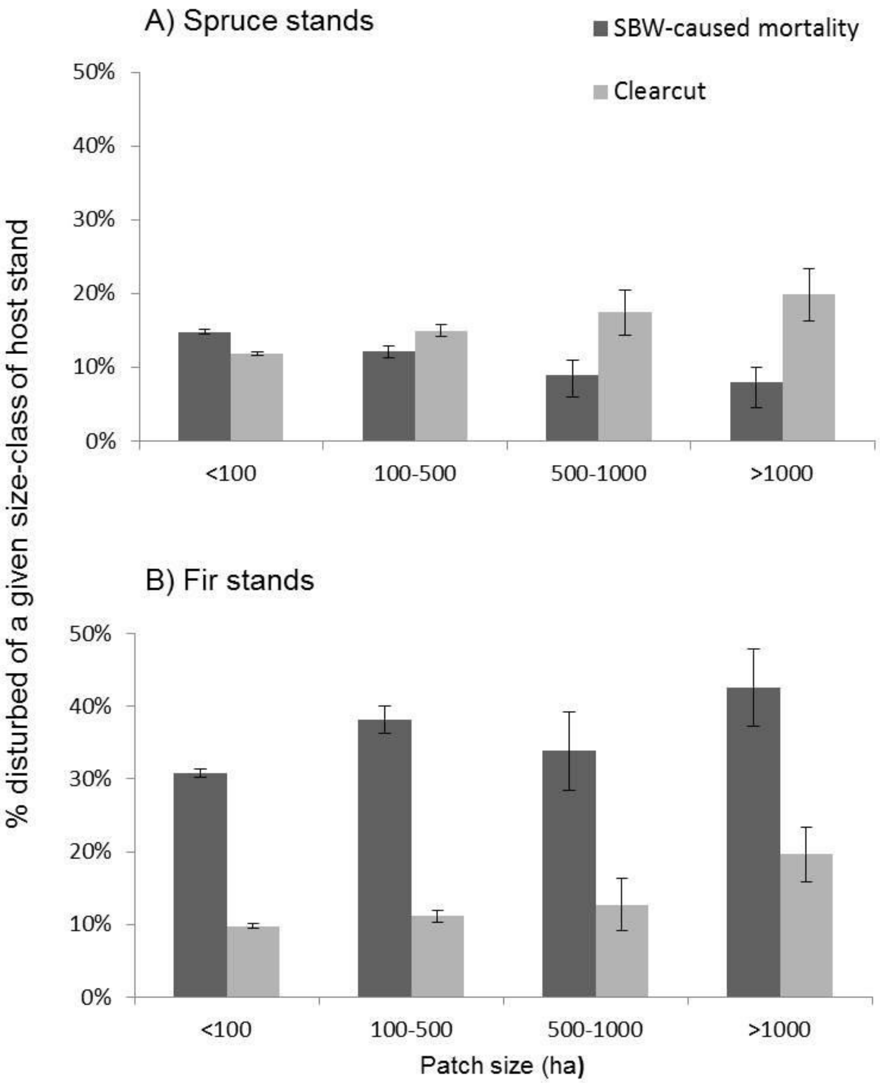


Figure 2. The percentage of spruce and fir stands that were harvested or attacked by SBW according to the size of the stand.

Dendrochronological analyses

The dendrochronological analyses suggest that the proportion of balsam fir trees affected (showing a growth decrease in tree-ring cores) does not change based on the percentage of a landscape dominated by host species (Figure 3). In other words, as long as there are host species present in a landscape the SBW will find them. A dilution effect does not occur in terms of defoliation and growth reduction. Associational resistance may however still occur in terms of survival (Su et al. 1996). In figure 4, the percentage of a landscape that is harvested affects defoliation (as measured by growth reductions in tree rings) of black spruce trees. The proportion of black spruce showing growth reductions decreases with landscape fragmentation whereas the severity (number of years of growth reduction) peaks in moderately fragmented landscapes. This is consistent with results presented two years ago showing greater spruce mortality in moderately (50%) fragmented landscapes. Our work thus consistently demonstrates that landscape configuration has only a small effect on fir but that landscape configuration has a much stronger influence on defoliation, growth reductions and mortality of black spruce (Kneeshaw et al. In-review).

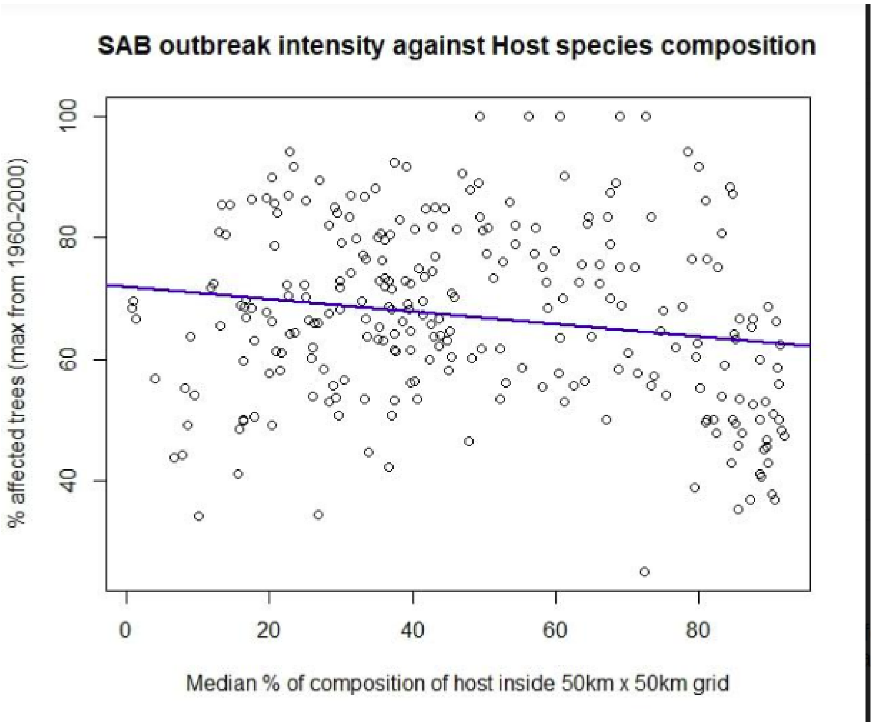
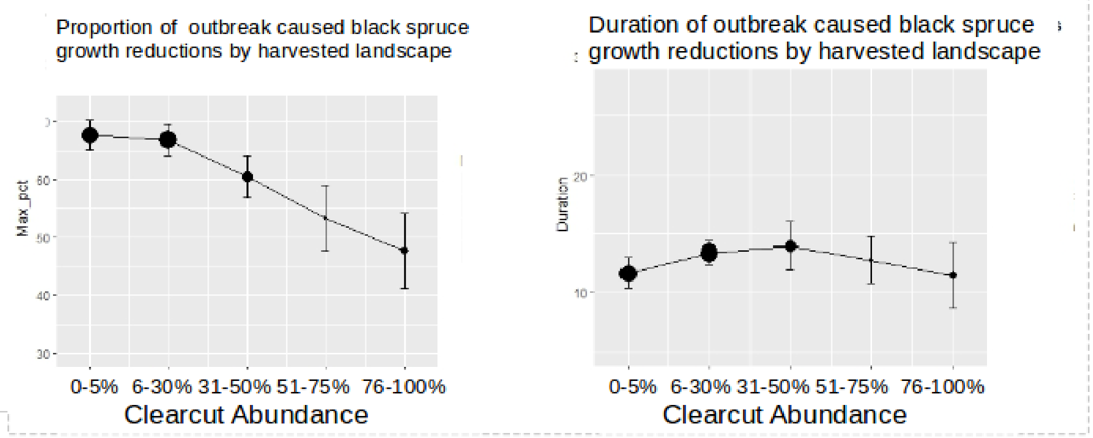


Figure 3. Proportion of fir trees registering a growth decrease as a function of the landscape level proportion of host trees as measured per 250km^2 grid. Although the relationship is significant $p<0.001$, the variance explained is small (R2= 0.03). Analyses with proportion of fir only as the indepent variable were not significant and with spruce only the same slightly negative relationship was observed.


Proportion of outbreak caused black spruce growth reductions by harvested landscape

Duration of outbreak caused black spruce growth reductions by harvested landscape

Figure 4. (A).Proportion of black spruce trees showing SBW caused growth reduction and (B) duration of SBW caused growth reduction in landscapes in the white birch-balsam fir climatic subdomain with different degrees of clearcut caused openings. Bars represent standard errors

The potential influence of climate change on the interaction between drought and insect defoliation

Methods

In last years report we assessed how climate change may affect the risk of SBW mortality using BIOSIM to calculate climate conditions at the beginning of the last outbreak i.e. for 1965-1976 and also for 2003-2014 i.e. for the conditions at the time of the current outbreak and using MODIS images and showed that there is support for central Nova Scotia to be less at risk in the future while the Cape Breton highlands could be more at risk.

In the current report we sought to test whether moisture stress predisposes trees to defoliation using SBW and forest tent caterpillar (FTC) as test organisms. We also tested whether time lagged effects influence the relationship. We quantified ecosystem memory (time lags) to past water and insect defoliation stress and empirically tested for their interactive effects on tree growth in western (Alberta) and eastern (Quebec) regions of the Canadian boreal forest. The two study regions are characterized by different climates, species compositions, and primary defoliating insect species (SBW and FTC). We used a Bayesian hierarchical model to estimate ecosystem memory to environmental stressors and to quantify their antecedent effects on current ecological processes. We applied the model separately to tree-ring, interpolated climate, and aerial defoliation datasets from the two study regions. Using two contrasting boreal study systems allows us to highlight the generalizability of the model to two different systems and allows for a more robust interpretation of interactions between drought and insect defoliation (i.e. a model was tested for the FTC in the west and a separte model for the SBW in the east).

Results

Study stands in the East exhibited a more prolonged response to past climatic water deficit than stands in the West (Figure 5). However, the estimated memory function for the West exhibited a faster response to climatic water deficit than in the East. Similar to climatic water deficit, there was weak evidence study stands in the East exhibited more prolonged memory to past insect defoliation events than stands in the West.

The estimated ecological memory functions for climatic water deficit indicate memory to moisture stress for up to 5 (West) to 7 (East) years in the past (Figure 3A). Water deficits in the growing season prior to the year of growth had the highest antecedent weight in both study regions. These results are consistent with previous studies indicating water availability in preceding years has greater impact on tree growth and mortality than current growing season conditions (Michaelian et al., 2011; D’Amato et al., 2013).

Persistent effects of insect defoliation on stands in both study regions were also observed in which effects lengthen and magnitude increases the longer a defoliation event lasts. Additionally, the increase in cumulative effects over consecutive years of defoliation shows maximum antecedent values one or more years after the initiation of a defoliation event. While the ecological memory functions for insect defoliation were similar for the two study regions (as evidenced by overlapping 95 percent credible intervals in Figure 5B), there is evidence the memory of eastern stands to past SBW defoliation events is longer than the memory of western stands to past FTC defoliation events (as indicated by the larger range parameter, $1 / \varphi$, in the East).

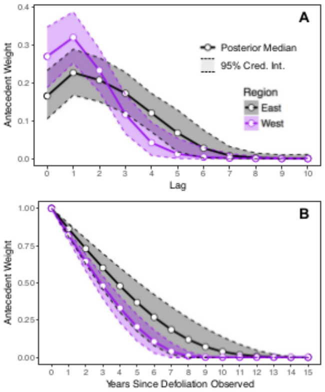


Figure 3: Ecological memory functions for climatic water deficit (A) and insect defoliation (B) based on annual radial tree growth in eastern and western study regions. Lag indicates the number of years before current year growth.

Figure 6 presents the sum of estimated effects for antecedent insect defoliation, antecedent climatic water deficit, and their interaction relative to regional mean annual tree growth over the study period after controlling for tree size.
Thus even when investigating lagged effects in response (antecedent effects) of mean annual tree growth in stands from both study regions we were not able to identify a negative interaction between insect defoliation and climatic water deficit (γ 3 in Table 2). This lack of a negative interaction between defoliation and drought stress was surprising but is consistent with other studies (Kolb et al. 2016, Jacquet et al., 2014). The lack of interactive effects on host-tree growth may be attributable to the absence of severe drought conditions in either region during the study period. The climate was drier, on average, in both regions prior to circa 1960. More severe water stress may trigger stronger tree growth responses leading to interactions with antecedent defoliation not observed in the current study. Indeed, ecological theory indicates there are likely drought thresholds beyond which (tree responses occur) tree stress occurs, but below which, trees are able to maintain basic physiological function (Allen et al., 2015).

We did, however, observe an increase in the mean annual growth of large-diameter, non-host trees in the East when both defoliation and drought occurred. This is likely due to reduced evapotranspiration from defoliated trees permitting non-host trees to benefit from increased moisture in defoliated vs non-defoliated stands. it is also consistent with studies indicating increases in the growth of non-host trees following insect defoliation events in the eastern Canadian boreal forest (Duchesne and Ouimet, 2008).

These initial results are thus promising in that although effects persist, the lack of negative interactions under the observed conditions and even when considering lagged effects means that forests are more resilient than we anticipated. Negative interactions could still be expected to occur beyond a given threshold or for tree mortality.

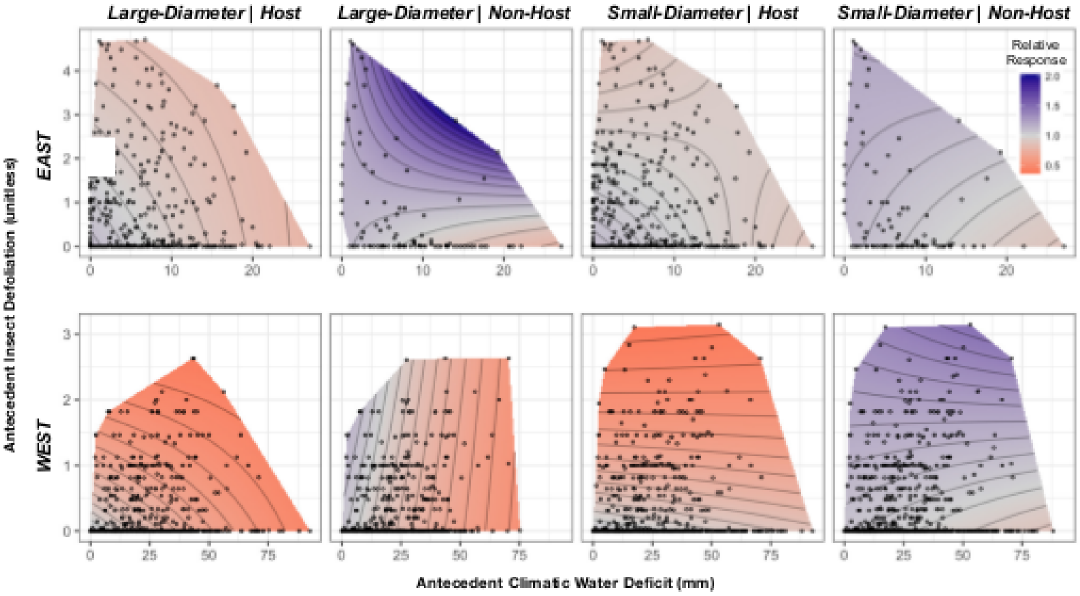


Figure 5: Estimated effects of antecedent insect defoliation, antecedent climatic water deficit, and their interaction on tree growth in eastern and western study regions by species (defoliator host vs. non-host) and size (large- vs. small-diameter) categories. Points represent posterior median antecedent variable values based on study data. Relative response surfaces correspond to mean tree growth under antecedent conditions relative to regional mean tree growth over the study period (East: 1968-1998, West: 1968-2010) after controlling for tree size. Response surfaces were generated by imposing a dense grid over the range of modeled antecedent variable values.

Table 1: Posterior summary of stand-level, antecedent variable coefficients. Posterior median coefficient values are provided for antecedent insect defoliation, antecedent climatic water deficit, and their interaction for each study region by species (defoliator host vs. non-host) and size (large- vs. small-diameter) categories. 95 percent credible intervals are given in parentheses. Coefficients for which credible intervals do not include zero are bolded.

Parameter	Category	East	West
		Antecedent Defoliation	
γ_1	Host	**-0.128 (-0.187, -0.077)**	**-0.467 (-0.602, -0.335)**
	Non-Host	0.026 (-0.055, 0.106)	0.083 (-0.03, 0.201)
		Antecedent Water Deficit	
γ_2	Large	**-0.034 (-0.043, -0.026)**	**-0.012 (-0.014, -0.01)**
	Small	**-0.02 (-0.029, -0.011)**	**-0.003 (-0.004, -0.001)**
		Antecedent Defoliation × Antecedent Water Deficit	
γ_3	Host|Large	0.005 (-0.003, 0.012)	0.004 (-0.001, 0.008)
	Host|Small	0.007 (-0.001, 0.015)	0.001 (-0.003, 0.005)
	Non-Host|Large	**0.032 (0.012, 0.067)**	-0.001 (-0.005, 0.003)
	Non-Host|Small	0.005 (-0.014, 0.029)	0.002 (-0.002, 0.005)

Evaluation of the impact of microbes on SBW survival and growth

METHODS:

At last year’s meeting we presented preliminary results and analyses on microbial effects on SBW health that showed a possible effect of microbes in digestion. Since then we have pursued the study of the effect of microbial communities on SBW health in a laboratory and have begun a field study. have been shown to be play an important role for host health in mammals, especially humans. It is thought that microbes could play an equally important role in other animals such as insects. On the other hand other bacteria have been associated with disease and reductions in health..We studied the effects of the gut-associated microbial community in the eastern spruce budworm, *Choristoneura fumiferana*, by studying the effects of an antibiotic treatment and diet on larval *C. fumiferana* growth. We acquired approximately 1,000 spruce budworm second instar larvae that had completed diapause from the Insect Production Services at the Great Lakes Forestry Centre, (Sault Ste. Marie, ON, Canada). For the completed experiment reported here, 200 larvae were randomly selected and split equally among 5 treatments (n=40): artificial diet with antibiotics, black spruce (*Picea mariana*) foliage treated with antibiotics, untreated black spruce foliage, balsam fir (*Abies balsamea*) foliage treated with antibiotics, and untreated balsam fir foliage.

Artificial diet was acquired from the insect production services (as above) Spruce foliage was collected from saplings housed in the greenhouse at the Université du Québec à Montréal and stored at -20°C for ~4 weeks. Additionally, fir foliage was collected from trees near Baie Comeau, Québec and stored in sterile bags at -20°C for ~4 weeks. For antibiotic treatments, a 1500 ppm solution of methyl paraben and a 50 µg/ml solution of streptomycin were each sprayed on the foliage every other day. Larval health was assessed every other day by measuring larval weight. We chose larval weight as a proxy for health because it is often used as a proxy for fitness in pupae and therefore can also be used as a proxy for overall health (Hammer et al., 2017). We extracted DNA from all midguts of surviving larvae (n=96). In addition 10 random individuals were selected randomly from each of the 5 treatment groups to extract DNA from foliage or synthetic diet (n=101) and frass (n=99), both collected at each of the two time points. All genomic DNA was extracted using the MoBio PowerSoil DNA extraction kit (Qiagen)

The effects of antibiotic treatment and diet (spruce or fir) on spruce budworm growth were tested using a mixed-effects model in the R package nlme (Pinheiro et al., 2017). Survival was tested with a logistic model.

In parallel with this laboratory study we have also begun a field study. This is being done as foliar microbe communities are known to change not only with tree species but also with geographic location.

Results

In terms of growth (Figure 7), the most relevant comparisons show that individuals feeding on fir treated with antibiotics grew less than those feeding on antibiotic treated spruce (-0.020 ± 0.005(mean ± SE); p<0.0001), and larvae feeding on untreated fir grew less than those feeding on untreated spruce (-0.017 ± .006; p=0.032). There was also no significant effect of diet (logistic regression, z= -0.897, p=0.3695) on larval survival rates, however the antibiotic treatment tended to favor survival (logistic regression, z= -1.810, p=0.0702). This could be interpreted as some members of the microbe community have deleterious effects on SBW. Comparisons of growth between larvae feeding on antibiotic treated and untreated foliage of the same type (i.e fir or spruce) were not significant however. This suggests that the differences in growth attributed to antibiotic treatment are driven by the differences in growth rate observed between larvae feeding on the two tree species (Figure 7). Alternatively, these differences may be due to the provenance of the seedlings. Spruce seedlings were nursery grown and thus boosted with nutrients while fir (seedlings ??? foliage) were taken from the field and thus should be expected to have much higher C:N ratios. Unfortunately foliar nutrient analyses were not conducted.

To further the analysis, we also tested whether microbial communities were different between fast and slow growing SBW. Gut communities of larvae feeding on antibiotic treated fir, on untreated fir foliage, on antibiotic treated spruce foliage and n untreated spruce foliage were not significantly different between fast and slow growers (PERMANOVA, p=0.100). Again these supplementary laboratory analyses do not show any promising results.

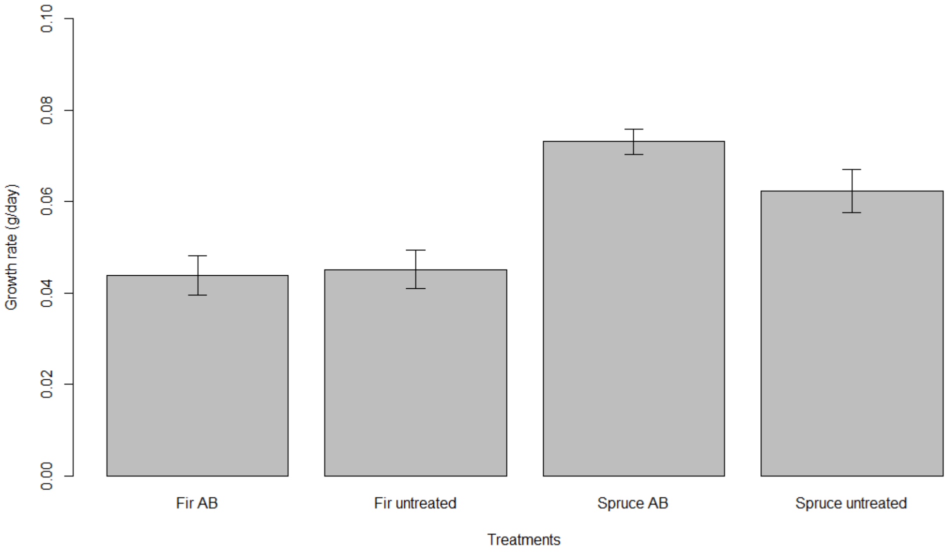


Figure 7. Growth rate (grams/day, ± S.E) of spruce budworm larvae among different diets (spruce versus fir foliage) and antibiotic treatments (AB = antibiotic treated). Letters indicate treatment combinations that differed significantly (p < 0.05) according to a Tukey’s Honest Significant Difference post-hoc test, based on a mixed model.

We show that the antibiotic treatment was sufficient to cause shifts in the microbial communities associated with balsam fir and black spruce foliage as well as in the guts of *C. fumiferana* larvae under laboratory conditions. Contrary to our expectations, we found that the observed antibiotic treatment did not significantly alter larval growth. We did find, however, that under laboratory conditions *C. fumiferana* larvae performed better when feeding on black spruce foliage compared to balsam fir which is widely understood to be the preferred food of *C. fumiferana* due to phenology.

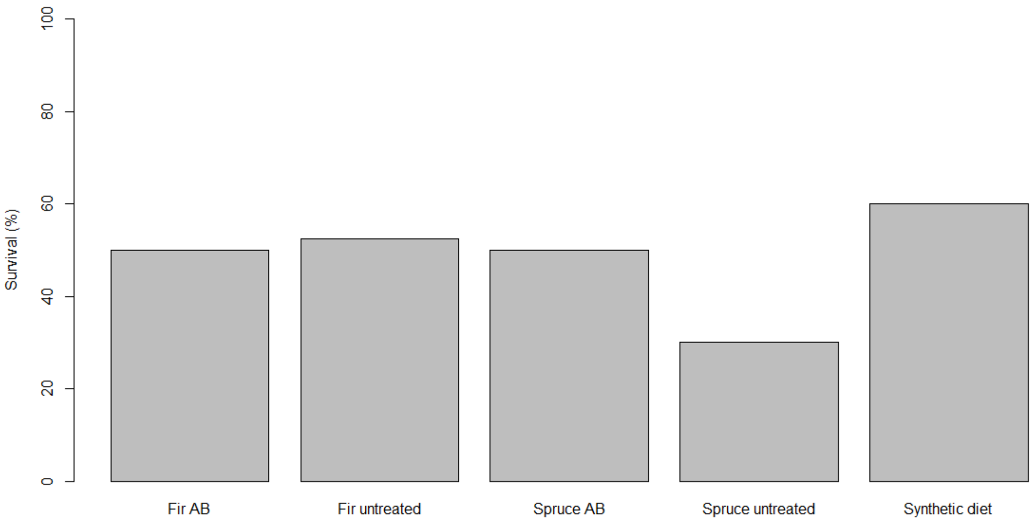


Figure 8. Percent survival of spruce budworm larvae among treatment diets (spruce versus fir foliage and synthetic diet) and antibiotic treatments (AB = antibiotic treatment).

In the coming year, to respond to limitations of the first lab study we will work on characterizing the microbiome of the SBW along various natural gradients to assess which factors determine the composition of its microbial community. We will also measure the capacity of the spruce budworm to harbor and retain a stable microbiome during digestion and development. Samples from the field will be reared and analyzed with metagenomic pipelines to assess the functional diversity and importance of the microbiome and its relationship to parasitoid predators across a large climatic, geographic and compositional gradient. In the lab experiment there was no interaction between parasitoids and microbes. However, microbes could help protect SBW larva or accelerate damage from parasitoids. This follow-up project has the goal of bringing a better understanding of the relationship between the SBW and its microbiome, outbreak status and parasitoid predators.

CONCLUSIONS

- The landscape level effects of logging on forest configuration influence tree response to spruce budworm outbreaks. Much earlier work has focused on stand level effects (MacLean 1980, Bognounou et al. 2017) but our work at the landscape level in the Border Lakes area as well as in more maritime conditions is consistently showing the influence of landscape level patterns. The most vulnerable species, balsam fir, responds only weakly to landscape level effects i.e. when it is present it is affected. The more valuable black spruce responds more strongly.

- The largest spruce stands are much more affected by harvesting than by SBW outbreaks whereas the opposite is true for fir. Fir is twofold more affected by SBW than by harvesting. As large blocks of spruce show little vulnerability to SBW, foresters should be making every effort to maintain large blocks of spruce.

- Black spruce seems to be more severely affected by SBW in moderately fragmented landscapes (30-75% of a landscape in clearcuts). Further work is needed to refine our understanding.

- Climatically, last year we showed the recent climatic shift over the last 30 to 40 years should reduce the risk of mortality in areas such as Nova Scotia, whereas traditional hot spot such as the Gaspé Peninsula will continue to be a high risk area. This year we have shown that risks of increased negative effects of defoliation (in terms of growth reductions) due to interactions with moisture stress may not be as problematic as previously conceived. Further work should pursue using tree mortality and higher levels of moisture stress.

- The knowledge presented here can be used to better target forest protection strategies, i.e. spruce stands in moderately harvested landscapes are more vulnerable to SBW than in landscapes with low harvesting or in landscapes with very high harvesting and thus with insufficient food to support an outbreak. Small droughts do not seem to increase the need for supplementary interventions to protect standing timber (at least in terms of growth reductions). Although the initial laboratory evaluations of microbial effects on SBW were not promising, further work in the field is required to further evaluate its potential. Continued funding will permit us to further evaluate landscape level effects, climate induced effects, as well as to address potential carbon impacts.

ACKNOWLEDGEMENTS

Financial support for this research was provided by the the Spray Efficacy Research Group (SERG) through the SOPFIM, NSDNR, AlbertaAAF and the OMNRF. UQAM also contributed to support this work. Data was provided as in-kind support by the Quebec Ministère des Forêts, de la Faune et des Parcs. We thank Guillaume Sainte-Marie, Mathieu Neau, Louis Etienne Robert, Jeanne Moisan Perrier and Ariane Breault for their work structuring the data and performing the analyses.

REFERENCES

Anderegg, W. R. L. and Callaway, E. S. (2012). Infestation and hydraulic consequences of induced carbon starvation. Plant Physiology, 159(4):1866–1874.

Blais, J. 1958. The vulnerability of balsam fir to spruce budworm attack in northwestern Ontario, with special reference to the physiological age of the tree. For. Chron **34**: 405-422.

Blais, J.R. 1983. Trends in the frequency, extent, and severity of spruce budworm outbreaks in eastern Canada. Can. J. For. Res. **13**(4).

Bognounou, F., De Grandpré, L., Pureswaran, D.S. and Kneeshaw, D., 2017. Temporal variation in plant neighborhood effects on the defoliation of primary and secondary hosts by an insect pest. *Ecosphere*, *8*(3) , pages ??.

D'Amato, A. W., Bradford, J. B., Fraver, S., and Palik, B. J. (2013). Effects of thinning on drought vulnerability and climate response in north temperate forest ecosystems. Ecological Applications, 23(8):1735–1742.

Duchesne, L. and Ouimet, R. (2008). Population dynamics of tree species in southern Quebec, Canada: 1970-2005. Forest Ecology and Management, 255(7):3001–3012

Foster, J. 2011. Forest insect defoliation patterns and carbon dynamics: linking remote sensing with simulation modeling. *In* Ph.D. Thesis, Forest and Wildlife Ecology Dept. University of Wisconsin-Madison. 203 p.

Jacquet, J.-S., Bosc, A., O'Grady, A., and Jactel, H. (2014). Combined effects of defoliation and water stress on pine growth and non-structural carbohydrates. Tree Physiology, 34(4):367–376.

James, P.M., Sturtevant, B.R., Townsend, P., Wolter, P., and Fortin, M.-J. 2011. Two-dimensional wavelet analysis of spruce budworm host basal area in the Border Lakes landscape. Ecol. Appl. **21**(6): 2197-2209.

Kolb, T. E., Fettig, C. J., Ayres, M. P., Bentz, B. J., Hicke, J. A., Mathiasen, R., Stewart, J. E., and Weed, A. S. (2016). Observed and anticipated impacts of drought on forest insects and diseases in the United States. Forest Ecology and Management, 380:321–334.

MacLean, D.A. 1980. Vulnerability of fir–spruce stands during uncontrolled spruce budworm outbreaks: a review and discussion. For. Chron. **56**: 213-221.

McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D. G., and Yepez, E. A. (2008). Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist, 178(4):719–739

Ministère des Forêts, de la Faune et des Parcs. 2016. Aires infestées par la tordeuse des bourgeons de l'épinette au Québec en 2016 - Version 1.0. Gouvernement du Québec, Direction de la protection des forêts. 17p.

Pelletier, G., Dumont, Y., and Bédard, M. 2007. SIFORT: Système d'Information forestière par tesselle. Manuel de l'usager. Ressources naturelles et Faune, Québec.

Pureswaran, D. S., De Grandpré, L., Paré, D., Taylor, A., Barrette, M., Morin, H., Régnière, J., and Kneeshaw, D. D. (2015). Climate-induced changes in host tree insect phenology may drive ecological state-shift in boreal forests. Ecology, 96(6):1480–1491.

Robert, L.-E., Kneeshaw, D., and Sturtevant, B.R. 2012. Effects of forest management legacies on spruce budworm (*Choristoneura fumiferana*) outbreaks. Can. J. For. Res. **42**(3): 463-475.

Roland, J. 1993. Large-scale forest fragmentation increases the duration of tent caterpillar outbreak. *Oecologia*, ***93*: 25-30.**

Sanderson, E. W., et al. 2002. The Human Footprint and the Last of the Wild *BioScience **52**:* 891-904.

Sainte-Marie, G.B. 2016. Passer la sylviculture dans la tordeuse : évaluation des traitements préventifs face aux pertes de bois liées à la tordeuse des bourgeons de l'épinette. *In* Ph.D. Thesis, Institut des sciences de l'environnement. Université du Québec à Montréal, Montreal, QC, Canada. 127 p.

Su, Q., MacLean, D.A., and Needham, T.D. 1996. The influence of hardwood content on balsam fir defoliation by spruce budworm. Can. J. For. Res. **26**(9): 1620-1628.

Funding requested. (‘000 $)

Item	**2015-16**	**2016-17**	**2017-18**
Field work	30k	30k	30k
Graduate student salaries (1 PhD)	20k	20k	20k
Research professional	10k	10k	10k
Student/Casual labour	7k	7k	7k
Supplies	2.5k	2.5k	2.5k
Total requested	**69.5k**	**69.5k**	**69.5k**
Proposed sources			
CFS – Forest Productivity / Disturbance	15 k	15k	15k
University graduate student support	5k	5k	5k
SERG-I Partners	49.5k	49.5k	49.5k
Total	**69.5k**	**69.5k**	**69.5k**

Total requested from SERG-I partners for 2017-2018: $50k

Funding received (‘000 $)

Item	**2015-16**	2016-17	2017-18
CFS – Forest Productivity / Disturbance	0 k	0k	0k
University graduate student support	5k	5k	6k
SERG-I Partners	6k	6	23k
Total	**11 K**	11k	29k

We also received in-kind support of access to data from the Quebec MFFP, from OMRNF and from Alberta Ministry of Agriculture and Forestry.

Numerical descriptors of spruce budworm immigration on the west coast of Newfoundland

Marc Rhainds [1*], *Dan Lavigne* [2] *and Troy Rideout* [2]

[1] *Natural Resources Canada, Canadian Forest Service - AFC, P.O. Box 4000, Fredericton, New Brunswick, E3B 5P7.* [*] *Corresponding author:* marc.rhainds@canada.ca

[2] *Ministry of Natural Resources, Government of Newfoundland and Labrador, Herald Towers, P.O. Box 2006, Corner Brook, Newfoundland*

Abstract

A simple mathematical tool is proposed to detect the incidence and intensity of budworm immigration on the west coast of Newfoundland, based on variables related to the statistical mode of abundance (nights with highest number of budworms) as derived with captures at light traps. An abductive approach is privilgeged (inference to the best explanation, or “I know it when I see it”) to pinpoint instances where immigrations provide the simplest and most logical explanation about a given frequency distribution of budworm abundance. This report illustrates a likely immigration event that took place on 26 – 27 July 2017, and for which post-migration survival of budworms resulted in 3-d clusters of high adult abundance.

Résumé

Une approche mathématique est développée afin d’identifier l’incidence et intensité de mouvements migratoires de la tordeuse sur la côte ouest de Terre-Neuve; l’approche est basée sur des paramètres associés au mode statistique d’abondance (nuits avec des nombres élevées de tordeuses) et dérivés à l’aide de captures dans les pièges lumineux. Les données sont analysées sur une base abductive (inférence à l’explication la plus probante, ou “Je reconnais un phénomène quand je le vois”) pour identifier des cas d’espèce pour lesquels l’immigration procure l’explication la plus simple et logique à une distribution spécifique d’abondance. Dans ce rapport, nous évaluons les attributs numériques d’un mouvement migratoire dans la nuit du 26 au 27 Juillet 2017, pour lequel la survie post-migratoire des tordeuses est liée à une période résiduelle de 3 jours avec des captures élevées dans les pièges lumineux.

Background and Objectives

Spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae), is a univoltine defoliator of balsam fir and spruces in Nearctic boreal forests. Adults emerge in late June – July over a span of 10 - 14 d, with a level of protandry of about 2 d. Gravid females with heavy abdomen are incapable of flight and oviposit in their natal patch early in life. Thereafter, partly spent females may migrate by ascending above tree canopies to be carried downwind.

The abundance of budworms has been monitored for decades with light traps, generally to predict future population abundance; few studies have focussed on day-to-day variation in abundance. Advantages of light traps over pheromone traps are twofold: (1) they capture adults of both sexes (though males are usually more abundant than females); and (2) detection of female migrants improves demographic forecasting.

Radar observations and aerial collections of windborne budworms have been used to document weather conditions conducive to migrations and sex ratios (males migrate but less frequently than females). One challenge in documenting immigration is the 'homogeneous' niche of budworm. Local populations of budworms in vast forest stretches are spatially autocorrelated to an extent that makes it hard to separate short-range local flights from long-range migrations.

The geographic setting of Newfoundland (NL), a large island in eastern Canada isolated from the mainland by a an oceanic barrier > 100 km across, provides an opportunity to detect immigrations. Budworm populations in NL may also be isolated in time at the northern edge of the distribution range: in the absence of a bridge in time between source and sink populations (*i.e.*, late flight in NL relative to the mainland where migrants originate), all budworms at light traps can be classified as residents.

This study aims at detecting major immigrations of budworms characterized by large influx of adults. Minor immigrations involving few individuals are outside the scope of the study as they are difficult to tract, have limited demographic consequences, and lead to a teleological trap: if immigrations occur everywhere they become differentiable nowhere.

Immigrations are defined as pulsed, external depositions of budworms that are delimited in time relative to local flight of resident moths. Immigrations can be ascertained when they occur outside (before or after) expected flight in sink populations (asynchronic immigration). Synchronic immigrations (those that coincide with local flight) are difficult to detect because they are superposed onto temporal landscapes in sink populations. Large influx of immigrants imply that variables related to statistical mode (contiguous nights with high number of moths) reflect the timing and intensity of immigration.

We explore challenges and opportunities of detecting immigration of budworms on the west coast of NL based on temporal variation in abundance of adults at light traps.

Materials and Methods

The study was conducted between 2014 – 2017 at two balsam fir stands with low budworm density (< one larva per branch; Lavigne 2016) located ca 1.5 km inland (Rocky Harbor, 49.57^{o}N, 57.88^{o}W) and 150 m from the coastline (Sally's Cove, 49.73^{o}N, 57.93^{o}W). Adult budworms were captured with stainless steel vane traps with a 15-W neon tube as light source (Leptraps, Georgetown, KY); the traps were suspended on a rope 3-4 m above ground between the trunks of two firs, 50 m inside the forest stand. Each trap was wired to a 12 V marine battery that was replaced every 4 – 6 day to prevent discharge. Moths captured at light traps were killed using Vapona strips. Number of traps for different sites and years are reported in Table 1. Moths were collected at light traps daily in 2014 – 2015, and at intervals of 1 – 4 d in 2016 – 2017. The moth samples were transferred into plastic containers labeled by site, date, and trap number before being sorted from other species (bycatch); genitalia of specimens were inspected under a stereoscope to separate males and females.

The schedule of budworm emergence was simulated using the BioSIM phenology software at Rocky Harbor and Sally's Cove, as well as three potential sources of migrants in the province of

QC: known forest stands with high densities of budworms near Gaspé (49.05°N, 64.62°W), Baie-Comeau (49.25°N, 68.22°W), and Sept-Îles (50.32°N, 66.46°W).

The following rules of thumb were utilized to detect major immigration events in NL:

(1) Immigrations are by nature pulsed events, hence the primary parameter of interest relates to the statistical mode of abundance; more precisely, post-migration survival of budworms is expected to bring about high captures of adults at light traps for a period of t d. The timing and intensity of immigrations can thus be quantified using t-d clusters of high budworm abundance (n_t / t) relative to the entire period of flight (N / T), in which N represents the total number of budworms at light traps and T the interval of sampling:

$$K = (n_t / t) / [(N - n_t) / (T - t)] \qquad (1)$$

Low and high values of K are expected in the absence / presence of immigration, respectively. K thresholds linked with immigration are currently unknown but can be infered using empirical data reported in this study.

(2) Due to distinct emergence time and dispersal behavior of male and female budworms, immigrations need to be calibrated separately along sex lines:

$$K_f = (f_t / t) / [(F - f_t) / (T - t)]; \text{ and} \qquad (2)$$

$$K_m = (m_t / t) / [(M - m_t) / (T - t)] \qquad (3)$$

For each seasonal record of abundance at a given light trap, an iterative approach was used to identify intervals m_t and f_t (t bounded between 2 and 5 d) that maximimze estimates of K_m and K_f. Iterations were not constrained to intervals including the statistical mode.

(3) Because both males and females migrate, the timing of ‘true’ immigrations is expected to coincide (or largely overlap) for males and females:

$$H_o = \text{immigration if interval } f_t \approx \text{interval } m_t \qquad (4)$$

Statistical analyses were conducted with the SAS software (SAS Institute, Cary, NC). Generalized linear model was used to evaluate the effect of site and year (including two way interaction term) on number of budworms (y’ = log y), sex ratios (proportion of females, subjected to arcsine transformations), average date of capture (day after June 30th), and extent of protandry (average date of capture of females – average date of capture of males).

Results

A total of 13 952 budworms were sampled during the four year study, with consistently higher densities at Sally’s Cove than Rocky Harbor ($F_{site} = 58.01$, $df = 1, 11$, $P < 0.0001$) and in 2014 – 2017 than 2015 – 2016 ($F_{year} = 29.96$, $df = 3, 11$, $P < 0.0001$); the interaction term was not statistically signficant ($F_{site*year} = 1.40$, $df = 3, 11$, $P = 0.2944$) (Fig. 1).

Males vastly outnumber females throughout the experiment (Fig. 2). Proportions of females were higher at Sally's Cove than Rocky Harbor (F_{site} = 9.49, df = 1, 11, P = 0.0105) and lowest in 2017 (F_{year} = 4.87, df = 3, 11, P = 0.0215); the interaction term was not statistically signficant ($F_{site*year}$ = 0.90, df = 3, 11, P = 0.4706) (Fig. 1).

Captures of budworms at light traps occurred earlier in 2014 than in other years (F_{year} = 796.17, df = 3, 11, $P < 0.0001$) (Fig. 2). Timing of capture did not consistently varied among sites (F_{site} = 2.35, df = 1, 11, P = 0.1532); the significant interaction term ($F_{site*year}$ = 8.20, df = 3, 11, P = 0.0038) was due to budworms occuring earlier seasonally at Rocky Harbor than Sally's Cove in 2014 and 2015, followed by an opposite trend in 2016 (Fig. 1).

With the exception of Sally's Cove in 2017, males occurred on average 2 to 4 d earlier than females (Fig. 1). The extent of protandry was higher in 2014 and lower in 2017 (F_{year} = 4.54, df = 3, 11, P = 0.0264) but did not vary between sites (F_{site} = 3.06, df = 1, 11, P = 0.1081; $F_{site*year}$ = 2.13, df = 3, 11, P = 0.1539).

Major immigrations are unlikely to have taken place in 2014 and 2015, as indicated by low values of K_m and variable intervals of f_t and m_t between light traps (Fig. 2, Table 1). It is impossible to assess whether or not immigrations took place in 2016 because light traps were sampled after a four d interval during the period of peak abundance (Fig. 2); however, variation in m_t at Rocky Harbor and Sally's Cove (< 20 km apart) is inconsistent with the occurrence of a broadscale immigration (Table 1). The most likely immigration event during the course of the study occured at both Rocky Harbor and Sally's Cove on the night of 26 – 27 July 2017: for the interval 27-29 July (including post-migratory survival of budworms; Fig. 2), values of K_m and K_f exceeded 10 for all traps, meaning that captures over the 3 d interval were on average 10 to 25 higher than during the remainder portion of the sampling season (Table 1, Fig. 2)

Discussion

This study illustrates challenges in documenting synchronic immigrations of budworms (those that coincide in time with local flight in sink populations), even at locations such as NL that are geographically isolated. Because immigrations are by nature pulsed events, their detection requires frequent collections of moths captured at light traps, ideally on a daily basis. The importance of sampling constraints is examplified by the statistical mode in 2016 (Fig. 1, Table 1) which occurred during a four d interval between trap collections; in that instance, it is near impossible to conclude as to whether or not an immigrations took place. Spatial variation in K_m and K_f indices among light traps (*i.e..* K_f varied between 4.82 to 22.50 at different light traps in 2016; Table 1) illustrate pitfalls in attempts to monitor migration using a single trap per location.

The simulated period of emergence on the west coast of NL, as derived with BioSIM, provided a broad phenological window encompassing temporal variation in budworm abundance at light traps – yet with an apparent early season bias (Fig. 2). In any event, temporal coincidence of expected – observed abundance of budworms does not imply the absence of immigration, however, due to a broad overalp in phenological windows in NL and on the mainland (Fig. 3).

Female budworms were most abundant during late phases of expected flight between 2014 and 2016 (Fig. 1). The phenomenon, which is reported here for the first time, may be due to unknown interactions between the phenology and physiology of female reproduction. While late season immigrations may be responsible for the trend above, this scenario appears unlikely considering that major migration events are expected to bring about high numbers of males *and* females while n_t modes diverged along sex lines ($f_t \neq m_t$; Table 1; see also Eq. 4).

If an immigration event took place during the course of the study, the most likely candidate would be the early season 3 d peak of abundance between the 27 – 29 July 2017 which included high proportion of adults at both Sally's Cove and Rocky Harbor (Fig. 1, Table 1).

Protandry in budworms leads to delayed flight of females relative to males in closed populations with limited immigration input (Figs. 1, 2); similar phenology for males and females at Sally's Cove in 2017 (Figs. 1, 2) strongly suggests early season deposition of extraneous moths. Further studies are needed to validate the hypothesis that the absence of protandry in seasonal patterns of budworm abundance can be used (in combination with other factors) to infer long-range immigration events.

In conclusion, empirically-derived mode-related variables provide a tool to characterize the incidence – intensity of asynchronic immigration - such as was apparently the case on the night of 26 – 27 July 2017, a finding that remains to be validated with radar technology. Logistically, rigorous determination of immigration necessitates daily record of moth abundance using more than one trap for any given location.

Funding

SERG:	$ 15 000
CFS-AFC	$ 5 000

Acknowledgements.

We are grateful to K Young, R Graves and G Brodersen for field assistance. Funding was provided by DNR-NL and SOPFIM.

Table 1. Estimates of parameters utilized to detect the incidence of immigration among spruce budworms captured at light traps (see Eqs. 1 – 4 in text for description of parameters)

Year	Site	Trap	Mode-related variables [t]		K	
			♂	♀	♂	♀
2014	RH	1	23-24	27-30	1.86	6.60
		2	15-19	23-24	1.91	6.36
		3	18-19	23-24	2.70	6.81
	SC	1	18-19	27-30	1.82	5.45
2015	RH	1	35-36	43-47	3.77	3.81
		2	35-36	43-46	6.74	7.30
		3	35-36	46-49	5.13	9.58
	SC	1	43-44	-	5.84	4.87
		2	43-44	43-47	5.59	8.02
		3	40-44	43-47	3.19	11.29
2016	RH	1	37-40	37-40	7.17	10.36
		2	37-40	37-40	3.07	22.50
		3	37-40	37-40	4.50	7.33
	SC	1	31-33	36-39	5.84	11.50
		2	31-33	36-39	6.34	4.82
2017	RH	1	27-29	27-29	19.76	10.50
		2	27-29	28-29	25.77	16.94
	SC	1	27-29	27-29	10.74	17.67
		2	27-29	27-29	12.59	16.47

[t] Day after June 30th.

Fig. 1. Variation in abundance, sex-ratio, timing of capture, and level of protandry for spruce budworms captured at two locations on the west coast of NL.

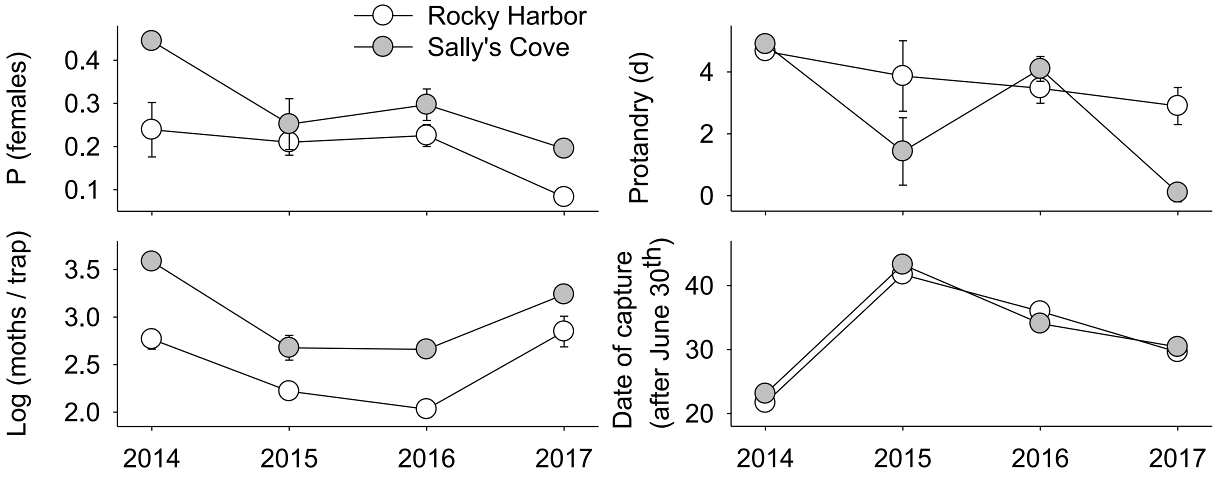


Fig. 2. Abundance of spruce budworms captured at light traps deployed along the coastline of western Newfoundland, at Rocky Harbor and Sally's Cove. For each year, dashed lines represent simulated emergence patterns of budworms as determined with the BioSIM phenology software.

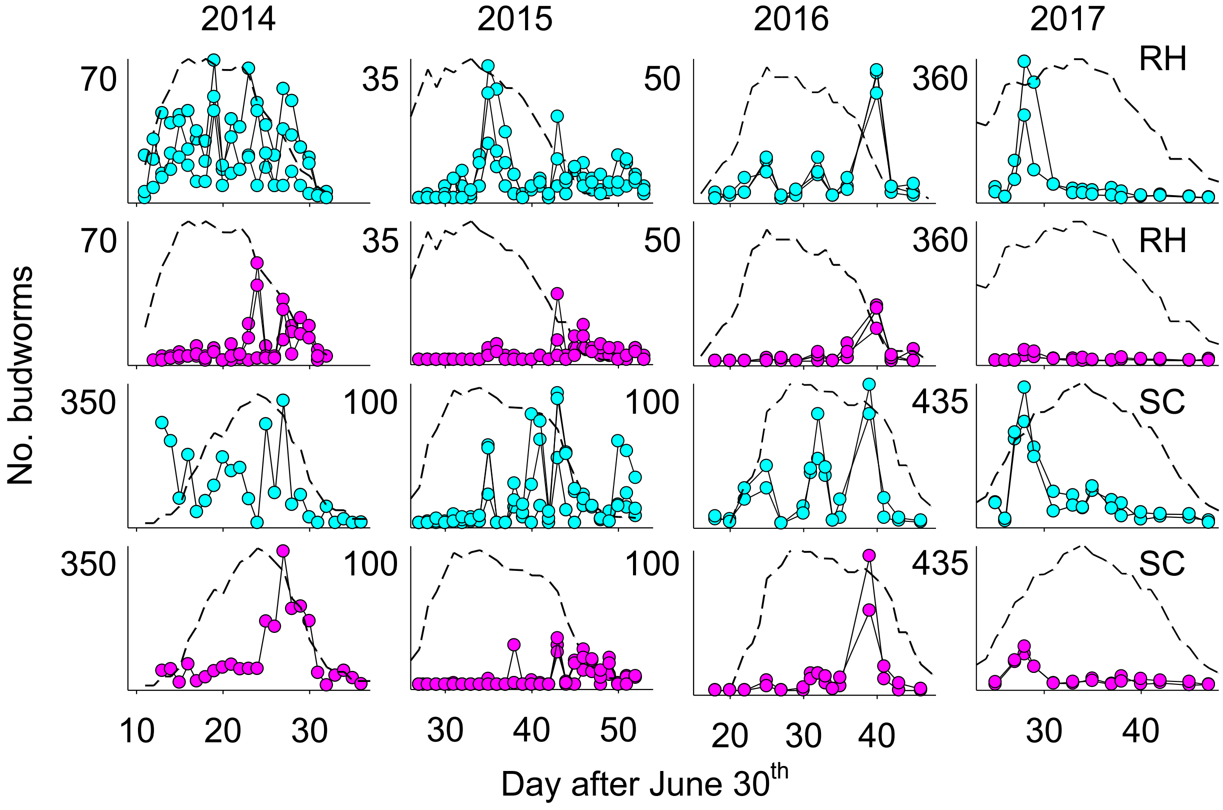
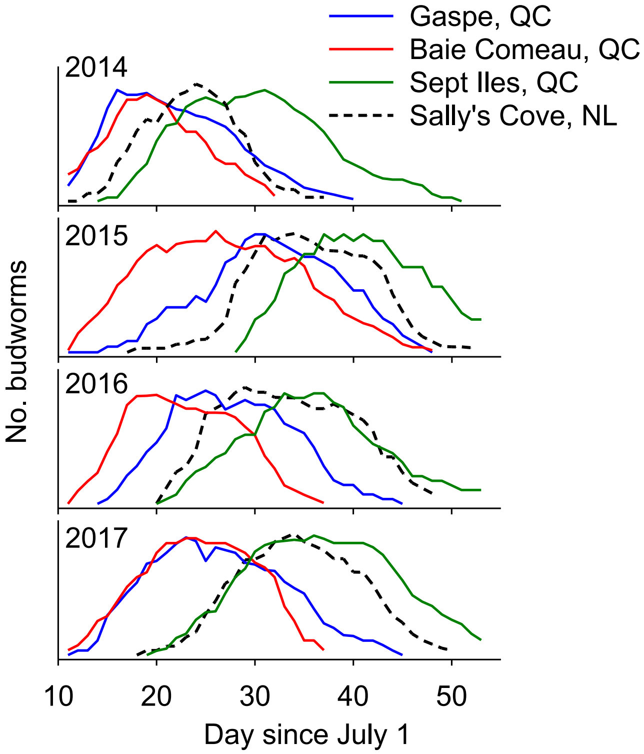


Fig. 3. Predicted phenology of spruce budworm emergence as derived with the BioSIM phenology software at Sally's Cove on the west coast of Newfoundland as well as three potential sources of migrants: known forest stands with high densities of budworms in the province of QC near Gaspé, Baie-Comeau , and Sept-Îles.



Measuring the active space of a spruce budworm pheromone trap: bridging the gap between chemical ecology and estimates of absolute population density

C J K MacQuarrie[1], *J D Allison*[1], *M C Bouwer*[2], *R Johns*[3], *D Pureswaran*[4]

1) NRCan Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario.
2) Department of Chemistry/Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.
3) NRCan Canadian Forest Service, Atlantic Forestry Centre, Fredericton, New Brunswick.
4) NRCan Canadian Forest Service, Laurentian Forestry Centre, Ste Foy, Quebec.

Abstract:

This report presents the results from experiments to determine the relationship between number of spruce budworm (SBW) moths caught in pheromone-baited traps, and the absolute number of moths present in a stand. In 2017 we quantified the relationship between when a moth responds to the pheromone plume emitted by a trap and the distance at which the response occurs using a walking bioassay in a large wind tunnel. We obtained lab-reared male SBW pupae from the insect production facility in Sault Ste. Marie, emerged them under two light-dark regimes, placed them in small wire-mesh cages and then exposed them to a pheromone plume. We then observed the wing fanning behaviour of the moths in the cages as an indication of a positive response to the pheromone. These experiments were repeated at different times of day and with different concentrations of pheromone. We found that the probability of response was less than 0.5 at all distances > 10 m regardless of the pheromone concentration or time of day. However, the probability of positive response at a given distance from a trap increased with higher concentrations of pheromone. These responses were also mediated by temperature, with lower probability of response (or no response) found when experiments were run in the evening (when it was cooler) than during the late afternoon. Previous experiments in this study have suggested the sampling radius of traps is small and that the plume structure emerging from the trap is complex. These data add to these earlier findings to suggest that SBW moths only begin to exhibit a positive response to the trap when they are in relatively close proximity to it.

Résumé

Le rapport présente les résultats d’expériences visant à déterminer le rapport entre le nombre de papillons de la tordeuse des bourgeons de l’épinette (TBE) capturés dans des pièges appâtés aux phéromones et le nombre absolu de papillons présents dans un peuplement. En 2017, nous avions quantifié le rapport entre le moment où un papillon réagit au panache de phéromone émis par un piège et la distance à laquelle la réaction se produisait à l’aide d’un essai biologique de marche dans un grand tunnel aérodynamique. Nous avons obtenu des nymphes mâles de la TBE élevées en laboratoire aux installations de production d’insectes de Sault Ste. Marie, les avons fait émergé sous deux modes de lumière-obscurité, placé dans de petites cages en treillis métallique puis exposé à un panache de phéromone. Nous avons ensuite observé le comportement des ailes des papillons dans les cages, leur déploiement était une indication de réaction positive à la phéromone. Ces expériences ont été répétées à différents moments de la journée et avec différentes concentrations de phéromone. Nous avons constaté que la probabilité de réaction était de moins de 0,5 pour toutes les distances de > 10 m, peu importe la concentration de phéromone ou le moment de la journée. Cependant, la probabilité d’obtenir une réaction positive à un piège à une distance donnée augmentait avec des concentrations plus élevées de phéromone. De plus, la température avait un effet modérateur sur les réactions, la probabilité d’une réaction (ou d’un manque de réaction) étant plus faible lors des expériences conduites le soir (lorsqu’il fait plus frais) qu’à la fin de l’après-midi. Des expériences menées antérieurement dans le cadre de cette étude avaient semblé montrer que le rayon d’échantillonnage des pièges est petit et que la structure du panache qui émerge du piège est complexe. Les nouvelles données s’ajoutent aux constatations antérieures pour laisser entendre que les papillons de la TBE commencent à manifester une réaction positive au piège seulement lorsqu’ils se trouvent relativement proches.

Introduction

The objective of this project is improve the utility of pheromone-baited spruce budworm (SBW) traps by determining the relationship between the number of moths captured and the absolute local density (i.e., number of adults per unit area). We have four research objectives: 1) Develop and test field and laboratory methods to test SBW response to pheromone baited traps and behavioural responses to odor plumes. 2) Use methods developed in (1) to determine the baseline relationship between absolute density of SBW and pheromone-baited trap catches 3) Examine variation in (2) among different stand types; and 4) Use relationships developed in (2 & 3) to provide quantitative estimates of SBW throughout eastern Canada from trap-catch data collected by provincial agencies and studies in ACOA 2.2.5. Research conducted in 2017 and reported below addresses objective 1.

Pheromone-baited traps are widely used to sample insect populations. In addition to their high degree of specificity, sex and aggregation pheromone-baited traps have the advantage of being able to sample low density populations because they induce orientation of the target taxa towards the trap. Despite these significant advantages, the application of pheromone-baited traps is limited in forest settings by our ignorance of their active space (the zone where pheromone concentration is at or above the insect’s threshold for response). The utility of pheromone-baited traps would be extended dramatically if instead of providing an estimate of relative density, they could be used to generate an estimate of absolute density (i.e., # of adults per unit area). Knowledge of the active space of the pheromone trap is required in order to estimate absolute density from a pheromone-baited trap catch.

The SBW is one of the most damaging forest insects in North America. Estimates of population size are used to inform management decisions for SBW. Currently these estimates are based on pheromone trap catches and estimates of the density of early instar larvae on branches. There is a positive relationship between early and late-instar larval density on branches and the number of adult moths in traps. However, both relationships are weak when populations are small. This is because estimates of larval density are difficult to obtain when populations are small, and larval density and trap catches tend to be highly variable. This suggests that estimates of population size made from both trap catch and larval density are decent proxies for population size when populations are large, but not when populations are small. This is potentially a problem because the ability to detect and estimate the size of small populations of SBW is required for tactics targeting incipient populations (e.g., ‘early intervention’). These estimates would be improved if the relationship between the number of moths in a trap and the density of adults within a stand was known. Determining the size and shape of the active space of a trap will also aid in the design of sampling plans for SBW by optimizing the distance between traps in an array. The inability to generate estimates of absolute density is not limited to the SBW. If successful, the deliverables will facilitate similar applied research with additional forest pests.

The two previous reports (MacQuarrie et al 2016, MacQuarrie et al 2017) documented field and wind tunnel studies designed to determine the active space and plume structure emitted from budworm traps baited with artificial lures. Here we report on the last component of the project where we test the behavioural response of male moths to a lure in a large wind tunnel.

Methods

We obtained male SBW pupae from the colony at Natural Resources Canada Insect Production Services in Sault Ste. Marie, Ontario. The pupae were placed individually inside creamer cups then placed into one of two controlled environment chambers (Conviron; 20 C; 16:8 L:D). The chambers were identical, except that one was programmed to enter the dark phase at 17:00 EDT and the other 21:30 EDT. Moths reared in these chambers are referred to as ‘day’ and night’, respectively. Moths in the ‘day’ group were tested during the daytime (beginning at 18:00 EDT) and moths in the ‘night’ group were tested during the nighttime (beginning at 10:30 EDT) in the walking bioassay. Thus for both bioassay periods, male moths would have been in the same phase of their L:D cycle.

The wind tunnel was constructed in a large greenhouse in Sault Ste. Marie, Ontario (New North Greenhouses). Airflow through the tunnel was by means of large fans mounted on the rear wall of the greenhouse that drew air from outside. The greenhouse fans were fitted with a laminizer constructed from a wooden frame and aircon filters (Duststop air filters product # 64-3416-2) immediately in front of the fan. To determine a location in the tunnel to conduct our experiments we searched for a spot where the air flow rate was relatively constant by measuring windspeeds throughout the tunnel at 1.6 m above the ground and then visualizing these data with contour and box plots. Once we determined a location we hung a white bucket funnel trap from a metal stand such that a lure placed in the trap was positioned 1.6 meters above ground. For each experiment we positioned either red rubber septum lures loaded with 3, 30 or 300 ug of a pheromone blend (95:5% E11-14:Ald: Z11-14:Ald) (i.e., 1,10 or 100 female equivalents) or a commercially available PVC ‘flexlure”, or an empty ‘blank’ trap (as a control).

To test the response of males of male moths, each was placed individually inside a metal wire cage (5 cm by 1.5 cm radius cylinders). The cages were attached to a wooden pole 1.6 m long, such that when the end of the pole was placed on the ground the caged moth was at the same height as the lure inside the trap. The pole was held by an observer who documented the response of each moth. The observer began their observations by placing the pole 22m downwind and in direct line with the trap then the behaviour of the moth was observed for a period of 30 seconds before moving to the next position that was two meters closer to the trap. The behaviour that was scored as an activity stimulated by pheromone presence was wing fanning followed by flight within the wire cage. This behaviour needed to be observed for at least 5 consecutive seconds to be scored as positive. Once this behaviour was observed the distance to the trap was recorded and the moth was removed from the experiment. If the behaviour was not observed the moth was recorded as ‘not responding’.

We made air flow rate measurements at each of the positions downwind (i.e., 0–22 m, at 2 m intervals) from the trap both before and after each experiment using a hotwire anemometer, (OMEGA Model HHF-SD1). We also recorded temperature data at each position for the last two night time experiments using a series of temperature data loggers (Onset HOBO) that recorded the temperature at each position every 30 seconds.

Pheromone release rate estimation

We determined the release rate for the red rubber septa lures as a function of temperature. To do this a repeated measures experiment was performed on a red rubber lure loaded with 300 ug of the pheromone blend (95:5% E11-14:Ald: Z11-14:Ald). The lure was placed in a 20 ml glass chamber fitted with a 20 mm PTFE/Silicone (Supelco) septum. The glass chamber was partially submerged in a water bath with a controlled temperature. Samples were taken with a conditioned SPME fiber (PDMS/CAR/DVB) by exposing the fiber to the inside of the chamber for a period of 50 minutes at a known temperature. Two sequential replicate samples were taken at each of the sampling temperatures starting off at 24 degrees Celsius then increased by 2 degrees Celsius sequentially up to 32 degrees Celsius. The chamber was opened between each sampling event to release the volatile fraction of the pheromone inside the glass chamber. The fibers were immediately analyzed on the 6890 GC-FID system equipped with a DB-WAX column (ID 0.25mm). This experiment was repeated 5 times on day 2, 3, 8 10 and 15 after the lure was loaded.

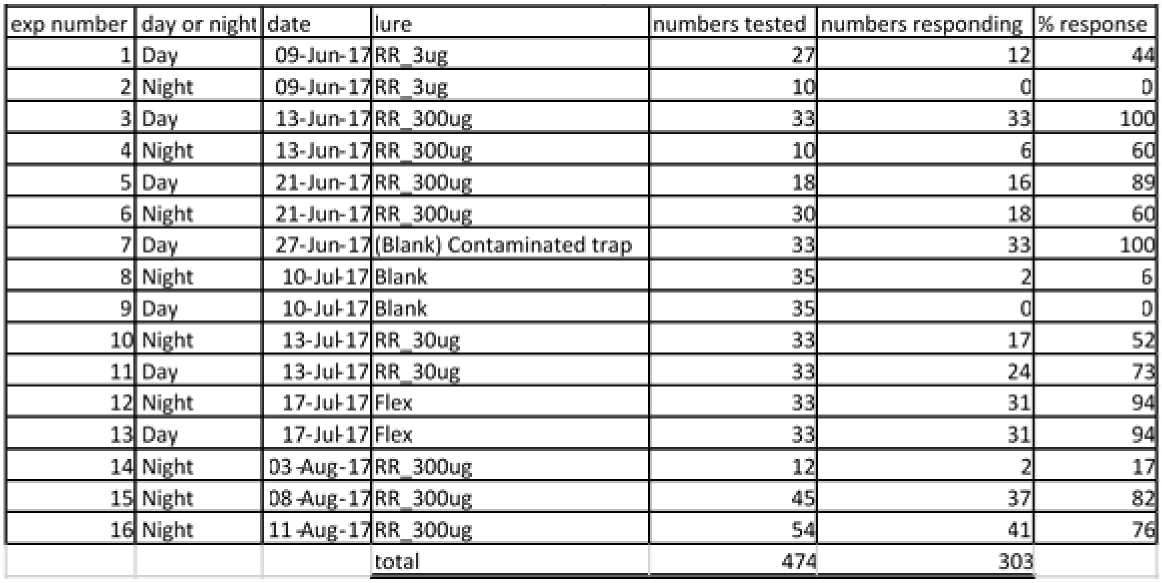
Results

Walking bioassay:

A total of 474 male SBW moths were tested with 303 (64%) exhibiting the a response to the pheromone plume (Table 1). Data from experiments 1-6 and 8-16 are included in analyses of flight response. Data from experiment 7 was not used as we later determined that the ‘blank’ trap had been previously used and was therefore contaminated with SBW pheromone and thus not a true ‘blank'.

Overall, most moths responded at very short distances from the trap (Figure 1). We used the number of moths responding at each distance to test of the effect of pheromone loading and time of day and found a significant effect of both predictors (Table 2), with more SBW responding in the daytime flights. This suggested that temperature was likely affecting the response of the insect. Due to these observations 99 three-day old individuals were tested only for the night time experiment on 8 and 11 Aug. Temperature was recorded at each location with eleven data loggers during these night experiments.

Table 1: Summary of sixteen walking bioassays.



exp number	day or night	date	lure	numbers tested	numbers responding	% response
1	Day	09-Jun-17	RR_3ug	27	12	44
2	Night	09-Jun-17	RR_3ug	10	0	0
3	Day	13-Jun-17	RR_300ug	33	33	100
4	Night	13-Jun-17	RR_300ug	10	6	60
5	Day	21-Jun-17	RR_300ug	18	16	89
6	Night	21-Jun-17	RR_300ug	30	18	60
7	Day	27-Jun-17	(Blank) Contaminated trap	33	33	100
8	Night	10-Jul-17	Blank	35	2	6
9	Day	10-Jul-17	Blank	35	0	0
10	Night	13-Jul-17	RR_30ug	33	17	52
11	Day	13-Jul-17	RR_30ug	33	24	73
12	Night	17-Jul-17	Flex	33	31	94
13	Day	17-Jul-17	Flex	33	31	94
14	Night	03-Aug-17	RR_300ug	12	2	17
15	Night	08-Aug-17	RR_300ug	45	37	82
16	Night	11-Aug-17	RR_300ug	54	41	76
			total	474	303	

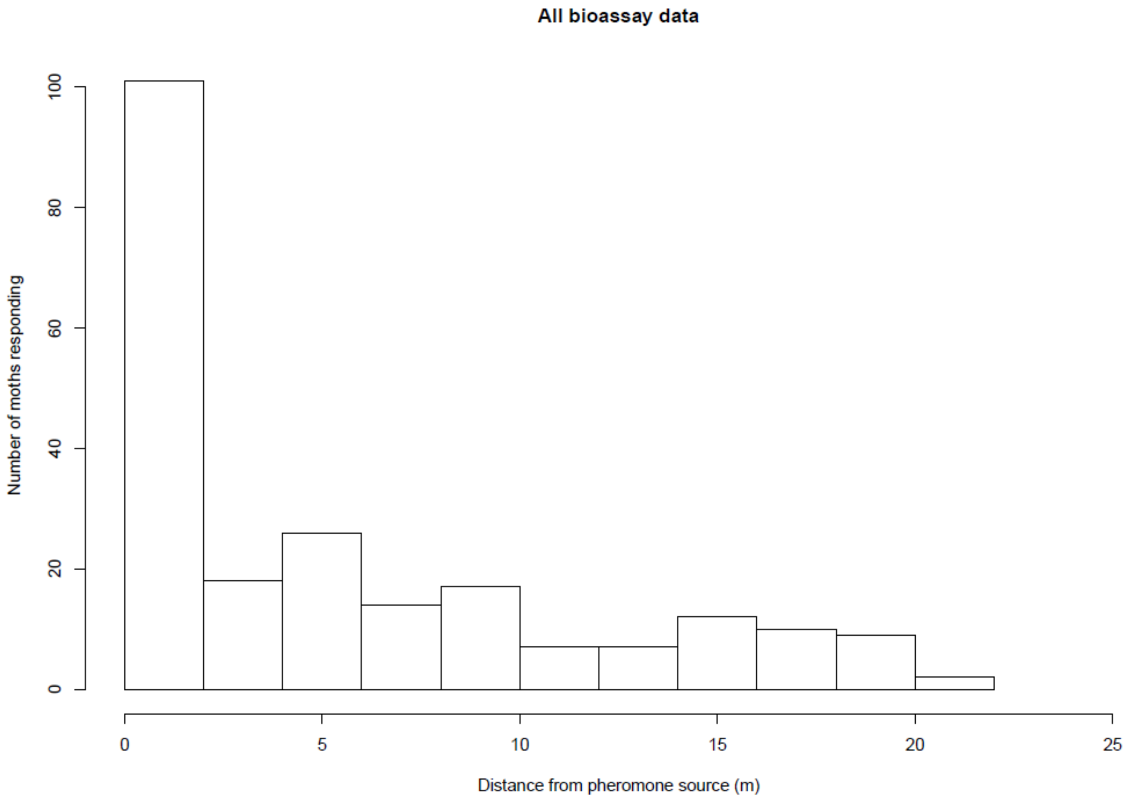


Figure 1: Frequency distribution of moth responses versus distance from the pheromone source. Most moths responded within 5 m of the source.

Table 2: ANOVA of effect of lure and time of day on distance at which moths responded

Term	df	SS	MS	F	p
Lure	3	1361	453.6	17.100	7.82×10^{-10}
Day	1	153	153.3	5.778	0.0172
Residual	183	4855	26.5		

Lastly, we examined the probability of moths exhibiting a behavioural response at a given distance. For these data we expanded the observed data set by making the assumption that a moth's response, y, at a distance x_r would continue to exhibit a response at all distances $< x_r$. By applying this assumption to all records for all male SBW moths used in the experiment we were able to produce a complete flight record for each individual for all distances from 0 to 22 m from the source, where $y = 0$ for all $x > x_r$ and $y = 1$ for all $x <= x_r$. We then used these data in a logistic regression as the response to examine the effect of distance, lure composition and time of day on the probability of response. We found that the probability of a moth responding was < 0.5 (or 50%) at all distances > 10 m regardless of lure type or pheromone concentration (Figure 2). As seen in the earlier analysis time of day was important with more response observed in the 'day' moths than the 'night' moths, likely owing to the temperature being warmer. Further analyses will test this assumption.

Lure release rate:

Temperature and lure age has a significant effect on the release rates of the pheromone constituents of *C. fumiferana* from the red rubber septum (Table 3). A linear decline in release rates was observed as the lure aged for both components and an increase in temperature increased the release rates of the lure (Figure 3).

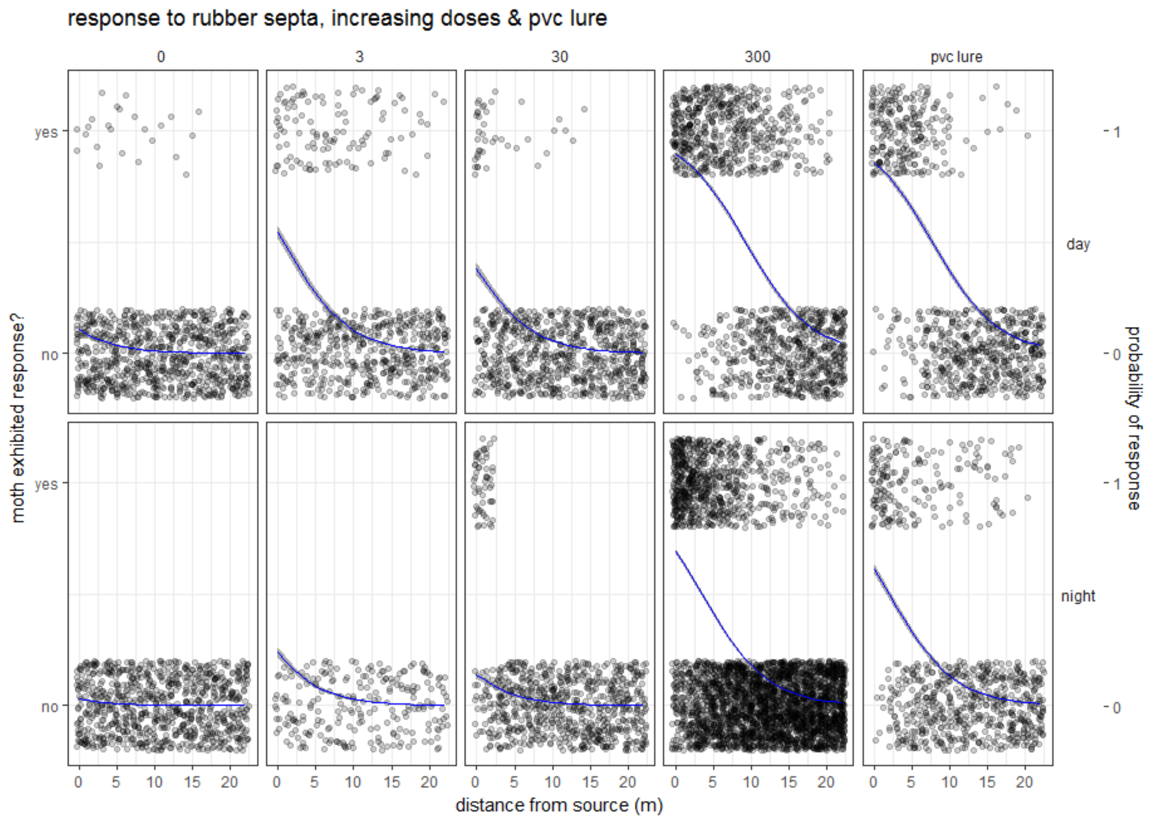


Figure 2: Probability of moth exhibiting positive response to lure increases with dose and varies somewhat with time of day. Response of moth to PVC lure is similar to that of high-dose rubber septa lure (right two columns).

Table 2: ANOVA of effect of lure age and temperature on release rate of lure

Response: Release rate of E11-14:Ald					
Term	df	SS	MS	F	p
Lure age	1	67149	67149	63.546	2.809×10^{-10}
Temperature	1	299843	299843	283.756	$< 2.2 \times 10^{-16}$
Residual	47	49665	1057		
Response: Release rate of Z11-14:Ald					
Lure age	1	0.364	0.364	0.3292	0.5688
Temperature	1	284.941	284.941	257.8912	$< 2.2 \times 10^{-16}$
Residual	47	51.930	1.105		

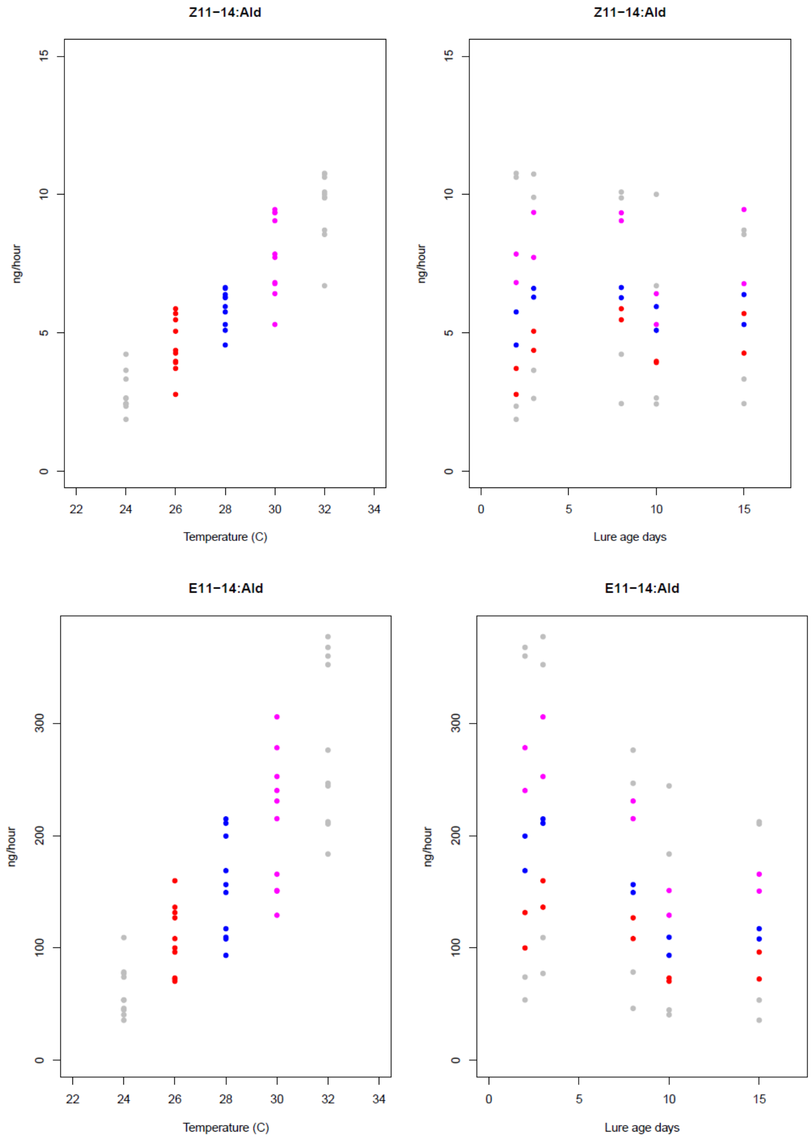


Figure 3: release rate increases with temperature but decreases with lure age.

Discussion

Our previous experiments in this study suggested that the attractive radius of SBW traps is small and the pattern of airflow emerging from the trap is highly variable. The results of this experiment support those earlier findings by showing that the probability of a male moth responding to a trap is very low, even at very short distances from the lure. These experiments also show that the release rate of lures is, not surprisingly, positively related to temperature but not very (strongly?) correlated to the age of the lure. This suggests that release rates of rubber septa lures are somewhat constant over time, independent of temperature.

Final analyses of all components of this study is ongoing. However, we can likely make the preliminary conclusion that if there is a relationship between moth catch and local density of SBW larvae the area over which the relationship holds is likely very small. Our data would suggest that traps are effective at sampling a small airspace immediately around the trap. Thus if there is a relationship between trap catch and local density it would likely only hold for the area immediately surrounding the trap. This would tend to suggest that it may not be possible to calibrate low trap catch values to absolute density of moths or larvae within a stand. Thus we have accomplished objectives 1 and 2 but is likely not possible to accomplish objectives 3 and 4 as the present technology available is not sufficient for the task.

References

MacQuarrie CJK, JD Allison, R Johns, D Pureswaran. 2016. Measuring the active space of a spruce budworm pheromone trap: bridging the gap between chemical ecology and estimates of absolute population density. SERG International project report.

MacQuarrie CJK, JD Allison, MC Bouwer R Johns, D Pureswaran. 2017. Measuring the active space of a spruce budworm pheromone trap: bridging the gap between chemical ecology and estimates of absolute population density. SERG International project report.

Financial report 2016-2017

Funding sources	**requested**	**received**
NRCan CFS*****	$4,000	**4,000**
SERG-I		**$10,000**
Total		**14,000**

*Includes in-kind (travel expenses, staff time, administrative support and lab supplies).

Optimization of trapping parameters for spruce budworm sampling

C J K MacQuarrie[1], J Allison[1], D Carleton[2], R. Johns[3]

1) NRCan Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario.
2) New Brunswick Energy and Resource Development
2) NRCan Canadian Forest Service, Atlantic Forestry Centre, Fredericton, New Brunswick.

Abstract:

This report presents the results of lab and field experiments on the effective trapping radius of baited spruce budworm (SBW) traps, and the aging rate of SBW pheromone lures. In 2016 and 2017 we tested the correlation and effect of trap interference in trap catch using 4 x 4 trap arrays with different inter-trap spacings replicated in Ontario and New Brunswick. These data show that at intertrap spacings of > 10 m there is likely no interference between bucket traps baited with PVC flex lures. We also found that low auto correlation among trap catch at very short distances (i.e., < 5 m). This is contrary to other trapping designs where autocorrelation was found at distances of up to 30 m, and greater. This result suggests that the overall sampling radius of SBW traps is likely small. We determined the relationship between lure mass, time and temperature by aging lures under field and lab conditions. The small mass of SBW lures made determining changes in weight to be difficult, even using a sensitive balance. Lures also tended to increase in mass at times, often associated with high humidity. This suggests that the lure matrix absorbs water during the period they are deployed. If so, this means that in order to predict the aging rate of lures we would need to develop a model that both accounts for weight change in the physical structure of the lure and a component that models the loss of pheromone from the lure.

Résumé

Le rapport présente les résultats d'expériences menées en laboratoire et sur le terrain pour déterminer le rayon de piégeage efficace des pièges appâtés pour attirer la tordeuse des bourgeons de l'épinette (TBE), et le rythme auxquels vieillissent les appâts de phéromone de la TBE. En 2016 et en 2017, nous avons étudié la corrélation et l'effet de l'interférence du piège dans les prises en positionnant des pièges selon un modèle 4 x 4 avec des distances différentes entre les pièges, expérience que nous avons reproduite en Ontario et au Nouveau-Brunswick. Les

données montrent qu'à des distances de > 10 m entre les pièges, il n'y a probablement pas d'interférence entre les pièges contenant des appâts flexibles en PVC. Nous avons également observé une faible autocorrélation parmi les prises de chaque piège à des distances très courtes (< 5 m). Voilà qui va à l'encontre d'autres dispositions de pièges où une autocorrélation a été observée à des distances pouvant allant jusqu'à 30 m ou plus. Un tel résultat laisse croire que le rayon d'échantillonnage global des pièges de TBE est probablement faible. Nous avons déterminé le rapport entre la masse, la durée et la température de l'appât en vieillissant les appâts sur le terrain et en laboratoire. La faible masse des appâts de TBE a fait qu'il a été difficile de déterminer les changements dans le poids, même à l'aide d'une balance très sensible. De plus, les appâts avaient parfois tendance à gonfler, par exemple quand le taux d'humidité était très élevé, ce qui fait penser que la matrice de l'appât absorbe de l'eau durant la période de déploiement. Si cela s'avère, cela signifie qu'il faudra, pour prédire le taux de vieillissement des appâts, mettre au point un modèle qui tient compte à la fois du changement de poids attribuable à la structure physique de l'appât et de la perte de phéromone de l'appât.

Introduction

Present trapping guidelines for spruce budworm (e.g., Houseweart et al 1981, Sanders 1986, 1988) were developed using insect traps and lures that are no longer used in provincial monitoring programs. Most provincial monitoring programs use the bucket-style non-saturating pheromone trap (multi-Pher Trap or Uni-Trap) or provisioned with either a PVC 'flex lure' or a rubber septa impregnated commercially produced budworm pheromone blends. However, the guidelines for SBW trapping were developed using saturating sticky delta or wing traps and pheromone lure blends baited with lures produced in a research laboratory. Trapping for adult male budworm is sufficient to make reasonable predictions about the likely size of the following years budworm population in a stand, and make relative comparisons among traps deployed by the same agency. However, three issues have been identified by provincial agencies that do spruce budworm trapping that, if resolved, would lead to improved utility of budworm traps. These issues are:

1) The quality and composition of lures used to trap SBW can vary among years and between batches of lures.

2) Release rates of commercially produced lures, and change in attractiveness over time to attract SBW has not been recently assessed.

3) The variability among lures can prevent quantitative year-over-year comparisons of trap catch.

To address these issues some budworm trapping programs perform calibration tests of lures from different batches or years. In these tests lures from different batches are deployed in a paired design during the trapping season. Then by comparing the catch for both lure types a correction factor can be computed to permit an 'apples-to-apples' comparison of trap catch among traps baited with different lures. These field comparisons of lures are predicated on the assumption that two traps, as long as they are hung far enough apart from each other, constitute independent samples of the same population. However, it is known that insect traps baited with lures, sometimes called "attractive traps", can interfere with each other's catch if placed too close together. Associated with these questions are the effect of lure age on trap catch and how environmental condition (e.g., temperature) influences the release rate of the lure, and thus how many insects are collected.

Recently, methods have been developed to assess trap interference using statistical tools (Bacca et al 2006; Suckling et al 2015). In these methods, grids of traps (e.g., 3x3, 3x4 or 4x4) are set out in a replicated design with increasing distance among traps in the grid. By comparing either the ratio of catch in traps in different places in the grid and among grids of different sizes (Suckling et al 2015) or examining spatial auto-correlation of trap catch (Bacca et al 2006) the intertrap distance at which trap interference occurs, or ceases to occur, can be determined. These analyses can infer the optimal distance between traps that should be used when testing different lure types or to quantify year over year differences among batches of lures.

Here we used a 4x4 trapping grid of baited spruce budworm traps deployed over two years in New Brunswick (2016 & 2017) and Ontario (2017) to test the effect if inter-trap distance on correlation and interference among adjacent traps. In related experiments we determined the relationship between lure weight and age for both lures aged in an controlled environment chamber and under ambient outside conditions.

Methods

Study 1 - Trap interference We selected one site in each of Ontario and New Brunswick within which we installed 4x4 grids of baited multi-Pher spruce budworm traps provisioned with PVC flexlures. Each site had a homogenous composition of balsam fir and white spruce over the area which the traps were deployed and were situated adjacent to a regions with moderate to high spruce budworm density. Spruce budworm density was determined using provincial budworm survey data (i.e., aerial survey data for Ontario, provincial L2 survey for New Brunswick). At each site we establish five, 4 x 4 trap grids at 3 m, 10m, 20 m, 40 m and 60 m intertrap spacing. The traps were deployed before flight season (ca. mid-June) in each region and recovered in mid-August. The placement of each trap was determined using a handheld GPS unit or a tape (in the case of the 3m grid in New Brunswick). In 2017 traps in New Brunswick were checked twice, but catches were pooled over the entire season for our analyses.

Following methods in Bacca et al. (2006) and Suckling et al (2015) we completed the following analyses using the season trap catch as the response variable:

1) We compared the ratio of trap catch between corner and edge traps; edge and centre traps and corner and centre traps. Theory predicts that when traps are placed close together corner traps should catch more insects because of the combined effect of multiple, overlapping lures and thus interfere with the catch of interior traps. By determining the inter-trap distance at which the ratio between catch in corner vs. inner traps is maximized we can predict the minimum trap spacing at which traps should be deployed to minimize this interference (See Suckling et al 2015).

2) We computed a metric of trap disruption by comparing the catch in the centre four traps of the largest grid (60 x 60 m spacing) to the catch in the centre of the other grids to determine a relationship between disruption and inter-trap spacing. We fit a linear relationship to these data to determine a predictive relationship between the trap disruption and spacing.

3) We determined the number of budworm males caught per unit area, assuming that the sampled area of the trap was equal to the area of a circle centre on the trap with diameter equal to that of the trap spacing. We fit a non-linear model to these data to determine the rate at which trap catch per unit area stabilizes relative to the trap spacing.

4) We used variograms and fit spatial covariance models to assess the spatial autocorrelation among all traps. In this analysis the inter-trap distances with trap catch numbers that are highly correlated have low semivariance. Semivariance measures the correlation of trap catch among all pairs of traps at a given distance and is expected to be low where trap catch is highly correlated, and increase to an a asymptote (the ‘sill’) at some intermediate distance. The location of the sill

is the range at which the trap catch are determined to be independent.

For analyses 1 and 2 we used the relative position of each trap in the grid to determine its position in space (i.e., edge, centre, corner). However, some traps were misplaced in the grid owing to errors in reading the GPS unit and so were not used in these analyses. For analysis 4 we used the absolute spatial coordinate of the trap (i.e., its latitude and longitude) and so all traps were used. However, in New Brunswick the 3m grid was laid out using a tape and GPS coordinates were unavailable for this site at the time of this report, as were the spatial positions of the trap grid in 2017.

Study 2. Lure release rates.

This experiment quantified the release rate of SBW lures under both field and laboratory conditions. In the field test we obtained commercial PVC flex lures (Synergy) and rubber septa (aPhinity SB) impregnated with budworm pheromone. These lures were placed in either direct sun or shade at a site on the grounds of the Great Lakes Forestry Centre. Each lure was then measured once per day to evaluate the change in weight over time as the pheromone component was lost. This experiment was repeated twice in 2016 and 2017 using samples from the same batches purchased in 2016. We also recorded the temperature during the release period using a data logger (2016) or obtained temperature data from Environment Canada (2017). In this analysis we only model the rate of change in lure weights versus exposure days. We fit a rate equation to these data to describe the change in pheromone weight over time which we used to calculate the half-life of the lure.

The lab test employed a similar procedure, but maintained lures at a constant temperature and humidity over the course of the experiment. This experiment used an apparatus designed to test release rates of insect pheromone lures over time and consists of a sensitive balance enclosed in a climate controlled chamber (Zhu et al). Three lures were tested simultaneously for each temperature to ensure there was sufficient mass on the scale to register a change in mass and 5 temperatures were tested.

Results

Discussion

Study 1:

Analysis 1 – Ratio of trap catch by position

The ratio of catch between groups of traps in different positions in the grid showed the expected pattern. There was a low ratio between corner and edge traps (Figure 1; left column) indicating these traps were catching similar numbers of insects. The ratio increased as we compared edge to centre (Figure 1; centre column) and corner to centre traps (Figure 1; right column) peaking in the either the 10 m or and 20 m intertrap spacing. This pattern was consistent over the 2 years and 2 sites used to examine traps. In mass-trapping studies to avoid interference among traps Sucking et al propose the equation of 2.3d, where d is the inter-trap distance where the corner vs. centre ratio is at a maximum. Applying that equation here suggests that trap interference (i.e., competition for catch) is diminished at either 23 m or 46 m.

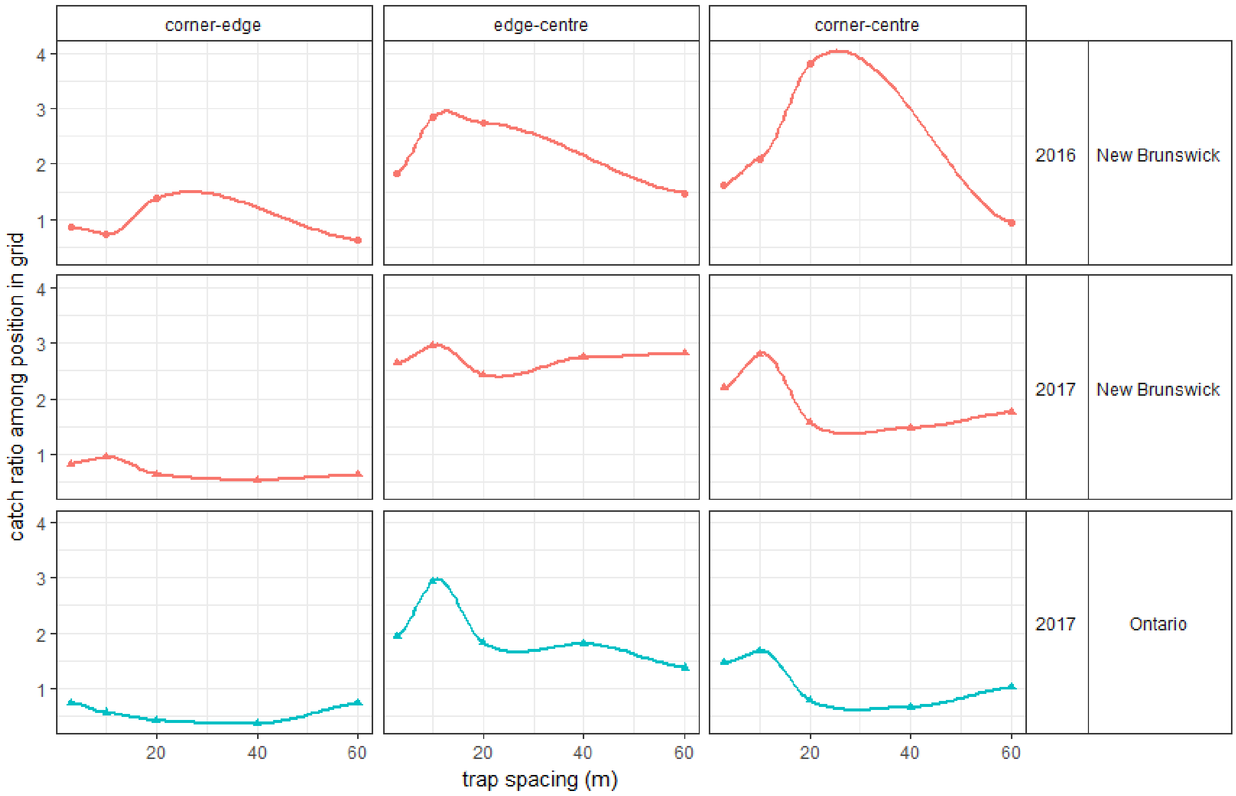


Figure 1: Ratio among trap catch for various trap positions in the 4 x4 grid. Trap interference is predicted to be maximized in the corner-centre ratio (right column) and minimal in the corner-edge ratio (left column).

Analysis 2 – Disruption

Disruption is the difference in trap catch in a small grid experiencing trap competition compared to a large grid where trap interference should not occur. Using the data from both sites and both years we see the expected negative relationship between spacing and disruption. The model predicts that disruption does not occur at spacings greater than 50 m. However, there is a strong discontinuity in the data between 10 m and 20 m spacing, where disruption drops off sharply. This is consistent with the observation of low interference below 20 m inter-trap spacing.

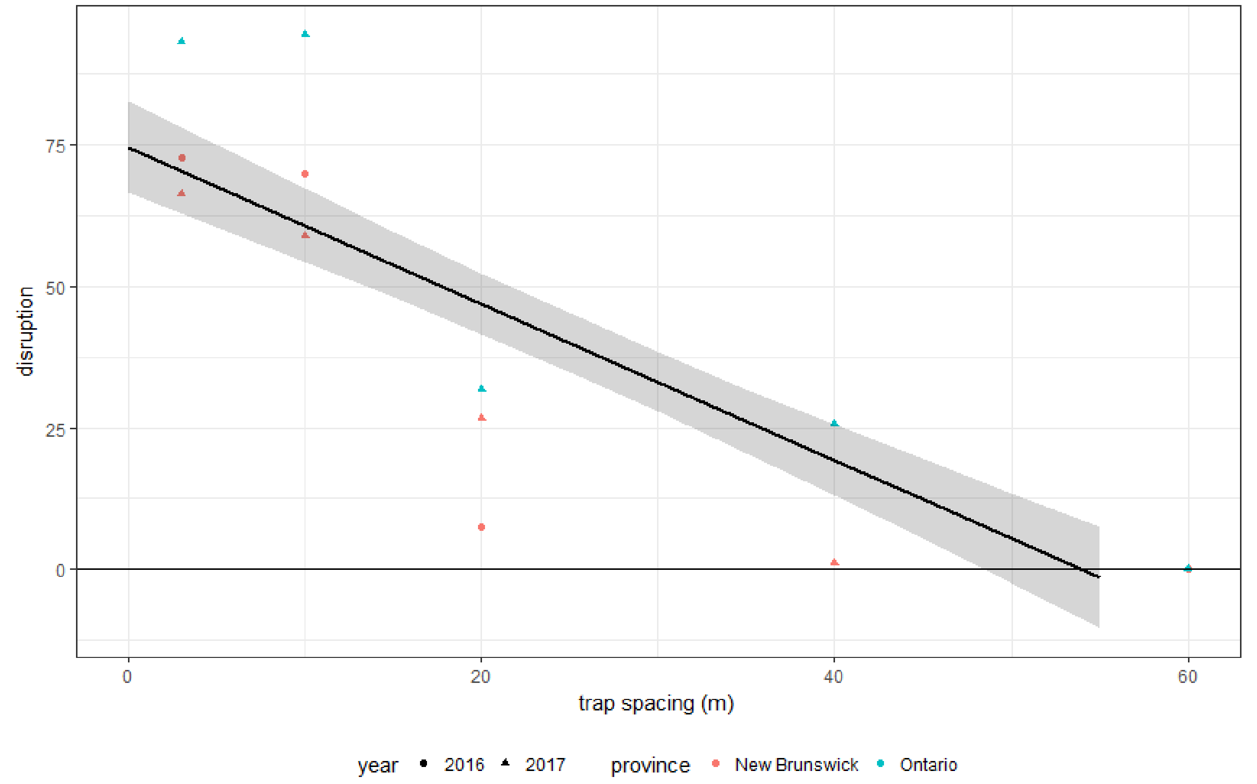


Figure 2: Trap disruption decrease with increasing trap spacing. Disruption is computed as the percent difference between the catch of the centre four traps in each 4 x 4 trapping grid when compared to the catch in the largest grid. $y = 74.5 – 1.3x$, $R^2 = 70.5$

Analysis 3 – Density vs. spacing

Traps that are closer together are predicted to catch more insects per unit sampling area. The explanation being that many traps placed together act like a ‘super trap’ compared to those farther apart. As traps are placed at increasing distances between them this effect drops off and catch per unit area becomes more consistent. In our arrays, the number of budworm per sampled area stabilized between the 10 and 20 m spacing (Figure 3). This observation is consistent with the pattern seen in analyses 1 and 2 (Figures 2, 3).

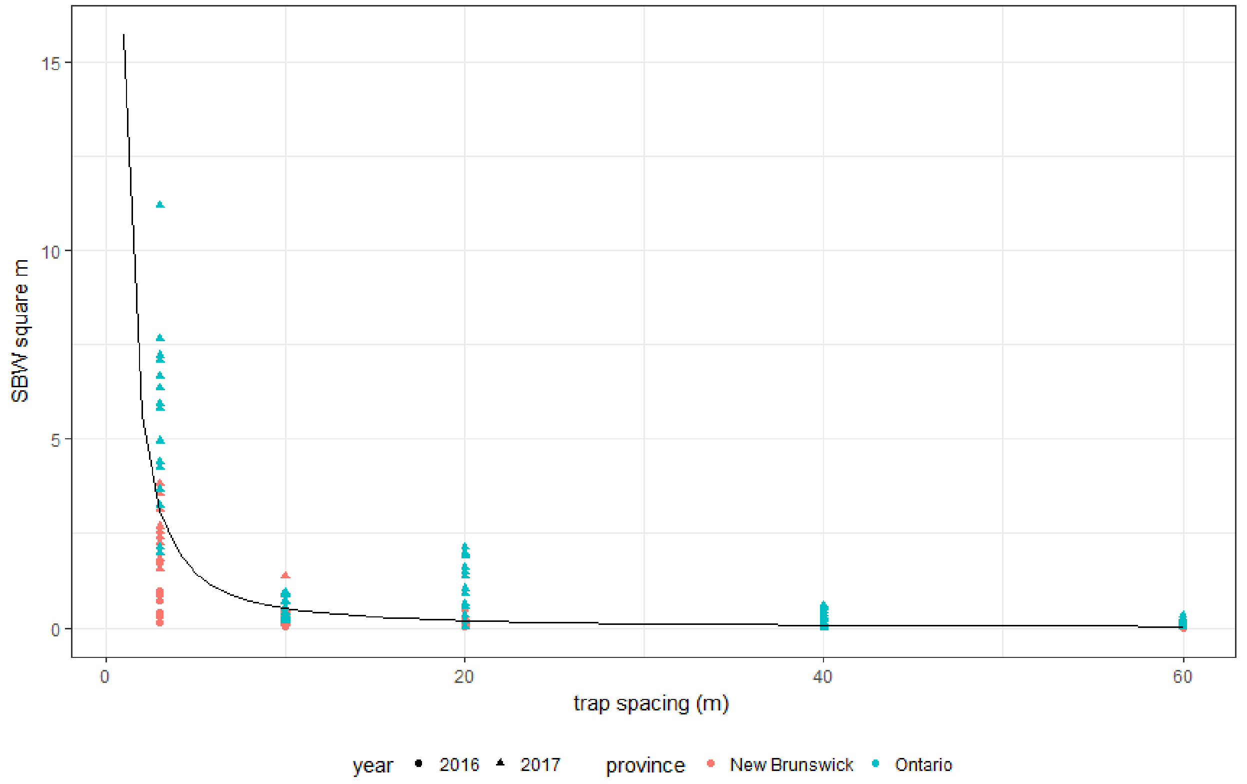


Figure 3: Number of adult male spruce budworm decreases exponentially with increasing distance among traps in a 4 x 4 grid. $y = 15.6x^{-1.48}$

Analysis 4: Spatial autocorrelation

We found here that there was no spatial autocorrelation among the trap catch from New Brunswick in 2016 and thus the trap catches were not spatially autocorrelated. We did detect a pattern in the 2017 Ontario trap catch that was fit equally well by both an exponential model and a spherical model (Figure 4) with the range predicted to be between 2 and 3 metres. The lack of spatial autocorrelation in the New Brunswick data may be attributed to the lack of the 3 m trap array, as it appears it is within that range that traps are autocorrelated.

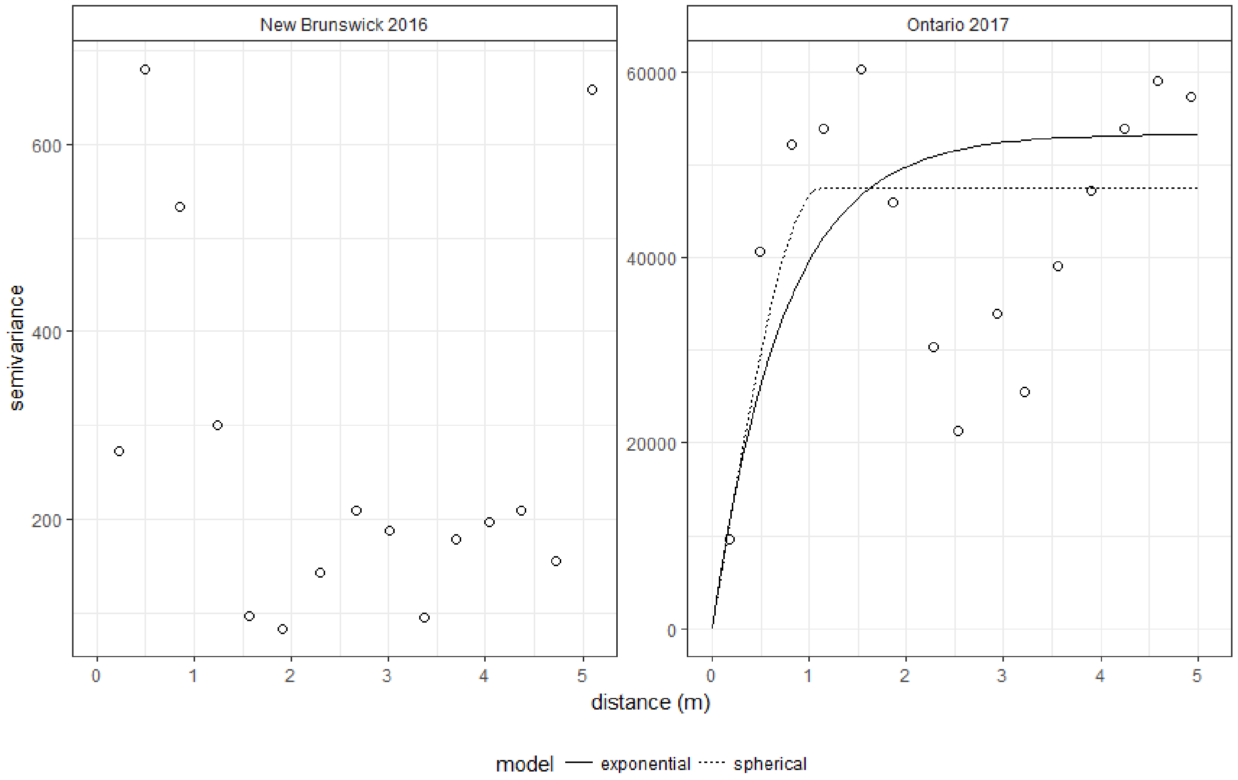


Figure 4: Spatial correlation among trap was not found beyond 5 m in the Ontario 2017 data. The Ontario 2017 trap catch data was reasonably fit by both an exponential and spherical spatial correlation structure. There was no spatial correlation structure in the New Brunswick 2016 data.

Study 2 Lure release rates

Field aging

The intended plan of analysis for these data was to determine the half-life of the entire lure. However during the analysis we determined that the lures maintained considerable weight, as a percentage of the original mass, at the end of the aging experiment. Such that when computing half-lives we determined values in excess of 5000 days. We then attempted to determine the rate equation for the pheromone component by correcting for the final mass of the lure, on the assumption that all the pheromone component would have been released and therefore any mass loss we detected was attributed to loss in the pheromone. However, both lure types occasionally increased in weight, likely owing to the absorption of humidity. Nevertheless, we performed the analysis as intended using the estimated pheromone mass as the response variable. When we did this we fit negative relationships for each of the lure types and exposure conditions (sun, shade) and from these equations determined predicted lure half-lives of between 35 and 45 days for PVC flexlures, and 45 to 50 days for rubber septa. Rubber septa hung in the shade exhibited no weight change after the first few days of the experiment. We were still able to fit the desired model, but when we did we resolved a half-life of only a few days (Figure 5).

We repeated the measurements and analysis in 2017 and found no change in the weight for most of the lures over >60 days and thus were not able to fit any model. Further analyses of these data are required.

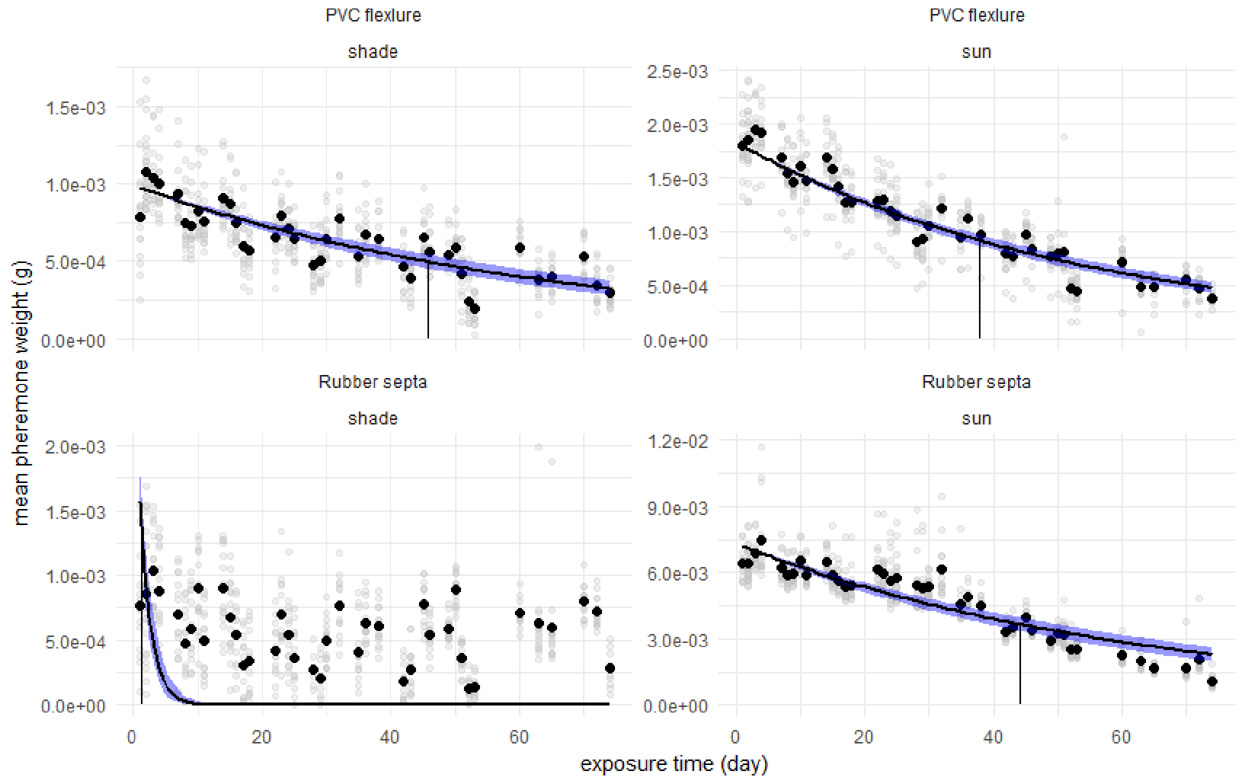


Figure 5: Weight of pheromone lures in shade (left) and sun (right) decrease with exposure time. Black vertical lines indicate the predicted lure half-life based on the fit of the rate function (black lines within blue shading), each grey point represents one observation of lure mass, each black point is the daily mean.

Lab aging

The observed behaviour of weight change in lures did not match the predicted behaviour (Figure 6). Under controlled conditions the rubber septa experienced a rapid decrease in mass within the first few hours of the experiment then levelled off. The PVC lures experienced a rapid loss at 20 C, but gained weight in the 25 C treatment before decreasing, and experienced no change in the higher temperatures. Any weight gain was attributed to absorption of humidity over the course of the experiment by the lures. Both lures also exhibited high variability in weigh over time. Some of this is owed to very small mass of the active ingredient relative to the size of the lure.

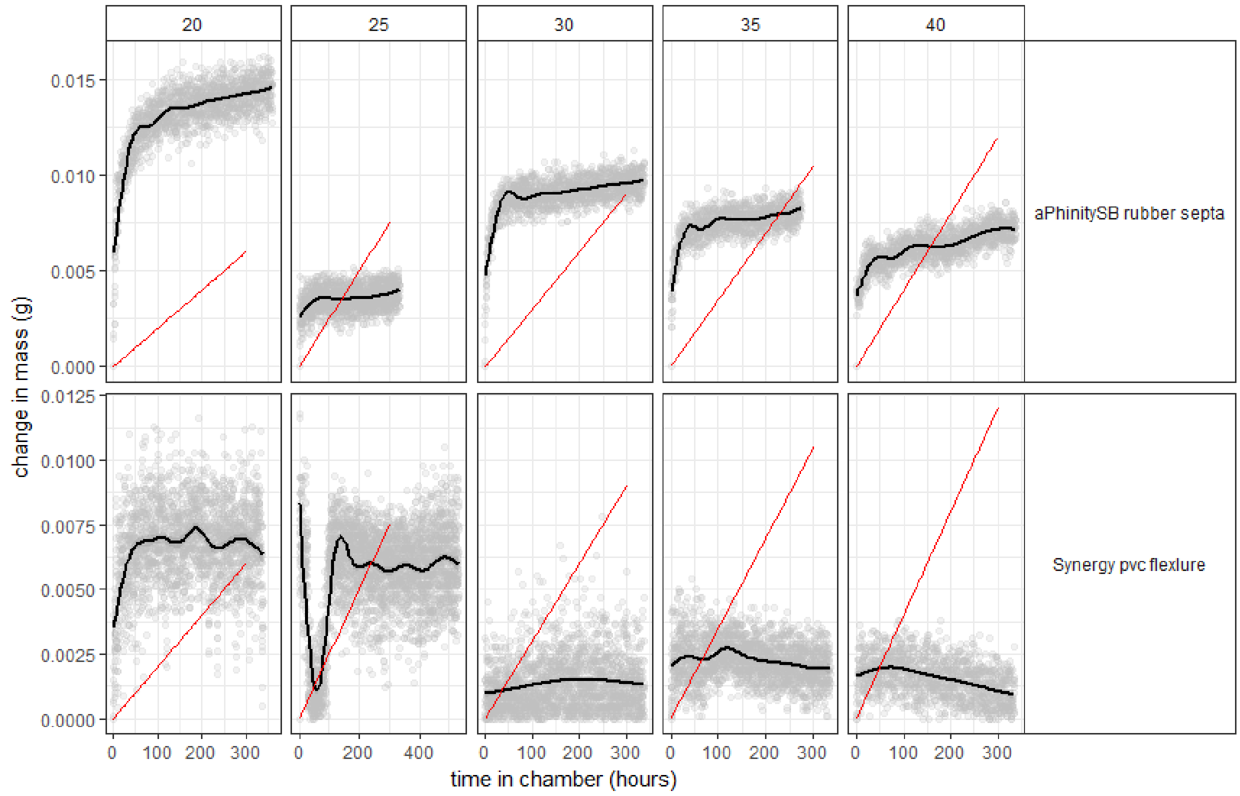


Figure X: Change in mass of two spruce budworm lure types over time in a controlled environment chamber. Each column represent the standard temperature each lure was exposed to The red line shows the expected pattern in the data, each point represents one observation of lure mass.

Discussion

The analysis of the spatial trap capture data indicates that beyond 10 m there is likely no interference between bucket traps baited with PVC flex lures. There may be some interference between 10 and 20 m but this appears to be minimal, to non-existent. Beyond 20 m of trap spacing there appears to be no appreciable effect on catch of adjacent traps. When we examine how correlated trap catch is, we find that there exists low auto correlation at very short distances. This is contrary to other trapping designs where autocorrelation was found at distances of up to 30 m, and greater. However, in those systems the traps being tested were deployed in agroecosystems (field crops or nurseries). Forest ecosystems are more complex and therefore the opportunity for forest structure to interfere with trap catch likely exists. One corollary of our finding is that it suggests that the overall sampling radius of these traps is likely small.

Determining aging rates for lures proved to be more difficult than anticipated. The small mass of the lures made determining changes in weight to be difficult, even using a sensitive balance. We also found that lures tended to increase in mass at times, often associated with high humidity. This suggests that the lure matrix (e.g. the PVC or rubber) is likely absorbing water during the period they are deployed. If so, this means that in order to predict the aging rate of lures we would need to develop a two-part model. One that accounts for weight change in the physical structure of the lure with a component (i.e., a rate model) that models the loss of pheromone from the lure.

The difficulty in determining a rate model using the laboratory aging technique may have owed to the temperature profile that we used. The laboratory aging method was developed to determine aging rates for bark beetle lures deployed in forests in the United States where temperatures during the sampling period are higher (i.e., 20-40 C) than expected in Canadian forests during sampling for spruce budworm (10-20 C). To address this we plan to age lures at lower temperatures to determine if less rapid rates can be observed. However, regardless of temperature we did have the same problems related to humidity and measuring small changes in mass.

This work was also complicated by the fact that some lures appeared to show no change in weight during the entire experiment. This suggests that here was no appreciable pheromone component loaded onto the lure. In other lures we observed that there was no change in mass for the initial period of the experiment, then a rapid loss of mass over a few days. This inconsistency in lure performance will make producing accurate models difficult.

References

Financial report 2017-2018

Funding sources	**requested**	**received**
NRCan CFS*	$2,000	**$2,000**
SERG-I	$6985	**$5,000**
Total		**$7,000**

*Includes in-kind (staff time, administrative support and lab supplies).

Testing the efficacy of *Trichogramma minutum* in the context of an 'Early-intervention Strategy' against the spruce budworm using different release methods

Véronique Martel[1], Simon Trudeau[1], Rob Johns[2], Emily Owens[2], Sandy Smith[3], Guy Boivin[4]

[1]Laurentian Forestry Centre, Canadian Forest Service, Natural Resources Canada, Qc; [2]Atlantic Forestry Centre, Canadian Forest Service, Natural Resources Canada, NB; [3]Department of Forestry, University of Toronto, ON; [4]Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, Qc

Industry Partners: Anatis Bioprotection; Canopée dronautique

Abstract

This report presents the second year's results (2017) of the research project aiming to assess the efficacy of *Trichogramma* releases against SBW. In 2017, eleven blocks were set up in three areas in Quebec: seven blocks had a control, a release by card and a release by drone plots. The other four blocks had a control and a release by card plot. In each plot, branches were collected in the spring and fall to evaluate population densities using L2, and in summer for SBW egg parasitism. Moths were collected using pheromone traps from the Budworm Tracker project (http://budwormtracker.ca) in each region to synchronize the release with oviposition. While in 2016, results showed that egg parasitism was higher in all plots treated with cards, in 2017, the results were not as clear. The block with the lowest egg density showed a higher egg parasitism for both treated plots (cards and drones) compared to the control. However, parasitism was low in the other blocks. Several factors could have decrease the impact of our parasitoids' releases: the cold and rainy weather, the presence of another moth species in abundance that could have come into competition with SBW eggs for parasitism, and predation by slugs.

Résumé

Ce rapport présente les données de la 2^{e} année (2017) du projet visant à évaluer l'efficacité des lâchers de trichogrammes contre la TBE. En 2017, onze blocs ont été installés dans trois régions du Québec : sept blocs avaient un site témoin, un site avec lâchers par cartes et un site avec lâchers par drone. Les quatre autres blocs avaient un site témoin et un site avec lâchers par cartes. Dans chaque site, des branches ont été collectées au printemps et à l'automne afin d'évaluer les densités de populations à l'aide des L2, et à l'été pour les taux de parasitisme des œufs. Les papillons étaient piégés par des pièges à phéromone du projet Pisteurs de tordeuses (http://pisteursdetordeuses.ca/) pour synchroniser les lâchers. Alors qu'en 2016, les résultats montraient que le parasitisme était plus élevé dans les sites traités, en 2017, les résultats étaient moins clairs. Le bloc avec la plus faible densité d'œufs a obtenu un parasitisme plus élevé dans les sites traités (drone et tricho-cartes) que dans le témoin. Par contre, le parasitisme était bas et non concluant dans tous les autres blocs. Plusieurs facteurs semblent avoir diminué l'impact de nos traitements : la météo froide et pluvieuse, la présence d'une autre espèce de papillons abondantes qui pourrait avoir été en compétition pour le parasitisme des œufs, et la prédation par des limaces.

SERG Interim Report (Year 2, Jan. 2018): *T. minutum* against SBW

Introduction

With a spruce budworm outbreak currently spreading through eastern Canada, there has been increased interest in developing new approaches to mitigate potential damage. The so-called 'Early-intervention strategy' (EIS) has been proposed as an alternative/complementary approach to the traditional 'Foliage protection strategy'. EIS focuses on using products to treat spruce budworm 'hot spots' while populations are still relatively low, ideally halting or slowing the local rise and spread of outbreak. A significant challenge to developing the EIS is that female moths often migrate in from surrounding unsprayed areas to lay eggs, potentially offsetting local population reductions caused by insecticide application [1]. None of the currently available control agents (i.e., Mimic, Btk, and pheromone) can be used to target spruce budworm eggs and it remains uncertain whether additional egg mortality could aid in managing low-density populations near to ongoing outbreaks.

Trichogramma minutum is a generalist egg parasitoid commonly used in agroecosystems [2] and has been tested previously as a biological control agent against spruce budworm. Among the advantages of using this biocontrol agent is the ease of its rearing for commercial purpose and the fact that it kills its host before the damaging stage, i.e. before caterpillars. Although it is used in orchards [3] and has been previously tested as a biological control agent against spruce budworm, it is not currently used in forestry in Canada. Past studies found that proper deployment of *T. minutum* in areas of high spruce budworm density increased egg parasitism by 14-83% and reduced larval populations from 42 to 82%, thus providing significant foliage protection benefits [4]. In the context of EIS, *T. minutum* application densities would be much lower and cost less than those required for treating high-density populations. Moreover, the wide range of established deployment methods (e.g., aerial application, ground application, etc.) [5] and negligible environmental impact could make it a versatile tool for application in a conventional spray program as well as in areas not suitable for chemical insecticide application, such as some woodlots, conservation and residential areas, and parks. As a complementary extension of the ongoing ACOA Early Intervention project (ACOA 2.2.5), we propose to examine the potential of *T. minutum* as a biological control agent for reducing egg densities in low-density spruce budworm populations in small private wood lots, using two different release methods. This control strategy would be the only one currently available where the immigrating moths could be targeted through their egg progeny and one of the few control methods available for private owners. This mortality would also be added to the natural parasitism, and eventually to any larval insecticide used.

For the second year of this project, we focused on comparing the efficacy of two release methods: the installation of cards on the ground vs. drone.

Objectives

1) Determine whether the use of *T. minutum* significantly increases egg mortality in low to moderate density spruce budworm populations.
2) Determine the impact of *T. minutum* releases on the populations' growth (L2-L2).
3) Compare the efficacy of both release methods (cards vs. drones).

Methodology

Study sites

Based on the L2 and defoliation levels evaluated by the MFFP, we identified three areas (Charlevoix, Mauricie and Laurentides – Sainte-Adèle) where SBW is present with either low defoliation or in a restricted area. Within each area, we selected respectively four, six and one 1 ha blocks with one (Charlevoix) or two (Mauricie and Laurentides) treated plots (30m x 30m) and one control plot (30m x 30m) (Fig. 1). It is important to not that in Sainte-Adèle, the municipality had decided to treat all their forest and wood lots with Btk to decrease SBW populations.

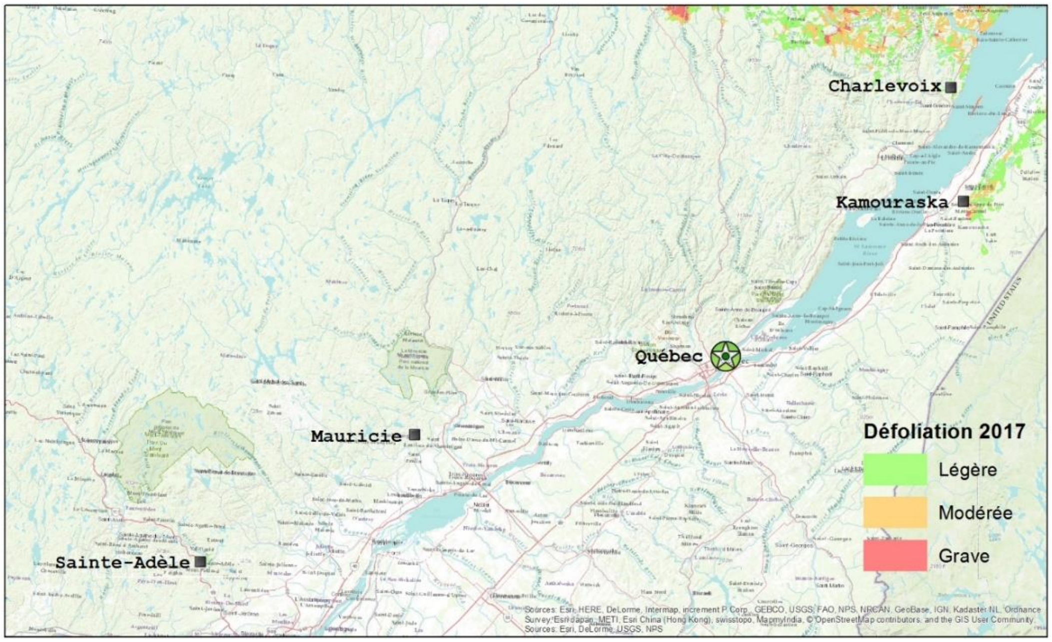


Figure 1. *Map showing the four study areas in Quebec: Sainte-Adèle (2017), Mauricie (2017), Charlevoix (2016-17) and Kamouraska (2016). Coloured areas represents the observed defoliation by aerial surveys made by MFFP in 2016.*

Trichogramma releases

Release timing is of prime importance in biological control, and especially when releasing *Trichogramma* [4]. To make sure that the first *Trichogramma* release was carried out at the beginning of the moth flight periods, we were in contact with citizens from the Budworm Tracker project to be informed of when the first males were captured. The first release was conducted a few days after the first capture and the second release one week after the first one.

Ground releases (using commercial cards provided by Anatis Bioprotection, thereafter called 'Tricho-cards' on which about 6,000 *Ephestia kuehniella* eggs parasitized by *T. minutum* are glued – Fig. 2) were made in each treatment plot. For each release, 100 Tricho-cards were installed at eye level on balsam fir or spruce branches, as evenly as possible within the plot. At the second release, the first card was left in place to allow for late emergences and a second card was installed next to the first one. The Tricho-cards were removed about a month later, during branch sampling.

Figure 2. *Tricho-cards, provided by Anatis Bioprotection, containing about 6,000 eggs parasitized by* T. minutum

Drone releases (using a modified drone developed by Canopée Dronautique; Fig. 3) were done in one plot/block in Mauricie and Ste-Adèle (but not Charlevoix), with the same release rate (3M/ha, i.e. 1.5M females/ha) and on the same day as the cards installation.

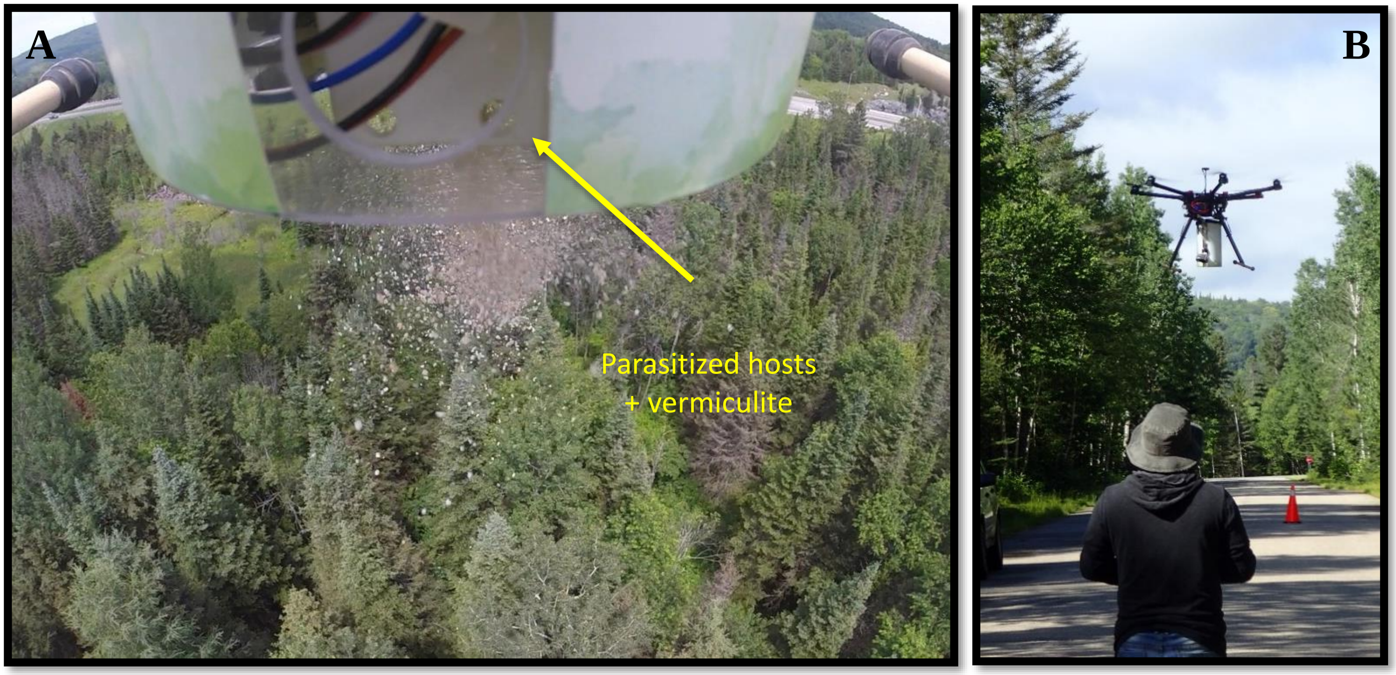


Figure 3. A. *Picture taken from the drone, by Canopée Dronautique, showing the parasitized eggs mixed with vermiculite being released in Sainte-Adèle;* ***B.*** *Drone take-off for the release in Mauricie (Alexandrais Maltais piloting the drone).*

Egg parasitism and population growth

Local population densities were assessed using L2 assessment by branch collection (15/plot) before (April 2017) and after (October 2017) the growth season as well as egg assessment by branch collection (25/plot) about 10 d after the end of oviposition period as predicted by BioSIM [6]. The egg collection was also used to assess the effectiveness of the *T. minutum* treatment and the natural occurrence of *Trichogramma* in the control blocks by measuring parasitism rates.

Results

Population levels

The L2 density before the treatments varied between a little below 1 to 15 L2 per branch in the different sites (Fig 4). The L2 density after the treatments are not yet available as SBW is currently in diapause – results will be available in spring 2018.

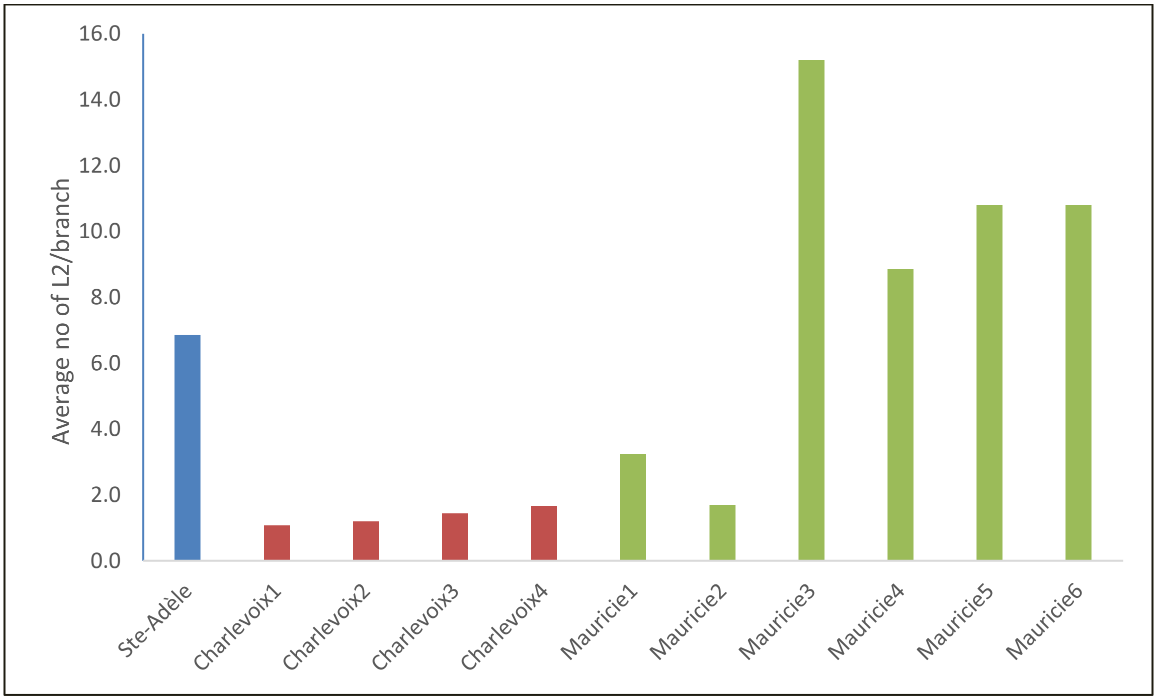


Figure 4*. Average number of second instar larvae (L2) per branch in the study sites.*

Egg parasitism

There was some parasitism in a few plots, but there is no clear pattern (Fig. 5A-C). When putting the percentage of parasitism in relation with the egg density on the site, it appears that the highest parasitism rates were found when egg density was lower, which would be expected (Fig. 6).

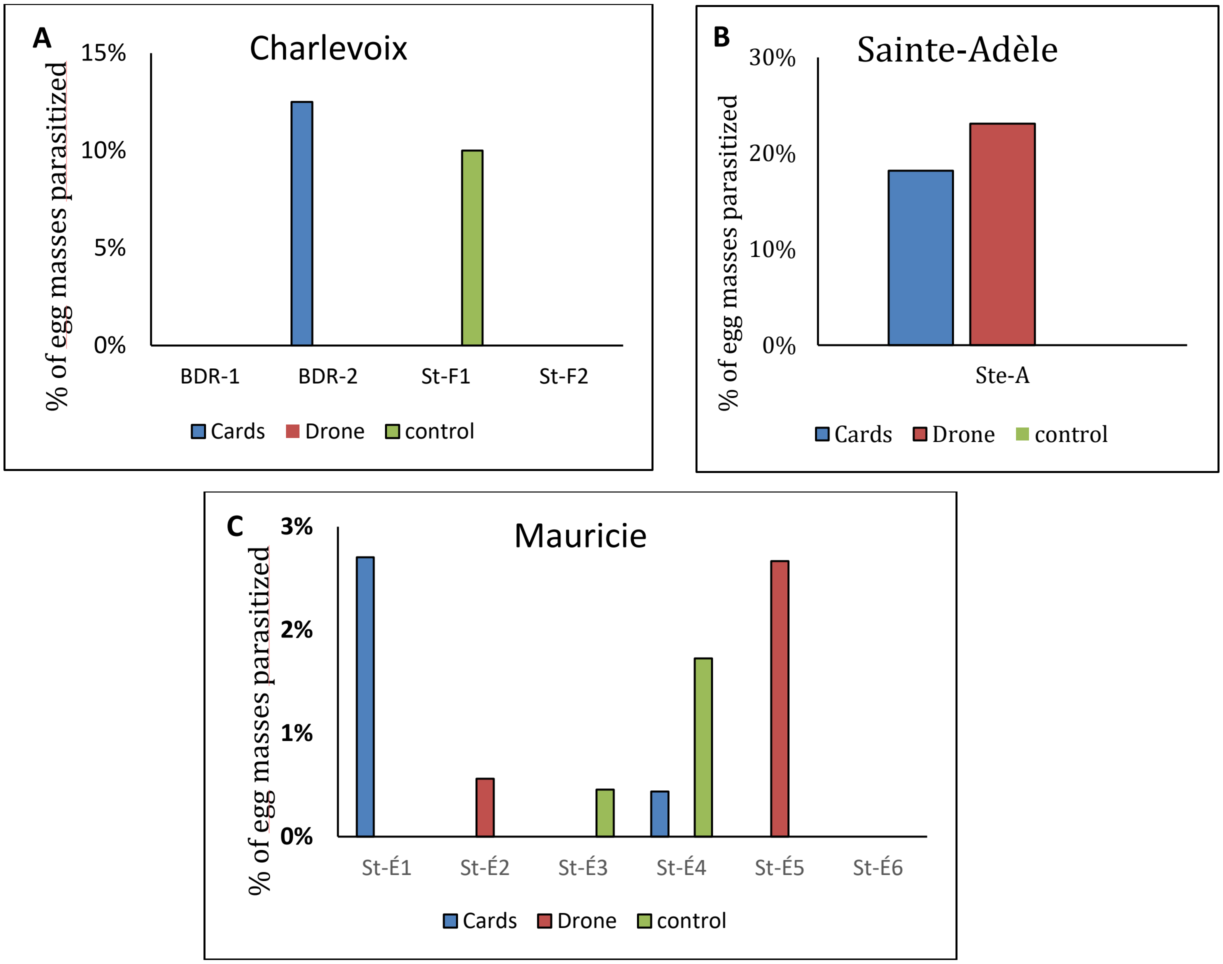


Figure 5*.Percentage of SBW egg masses parasitized by* T. minutum *in the control and treated plots for each area.*

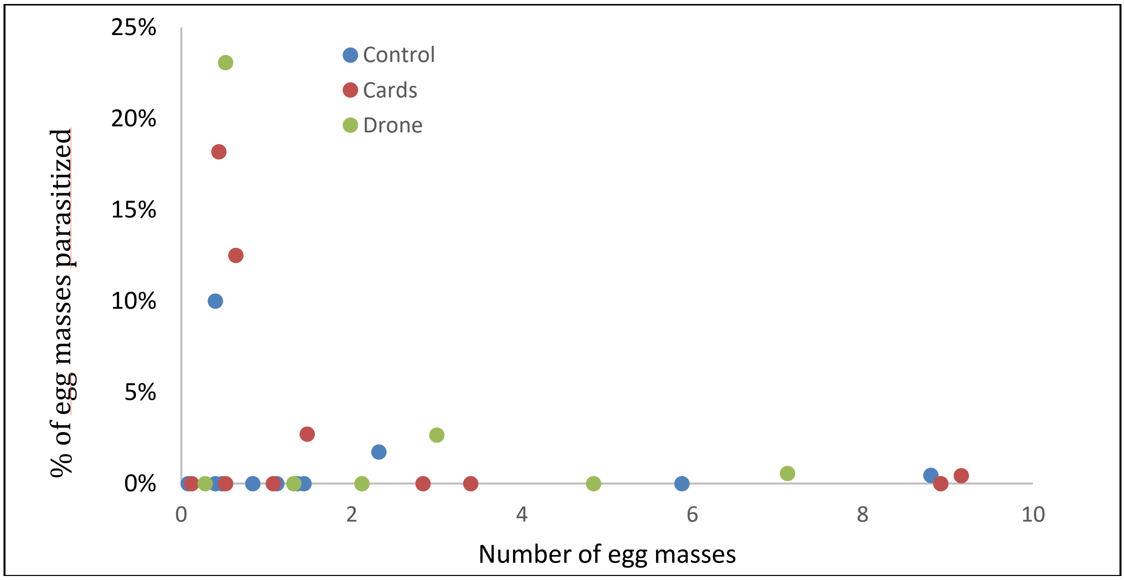


Figure 6*. Percentage of SBW egg masses parasitized by* T. minutum *in both 2016 and 2017 in relation with the density of eggs on the site*

Discussion and tentative conclusions

The results make it difficult to draw clear conclusions on the efficacy of *Trichogramma* releases against SBW. In both year (2016 and 2017), the block with the highest efficacy was the one with the lowest egg density. That probably indicates that the release rate should be increased for plots with higher SBW density, to compensate for the lowest efficacy of *Trichogramma* at high host density. In 2017, for the Sainte-Adèle block where the egg density was the lowest, plots with releases by both drone and tricho-card show similar efficacy, with about 20% of SBW egg masses parasitized, while there was no parasitism in the control plot. Although only on one site, these results indicate that this method, under optimal conditions, can give promising results. Sainte-Adèle probably had the lowest egg density, even though the L2 density was initially higher than in Mauricie, because of the Btk treatment performed at the larval stage. This suggest that the use of two control methods might increase the overall efficacy in decrease SBW densities. The L2 data after treatments will confirm, or infirm, this.

However, results from the Charlevoix and Mauricie blocks are not as clear. In Mauricie, there is some parasitism in different plots, with no clear pattern between treated and control sites. Nevertheless, the important aspect is that no parasitism was above 3%, which mean that it was negligible everywhere. In Charlevoix, where no drone release were done, we had parasitism in only 2 plots: one treated and one control. Among the possible factors that could have decrease our efficacy: (i) the cold and rainy weather during the release period could have delay the *Trichogramma* emergence, extending the duration of their exposure to predation; (ii) in Mauricie, at the time of the release, two other moth species were found in higher abundance than SBW – these moths' eggs were probably in competition with SBW eggs to be parasitized by *Trichogramma*, decreasing our efficacy; (iii) in Charlevoix, some of the Tricho-cards collected after the emergence show important signs of slug predation – if the predation occurred before the *Trichogramma* emergence, the actual release rate was much lower than intended.

The L2-L2 population growth data, available next spring, will be necessary to confirm if the treatment had an impact on the population level for the Ste-Adèle block. Although not so clear, these preliminary data show that it is possible to increase egg parasitism using *T. minutum* releases under good conditions (SBW density, *Trichogramma* release rate, weather, etc.), and that it might be more efficient in low-density populations. However, additional study is necessary to determine more precisely these conditions. For any further study, I would recommend using the drone release method instead of the Tricho-cards one: the release are much faster and show the same efficacy for the block where it worked.

Acknowledgements

We thank the Ontario Ministry of Natural Resources and Forestry for financial support and the Ministère de la forêt, de la faune et des parcs du Québec (MFFP) for their support and for providing data from the L2 and defoliation surveys. We also thank the Canadian Forest Service, especially the SMA Innovation Funds for providing financial and in-kind support, Anatis Bioprotection, especially Silvia Todorova and Mylène St-Onge, for providing insects and precious advices, as well as Canopée Dronautique (Frédéric Jean et

Alexandre Maltais) for the drone services. We are also grateful to the private lot owners and the Saint-Siméon and Sainte-Adèle municipalities for providing us with access to their land and property during this study. Numerous people also provided support in the lab and the field: we thank S. Bélanger, P. Huron, M. Labrecque, M. Gorla, L.-P. Gélinas and C. Rochon.

References

1. Béchard, A., Delisle, J., Johns, R., Labrecque, A., Martel, V., Pureswaran, D., Régnière, J., Royer, L., Thompson, D., van Frankenhuyzen, K. (2014) SERG-I Project FPL-786 Dynamics and management of rising spruce budworm outbreak. Interim Report.
2. Consoli et al. 2010. *Egg parasitoids in agroecosystems with emphasis on* Trichogramma. Springer.
3. Smith SM (1996) Biological control with *Trichogramma*: advances, successes, and potential of their use. *Annual Reviews in Entomology* **41**: 375–406.
4. Smith, S.M., Wallace, D.R., Howse, G., Meating, J. (1990) Suppression of spruce budworm populations by *Trichogramma minutum* Riley, 1982-1986. *Memoirs of the Entomological Society of Canada* **153**: 56-80.
5. Smith, S.M., Wallace, D.R. (1990) Ground systems for releasing *Trichogramma minutum* Riley in plantation forests. *Memoirs of the Entomological Society of Canada* **153**: 31-44.
6. Régnière, J., Saint-Amand, R., Béchard, A. (2014) BioSIM 10 – User's manual.
7. Quayle, D., Régnière, J., Cappuccino, N., Dupont, A. (2003) Forest composition, host-population density, and parasitism of spruce budworm *Choristoneura fumiferana* eggs by *Trichogramma minutum*. *Entomologia Experimentalis et Applicata* **107**: 215-277.

Optimization of SBW pheromone application for mating disruption

C. M. Riley

Agrifor Biotechnical Services Ltd.,
151 Queen Street, Fredericton, New Brunswick E3B 7J2, Canada

Abstract

A controlled-release formulation, Confound SBW, containing 95E:5Z (E,Z)-11-Tetradecenal was approved and registered in July 2017. A second project funded by the Atlantic Canada Opportunities Agency under the Atlantic Innovation Fund was approved in 2017 but not in time to procure product for field testing. Additional results from a field trial reported in 2017 are presented. Populations of second instar larvae (L2) in the area treated with Confound SBW were significantly lower than in three of the four untreated control areas. Post-treatment L2 values in the treated area decreased by 36.3% compared to pre-treatment assessments whereas corresponding values in untreated control areas increased by 242% to 461% with an average of 264%. Larval populations per square meter of branch area in the treated area ranged from 11.1% to 40.0% of values in control areas i.e. a relative reduction of 60% to 88.9% as a result of mating disruption with the unregistered Confound SBW product. The relative reduction calculated using the combined data for all four control areas was 81.3%. The treatment effects observed in 2016 did not lead to a significant reduction in the number of moths caught in pheromone traps that were deployed in the same area in 2017. This is not too surprising given that the area treated was only 100 ha. Moth populations in all study areas were greater in 2017 than in 2016.

Résumé

Une formulation à libération contrôlée, Confound SBW, contenant 95E: 5Z (E, Z) -11-tétradécénal a été approuvée et enregistrée en juillet 2017. Un deuxième projet financé par l'Agence de promotion économique du Canada atlantique dans le cadre du Fonds d'innovation de l'Atlantique a été approuvé en 2017 mais pas à temps pour se procurer le produit pour les essais sur le terrain. Des résultats supplémentaires d'une étude rapportée en 2017 sont présentés. Les populations de larves de deuxième stade larvaire (L2) dans la zone traitée avec Confound SBW étaient significativement plus faibles que dans trois des quatre zones témoins non traitées. Les valeurs de L2 post-traitement dans la zone traitée ont diminué de 36,3% par rapport aux évaluations avant traitement alors que les valeurs correspondantes dans les zones témoins non traitées ont augmenté de 242% à 461% avec une moyenne de 264%. Les populations larvaires par mètre carré de surface de ramification dans la zone traitée variaient de 11,1% à 40,0% des valeurs dans les zones témoins, soit une réduction relative de 60% à 88,9% par suite de la perturbation de l'accouplement avec le produit Confound SBW non enregistré. La réduction relative calculée avec les données combinées pour les quatre zones témoins était de 81,3%. Les effets du traitement observés en 2016 n'ont pas entraîné de réduction significative du nombre de papillons capturés dans les pièges à phéromones déployés dans la même zone en 2017. Ce n'est pas surprenant compte tenu du fait que la superficie traitée n'était que de 100 ha. Les populations de papillons dans toutes les zones d'étude étaient plus élevées en 2017 qu'en 2016.

Background and Objectives

The field efficacy of a prototype sprayable, controlled-release, formulation of 95E:5Z (E,Z)-11-Tetradecenal for potential use as a mating disruptant was successfully demonstrated in 2015[1]. Results were considered by stakeholders in the forest industry to be sufficiently encouraging to justify the pursuit of product registration and in June 2016 a data package was submitted by Sylvar Technologies Inc. to the Pest Management Regulatory Agency. In July 2017 registration was approved and the product is now available as Confound SBW.

In July 2016 a 100 ha, field trial with formulation was conducted under a Research Authorization as part of SERG-I Project 2009/08-2016-759A to generate additional efficacy data. Results in terms of trap shutdown were slightly higher than those seen in 2015[2] but results in terms of second instar larval populations based on branch samples that were collected in November 2017 were very encouraging. Larval populations in the area treated with 95E:5Z (E,Z)-11-Tetradecenal were significantly lower than in three of the four untreated control areas as well as in an area treated with the 83E:17Z (E,Z)-11-Tetradecenal formulation (Table 1). Post-treatment L2 values in the treated area decreased by 36.3% compared to pre-treatment assessments whereas corresponding values in untreated control areas increased by 242% to 461% with an average of 264%. Post-treatment larval populations per square meter of branch area in the treated area ranged from 11.1% to 40.0% of values in control areas i.e. a relative reduction of 60% to 88.9% as a result of mating disruption with the unregistered Confound SBW product. The relative reduction calculated using the combined data for all four control areas was 81.3% (Table 2). This may be the first field trial in which the efficacy of pheromones for mating disruption has been demonstrated for spruce budworm based on pre- and post-treatment larval counts.

Confound SBW has been developed over the last three years with funding from the Atlantic Canada Opportunities Agency (ACOA), SERG-I and other sources. In July 2016 a proposal was submitted by Acadia University for a second, five-year, AIF project.

Mating disruption is not a new concept but it is a concept that has never been put into practice as part of a long-term operational spruce budworm management program. Technical knowledge for the effective use of the product will be needed to provide

---

[1] The ability of a broadcast application of sex pheromone to reduce the attractiveness of pheromone traps is used as a proxy to evaluate the potential of products for mating disruption and population management. The total number of moths caught in pheromone traps within the treated area (74ha) was initially reduced by 100% compared to control areas and the formulation continued to be resilient and effective despite 120mm of rain within the first 10 days of application. After 10 days numbers were still reduced by >99% and by >98% after 25 days. Branch samples for L2 assessment were collected but there was no obvious treatment effect. This was not unanticipated given that the pheromone was not applied until ten days after the first male moths were found in the pheromone traps.

[2] An average cumulative total of 57 moths per trap were found over the duration of the flight period in the area treated with the 95E:5Z formulation. The corresponding values in the control areas ranged from 782 to 1436. The average cumulative total of moths caught in all of the control areas combined was 1025 per trap. In 2016 the product was applied around 12 days before male emergence and so the treatment was losing potential effectiveness and had a reduced effect when moths did emerge and populations were at their peak.

specialized technical support to product users and will be a critical requirement for a forest industry that has no experience of population management using mating disruption. Efforts in the second AIF project are largely concentrated on the development of use strategies that optimize the cost-effectiveness of sprayable formulations for mating disruption and the development of data that demonstrates mating disruption as an effective tool for forest protection.

The objective for the field season of 2017 was to evaluate one or more aerial application practices and use strategies for reducing operational costs and optimizing the effectiveness of sprayable pheromone formulations for the management of spruce budworm populations.

The second AIF project to continue the development of pest management product based on semiochemicals was approved in July 2017 but unfortunately, athough the necessary approvals for aerial application were in place, this was too late to procure product for field testing. The work in 2017 was largely based on assessing the carry-over effect of the 2016 treatment with Confound SBW on moth populations (i.e. pheromone trap catches) in the 2017 season and the identification of potential sites for product testing in 2018. Field testing with a blank formulation to advance the optimization of aerial application practices was proposed at short notice but the approval to work on Crown land was not granted.

Methodology

Sixty-five Multipher® pheromone traps with a 3 mg spruce budworm lure were deployed on July 4 at the same locations that had been used in 2016. The traps were spaced approximately 100 m apart in the canopy at a height of approximately 7 m. Moths were found in the traps when they were first assessed on July 8. Moth catches in pheromone traps were initially assessed every two days but the time interval was later extended to three or more days according to weather conditions and the numbers of moths encountered.

The spruce budworm infestation in New Brunswick is expanding and populations are increasing. In early November the New Brunswick Department of Energy and Resource Development was consulted regarding the choice of sites for the testing of Confound SBW in 2018. The treatment and control sites used to date may fall within the operational treatment program for 2018. Potential new areas for field testing were identified. Branch samples for the assessment of second instar populations and the estimation of baseline values were collected from six different areas and in mid-November a total of 270 branch samples were delivered to the Forest Protection offices of Nova Scotia Department of Natural Resources for processing.

Results and Discussion

The cumulative catches (in 2017) of moths in traps from control areas and treated areas that were used in the 2016 field study are shown in Figure 1. The lowest total number of moths, 1071 per trap, were caught in the area that had been treated with the prototype Confound SBW (95E:5Z) formulation. The corresponding number in the area that had been treated with the 83E:17Z formulation was 1497. Values in the untreated control areas ranged from 1396 to 1961 per trap. There was no significant differences between the cumulative totals in the Confound SBW treatment and the control areas (ANOVA, P = 0.246). This is not unexpected given the relatively small treatment area and the potential for the influx of moths into the treatment area from areas that were not treated.

The total number of moths caught per trap in 2017 reflects an increasing population compared to 2016. In 2016 the average numbers per trap in control areas ranged from 782 to 1436 (Figure 2).

Acknowledgements

Total funding for this work and the purchase of supplies for the 2018 field season was approximately $40,000. This work was supported by the Atlantic Canada Opportunities Agency and through SERG International, with a financial contribution from the Newfoundland and Labrador Department of Natural Resources ($10,000). In-kind contributions were made by the Nova Scotia Department of Natural Resources, Forest Protection Limited and Agrifor Biotechnical Services Ltd. The contributions of all collaborators are gratefully acknowledged.

Table 1. Second instar larval population data.

Block	No. samples (75 cm mid-crown branches)	Branches with L2 (%)	Total no, of L2 found	Mean L2/branch	Mean L2/m2 branch[3]
Control A	60	10.0	10	0.17	0.74a
Control C	15	40.0	9	0.60	2.99b
Control D	30	26.7	17	0.57	2.92b
Control F	30	30.0	14	0.47	2.01b
Combined controls	135	22.2	50	0.37	1.76b
E (95/5)	45	6.7	3	0.07	0.33a
B (83/17)	45	20.0	20	0.44	2.35b

Table 2. Treatment efficacy based on populations of second instar larvae.

Block	L2 count / branch 2015 (2016)	Mean L2/m2 branch	95/5 treatment L2/m2 (% of control value)	Relative reduction – treatment versus control (%)
Control A	0.07 (0.17)	0.74	40.0	60.0
Control C	0.13 (0.60)	2.99	11.1	88.9
Control D	0.23(0.57)	2.92	11.8	88.2
Control F	0.13 (0.47)	2.01	14.3	85.7
Combined controls	0.14 (0.37)	1.76	18.7	81.3
E (95/5)	0.11 (0.07)	0.33		

---

[3] Means with the same letter are not significantly lower than the 95E:5Z (E,Z)-11-Tetradecenal treatment. ANOVA followed by the Two-Sample t-Test ($P < 0.05$)

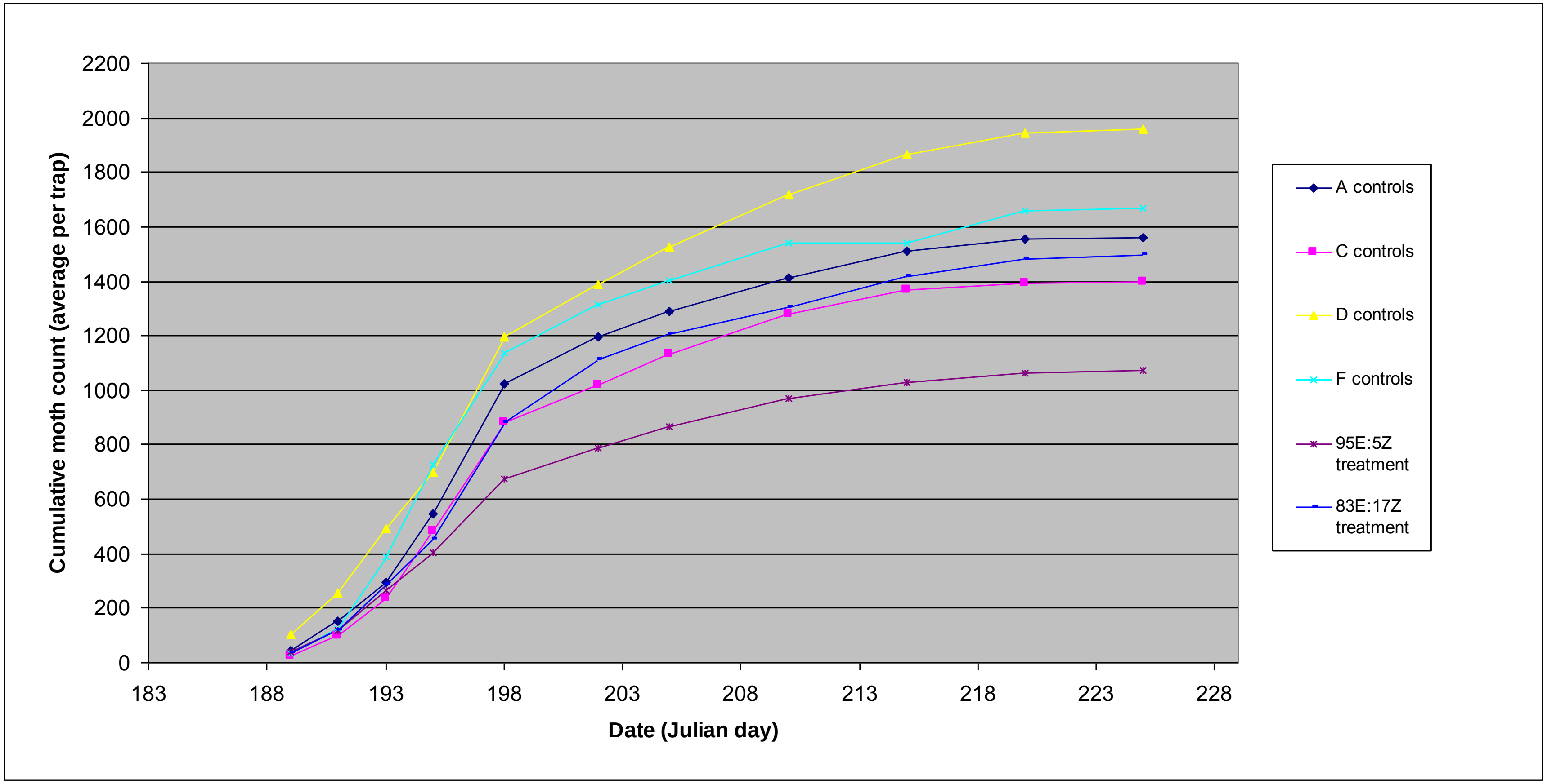


Figure 1. Cumulative catches of moths in pheromone traps located within treated and control sites as a function of time during the 2017 SBW flight season.

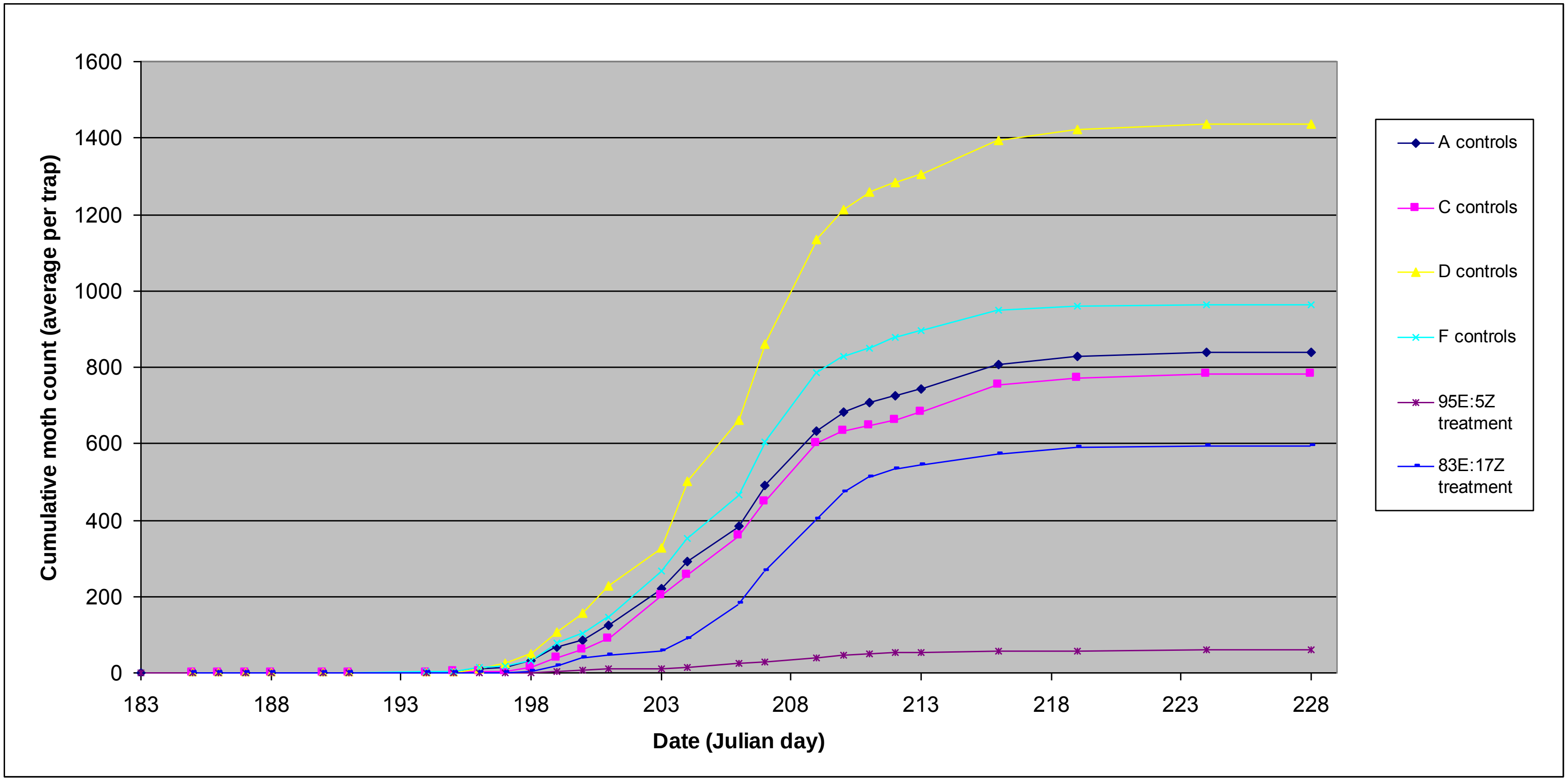


Figure 2. Cumulative catches of moths in pheromone traps located within treated and control sites as a function of time during the 2016 SBW flight season.

Comparisons of *Btk* aerial spraying strategies against the eastern spruce budworm, based on protection timing and intensity during a complete outbreak episode.

Alain Dupont[1], *Éric Bauce*[2], *Alvaro Fuentealba Morales*[2], *Christian Hébert*[3] *and Richard Berthiaume*[2]

[1] Société de protection des forêts contre les insectes et maladies, 1780 Semple, Quebec City (QC) G1N 4B8
[2] Consortium iFOR, Université Laval, Pavillon Abitibi-Price, 2405 de la Terrasse, Quebec City (QC) G1V 0A6
[3] Canadian Forest Service, Laurentian Forestry Centre, 1055 du P.E.P.S., Quebec City (QC) G1V 4C7

ABSTRACT

Large-scale aerial spraying operations against the spruce budworm (*Choristoneura fumiferana* Clem.) with the biological insecticide *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*), aims at maintaining trees alive during outbreak episodes. This objective is achieved when ≥ 50% of current-year foliage in balsam fir [*Abies balsamea* (L.) Mill.] stands is preserved. However, it is unknown if this standard approach used in Quebec province is always justified, or if less frequent interventions can provide similar results at lower cost. We conducted between 2010 and 2017 field experiments in Quebec's North Shore region to determine the efficacy of five different protection scenarios in protecting balsam fir, white spruce [*Picea glauca* (Moench) Voss], and black spruce [*Picea mariana* (Mill.) BSP] mixed stands. We hypothesized that less frequent *Btk* applications can provide an adequate level of protection. After nine years of defoliation, our results show a clear effect of protection scenarios on host tree species mortality caused by the spruce budworm. As the protection intensity decreases, the volume of balsam fir mortality increases (0% to 25%). Moreover, balsam fir mortality remains higher then spruce trees, but dead volumes are similar between white and black spruce species. At this stage of the outbreak, our observations suggest that balsam fir stands could be treated every 2 years, and white spruce every 3 years without significant mortality level (< 4%). Based on this study, it is possible to apply the required protection level at lower cost or on a greater forested area at a comparable cost.

RÉSUMÉ

Les pulvérisations aériennes d'insecticide biologique (*Bacillus thuringiensis* var. *kurstaki*) sur de vastes superficies infestées par la tordeuse des bourgeons de l'épinette (*Choristoneura fumiferana* Clem.), visent à maintenir les arbres vivants jusqu'à la fin de l'épidémie. Cet objectif est atteint lorsque ≥ 50% du feuillage annuel du sapin baumier [*Abies balsamea* (L.) Mill.] est maintenu. Toutefois, on ignore si cette approche standard utilisée au Québec demeure toujours justifiée ou si des interventions moins fréquentes permettent d'obtenir des résultats similaires à moindre coût. Entre 2010 et 2017, nous avons réalisé une étude dans la région de la Côte-Nord du Québec, afin de comparer l'efficacité de cinq différents scénarios de protection dans des forêts composées de sapins baumiers, d'épinettes blanches [*Picea glauca* (Moench) Voss], et d'épinettes noires [*Picea mariana* (Mill.) BSP]. L'hypothèse de départ stipule que des applications moins fréquentes de *Btk* peuvent fournir un niveau de protection adéquat. Après neuf années de défoliation, les résultats démontrent un net effet des scénarios de protection sur la mortalité des essences hôtes causée par l'insecte. À mesure que l'intensité de la protection diminue, le volume de sapin mort augmente (0 à 25%). De plus, la mortalité du sapin baumier demeure plus élevée que celle des épinettes, mais les volumes morts sont similaires entre l'épinette blanche et l'épinette noire. À ce stade de l'épidémie, nos observations suggèrent que les peuplements de sapins baumiers peuvent être traités aux 2 ans, tandis que les forêts d'épinettes blanches aux 3 ans sans subir une mortalité significative (< 4%). D'après les résultats obtenus jusqu'à

maintenant, il semble possible d'appliquer la protection requise à moindre coût ou de protéger une superficie plus vaste à un coût similaire.

INTRODUCTION

During the previous spruce budworm (SBW) outbreak (1967-1992), forest pest managers have adapted their protection strategy according to the infestation development. In the province of Quebec, aerial spraying programs involving insecticides have been increasingly targeted between the 1970s and the late 1980s (Dorais 1992). Indeed, approaches such as reducing the infestation intensity (1970-72), eradicating new epicentres (1971), slowing the spread of the outbreak (1973-74), protecting the current shoots (1975-84), and protecting the new foliage of the most vulnerable forests were implemented successively. The last strategy is still applied against the spruce budworm in Quebec. The first spray is carried out after one year of moderate to high defoliation level, to protect at least 50% of the annual foliage. The achievement of this objective throughout the outbreak results in a low risk of balsam fir mortality (Hardy and Dorais 1976).

However, this approach has major limitations and it also raises several issues. First, this strategy constitutes a form of annual subscription to the same infested areas for the whole outbreak. This allows limited room for additional area of forest requiring a protection, given the tight budgets. Thus, the average of protected / infested forest ratio stays around 4% since the beginning of this SBW outbreak. This is quite small regarding protection needs to maintain socioeconomic activities related to various forest resources. Currently, only a portion of the forest lands dedicated to wood production are included in spraying programs against the SBW in Quebec. According to the use of forest lands, the type of production, and the host tree species affected by the insect, it is important to determine if the protection target should always be 50% of the current foliage. Moreover, as current wood products available on the market require high quality fiber, lowering protection costs would allow protecting larger areas with the same budget, thus reducing tree mortality and increasing wood supply stability.

Considering the availability of financial resources dedicated to forest pest management, it is crucial to use these funds the most efficiently as possible. The major challenge remains to do more effective protection with the available financial resources. To achieve this, it becomes reasonable to work on aerial spray programs optimization from the beginning of an outbreak and until the end. Even if many authors have studied forest management and protection combinations in relation with intervention priorities and economical concerns, the results associated with variable protection regimes are not well known by forest pest managers. On the other hand, the current SBW outbreak occurs in a forest multiple use context, within which protection objectives should be established for other values than wood production.

In 2007, we initiated a long-term study in which we compare four spray scenarios, along a gradient of intensity, with unsprayed forest stands, in a region affected by a severe outbreak of the spruce budworm in Quebec (table 1). Our main goal is to evaluate and compare the efficacy of these protection scenarios against the SBW, using biological insecticide *Bacillus thuringiensis* var. *kurstaki* (*Btk*) aerial applications. The studied scenarios are defined as follows:

1) No protection or control (real SBW impacts);
2) *Btk* treatment every 3 years (extensive protection);
3) *Btk* treatment every 2 years (average protection);
4) Standard *Btk* protection (intensive protection or annual defoliation ≤ 50%);
5) *Btk* very intensive protection (Very light to no SBW impact).

TABLE 1 AREA OF DEFOLIATION BY SBW IN QUEBEC'S NORTH SHORE REGION FROM 2006 TO 2017 (MFFP 2017).

Year	Defoliation category (ha)			Total
	Light	Moderate	Severe	
2006	1 780	435	104	2 319
2007	18 109	18 795	17 086	53 990
2008	20 550	30 729	40 311	91 590
2009	47 937	59 167	82 177	189 281
2010	140 346	189 915	206 081	532 342
2011	474 310	450 877	397 425	1 322 612
2012	567 999	592 261	585 054	1 745 314
2013	287 502	712 711	1 465 507	2 465 721
2014	457 193	937 419	1 551 744	2 946 357
2015	914 548	1 652 384	1 187 673	3 754 605
2016	1 375 034	1 550 427	825 414	3 750 875
2017	809 955	1 378 952	1 197 007	3 385 915

The intensity of spraying interventions and associated costs are directly related to the protection scenarios included in the study (table 2).

TABLE 2 ANTICIPATED INTERVENTIONS AND TREATMENTS

Strategy	Year												
	0	1	2	3	4	5	6	7	8	9	10	11	12
	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
No protection	0	0	0	0	0	0	0	0	0	0	0	0	0
Every 3 Years		1			2			2			2		
Every 2 Years		1		2		2		2		2		2	
Standard			2	2	2	2	2	2	2	2	2	2	1
No SBW impact		1	2	3	3	3	3	3	3	3	3	2	1

The study will be conducted along five major perspectives: efficacy of *Btk* treatments, timber quality, wood losses (growth, mortality), effects on carbon balance, and protection investments. Using a benefit / cost analysis, the project will lead us to determine the more efficient scenario or complementary scenarios, that can be considered as an optimal strategy to minimize the impact of spruce budworm on susceptible forests.

The forest pest managers are interested in annual and long-term efficacy of *Btk* against the spruce budworm. In practice, this biological insecticide has been operationally introduced at the end of the previous outbreak. Therefore, several of them are speculating about its effectiveness in high SBW population levels. This is a key issue in this research because when *Btk* treatments are not applied every year, it is common to observe some SBW population buildup.

SBW impacts on softwood production are widely documented in unprotected forest stands, but in protected forests the literature is quite scarce. Regarding variable protection regimes against the SBW, the assessments of growth losses and mortality levels remains non-existent. The timber quality following a spruce budworm epidemic and woodboring insect attacks has become increasingly important for the forest industry.

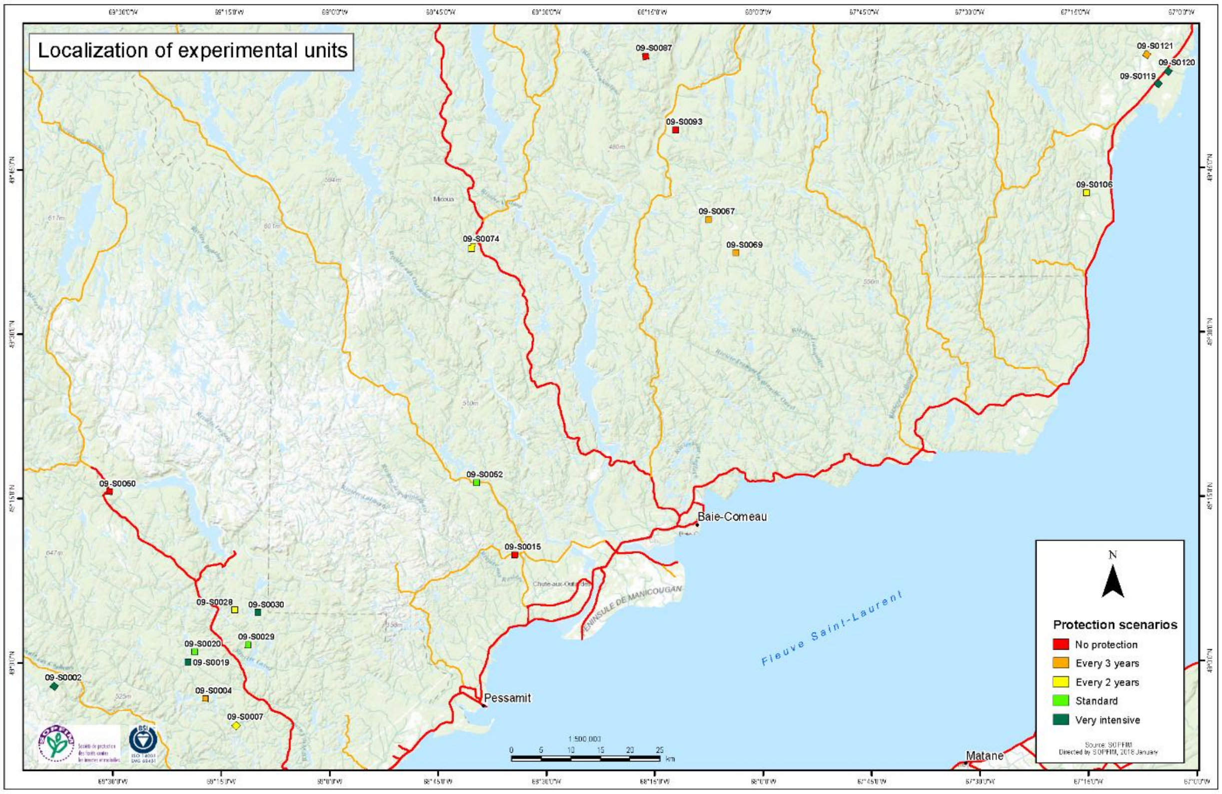
The carbon balance represents a new reality in forestry and in integrated forest pest management (Hennigar and MacLean 2010; Dymond *et al.* 2010). Natural disturbances like SBW outbreaks can create an additional carbon source, but forest protection with *Btk* could help to reduce CO_2 emissions. If this hypothesis is founded, it becomes possible to obtain more support from the environmental community opposed to the use of pesticide.

MATERIALS AND METHODS

Study area

The study area is located in Quebec's North Shore region, more specifically between Forestville and Rivière-Pentecôte, where the SBW has affected softwood stands over the last 10 years. The experimental design refers to 20 experimental units (100 ha each) established at least one year prior to spruce budworm defoliation in balsam fir – white spruce – black spruce mixed forests, belonging to 30, 50 and 70-year old stands, and divided into five (5) protection scenarios (figure 1).

FIGURE 1 LOCALIZATION OF EXPERIMENTAL UNITS



Btk application and efficacy

The insecticide applications were carried out on an operational basis using all the available resources in terms of aircraft and registered *Btk* products. Foray 76B^{TM} and Bioprotec HP^{TM} are *Btk* strain HD-1 commercial formulations at nominal potency of 20.0 billion international units per litre (BIU/L) (Abbott Laboratories, Chicago, IL; on behalf of Valent Bio-Sciences Corporation, Libertyville, IL and AEF Global Inc, Lévis, QC, respectively). Both *Btk* formulations were applied to the experimental blocks, which are described above. Over the years, many aircrafts were used (Cessna 188; Dromader M-18; Air Tractor 402, 502, 504, 602 and 802) with six or eight Micronair atomizers (Micronair Sprayers Ltd, Bromyard, UK), respectively. Micronair atomizers, spinning at 3,195 g, were located within 75 % of the total wingspan. These aircrafts were flown between 161 and 210 km/h, with 50, 80 or 100-m spray widths. Aerial treatment occurred early in the morning or at dusk under good weather conditions (wind speed < 6 km/h; no rain). The flow rate through the nozzles was calibrated to deliver 1.5 L/ha or 30 BIU/ha. *Btk* formulations were applied one to three times to the sprayed plots according to protection goals of each scenario. The first aerial application of *Btk* against the spruce budworm was targeted at third to early fourth-instar larvae (beginning to mid-June), whereas the second and third applications were carried out 5 and 10 later respectively. *Btk* treatments were timed to coincide with early flushing of balsam fir shoots. This timing provides optimal protection to balsam fir and does not reduce treatment efficacy in reducing final defoliation in white spruce and black spruce (Cadogan and Scharbach 1993, Carisey *et al.* 2004).

In each block, two 45-cm branch tip were collected from the upper midcrown of 15 balsam fir trees selected along three transects perpendicular to the flight lines. The first sample was collected 24-48 hours before the spray (pre-spray assessment) and the second, at the end of the insect feeding period (post spray at 85% pupae stage). For both samples, all the standard information has been collected (shoot development index, insect development index, insect count by branch and bud, current foliage defoliation).

Timber quality and growth losses

At the end of the outbreak, growth loss and timber quality assessments are planned on balsam fir, white spruce and black spruce belonging to 30, 50 and 70 years old. A subsample among 135 trees per experimental unit (15 trees-per species and age classes) will be cut down for the analysis.

Mortality of host species

In each block, tree plots (r = 11,28 m) is measured every year in order to evaluate the volume of mortality caused by the insect. The natural mortality is retrieved from the total amount of dead trees.

Carbon balance

All the information regarding defoliation, growth losses and tree mortality will be collected for the carbon balance evaluation. Available models and others in development by the scientific community are susceptible to be used for this issue.

Benefit-cost analysis

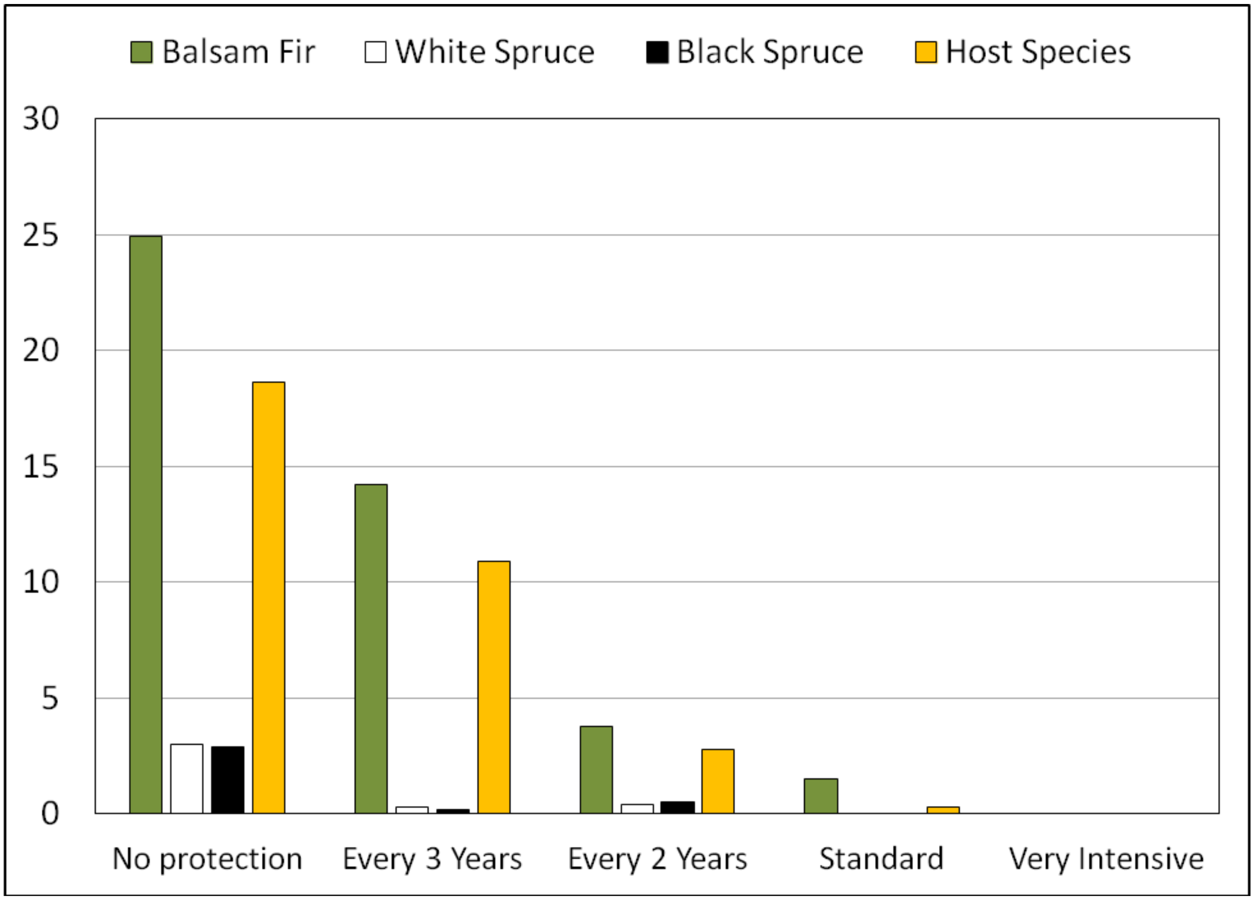
Every year, spraying operation costs are calculated and reported in actual cash value. At the end of the outbreak, the total investment for each protection scenario will be compared to the wood volume saved by the interventions.

PRELIMINARY RESULTS

Wood losses (mortality)

This year, SBW host tree species mortality assessment shows a clear increase in wood volume losses comparatively with 2016. Most of the insect impact remains on the most vulnerable species (balsam fir). At this stage of the outbreak, mortality of the spruce trees is very low. After 10 years of defoliation, we are entering in the most critical period of the outbreak because the mortality will increase very rapidly in the next five years.

FIGURE 2 SBW HOST SPECIES MORTALITY (% VOLUME) BY PROTECTION SCENARIO 2017



However, at the present stage of the outbreak, our results show that mortality of host tree species is related to the protection intensity represented by the different protection scenarios. At this time, it seems possible to suggest that balsam fir stands could be treated every 2 years and white spruce every 3 years without significant mortality levels.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support of SERG International (SERG-I), AEF Global Inc. (*Btk*), Valent Bioscience Corp.(*Btk*), Forest Protection Limited (aircraft), Nova Scotia Department of Natural Resources ($2k), USDA Forest Service ($1k), Ministère des Forêts, de la Faune et des Parcs (L2 processing), the Department of Natural Resources of the Government of Newfoundland and Labrador ($7k), and Canada Economic Development ($200k).

REFERENCES

Archambault, L., R.R. Gagnon, G. Pelletier, M. Chabot et L. Bélanger. 1990. *Influence du drainage et de la texture du dépôt sur la vulnérabilité du sapin baumier et de l'épinette blanche aux attaques de la tordeuse des bourgeons de l'épinette.* Can. J. For. Res. 20 : 750-756.

Bauce, É., N. Carisey, A. Dupont and K. van Frankenhuyzen. 2004. Bacillus thuringiensis subsp. kurstaki prescriptions for balsam fir stand protection against spruce budworm (Lepidoptera: Tortricidae). For. Entomol. 97(5): 1624-1634.

Bergeron, Y., A. Leduc, H. Morin and C. Joyal. 1995. *Balsam fir mortality following the last spruce budworm outbreak in northwestern Quebec.* Can. J. For. Res. 25: 1375-1384.

Carisey, N., É. Bauce, A. Dupont and S. Miron. 2004. *Effects of bud phenology and foliage chemistry of balsam fir and white spruce trees on the efficacy of Bacillus thuringiensis against the spruce budworm, Choristoneura fumiferana.* Agric. For. Entomol. 6: 55-69.

Clark, J. 1961. *Photosynthesis and respiration in white spruce and balsam fir.* State University, College of Forestry, Syracuse, NY. 72 p.

Dupont, A., L. Bélanger and J. Bousquet. 1991. *Relationships between balsam fir vulnerability to spruce budworm and ecological site conditions of fir stands in central Quebec.* Can. J. For. Res. 21: 1752 – 1759.

Dymond, C. C., E. T. Neilson, G. Stinson, K. Porter, D. A. MacLean, D. R. Gray, M. Campagna and W. A. Kurz. 2010. *Future spruce budworm outbreak may create a carbon source in eastern canadian forests.* Ecosystems 13:917-931.

Fettes, J.J. 1950. *Investigations of sampling techniques for population studies of the spruce budworm on balsam fir in Ontario.* Annual Tech. Rep. 4, Forest insect Lab., Canadian Forest Service, Sault Ste. Marie, ON, pp. 163-401.

Fournier, C., E. Bauce, A. Dupont and R. Berthiaume. 2009. *Wood losses and economical threshold of Btk aerial spray operation against spruce budworm.* Pest Manag. Sci. 66: 319-324.

Fox, G., J. Beke, T. Hopkin and D. McKenney. 1997. *A framework for the use of economic thresholds in forest pest management.* For. Chron. 73(3): 331-339.

Hennigar, C. R. and D. A. MacLean. 2010. *Spruce budworm and management effects on forest and wood product carbon for an intensively managed forest.* Can. J. For. Res. 40: 1736-1750.

MacLean, D. A. 1979. *Spruce budworm-caused balsam fir mortality on the Cape Breton Highlands, 1974-1978.* Can. For. Serv., Maritimes For. Res. Centre, Fredericton, N.B., Inf. Rep. M-X-97. 24 p.

MacLean, D. A. 1980. *Vulnerability of fir-spruce stands during uncontrolled spruce budworm outbreaks: a review and discussion.* For. Chron. 56: 213-221.

MacLean, D. A. 1981. *Impact of defoliation by spruce budworm populations on radial and volume growth of balsam fir: a review of present knowledge.* Mitt. Forstl. Bundes. Wien 142: 293-306.

MacLean, D. A. and T. A. Erdle. 1984. *A method to determine effects of spruce budworm on stand yield and wood supply projections for New Brunswick.* For. Chron. 60: 167-174.

MacLean, D. A., T. L. Hunt, E. S. Eveleigh and M. G. Morgan. 1996. *The relation of balsam fir volume increment to cumulative spruce budworm defoliation.* For. Chron. 72: 533-540.

MacLean, D. A., A. W. Kline and D. R. Lavigne. 1984. *Effectiveness of spruce budworm spraying in New Brunswick in protecting the spruce component of spruce-fir stands.* Can. J. For. Res. 14: 163-176.

MacLean, D. A. and D. P. Ostaff. 1989. *Patterns of balsam fir mortality caused by an uncontrolled spruce budworm outbreak.* Can. J. For. Res. 19: 1087-1095.

Ostaff, D. P. and D. A. MacLean. 1989. *Spruce budworm populations, defoliation, and changes in stand condition during an uncontrolled spruce budworm outbreak on Cape Breton Island, Nova Scotia.* Can. J. For. Res. 19: 1077-1086.

Ostaff, D. P. and D. A. MacLean. 1995. *Patterns of balsam fir foliar production and growth in relation to defoliation by spruce budworm.* Can. J. For. Res. 25: 1128-1136

Piene, H. and D. A. MacLean. 1984. *An evaluation of growth response of young, spaced balsam fir to 3 years of spruce budworm spraying with Bacillus thuringiensis.* Can. J. For. Res. 14: 404-411.

Raske, A. G. and W. J. Sutton. 1986. *Delayed decline and mortality of black spruce caused by spruce budworm and secondary organisms.* Environ. Can., Can. For. Serv., Nfld For. Res. Centre, Inf. Rep. N-X-236, St. John's, Nfld. 32 p.

Solomon, D. S. and T. B. Brann. 1992. *Ten-year impact of spruce budworm on spruce-fir forests of Maine.* USDA For. Serv., Gen. Tech. Rep. NE-165, 44 p.

Solomon, D. S., L. Zhang, T. B. Brann and D. S. Larrick. 2003. *Mortality patterns following spruce budworm infestation in unprotect spruce-fir forests in Maine.* North. J. Appl. For. 20(4): 148-153.

Sterner, T. E., D. G. Embree and G. D. Van Raalte. 1977. *Condition of balsam fir on Cape Breton Island following four years of uncontrolled spruce budworm infestation.* Can. For. Serv., Maritimes For. Res. Centre, Fredericton, N.B., Inf. Rep. M-X-80. 16 p.

Varty, I. W. and M. E. Godin. 1983. *Identification of some of the factors controlling aerial spray efficacy.* Information report M-X-142, Canadian Forestry Service, Department of the environment, Fredericton, New Brunswick. 29 p.

Testing new approaches for detecting and locating early increasing populations of the Spruce Budworm for implementing an Early Intervention Strategy

Christian Hébert, Jean-Michel Béland and Sébastien Bélanger

Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du P.E.P.S., P.O. Box 10380, Succ. Sainte-Foy, Quebec, QC, Canada, G1V 4C7

Abstract

We report that it is rapid and easy to install traps in the upper canopy of balsam fir with the BigShot technique and we confirm that traps placed in the upper canopy were much more efficient than those placed at 2-3 m high. Placing traps in the upper canopy of trees should be considered, particularly if large white spruces are present, at least in a subset of surveyed stands in non-outbreak areas, in an early intervention strategy as well as in moth dispersal studies. We also showed that combining pheromone to a portable light trap did not increased spruce budworm moth catches, contrary to other moths. As it increases sorting time, there is no advantage to combine these attractants for monitoring the spruce budworm. Pheromone trap catches in trace or low population density were similar, which is surprising as trace populations were located in a non-outbreak area, the Laurentian Wildlife Reserve, while the low populations were located in the Lower St-Lawrence region, at the south-western edge of the ongoing outbreak. However, light trap catches were better link with population density of these two regions. Light traps and pheromone traps may provide estimates at different spatial scales and they could thus provide complementary information on spruce budworm populations. The interpretation of these results should be clarified when upcoming L_2 population estimates will be available.

Résumé

Nous rapportons qu'il est facile et rapide d'installer des pièges dans la partie supérieure de la cime de sapins baumier à l'aide de la technique du BigShot, et nous confirmons que les pièges placés dans la cime supérieure étaient plus efficaces que ceux placés à 2-3 m de hauteur. L'installation de pièges dans la cime supérieure des arbres devrait être considéré, particulièrement si de grosses épinettes blanches sont présentes, au moins dans un sous-ensemble d'un réseau de surveillance en région non-épidémique. Cela serait particulièrement utile dans le contexte d'une stratégie d'intervention hâtive ou d'étude de la dispersion des papillons. Contrairement à d'autres espèces, il n'y a aucun avantage à ajouter une phéromone à un piège lumineux pour la tordeuse des bourgeons de l'épinette. Les pièges à phéromone sont des outils de détection utiles dans les populations basses ou à l'état de trace. Or, nous avons constaté avec surprise des niveaux de captures similaires entre les populations basses du Bas St-Laurent et les populations traces de la réserve des Laurentides. Cependant, les captures dans les pièges lumineux reflétaient bien les niveaux de population respectifs des deux régions. L'interprétation de ces résultats devraient être clarifiée lorsque les estimés de populations L_2 seront disponibles.

Introduction

A fundamental component of a successful Early Intervention Strategy (EIS) against the Spruce Budworm (SBW) is the ability to locate rapidly early rising populations or population hotspots, those released from endemic level or establishing after moth immigration. However, this is notoriously difficult to achieve in endemic populations. Pheromone traps are useful monitoring tools known to provide accurate predictions of SBW L_2 density (Rhainds et al. 2016). Pheromone traps are more efficient at capturing SBW moths when placed in tree canopy than when placed at 2 m high (Jobin et al. 1993) and predictions are slightly more accurate when placed in tree canopy (Rhainds et al. 2016). However, for practical reasons, pheromone traps are usually placed at 2 m high where their predictive potential varies more widely from year to year. Relationships between male moths and L_2 density can be weak in certain years ($R^2 < 0.08$) as seen in 2 out of 9 years of the Rhainds et al. (2016) study, even if they are still statistically significant. Pheromone traps capture only males while the next generation depend on females. Captures with these traps may not always be closely link with the extent of female moth dispersal/migration. Moreover, their range of action is unknown and thus the spatial scale at which pheromone trap data should be interpreted remains difficult to appraise.

New approaches using tools providing stand level estimates for detecting developing SBW hotspots may help improving detection of early rising SBW populations. Recent experiments using mark-release recaptures showed that the range of moth attraction of low-powered light traps remains most often within 10 m (Truxa and Fiedler 2012). Light traps can thus provide population estimates at the stand level. Moreover, light traps are also useful for studying moth dispersal and migration. In France, light traps were instrumental for highlighting massive migrations of the green oak tortrix, *Tortrix viridana* (Du Merle and Pinguet 1982). Light traps also capture moths of both sexes and thus provide a sex ratio that is useful to unravel the role of dispersal-migration on SBW population dynamics (Rhainds and Kettela 2014) or help in interpreting male catches in pheromone traps (Delisle et al. 1998). Rhainds and Kettela (2014) showed that light traps placed in the tree canopy provide accurate estimates of SBW egg densities and thus of the upcoming generation. We already showed that SBW moths, particularly females, were much more active in the upper canopy than in the lower canopy (Hébert et al. 2016).

Light trapping used in Maine has also showed that SBW outbreaks were detectable 4 to 7 years before defoliation occurs (Simmons and Elliott 1985). However, light traps used in this project were large, heavy and powered by large batteries that need to be recharge regularly (usually every 2 nights). Such traps are particularly useful for working in a low number of sampling sites with easy access. For example, studies of Du Merle and Pinguet (1982) and Simmons and Elliott (1985) were done respectively with 4 and 10 traps. To widen the utilisation of light traps, we need lighter and more autonomous models that could be used in numerous sites and in remote areas. The Luminoc® trap is a portable light trap (Jobin and Coulombe 1992) that respond to these characteristics and that was shown to be efficient to catch moths of various families, including the SBW. In 1994, when the SBW was at very low levels in Quebec, we collected at least one SBW moth in 9 out of 16 balsam fir stands sampled throughout the province. The highest catches (between 9 and 88 moths/stand) were recorded in the southwestern part of the province, where small and scattered patches of SBW defoliation (total of 2912 ha) were noticed. This indicates that the Luminoc® trap provides reliable estimates of SBW populations, even at endemic level. Moreover, a pheromone can be added to the Luminoc® trap (Delisle et al. 1998) and this often increases male moth captures, while allowing also catching females. The Luminoc® has also been combined with artificial oviposition substrates to sample eggs of the Hemlock Looper (Hébert et al. 2003) and it can be used as pitlight traps to catch forest litter insects (Hébert et al. 2000). Thus, the Luminoc® is a highly polyvalent tool for entomologists but the trap is no longer available commercially and thus, cannot fulfill our needs.

We thus developed a new version of the Luminoc® trap, the Luminoc 2.0, which uses LEDs as attractant rather than a 1.7 W fluorescent light as in the original Luminoc®. LEDs have a much longer life and consume far less energy than fluorescent tubes. Recently, LEDs have shown some potential when used in large light traps, even if they caught significantly less moths than mercury vapor traps (White et al. 2016). However, authors claim that the LED trap may be a viable alternative to the standard mercury vapor trap because of its lower cost. We confirm that this green technology lower the cost of the Luminoc® trap, which was a problem with the original version of the trap. However, a key issue with the SBW is to find ways to hang a trap on small balsam fir branches in the upper tree canopy, where SBW females are much more active (Hébert el al. 2016). One solution might be to lower trap weight as much as possible and this could be achieve by reducing the weight of batteries used to power the trap. Another important challenge is to find a way to rapidly install a device to climb the trap in the upper canopy.

Therefore, in 2017, our objectives were to 1) determine if the combination of a pheromone to a portable light trap could increase male moth catches of the SBW, 2) to test a device for climbing traps in the upper canopy of trees and 3) to compare the predictive potential of SBW L_2 populations for various moth abundances obtained using pheromone, light or light-pheromone traps, placed at 2 or 10 m high.

Materials and methods

Trap descriptions

The Multi-Pher® trap (model 1; Jobin and Coulombe 1988) was used as pheromone trap and two new models of the Luminoc traps were used in 2017. The Luminoc 2.0 trap was built while keeping in mind to lower the cost as much as possible, while maintaining its efficacy. As we targeted the SBW (a microlepidoptera), we used the container, funnel and plate of the Multi-Pher® trap without any modification and without baffles (see photos in Hébert et al. 2016). The upper container houses 8 C-alkaline batteries and an electronic circuit for controlling 4 LEDs. The upper container is a simple plastic recipient of 12-cm diameter and 11-cm high; the overall trap height is 31 cm, and thus the Luminoc 2.0 trap is 7 cm shorter than the original version (Luminoc®). The Luminoc 2.0 trap was described in our 2016 report (Hébert et al. 2016). A lighter version of the trap (Luminoc 3.0) was tested in 2016 and involved using a single LED powered by a 2 AA batteries.

2017 experiments

In 2017, our efforts aimed to 1) determine if combining SBW pheromone to the Luminoc traps could increase moth captures, 2) test a device for climbing traps in the upper canopy of trees and

3) improve forecasting of SBW L_2 populations. Thus, we compared Multi-Pher® pheromone traps (model 1) with two models of portable Luminoc traps (2.0 and 3.0) or a combination of the pheromone attractant with the light traps. The experiment was carried out in 36 mature balsam fir stands, 21 in the Lower St-Lawrence region and 15 in the Laurentian Wildlife Reserve (Figure 1). Stands of the Lower St-Lawrence region were located in areas where SBW populations were high, medium or low, according to the fall 2016 SBW L_2 density maps (Ministère des Forêts, de la Faune et des Parcs 2017). As a result, 6, 8 and 7 stands were located respectively in high, medium and low density areas. Stands located in the Laurentian Wildlife Reserve were considered at the trace level as no L_2 was found in the area (Figure 2). Two tests were carried out in parallel with the louder Luminoc 2.0 trap being hanged on lower balsam fir branches (2-3 m high) and the lighter Luminoc 3.0 trap being placed in the upper tree canopy. We used the SherrillTree BigShot system (Hughes et al. 2014) to hang a rope over a branch and climb a trap in the upper canopy of balsam fir trees. Thus, in each stand, we placed one Multi-Pher pheromone trap, one Luminoc 3.0 and one Luminoc 3.0 + SBW pheromone spaced by 50 m along a transect line and at least at 50 m from any road. On another transect 50 m apart, we placed one Multi-Pher pheromone trap, one Luminoc 2.0 trap and one Luminoc 2.0 + SBW pheromone at 2-3 m above ground, hanged on balsam fir branches. A strip of Vapor Tape II was placed in each trap. Samples were collected weekly between 29 June and 23 August to compare moth catches between various trap types. Anova was used to compare moth captures vs trap type at various SBW density, for each transect (Luminoc 2.0 and Luminoc 3.0) separately.

In early October, five balsam fir branches were collected to determine L_2 density. It has not been possible to process L_2 extraction in fall and thus, branches were placed in cardboard tubes to force L_2 emergence. These results will be available later during winter 2018. Linear regression will be use to assess the strength of the relationships between moth abundance in the various traps and L_2 abundance.

Results and discussion

Our results clearly showed that pheromone traps were far more efficient than portable light traps for catching SBW male moths, both in the upper canopy and at 2-3 m high (Figure 3). Moreover, adding SBW pheromone to the light traps did not increase male moth catches, contrary to other moth species (Jobin and Coulombe 1992). Therefore, there is no advantage to combine the two attractants as the sorting time is increased because the light source attract other moths, that are nearly absent in pheromone traps. Also, very few females were caught in light traps, some being even found in pheromone traps (Tables 1 and 2).

Our results also confirm previous results (Jobin and Bernier-Cardou 1988) that pheromone traps are more efficient when placed in the upper canopy than at 2-3 m high (Figure 4). For early detection, it would thus be advantageous to place traps in the upper tree canopy. The traps were installed rapidly and easily in the upper canopy of trees with the BigShot technique, even on balsam fir, which have small branches. Yet, if white spruce trees could be present in a stand, it would allow climbing traps much higher and it would probably allow using louder traps such as the Luminoc 2.0. Placing traps in the upper canopy, at least for a certain number of sites, would be useful for early detection of increasing populations and for moth dispersal/migration studies.

When used in outbreak areas (medium to high populations), the high efficacy of pheromone traps need counting thousands of moths, which is time-consuming and tedious. As pheromone traps are mainly detection tools, their use should be restricted to non-outbreak areas or at the front edge of an outbreak (low populations) in order to follow population spreading. However, our results of pheromone trap catches in trace or low population density suggest that these stands would have similar spruce budworm density, which is surprising as trace populations were located in a non-outbreak area, the Laurentian Wildlife Reserve, while the low populations were located in the Lower St-Lawrence region, at the south-western edge of the ongoing outbreak (Figure 2). It is interesting to note that light trap catches were better link with population density of the two regions. Light traps and pheromone traps may provide estimates at different spatial scales and they could thus provide complementary information on spruce budworm populations. For instance, even if they were rarely captured, some females were caught in the Luminoc 2.0 in the low populations of the Lower St-Lawrence region, while none was caught in the trace populations of the Laurentian Wildlife Reserve. The interpretation of these results should be clarified when upcoming L_2 population estimates will become available.

Sorting samples collected with the Luminoc 3.0 traps was much faster than for the Luminoc 2.0 traps because fewer non-target moths were caught in the upper canopy than with the Luminoc 2.0 traps at 2-3 m high. Moreover, the Luminoc 3.0 trap caught fewer SBW moths than the Luminoc 2.0. However, we cannot conclude on their respective efficacy as they used 1 vs 4 LEDs and they were placed at different heights. Finally, even if they catch lower numbers of moths, the final appraisal of the usefulness of portable light traps will be possible only when upcoming L_2 population estimates will become available.

Acknowledgments

We sincerely thank SERG-international members (SOPFIM, DNR-Newfoundland and Labrador and DNR-Nova Scotia) for their financial support. We also thank Anne Cotton-Gagnon and Jessica Girona, biologists who participated to field work and sample sorting/counting. Finally, thanks to Jon Sweeney, Robert Lavallée and Philippe Labrie, from CFS, for sharing ideas and for their help in using the BigShot technique in balsam fir.

Budget

	2017-18
Technician	15
Field work	15
Supplies	5
Total	**35**
Funding sources	
CFS	12
SERG-I partners	16
iFor	7

References

Delisle, J., West, R.J. and W.W. Bowers. 1998. The relative performance of pheromone and light traps in monitoring the seasonal activity of both sexes of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria*. Entomol. Exp. Appl. 89 : 87-98.

Du Merle, P. and A. Pinguet. 1982. Mise en évidence par piégeage lumineux de migrations d'adultes chez *Tortrix viridana* L. (Lep, Tortricidae. Agronomie 2 : 81-90.

Hébert, C., Jobin, L., Fréchette, M., Pelletier, G., Coulombe, C., Germain, C. and M. Auger. 2000. An efficient pit-light trap to study beetle diversity. J. Insect Conserv. 4: 189-200.

Hébert, C., Jobin, L., Auger, M. and A. Dupont. 2003. Oviposition traps to survey eggs of *Lambdina fiscellaria* (Lepidoptera: Geometridae). J. Econ. Entomol. 96: 768-776.

Hébert, C., Dubuc, Y., Jeffrey, O. and G. Pelletier. 2016. Testing new approaches for detecting and locating early increasing populations of the Spruce Budworm for implementing an Early Intervention Strategy. SERG International Workshop Proceedings, pp 233-250. Saskatoon, Saskatchewan.

Hughes, C.H., Johns, R.C. and J.D. Sweeney. 2014. A technical guide to installing beetle traps in the upper crown of trees. J. Acad. Entomol. Soc. 10: 12-18.

Jobin, L. and Coulombe, C. 1988. The Multi-Pher® insect trap. Forestry Canada, Quebec Region, Inf. Leafl. LFC-24.

Jobin, L. and Coulombe, C. 1992. The Luminoc® insect trap. Forestry Canada, Quebec Region, Inf. Leafl. LFC-26.

Jobin, L., Coulombe, C. and M. Bernier-Cardou. 1993. Use of Multi-Pher® trap to monitor spruce budworm populations. Forestry Canada, Quebec Region, Sainte-Foy, Quebec. Information Report LAU-X-103E. 24 p.

Ministère des Forêts, de la Faune et des Parcs. 2017. Insectes, maladies et feux dans les forêts du Québec en 2016, 52 pages.

Rhainds, M. and E.G. Kettela. 2014. Monitoring spruce budworm with light traps: the effect of trap position. Psyche 2014: 1-5.

Rhainds, M., Therrien, P. and L. Morneau. 2016. Pheromone-based monitoring of spruce budworm (Lepidoptera: Tortricidae) larvae in relation to trap position. Journal of Economic Entomology 109: 717-723.

Simmons, G.A. and N.C. Elliott. 1985. Use of moths caught in light traps for predicting outbreaks of the spruce budworm (Lepidoptera: Tortricidae) in Maine. J. Econ. Entomol. 78: 362-365.

Truxa, C. and K. Fiedler. 2012. Attraction to light-from how far do moths (Lepidoptera) return to weak artificial sources of light? European Journal of Entomology 109: 77-84.

White, P.J.T., Glover, K., Stewart, J. and A. Rice. 2016. The technical and performance characteristics of a low-cost, simply constructed, black light moth trap. J. Insect Sci. 16: 1-9.

Table 1: SBW moth catches in pheromone baited (P), light baited (L) and pheromone + light baited (P + L) traps in the upper canopy of balsam fir trees.

SBW L_2 density	**Males**			**Females**		
	Pheromone	**Luminoc[1]**	**Luminoc[1] + Pheromone**	**Pheromone**	**Luminoc[1]**	**Luminoc[1] + Pheromone**
High	4981.7 ± 640.6	267.0 ± 60.3	4092.7 ± 1035.7	1.7 ± 0.7	4.1 ± 1.0	4.7 ± 1.3
Moderate	1055.8 ± 163.7	92.2 ± 42.0	953.6 ± 228.0	0.3 ± 0.2	0.4 ± 0.3	0.4 ± 0.3
Low	131.0 ± 54.3	3.3 ± 1.7	114.0 ± 21.3	0.1 ± 0.1	0	0.1 ± 0.1
Trace	174.6 ± 36.7	0.4 ± 0.1	162.5 ± 38.3	0	0	0

[1] Luminoc 3.0

Table 2: SBW moth catches in pheromone baited (P), light baited (L) and pheromone + light baited (P + L) traps hanged on branches at 2-3 m high.

SBW L_2 density	**Males**			**Females**		
	Pheromone	**Luminoc[1]**	**Luminoc[1] + Pheromone**	**Pheromone**	**Luminoc[1]**	**Luminoc[1] + Pheromone**
High	1561.2 ± 256.6	172.5 ± 68.2	1155.3 ± 199.2	0.8 ± 0.8	5.8 ± 2.1	0.8 ± 0.4
Moderate	330.1 ± 63.5	73.8 ± 15.7	300.0 ± 33.0	0.1 ± 0.1	0.4 ± 0.3	0.9 ± 0.5
Low	24.9 ± 9.8	9.3 ± 2.7	37.7 ± 18.3	0	0.7 ± 0.3	0
Trace	55.2 ± 11.9	0.5 ± 0.2	69.5 ± 24.5	0	0	0

[1] Luminoc 2.0

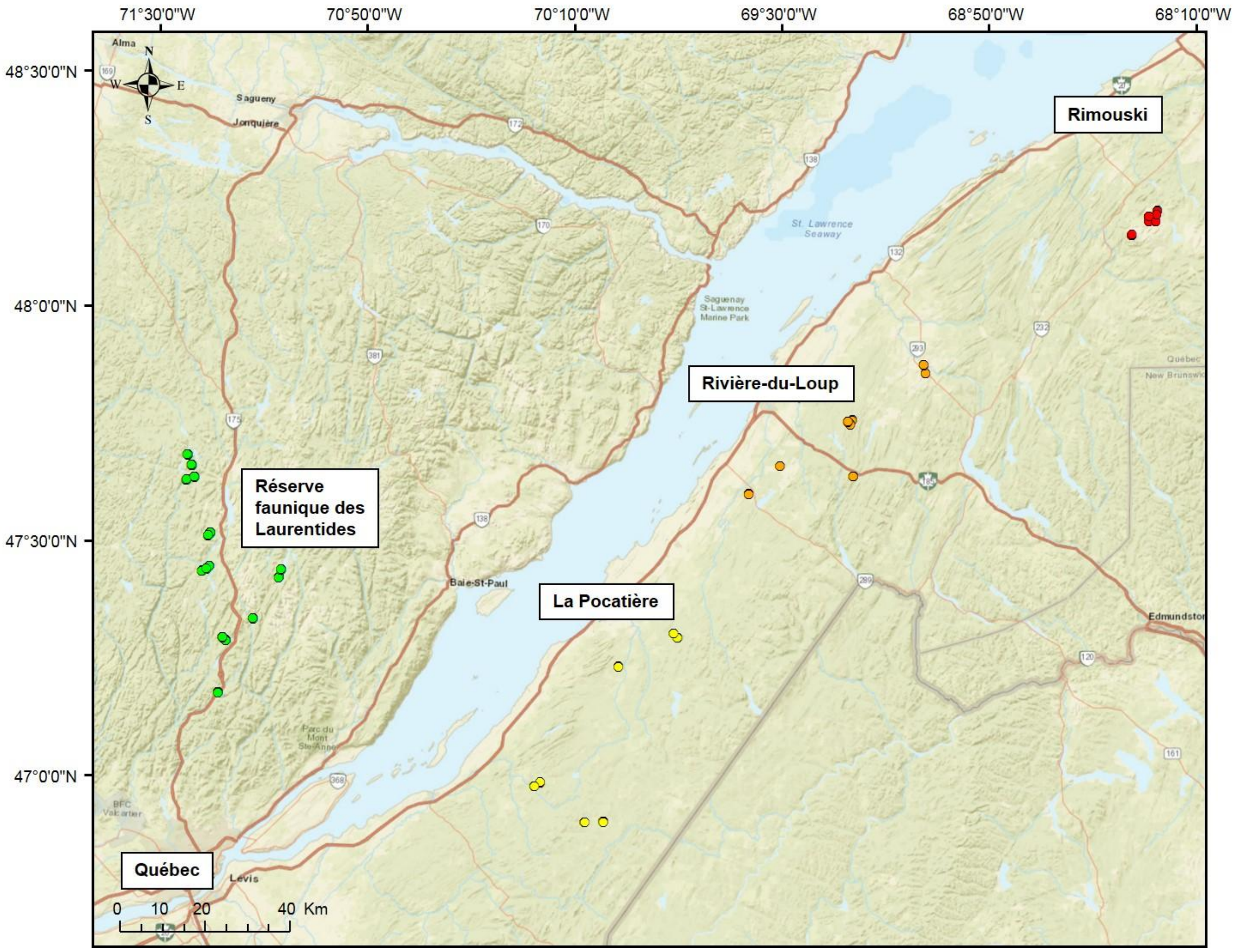


Figure 1: Stand locations in which pheromone, light and light-pheromone traps were installed. The red dots were in an area of high SBW density, the orange dots were in a medium SBW density, while the yellow and green dots were located respectively in areas of low and trace SBW populations.

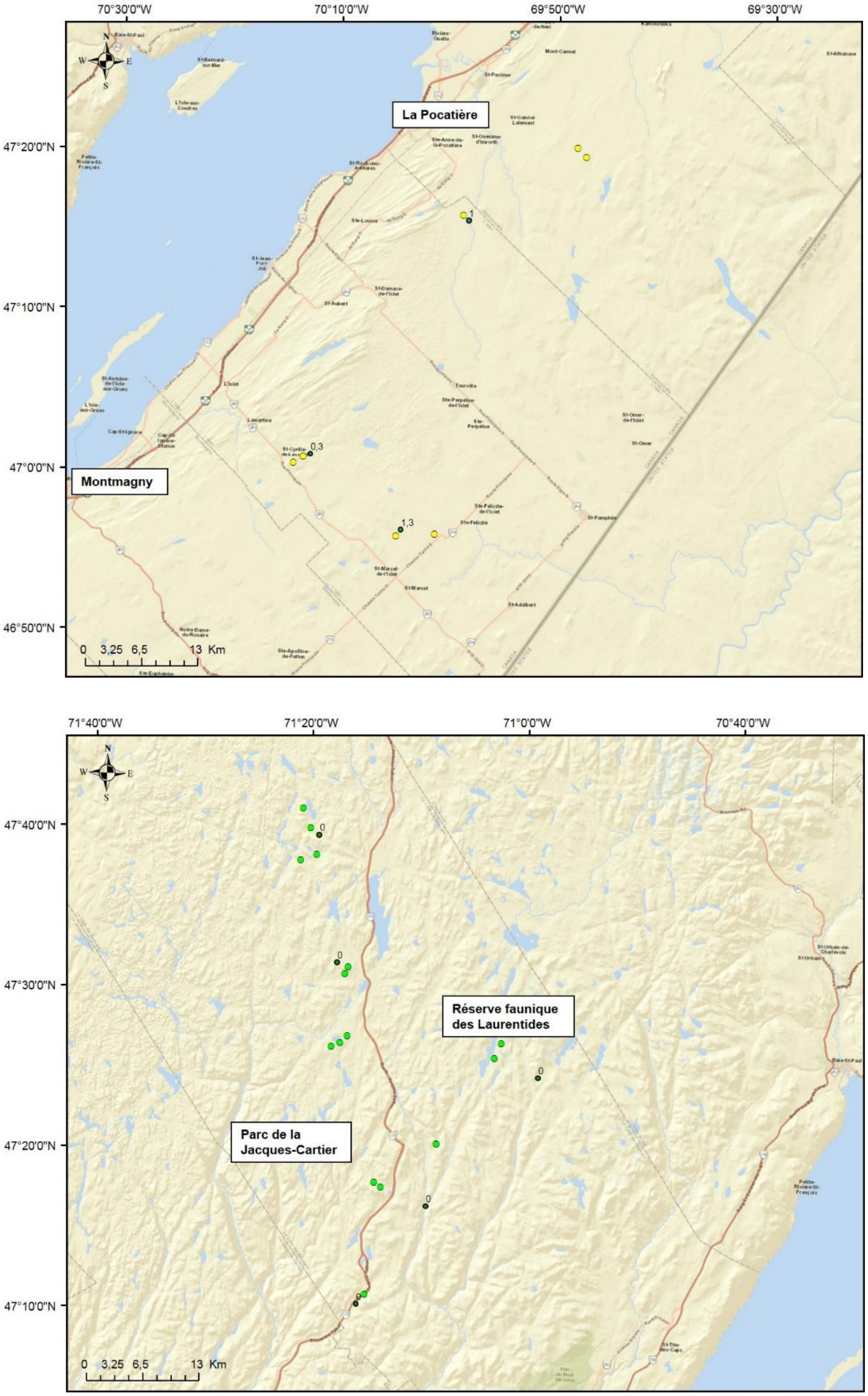


Figure 2: Stand locations and 2016 fall L_2 density showing the Lower St-Lawrence low SBW populations area (upper map) and the Laurentian Wildlife Reserve trace population area (lower map).

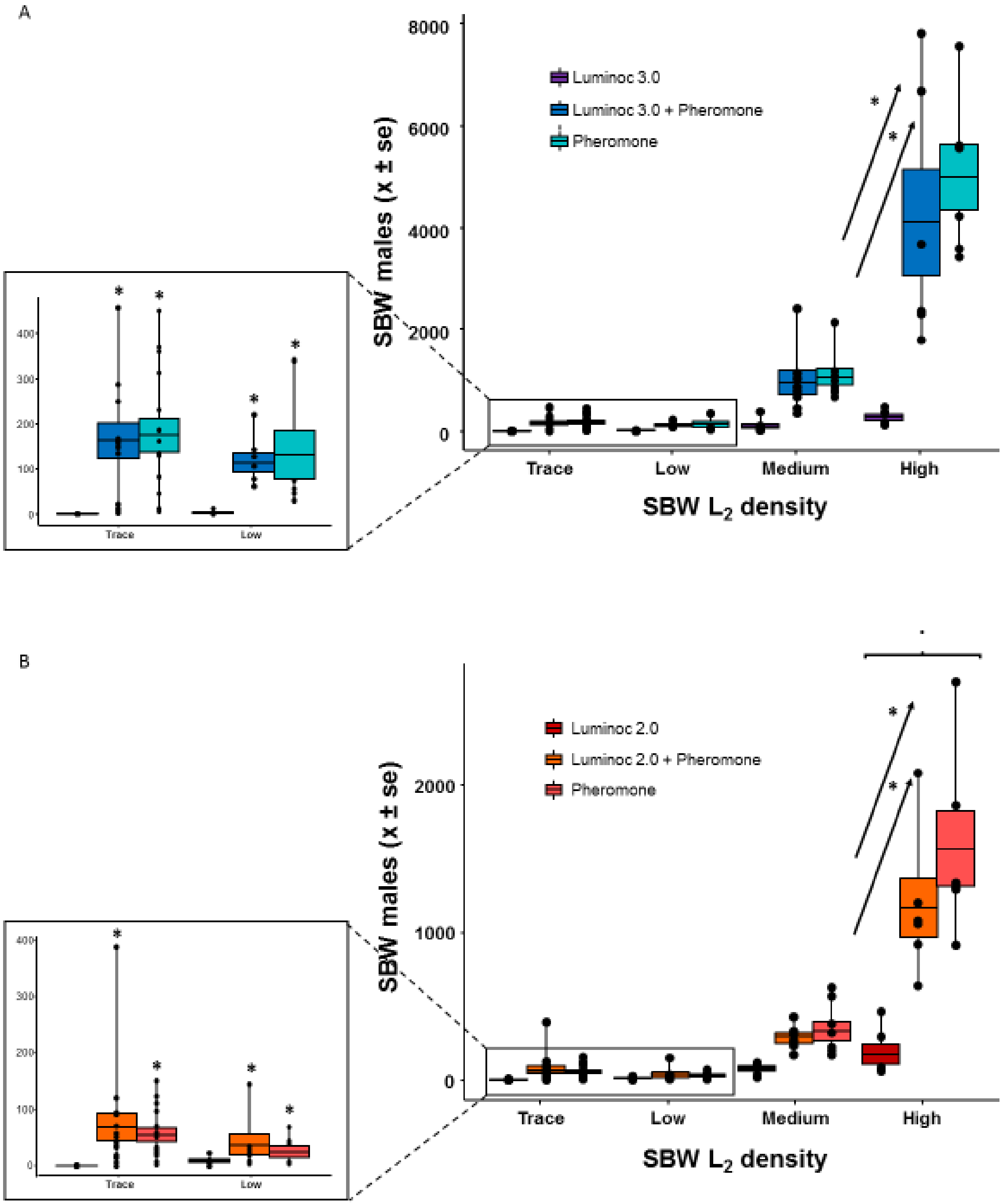


Figure 3: Spruce budworm male moths caught (A) in Luminoc 3.0 traps, pheromone traps and Luminoc 3.0 + pheromone traps placed in the upper canopy according to previous year SBW L_2 density, and (B) in Luminoc 2.0 traps, pheromone traps and Luminoc 2.0 + pheromone traps placed at 2-3 m high according to previous year SBW L_2 density.

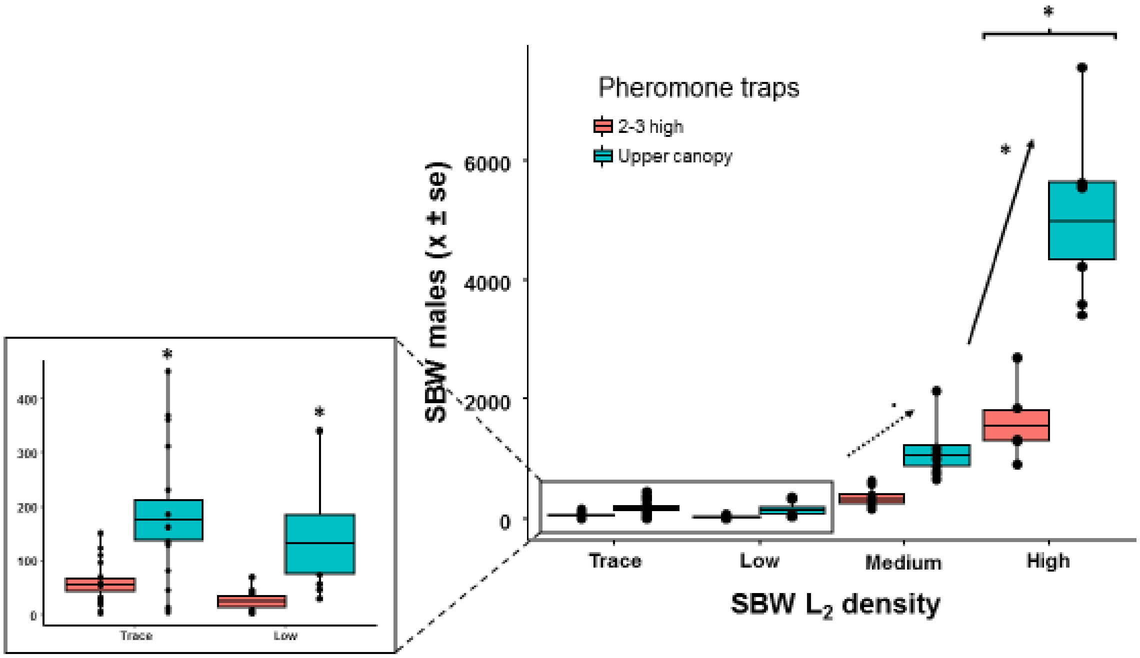


Figure 4: Spruce budworm male moths caught pheromone traps placed in the upper canopy or at 2-3 m high, according to previous year SBW L_2 density.

Improving monitoring tools to detect mountain pine beetle at low and high densities in novel habitats

Nadir Erbilgin[1], *Jennifer Klutsch*[1]

[1]Department of Renewable Resources, University of Alberta, Edmonton. Alberta, Canada

Summary: Mountain pine beetle (MPB) is an economically important bark beetle species that is currently expanding east into the boreal forest in Alberta. Pheromones are important in bark beetle management and in combination with tree volatiles can be used to attract beetles into traps. However, MPB attraction to traps baited with lures shows large variations depending on in part beetle densities in the field. Currently, a single type of lure (two beetle pheromones, *trans*-verbenol, *exo*-brevicomin, and one host tree compound, terpinolene) is used to attract MPB to traps, regardless of beetle densities. In 2014, we tested various combinations of beetle pheromones and host tree volatiles in the field in Alberta and found that a four-component lure (*trans*-verbenol, *exo*-brevicomin, terpinolene, and myrcene) caught the most number of beetles in low beetle densities. However, MPB exhibits dose-dependent response to the female aggregation pheromone in areas with low population numbers. In areas with high MPB population levels, the response is directly proportional to release rates of pheromones and host tree chemicals, suggesting that pheromones and host volatile chemicals have different functions in MPB attraction during host colonization, depending on its population levels. Having effective lures can be highly useful for predicting MPB population levels as tree mortality is driven by the number of beetles. Thus, we conducted a field experiment in 2017 and tested various release rates of pheromones and tree compounds to monitor MPB activities in both low and high density beetle populations in Alberta. We found differential attraction of beetles depending on their population densities. At the high density, standard release rates of MPB pheromones (*trans*-verbenol, *exo*-brevicomin) and increasing the release rates of host chemicals (terpinolene, myrcene) caught more beetles than standard release rates for both. In contrast, at the low density, all increases in release rates of pheromones-host tree chemical combinations caught more beetles than standard release rates. In particular, increasing the release rates of pheromones, both pheromones and host chemicals, and host chemicals caught more beetles. We are currently sexing the beetles, and identifying competitors and natural enemies. We will conduct a statistical analysis to determine the differences among treatments at each density.

Résumé: Le dendroctone du pin ponderosa (DPP) est un scolyte ayant une incidence économique importante qui connaît actuellement une expansion vers l’est dans la forêt boréale de l’Alberta. Les phéromones jouent un grand rôle dans la gestion des scolytes et peuvent être utilisées, en combinaison avec des volatiles d’arbres, pour attirer les DPP dans des pièges. Cependant, on constate de grandes variations dans l’attraction exercée sur le DPP par les pièges appâtés, variations qui dépendent en partie de la densité des scolytes sur le terrain. À l’heure actuelle, un seul type d’appât (deux phéromones de scolytes, *trans*-verbenol, *exo*-brevicomine, et un composé de l’arbre hôte, le terpinolène) est utilisé pour attirer le DPP dans les pièges, peu importe la densité des scolytes. En 2014, nous avions mis à l’essai sur le terrain en Alberta différentes combinaisons de phéromones d’agrégation et de substances volatiles des arbres hôtes et observé que c’était un appât contenant quatre composants (*trans*-verbenol, *exo*-brevicomine, terpinolène et myrcène) qui avait capturé le plus de scolytes là où leur densité était faible. Par contre, le DPP manifeste une réaction qui dépend de la dose à des phéromones d’agrégation femelles dans les endroits où la population est peu nombreuse. Dans les régions ayant des concentrations élevées de populations de DPP, la réaction est directement proportionnelle aux dates de relâchement des phéromones et des substances chimiques de l’arbre hôte, ce qui laisse entendre que les phéromones et les substances chimiques volatiles de l’hôte ont des fonctions différentes dans l’attraction exercée sur le DPP durant la colonisation de l’hôte, dépendant le niveau de population. Le fait d’avoir des appâts efficaces peut s’avérer extrêmement utile pour prédire les niveaux de populations du DPP, car la mortalité des arbres est reliée au nombre de scolytes. Par conséquent, en 2017, nous avons effectué une expérience sur le terrain et mis à l’essai divers taux de libération de phéromones et de composés des arbres pour surveiller les activités du DPP dans les populations de faible et de forte densité en Alberta. Nous avons constaté une attraction différentielle des scolytes qui dépendait de leur densité de population. À de fortes densités, les taux de libération habituels des phéromones du DPP (*trans*-verbenol, *exo*-brevicomine) et des taux de libération croissants de substances chimiques de l’hôte (terpinolène, myrcène) ont donné lieu, pour les deux, à plus de captures de scolytes qu’avec les taux de libération habituels. Au contraire, pour les populations de faible densité, toutes les augmentations des taux de libération des combinaisons de phéromones et de substances chimiques de l’arbre hôte ont permis d’attraper plus de scolytes que les taux standards. En particulier, l’augmentation des taux de libération de phéromones, aussi bien les phéromones que les substances chimiques de l’hôte, et des substances chimiques de l’hôte a permis d’attraper plus de scolytes dans les pièges. Nous procédons actuellement à la détermination du sexe des scolytes et à l’identification des compétiteurs et des ennemis naturels. Nous ferons une analyse statistique afin de déterminer les différences parmi les traitements pour chaque densité.

Introduction

Recent mountain pine beetle (MPB) *Dendroctonus ponderosae* Hopkins (Col: Curculionidae, Scolytinae) outbreaks have killed millions of hectares of mature pine trees, primarily lodgepole pine (*Pinus contorta* Douglas) in western North America (Safranyik et al. 2010). In western Canada, MPB outbreaks began in lodgepole pine forests in central British Columbia. Starting in 2006, the outbreak expanded beyond the Rocky Mountains and the eastern edge of the lodgepole pine range into north-central Alberta (Erbilgin et al. 2014).

The life history of MPB consists of dispersal, colonization, and development phases. Adult beetles emerge from dead brood trees, undertake a dispersal flight, and locate new hosts. The colonization phase, during which the beetles bore into the bark of selected trees, is characterized by rapid infestation triggered by aggregation pheromones. Due to the importance of pheromones in MPB biology, pheromones of MPB are known (Wood 1982; Seybold et al. 2006; Borden et al. 2008). However, we currently do not know whether lures currently used to monitor MPB populations in its historical range are effective for catching MPB in novel habitats, suggesting an urgent need for the development and efficacy of such lures.

In MPB, mass attack involves close interactions between host volatile chemicals mainly monoterpenes and beetle pheromones (Borden et al. 2008). When a female beetle initiates an attack on a tree, it releases *trans*-verbenol that is preferentially attractive to males, but females are also attracted. Males responding to the aggregation pheromone mate with females and release *exo*-brevicomin that attracts mainly females (Pureswaran et al. 2000). The mixture of both male and female aggregation pheromones serves as a powerful aggregation tool, which usually results in mass colonization on host trees. At this stage, volatile chemicals with beetle aggregation pheromones released from the trees under attack improve beetle attraction (Borden et al. 2008). This aggregation process is required for depletion of host tree defenses, successful host colonization, and reproduction (Safranyik et al. 2010). At the later stages of host colonization, female and male beetles reduce trans-verbenol and exo-brevicomin production respectively and instead they produce frontalin (male only) and verbenone as anti-aggregation pheromones to mediate the number of MPB arriving to the host

(Pureswaran et al. 2000; Erbilgin et al. 2014). Frontalin can be attractant or repellent depending on its concentrations (Borden et al. 1987).

Our original project supported by both fRI-Research and SERG-I consisted of two phases. Phase 1 focused on the improvement of commercially available MPB lures. We completed this phase in 2014 and showed that traps baited with a four-component lure (*trans*-verbenol + *exo*-brevicomin + terpinolene + myrcene) caught roughly 2.5 times more beetles than the same combination without myrcene (Klutsch et al. 2017). Based on these results, we conducted a large scale field experiments in 2015 and 2016 and baited trees with the four-component lure to monitor MPB activities in the Phase 2 of the project. We determined the optimum density of bait sites required to detect and monitor low MPB populations on the landscape. We tested three types of trap-tree formations: (1) Triangle (baited trees spaced 50 m apart arranged in a triangle formation); (2) Square (baited trees spaced 50 m apart arranged in a square formation); and (3) Rectangle (baited trees spaced 50 m apart arranged in a 50 m x 100 m grid formation). Each formation was arranged in a linear transect at 1 km, 4 km, 8 km to evaluate the optimum distance between each trap-tree formation. A total of 351 trees were baited over 16,000 km^2 in Alberta. We found that the square formation at 8 km distance seems to be the best trap-tree setup for the following reasons: (1) the number of mass attacked baited trees was relatively higher in the square formation than the other formations, and (2) the square formation at 8 km distance treatment had the least spill-over attacks on neighboring unbaited trees. These results suggest that this formation is highly effective at concentrating beetle attacks in a relatively small area. In 2016, we conducted a similar field experiment only with the square trap-tree formation with 8 and 12 km distances. Our preliminary results indicated that there were no statistical differences between 8 km and 12 km distances in terms of beetle colonization on baited and neighboring unbaited trees, suggesting that trap-trees can be set up 12 km away from one another to monitor MPB activities (Klutsch et al. 2017).

Since our results in the first Phase of our project were really promising, (adding an additional host monoterpene myrcene increased beetle catch), we continued this aspect of our project in 2017. Considering the importance of host chemicals in beetle aggregation on host trees our results are not surprising as it has commonly been reported that emissions of host tree monoterpenes can enhance beetle response to their pheromones (Borden et al. 1983; Byers 1989; Erbilgin et al. 2003, 2007). For example, myrcene in lodgepole pine is a potent synergist of *trans*-verbenol and *exo*-brevicomin (Borden et al. 1983; Conn et al. 1983). Myrcene is one of the most common monoterpene of western pines (Gijzen et al. 1993; Byers 1995).

Earlier work in BC clearly showed that increasing release rates of pheromones and host monoterpenes can improve MPB attraction (Miller and Borden 2000; Miller et al. 2005; Borden et al. 2008). These studies reported that (1) MPB exhibited dose-dependent response to *cis*- and *trans*-verbenol in areas with low population numbers. Increasing release rates of male aggregation pheromone *exo*-brevicomin had no effect on MPB attraction. In an area with a high population level of MPB, the response was directly proportional to release rates, suggesting that beetle attraction to its aggregation pheromone and host chemicals vary depending on its population size. (2) Increasing release rates of myrcene and terpinolene had synergistic effect on MPB attraction to its aggregation pheromone. In the field in 2017, we manipulated the release rates of MPB aggregation pheromones and host monoterpenes. Our aim was to determine which formulation attracts the most number of beetles at low and high densities of MPB populations in novel habitats.

Our primary objective is to develop two types of lures to monitor MPB activities at low and high density populations in novel habitats. Our research will answer the following four critical questions. (1) Do beetle responses to traps baited with a combination of beetle pheromones and host volatile chemicals vary with beetle density? (2) Do increasing release rates of pheromones or host volatiles increase beetle attraction to traps? (3) Do increasing release rates of both pheromones and host tree volatile chemicals increase beetle attraction to traps? (4) Can changes in release rates of lures affect attraction of beetles depending on their density?

Methodology

We tested the following four treatments in the field in 2017: (1) standard release rate of MPB pheromone (*trans*-verbenol, *exo*-brevicomin) + terpinolene + myrcene; (2) 2x release rate of (MPB pheromone) + standard release rate of terpinolene + myrcene; (3) standard release rate of MPB pheromone + 2x release rate of (terpinolene + myrcene); (4) 2x release rate of (MPB pheromone) + 2x release rate of (terpinolene + myrcene). Flight intercept traps baited with one of the four treatments were tested at both low and high density MPB populations in Swan Hills, Alberta. We selected three sites at each beetle density, with two replicates (blocks) per treatment in each site (Total 48 traps). Captured beetles were removed every seven days and traps were rotated at each collection. We did not include any blank control treatment in our experimental design as we previously demonstrated that traps without any lures did not attract any beetle.

Results

We found differential attraction of beetles depending on their population densities. At the high density, increasing the release rates of host chemicals (terpinolene and myrcene) in treatment 3 caught 7% more beetles than the standard release rate of MPB pheromones and host chemicals in treatment 1. Other treatments caught relatively less beetles than the standard release rate of MPB pheromones and host chemicals. In contrast, at the low density, all treatments caught more beetles than the standard release rate of MPB pheromone and tree chemicals in treatment 1. In particular, increasing the release rates of pheromone in treatment 2, both pheromone and host chemicals in treatment 4, and host chemicals in treatment 3 caught 67%, 41% and 22% more beetles, respectively. We are currently sexing the beetles, and identifying competitors and natural enemies. We will conduct a statistical analysis to determine the differences among treatments at each density.

Conclusions

Overall, our results show that (1) at high population densities where there are many potential mates and host trees available for colonization, beetles seek for host chemical cues to locate potential mates and hosts. The high release of host chemicals from trees under attack (high host chemical/pheromone ratios) increases attraction of MPB to its aggregation pheromone and also indicate healthy hosts, which release high amounts of host chemicals. (2) At low population densities where there are a few potential mates and a few host trees available for colonization, beetles seek for conspecific pheromones as much as host volatiles, indicating potential mates and host suitability.

Financials

Funding Sources	**2017**	**2018**
fRI Research (confirmed for 2018). Funding covers salary for one summer student/yr, a field truck, meals and accommodations, field and laboratory supplies.	$17,050	36,850
SERG-I (applied for 2018)**.** Funding for the second summer student ($9,600); one undergraduate lab technician ($2,000); funding to attend annual SERG-I meeting ($1,700).	$13,300	$14,000
Total	**$30,350**	**$50,850**

References

Borden JH, Chong LJ, Pratt KEG, Gray DR. 1983: The application of behaviour modifying chemicals to contain infestations of the mountain pine beetle, *Dendroctonus ponderosae*. Forestry Chronicle 59:235-239

Borden JH, Ryker LC, Chong Jr. LJ, Pierce HD, Johnson BD, et al. 1987. Response of the mountain pine beetle, Dendroctonus ponderosae Hopkins (Coleoptera: Scolytidae), to five semiochemicals in British Columbia lodgepole pine forests. Canadian J Forest Research 17: 118-128.

Borden JH, Pureswaran DS, Lafontaine JP. 2008. Synergistic blends of monoterpenes for aggregation pheromones of the mountain pine beetle. J Economic Entomology 101:1266-1275.

Byers JA. 1989. Chemical ecology of bark beetles. Experentia 45: 271-83

Byers JA. 1995. Host tree chemistry affecting colonization in bark beetles. In: Cardé RT, Bell WJ (eds) Chemical ecology of insects 2. Chapman and Hall, New York, USA, pp154-213.

Conn JE, Borden JH, Scott BE, Friskie LM, Pierce HD, Jr, Oehlschlager AC. 1983. Semiochemicals for the mountain pine beetle, Dendroctonus ponderosae (Coleoptera: Scolytidae) in British Columbia: field trapping studies. Canadian J Forest Research 13: 32011

Erbilgin N, Powell JS, Raffa KF. 2003. Effect of varying monoterpene concentrations on the response of Ips pini (Coleoptera: Scolytidae) to its aggregation pheromone: implications for pest management and ecology of bark beetles. Agricultural & Forest Entomology 5: 269-274.

Erbilgin N, Christiansen E, Krokene P. 2007. A host monoterpene influences Ips typographus responses (Coleoptera: Curculionidae, Scolytinae) to its aggregation pheromone: Implications for host colonization of bark beetles. Agricultural & Forest Entomology. 9: 135-140.

Erbilgin N, Ma C, Whitehouse C, Shan B, Najar A, Evenden M. 2014. Chemical similarity between historical and novel host plants promotes range and host expansion of the mountain pine beetle in a naïve host ecosystem. New Phytologist 201: 940-950.

Gijzen M, Lewinsohn E, Savage TJ, Croteau RB. 1993. Conifer monoterpenes: biochemistry and bark beetle chemical ecology. In: Teranishi R, Buttery RG, Sugisawa H (eds) Bioactive volatile compounds from plants, ACS Symposium Series 525. American Chemical Society, Washington, D.C. USA, pp 8-22

Klutsch JG, Cale JA, Whitehouse C, Kanekar SS, Erbilgin N. Trap trees: an effective method for monitoring mountain pine beetle activities in novel habitats. Canadian J Forest Research. 47: 1-6

Miller DR, Borden JH. 2000. Dose-dependent and species-specific responses of pine bark beetles (Coleoptera: Scolytidae) to monoterpene sin association with pheromone. Canadian Entomologist 132: 183-195

Miller DR, Lindgren SL, Borden JH. 2005. Dose-dependent pheromone responses of mountain pine beetle in stands of lodgepole pine. Environmental Entomology 34: 1019-1027.

Pureswaran DS, Gries R, Borden JH, Pierce HD, Jr. 2000. Dynamics of pheromone production and communication in the mountain pine beetle, Dendroctonus ponderosae Hopkins and the pine engraver, Ips pini (Say) (Coleoptera: Scolytidae). Chemoecology 10: 153-168.

Raffa KF, Berryman AA. 1983. The role of host plant resistance in the colonization behaviour and ecology of bark beetles (Coleoptera: Scolytidae). Ecological Monographs 53: 27-49

Safranyik LL, Carroll AL, Régnière J, Langor DW, et al., 2010. Potential for range expansion of mountain pine beetle into the boreal forest of North America. Canadian Entomologist 142:415-442.

Seybold S, Huber D, Lee J, Graves A, Bohlmann J. 2006. Pine monoterpenes and pine bark beetles: a marriage of convenience for defense and chemical communication. Phytochemistry Reviews 5: 143-178.

Wood DL. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. Annual Review of Entomology 27: 41146

SERG-I#782: Interim Progress Update, January 19, 2018

Mountain Pine Beetle Cold Tolerance and Seasonality: Implications for Population Dynamics and Range Expansion in Canada

Tolérance au froid et saisonnalité du dendroctone du pin ponderosa: implications pour la dynamique des populations et l'expansion de leur aire au Canada

K. Bleiker, G. Smith
Canadian Forest Service, Natural Resources Canada

Abstract

Given the presence of a suitable food source, temperature likely has the largest overall impact on mountain pine beetle's (MPB) distribution and population dynamics. Winter is usually the largest single source of mortality even in benign climates like southern British Columbia. Weather during the growing season affects development and MPB needs to maintain an adaptive seasonality – a synchronous one-year life cycle with the most hardy life stage entering winter – to be successful. This six-year project examines cold tolerance and seasonality (developmental regulation) for mountain pine beetle populations in the historic and expanded ranges. Our ultimate goals are to improve predictions of annual population trends based on annual weather; and provide the necessary empirical data to improve climatic suitability and spread models to identify areas regions at risk and the potential for eastward spread in Canada. For our interim progress report for this past year, we submit two recent publications to demonstrate our work. In addition, we provide a brief report below that highlights some results, which have not yet been published.

Résumé

Pourvu qu'il y ait une source de nourriture propice, c'est probablement la température qui a la plus grande incidence sur la répartition et la dynamique des populations de dendroctone du pin ponderosa. L'hiver est habituellement la principale cause de mortalité, même dans les climats plus doux comme ceux observés dans le sud de la Colombie-Britannique. La météo durant la période de croissance affecte le développement et, pour réussir, le dendroctone du pin ponderosa (DPP) a besoin de maintenir une saisonnalité adaptative – un cycle de vie synchrone d'un an, où l'étape du cycle de vie la plus robuste se situe au début de l'hiver. Ce projet d'une durée de six ans examine la tolérance au froid et la saisonnalité (régulation du développement) des populations de DPP dans leurs aires de répartition historiques et nouvelles. Nous visons ultimement à améliorer les prédictions des tendances annuelles des populations en nous fondant sur la météo annuelle, et à fournir les données empiriques nécessaires pour améliorer les modèles de viabilité climatique et de propagation permettant de déterminer les zones à risque et la possibilité d'une propagation vers l'est du Canada. Pour notre rapport d'étape, nous présentons deux publications récentes qui font état de nos progrès. Nous présentons aussi un court rapport ci-dessous qui met en évidence quelques résultats supplémentaires, qui n'ont pas encore été publiés dans des journaux avec comité de révision.

Bleiker KP, Van Hezewijk BH. 2016. Flight period of mountain pine beetle (Coleoptera: Curculionidae) in its recently expanded range. Environmental Entomology 45: 1561-1567.

Bleiker KP, Van Hezewijk BH. 2016. Période de vol du dendroctone du pin ponderosa (Coleoptera : Curculionidae) dans sa nouvelle aire de répartition. Environmental Entomology 45: 1561-1567

The ability to predict key phenological events, such as the timing of flight periods, is useful for the monitoring and management of insect pests. We used empirical data to describe the flight period of mountain pine beetle, Dendroctonus ponderosae Hopkins, in its recently expanded range east of the Rocky Mountains in Canada and developed a degree-day model based on the number of trapped beetles. Data were collected over four degrees of latitude and six years. The main flight period, when the middle 70% of the total number of beetles were caught, started during the second or third week of July, lasted 26 d, and peaked within 2 wk of starting. The best model accounted for 89% of the variation in the data. Mountain pine beetle's flight tended to start later and be more contracted at higher latitudes. The synchrony of mountain pine beetle's flight period in the expanded range appears to be comparable to the limited reports from the historic range, although it may start earlier. This suggests that conditions in the new range are suitable for a coordinated dispersal flight, which is critical for the beetle's strategy of overwhelming tree defenses by attacking en masse. Forest managers can use the model to support operational decisions, e.g., when to impose hauling restrictions to reduce the risk of spread through the transport of infested material, or the time frame for control programs. Understanding the flight period may also improve our ability to assess the response of mountain pine beetle to novel and changing climates in the future.

Résumé

La capacité de prédire des événements phénologiques clés, comme les périodes de vol, est utile pour surveiller et gérer les insectes ravageurs. Nous avons utilisé des données empiriques pour décrire la période de vol du dendroctone du pin ponderosa *Dendroctonus ponderosae* Hopkins dans sa nouvelle aire de répartition à l'est des Rocheuses au Canada et mis au point un modèle degrés-jours fondé sur le nombre d'insectes piégés. Les données ont été recueillies sur quatre degrés de latitude et six ans. La principale période de vol, celle pendant laquelle la tranche du milieu correspondant à 70 % du nombre total d'insectes a été capturée, a débuté durant la deuxième ou troisième semaine de juillet, duré 26 jours et atteint son sommet dans les deux premières semaines. Le meilleur modèle a représenté 89 % de la variation des données. À des latitudes plus élevées, le DPP avait tendance à commencer à voler plus tard et à avoir une période de vol plus courte. La synchronie de la période de vol du DPP dans la nouvelle aire de répartition semble comparable à celle des rapports limités provenant des aires historiques, bien qu'elle puisse commencer plus tôt. Donc, les conditions dans la nouvelle aire conviendraient à un vol de dispersion coordonné, ce qui est critique pour la stratégie du dendroctone de surcharger les défenses de l'arbre en attaquant en masse. Les aménagistes forestiers peuvent utiliser ce

modèle pour appuyer leurs décisions opérationnelles, p. ex., savoir quand imposer des restrictions afin de réduire le risque de propagation par le transport de matière infestée, ou décider du calendrier des programmes de lutte. Comprendre la période de vol pourrait aussi améliorer notre capacité d'évaluer la réaction du DPP à un nouveau climat ou à un climat changeant dans l'avenir.

Bleiker KP, Smith GD, Humble LM. 2017. Cold tolerance of mountain pine beetle (Coleoptera: Curculionidae) eggs from the historic and expanded ranges. Environmental Entomology 46: 1165-1170.

Bleiker KP, Smith GD, Humble LM. 2017. La tolérance au froid des œufs de dendroctone du pin ponderosa (Coleoptera: Curculionidae) dans les aires de répartition historiques et nouvelles. Environmental Entomology 46: 1165-1170.

Abstract

Winter mortality is expected to be a key factor determining the ability of mountain pine beetle, Dendroctonus ponderosae Hopkins (Coleoptera: Curculionidae), to expand its range in Canada. We determined the mortality rate and supercooling points of eggs from the beetle's historic range in southern British Columbia as well as the recently expanded range in north-central Alberta and tested if eggs require an extended period of chilling to reach their maximum cold tolerance. We found no effect of population source or acclimation time on egg cold tolerance. Although 50% of eggs can survive brief exposure to20.5-C (LT50), storage at 0.3-C and 7.5-C for 59 d resulted in 50% and 100% mortality, respectively. Our results indicate that eggs suffer significant prefreeze mortality and are not well-adapted to overwintering: eggs are unlikely to survive winter throughout much of the beetle's range. Our results provide information that can be used to help model the climatic suitability of mountain pine beetle, including how changes in seasonality associated with new or changing climates may affect winter survival. In addition to lower lethal temperatures, it is critical that the duration of exposure to sublethal cold temperatures are considered in a comprehensive index of cold tolerance and incorporated into survival and population models.

Résumé

La mortalité durant l'hiver devrait être un facteur clé dans la détermination de la capacité du dendroctone du pin ponderosa *Dendroctonus ponderosae* Hopkins (Coleoptera : Curculionidae), d'étendre son aire de répartition au Canada. Nous avons déterminé le taux de mortalité et les températures limites inférieures des œufs dans l'aire de répartition historique de l'insecte dans le sud de la Colombie-Britannique ainsi que dans la nouvelle aire de répartition dans le centre-nord de l'Alberta, et nous avons vérifié si les œufs exigent une période étendue de refroidissement pour atteindre leur tolérance maximale au froid. Nous n'avons constaté aucun effet attribuable à la source de la population ou à la durée de l’acclimatation sur la tolérance au froid des œufs. Bien que 50 % des œufs puissent survivre à une brève exposition à des températures allant jusqu’à -20,5C (LT50), un entreposage à 0,3C et à 7,5C durant 59 jours a donné lieu à des taux de mortalité de 50 % et de 100 %, respectivement. Nos résultats montrent que les œufs subissent une mortalité importante avant le gel et sont mal adaptés à survivre l’hiver : il est peu probable que les œufs survivent dans la majeure partie de l’aire de répartition de l’insecte. Nos résultats fournissent des renseignements qui peuvent servir à développer un modèle de viabilité climatique du dendroctone du pin ponderosa, y compris voir comment les changements dans la saisonnalité occasionnés par un nouveau climat ou des changements climatiques affecteraient la survie en hiver. Outre des seuils de température létale plus bas, il est essentiel que la durée de l’exposition à des températures froides sublétales soit prise en considération dans un indice global de tolérance au froid et intégrée aux modèles de survie et de population.

Background

MPB is subjected to a different climate in its recently-expanded range east of the Rocky Mountains in north-central Alberta. Temperature is a main factor limiting the beetle’s distribution and influencing its population dynamics. In the short-term, weather affects annual population trends and the likelihood of population increasing or declining. Over the long-term, climate determines the suitability of Canada’s boreal and eastern pine forests and the potential for outbreaks and continued spread east of the Rocky Mountains. Thus, we need to know the impact of temperature on insect survival to determine annual population trends in currently infested areas and to identify habitats that may be at risk to invasion in the future.

In its historic range west of the Rocky Mountains in British Columbia, MPB infestations have been limited by cold winter temperatures (e.g., -40°C isotherm) and cool summers where degree days are inadequate for the life cycle to be completed in one year (<833 degree days above 5.6°C, Safranyik and Carroll 2006). Even in benign winter climates like southern British Columbia, winter mortality is often the single largest source of mortality. Although we know the “-40°C for 2 weeks will kill them” rule of thumb, there are significant knowledge gaps on the lethal temperature thresholds for different life stages and the effect of cold severity and the duration of exposure. We also know little about the conditions that trigger the gain and loss of cold tolerance (acclimation and de-acclimation), which is necessary to predict early and late season mortality associated with extreme fall/spring weather events (Safranyik and Linton 1998). There is a population model for MPB, but it needs to be parameterized for Canada; for e.g., currently there is no difference in the overwinter survivorship of early versus late instar larvae in the model (Logan and Bentz 1999; Régnière et al. 2012). Our field observations to date indicate that only late instar larvae could survive most winters in central Alberta, Saskatchewan and Manitoba, while a number of life stages can successfully overwinter in southern British Columbia.

Temperatures during the growing season are also important because MPB needs to maintain an adaptive seasonality to be successful – specifically, MPB needs to maintain a synchronous, one-year life cycle with the most cold tolerant life stage entering winter. We tend to think of central Alberta, Saskatchewan, Manitoba and north-western Ontario as being cold, and they are in the winter; however, temperatures during the growing season in these provinces are warm compared to the beetle's traditional range in British Columbia. We have observed phenology to be advanced in the recently-invaded range in central Alberta compared to British Columbia. In some years, we found that MPB received 40% more thermal sums (degree days) in a year than it needed for a one-year life cycle – this could be potentially devastating to the beetle if they develop "too far" and pupate before winter. However, most insects maintained an adaptive seasonality, staying in the fourth instar larval stage and entering the winter in a cold hardy stage. We discovered a previously unknown cold-associated diapause that affects time to pupation for MPB. This control on development has significant implications for MPB success in regions like central Alberta and the southern boreal forest across Saskatchewan and Manitoba and north-western Ontario which have more degree days than needed for a one-year life cycle and are also expected to warm significantly between 1971-2000 and 2010-2039 (Agriculture Canada). In fact, areas of eastern Manitoba and much of north-western Ontario currently receive twice the number of degree days MPB requires for a one-year life cycle (Agriculture Canada). Understanding factor(s) that regulate MPB's development, and will ultimately determine the life stage that overwinters, is crucial to predicting the climatic suitability of a region for MPB.

To estimate annual beetle population trends and regions potentially at risk to invasion in the future in Canada, we need to know how beetle success varies with both winter and growing-season temperatures.

Project objectives and progress update

1) Determine cold temperatures lethal to the different life stages of MPB.

Almost complete: currently running final experiments for pupae and teneral adults, so should be complete by May 2018. All other life stages are complete. Results for eggs were published in 2017.

2) Examine the potential effect of host tree species (pure jack versus lodgepole pine) on the cold tolerance of MPB larvae (objective added in 2015).

A study was conducted in pure jack and lodgepole pine bolts and tree species had no effect on the cold tolerance of larvae reared at 22°C; this was reported in the 2015/2016 progress report. We would like to run this experiment starting in May or June 2018 using cold acclimated insects (vs. 22°C as in the first experiment). It is subject to a provincial partner providing the jack pine (to be discussed with Manitoba).

3 a) Define conditions required for cold acclimation in the fall and if de-acclimation in the spring is reversible (i.e., can larvae re-acquire cold tolerance after losing it).

3 b) Determine if a "chilling period" is required for the acquisition of maximum cold hardiness and the length of time that maximum cold hardiness can be maintained (added in 2015).

We have made significant progress on this objective in the last three years and results have been included in previous year's reports. As a bonus, we were able to compare insects from southern British Columbia to insects from north-central Alberta. Some results are highlighted below.

4) Determine mortality associated with cold events that vary in severity and duration of exposure.

Significant progress has been made on this objective. Results for eggs were published in 2017. The final experiments for pupae and teneral adults are wrapping up this spring. Late instar larvae have been completed: late instar larvae had surprisingly high survival even after being exposed to -21°C for several months. We are currently repeating this experiment with early instar larvae to test if early instar larvae are more susceptible to pre-freeze mortality (like eggs) or if they can survive extended exposure to cold (like late instar larvae).

5) Quantify MPB winter survival in the field and associated under-bark temperature regimes and tree moisture.

Data collection for this objective has been completed and preliminary results have been covered in previous reports.

6) *Examine factors affecting MPB's ability to maintain an adaptive seasonality (e.g., a one-year synchronous life cycle) in new habitats and climates (added in 2015).*

We have discovered a cold-associated diapause in MPB, which controls development and will be critical for MPB success in Canada's southern boreal forest. This is a novel and surprising result, which will require additional research in the future to fully describe and understand the implications of this finding.

As this is an interim progress report, detailed methods and results will not be presented. Below is the general approach used for the results highlighted here. Critical information is given in the detailed Figure headings.

Experimental material. Infested host trees were felled, cut into bolts (short logs ~35 cm long) and transported to the Pacific Forestry Centre in Victoria, British Columbia. The cut ends were sealed with hot paraffin wax to prevent desiccation. The material was then subjected to various temperature treatments using walk-in cold rooms and environmental chambers. Super-cooling points (see below) were used as a measure of cold tolerance. Results presented below are for late instar larvae.

Super-cooling points. The super-cooling point (SCP) is the lowest temperature immediately preceding the exotherm resulting from the latent heat of crystallization. It is the temperature that internal ice forms in the insect and the temperature at which they would die because internal ice formation is lethal to freeze intolerant insects such as the MPB. Thus, the median SCP is the temperature at which 50% mortality would be expected.

To determine the SCP, individual insects were secured with vacuum grease to an AWG30 Type T (copper/constantan) thermocouple attached to a USB TC-08 data logger (Pico Technology, Cambridgeshire, UK). Insects were inserted into individual shell vials and immersed in a Glacier G50 refrigerated bath controlled by a Haake PC200 circulator (Thermo Fisher Scientific, Waltham, MA, USA). The temperature of the bath was decreased at one degree per minute from the starting temperature (see below). The thermocouples attached to the insects recorded the temperature once per second and the lowest temperature recorded before the exotherm was recorded as the SCP.

Just prior to SCP determination, insects were excised from under the bark and placed in Petri dishes. Insects that were at treatment temperatures of 6°C or less at the time of sampling were held on ice for up to 1 h prior to SCP determination and the refrigerated bath was started at 5°C. Insects that were at treatment temperatures of 15°C or 22°C at the time of sampling were held at their respective temperature until their SCPs were determined and the bath was started at 15°C.

Control for mortality. Insects may suffer pre-freeze mortality and die at temperatures above their SCP. Insects held at 0°C or above were moving when they were sampled and thus obviously alive when their SCPs were determined. Insects held at colder temperatures (-7.5°C) for an extended period of time were often dormant when sampled. SCPs were only determined for larvae that looked healthy. Larvae with any gray or black discolouration were readily identified as dead. When insects were dormant at the time of SCP determination, an additional 50 larvae were placed on moistened filter paper in Petri dishes. Larvae were held at 22°C and checked daily until they either moved or became discoloured. Most larvae that were alive became active within 6 d; however, some larvae took up to 12 d to become active. Larvae that displayed normal vigorous movement were classified as alive; a bioassay demonstrated that these larvae were able to feed, pupate and successfully eclose to an adult. Some larvae displayed little movement, only quivering when prodded and moving their mandibles asynchronously; these larvae were classified as moribund as they were unable to feed or develop in the bioassay. They were added to the "dead" category. Data in the following figures are for larvae that were alive at the time of SCP determination.

Result Highlights

- ✓ Insects acclimated to the cold at 5°C, but the most effective temperature tested for cold acclimation was 0°C (Figure 1)
- ✓ 15°C was not cool enough to trigger the cold acclimation process (Figure 1)
- ✓ Larvae survived extended exposure to -7.5°C and their cold tolerance increased, but not to the maximum (Figure 1)
- ✓ Insects from southern BC were not as cold tolerant as insects from northern AB (this result was confirmed in repeat experiments with insects from two different sources in each region – Figures 1 and 2)
- ✓ It took ~60-70 days for larvae to acquire their maximum cold tolerance (this result was confirmed in repeat experiments with insects from two different sources in each region – Figures 1 and 2)
- ✓ Insects started to lose their cold tolerance after ~70 days at 0°C and 5°C, but not at -7.5°C (Figure 1)
- ✓ The rate of loss of cold tolerance increases with temperature (Figure 3)

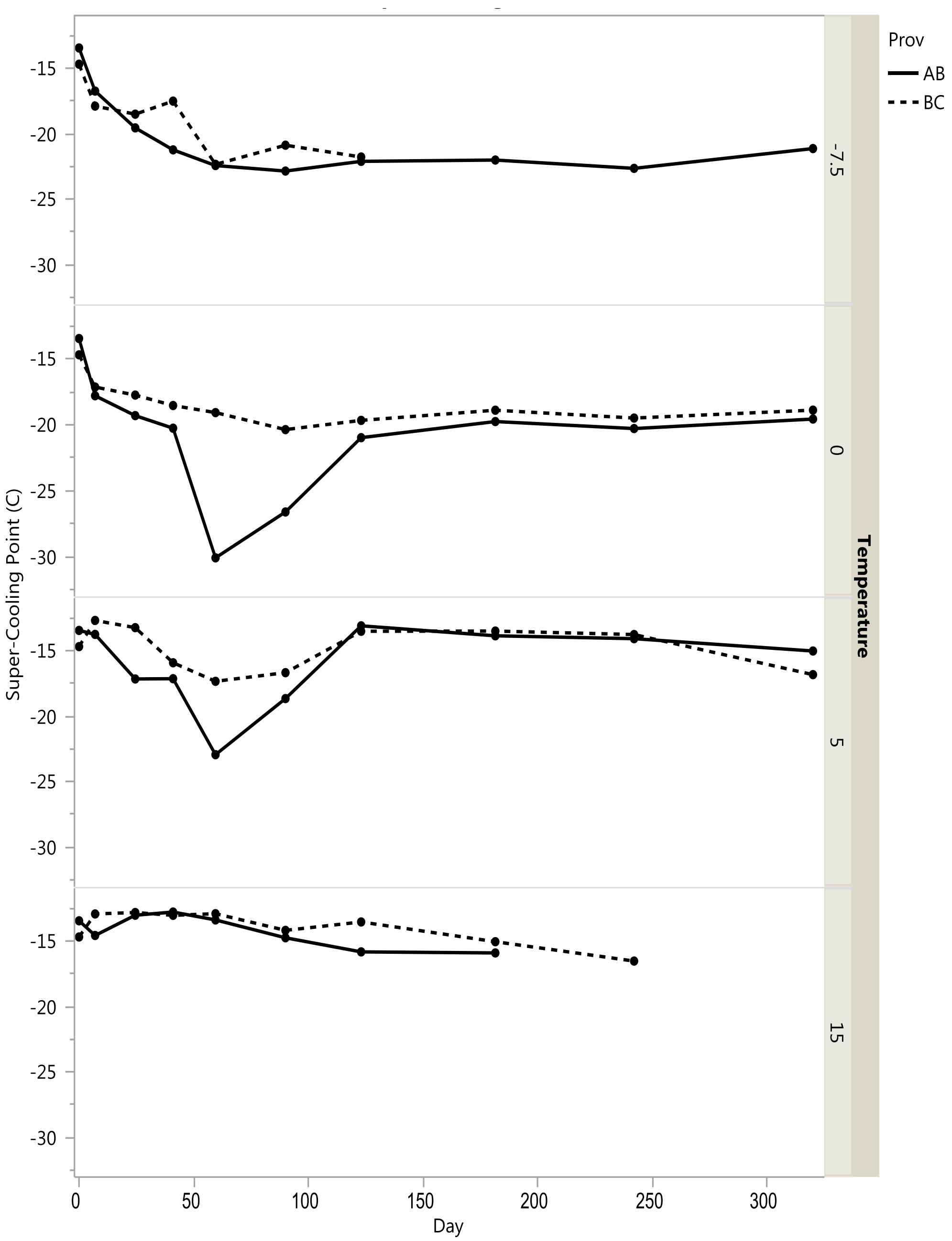


Figure 1. Median super-cooling points (SCPs) of mountain pine beetle larvae from a population near Grande Prairie in north-central Alberta (solid line) and a population near Greenwood in southern British Columbia (dashed line). The median SCP is the temperature at which 50% of the population would be expected to die. Infested wood from field sites was cut in late September (AB) or early October (BC), transported to the Pacific Forestry Centre and placed in walk-in cold rooms with a mean temperature of -7.5, 0, 5 or 15°C (Y_2 axis). SCPs were determined on Day 0, the day the wood was placed at the different temperatures, as well as after 7, 25, 41, 60, 90, 123, 182, 242, and 320 days at each temperature.

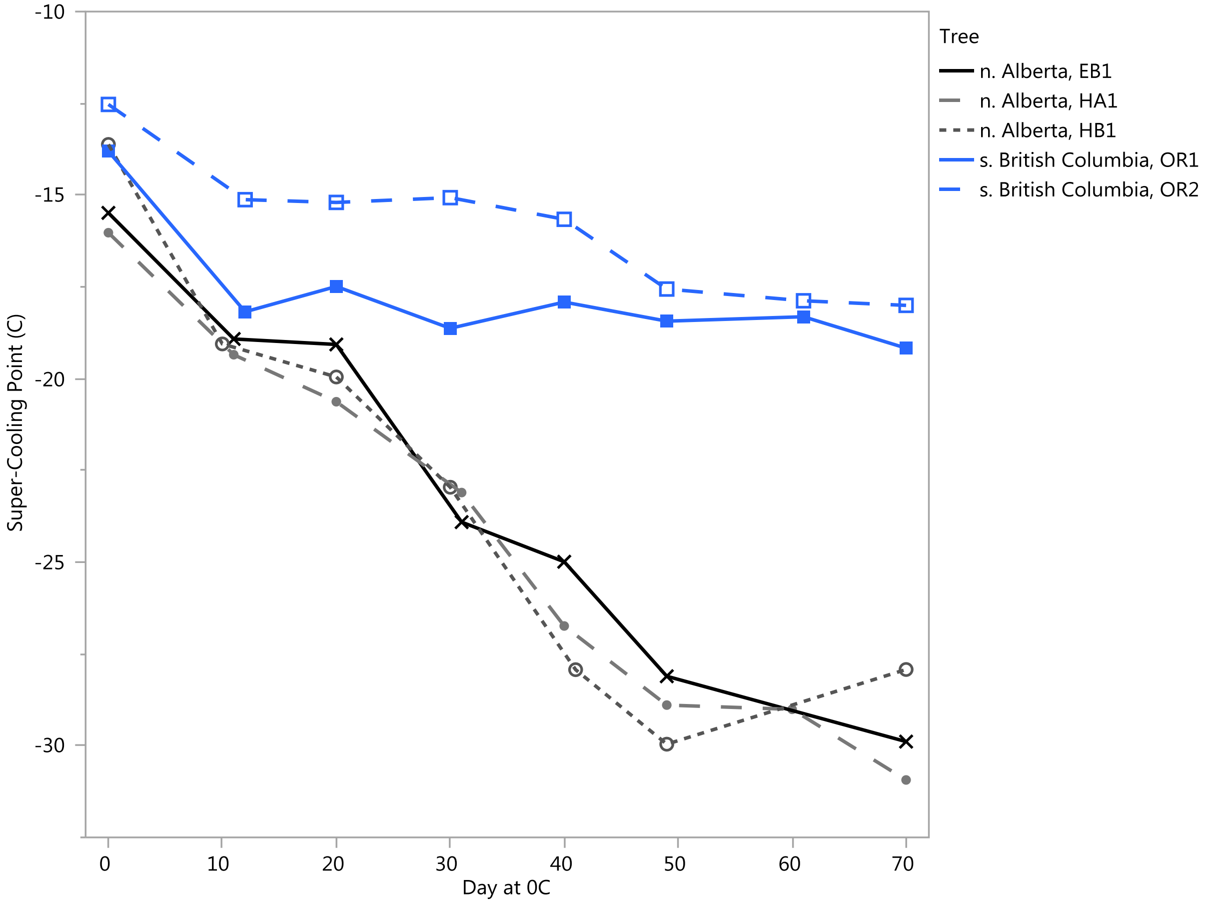


Figure 2. Median super-cooling points (SCPs) of mountain pine beetle larvae from three trees near Whitecourt in north-central Alberta (black and grey lines) and two trees near Campbell River on Vancouver Island in southern British Columbia (blue lines). The median SCP is the temperature at which 50% of the population would be expected to die. Infested wood containing late instar larvae was cut at field sites in late September (AB) and early October (BC), transported to the Pacific Forestry Centre and placed in walk-in cold room with a mean temperature 0°C. SCPs were determined on Day 0, the day the wood was placed at 0°C and then approximately every 10 days thereafter until Day 70.

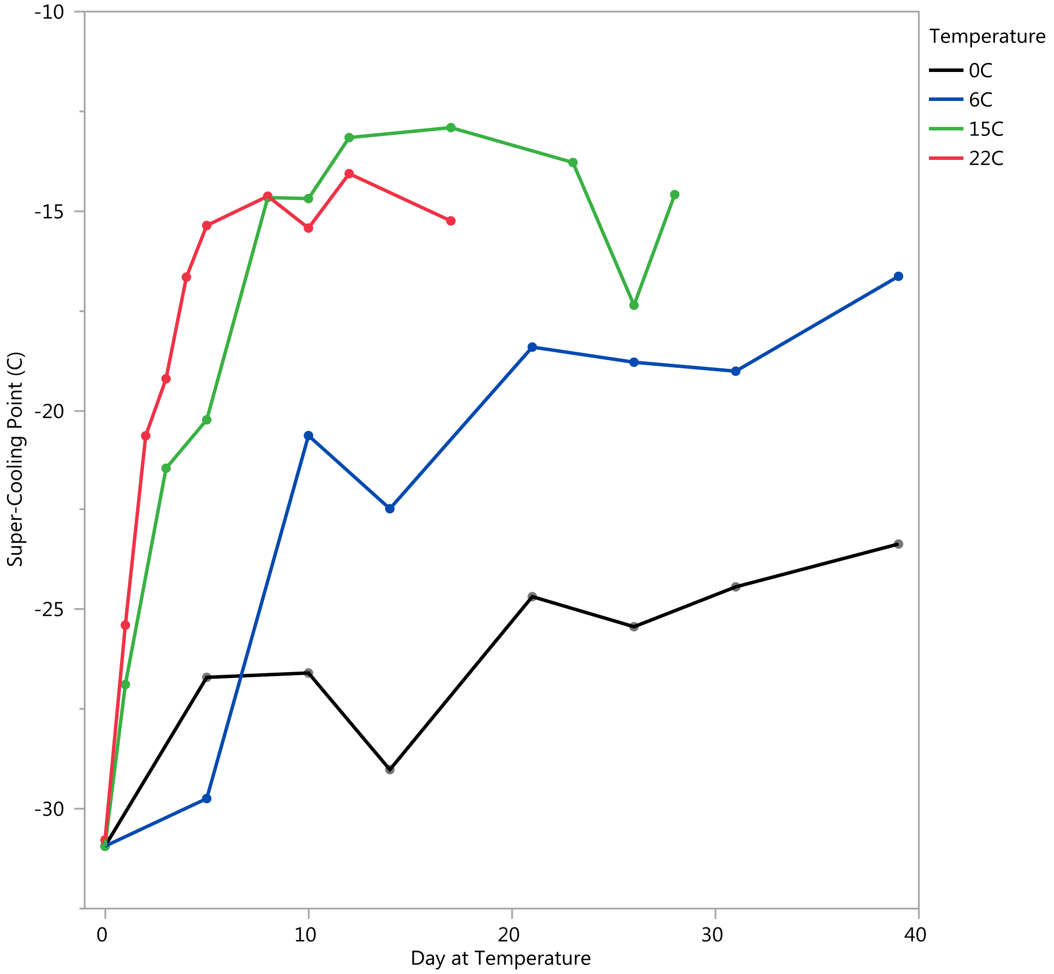


Figure 3. Median super-cooling points (SCPs) of mountain pine beetle larvae from one tree (HA1 in Figure 2) near Whitecourt in north-central Alberta. The median SCP is the temperature at which 50% of the population would be expected to die. The infested tree containing late instar larvae was cut in late September and transported to the Pacific Forestry Centre and placed in a walk-in cold room with a mean temperature of 0°C for 70 days to allow insects to acclimate to the cold (see Figure 2). After 70 days at 0°C, Day 0, the wood was placed at 6, 15 and 22°C, or left at 0°C, and SCPs were measured over time.

Acknowledgements

We thank K. Meyers, C. Dunbar, D. Wertman, M. Cassels, J. Liu, P. Bilek, E. McLeod, B. Wilmot, V. Waring, K. Hogg, G. Thandi, M. Cooper and B. Tomm for laboratory or field assistance. We are indebted to Manitoba Conservation (F. Ross) for jack pine logs and Alberta Agriculture & Forestry (D. Letourneau, J. MacCormick, M. Feser, C. Briggs, A. Sharpe, C. Whitehourse) for field assistance, site location or permits. This project is funded by fRI Research, the provinces of Alberta, Saskatchewan, Manitoba and Ontario through SERG-International and Forest Protection Limited, and Natural Resources Canada.

References

Agriculture Canada. Effective Growing Degree Days. http://www.agr.gc.ca/eng/science-and-innovation/agricultural-practices/climate/future-outlook/climate-change-scenarios/?id=1362684401064.

Logan JA, Bentz BJ. 1999. Model analysis of mountain pine beetle (Coleoptera: Scolytidae) seasonality. Environmental Entomology 28: 924-934.

Régnière J, Powell J, Bentz B, Nealis V. 2012. Effects of temperature on development, survival and reproduction of insects: experimental design, data analysis and modeling. Journal of Insect Physiology 58: 634-647.

Safranyik L, Carroll AL. 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. *In* The mountain pine beetle: a synthesis of its biology, management and impacts on lodgepole pine. *Edited by* L Safranyik and B Wilson, Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, Victoria, British Columbia. Pp. 3-66.

Safranyik L, Linton DA. 1998. Mortality of mountain pine beetle larvae, *Dendroctonus ponderosae* (Coleoptera:Scolytidae) in logs of lodgepole pine (*Pinus contorta* var. *latifolia*) at constant low temperatures. Journal of the Entomological Society of British Columbia 95:81-87.

SERG-I#782: Financial Report, January 19, 2018

Mountain Pine Beetle Cold Tolerance and Seasonality

Projected budget for the last 2 years of project

Projected Cash Costs	**2017/2018**	**2018/2019**
Student salaries (Co-op, M.Sc.)	45,000	20,000
Field work (vehicles, meals, lodging)	8,000	5,000
Supplies & laboratory analysis of samples	2,000	--
Info transfer (meetings or publications)	5,000	5,000
Total Cash Costs	**60,000**	**35,000**

Cash Contributers	**2017/2018 rec'd.**	**2018/2019**
NRCan/CFS	25,000	10,000
SERG-I	15,000	15,000
Foothills Research Institute	20,000	10,000
Total Cash Contributions	**60,000**	**35,000**

Original Budget – To date, project funding has been received as requested. The amount required to complete the research in 2017/2018 and 2018/2019 decreased because the study was able to take advantage of some additional NRCan funds that became available (let's call it internal "slippage").

Projected Cash Costs	**2013/2014**	**2014/2015**	**2015/2016**	**2016/2017**	**2017/2018**	**2018/2019**
Student salaries[a] (Co-op, M.Sc.)	55,000	55,000	76,000	50,000	50,000	33,000
Field work (vehicles, meals, lodging)	62,000	60,000	53,000	15,000	15,000	10,000
Supplies & laboratory analysis of samples	10,000	10,000	13,000	6,000	5,000	2,000
Info transfer (meetings or publications)	3,000	5,000	7,000	5,000	5,000	5,000
Total Cash Costs	**130,000**	**130,000**	**149,000**	**76,000**	**75,000**	**50,000**

[a] The proposed research involves laborious insect manipulations, assessments and measurements. M.Sc. student is working on seasonality study.

Cash Contributers	**2013/2014**	**2014/2015**	**2015/2016**	**2016/2017**	**2017/2018**	**2018/2019**
NRCan/CFS	25,000[a]	25,000	30,000	30,000	25,000	20,000
SERG-I	40,000	30,000	45,000	40,000	30,000	20,000
Foothills Research Institute [b]	65,000	75,000	65,000	6,000	20,000	10,000
Total Cash Contributions	**130,000**	**130,000**	**149,000**	**76,000**	**75,000**	**50,000**

[a] NRCan/CFS covered the purchase ($70,000) and installation ($30,000) costs of two environmental chambers at PFC

[b] Collaborative Research Agreement for 2013/14 through 2016/17 signed in July 2013. Submitted proposal for additional work in Oct. 2015. fRI funding goes towards cold tolerance objs. 1-5. SERG-I and NRCan funds goes towards cold tolerance and seasonality/development objs. 1-6.

In-kind Contributors: AB AAF and companies (Canfor, Weyerhaeuser) to provide assistance with locating field sites and permit applications. *CFS in-kind*: Facilities and salaries (0.9 Bleiker's research time, 0.9 Smith's research time, and we anticipate 0.3 FTE of technician time from a pool).

Simulating MPB invasive spread control in Saskatchewan using SpaDES

Alex M. Chubaty. Barry J. Cooke, Eliot J. B. McIntire

Natural Resources Canada, Canadian Forest Service

Abstract

The continued eastward spread of mountain pine beetle (*Dendroctonus ponderosae* Hopk.; MPB) now threatens the boreal forests of eastern Alberta, Saskatchewan, and beyond. Predicting the outbreak and spread dynamics of this insect in jack pine, and to evaluate control measures to mitigate a potentially devastating loss of forest habitat and timber supply requires not only a complex understanding of the various inputs to this system and their interactions (e.g., MPB population dynamics, climate impacts, landscape features) but also the technical capacity to run large-scale spatial simulation models, and to update them quickly as new data are acquired and new models are developed.

Nonlinear density-dependent dynamics of mountain pine beetle (MPB) recruitment means that MPB spread to Saskatchewan and beyond is primarily a “numbers game”, and this has sharp consequences for pest management thresholds. The nonlinear dependency of survival rate on temperature means that eruptive potential is regulated secondarily by climate, and this has consequences for the efficacy of pest management through “direct control”. Through simulation we distinguish the conditions under which MPB spread can be significantly slowed. Preliminary results indicate that the prognosis for pest management in the lodgepole pine of the Rocky Mountains Foothills region is distinctly different that of boreal jack pine.

Simulations of MPB population recruitment and spread were developed using the SpaDES (Spatial Discrete Event Simulation) platform. SpaDES facilitates a tight coupling of simulation and data within a modular framework that allows easy updates to model components. Furthermore, our development cycle follows current best practices for reproducible computing workflows and, where possible, we have followed an “Open-Access” model for software platform and simulation model development.

La propagation continue du dendroctone du pin ponderosa (*Dendroctonus ponderosae* Hopk, DPP) vers l'est menace maintenant les forêts boréales de l'est de l'Alberta, de la Saskatchewan et plus à l'est. Prédire l'éclosion et la dynamique spatiale de cet insecte dans le pin gris, et évaluer les mesures de contrôle pour atténuer une perte potentiellement dévastatrice de l'habitat forestier et de l'approvisionnement en bois nécessite non seulement une compréhension complexe des divers intrants de ce système et de leurs interactions (p. la dynamique des populations, les impacts climatiques, les caractéristiques du paysage) mais aussi la capacité technique d'exécuter des modèles de simulation spatiale à grande échelle, et à les mettre à jour rapidement au moment que de nouvelles données sont acquises et de nouveaux modèles développés.

La dynamique densité-dépendante non-linéaire du recrutement du DPP signifie que la distribution du DPP en Saskatchewan et au-delà est avant tout un «jeu des chiffres», ce qui a de conséquences importantes sur les seuils de lutte antiparasitaire. La dépendance non linéaire du taux de survie sur la température signifie que le potentiel éruptif est régulé secondairement par le climat, ce qui met en question l'efficacité de la lutte antiparasitaire par un «contrôle direct». Avec nos modèles de simulation, nous distinguons les conditions dans lesquelles la propagation du DPP peut être considérablement ralentie. Les résultats préliminaires indiquent que le pronostic de la lutte antiparasitaire dans le pin tordu dans la région des Rocheuses est nettement différent de celui du pin gris boréal.

Des simulations du recrutement et de la propagation de la population DPP ont été développées en utilisant la plateforme SpaDES («Spatial Discrete Event Simulation»). SpaDES facilite un couplage étroit de la simulation et des données dans un cadre modulaire qui permet de mettre à jour facilement les composants du modèle. De plus, notre cycle de développement suit les meilleures pratiques actuelles en matière de «workflows» informatiques reproductibles et, où possible, nous avons suivi un modèle d'accès "ouvert" pour le développement de modèles de simulation.

Introduction

Addressing the problems of anthropogenic environmental changes requires predicting the future state of ecological systems which have not yet been observed, and this requires robust predictions produced using a variety of ecosystem modelling approaches (Evans et al. 2012). Ecologists are frequently faced with addressing additional complexities of ecosystems including component feedback interactions, and are constrained not only by the availability of data but also their own expertise in constructing submodels of appropriate complexity for components outside of their domain of expertise (Evans et al. 2013). To make robust, high-quality predictions, we require that 1) data and dynamic models that can be updated frequently as needed; 2) models are tested against data and models should reproduce (qualitatively and quantitatively) the patterns observed in the data (e.g., Pattern Oriented Modelling: Grimm & Railsback 2012); and 3) uncertainty in data and models as well as error propagation are adequately addressed (Polasky et al. 2011; Evans et al. 2013).

The most critical threshold influencing eruptive dynamics is the point at which populations in the endemic niche are willing and able to successfully mass attack trees in the epidemic niche. This point is defined by the intersection between the endemic and epidemic niche recruitment curves. The key problem in mountain pine beetle (MPB) ecology is how changing environmental conditions (e.g., climate warming and water stress) tends to shift and bend these recruitment curves, allowing endemic populations subsisting on suppressed trees to suddenly access the epidemic niche comprised of healthy, vigorous trees. These are the fast (or “twitch”) dynamics of eruption. Simulating long-range MPB eruptive dynamics requires a set of host forest dynamics equations that allows for the existence and evolution of separate endemic and epidemic host tree niches, defined as distinct forest age and health classes that support endemic and epidemic populations of MPB. Additionally, a comprehensive simulation model would include slow (or “refractory”) dynamics of population regrowth post-epidemic collapse, with the natural emergence of endemic niches through the self-thinning process and stochastic perturbations (e.g., through fires or lightning strikes).

Back in 2013, global mean temperatures had been stable for a decade, and were so cool that they were were falling out of line with global circulation model predictions (Figure 1). At that time, MPB had not yet exploded in Jasper National Park, and the density of attacks in Hinton district was low enough that the cost of suppressing those populations to stable was reasonable. Four years later, after three consecutive years of record-breaking temperatures 2015-2017, Cooke & Carroll (2017) was published, and the warnings outlined there about (i) the importance of beetle numbers for initiating outbreak and (ii) the potential for critical change under climate change have come to pass. MPB populations in the backcountry of the Rocky Mountains and Foothills have exploded in the last three years. Jasper population are now, in 2018, well beyond control, and populations endemic to Hinton district are in eruptive mode (Figure 2).

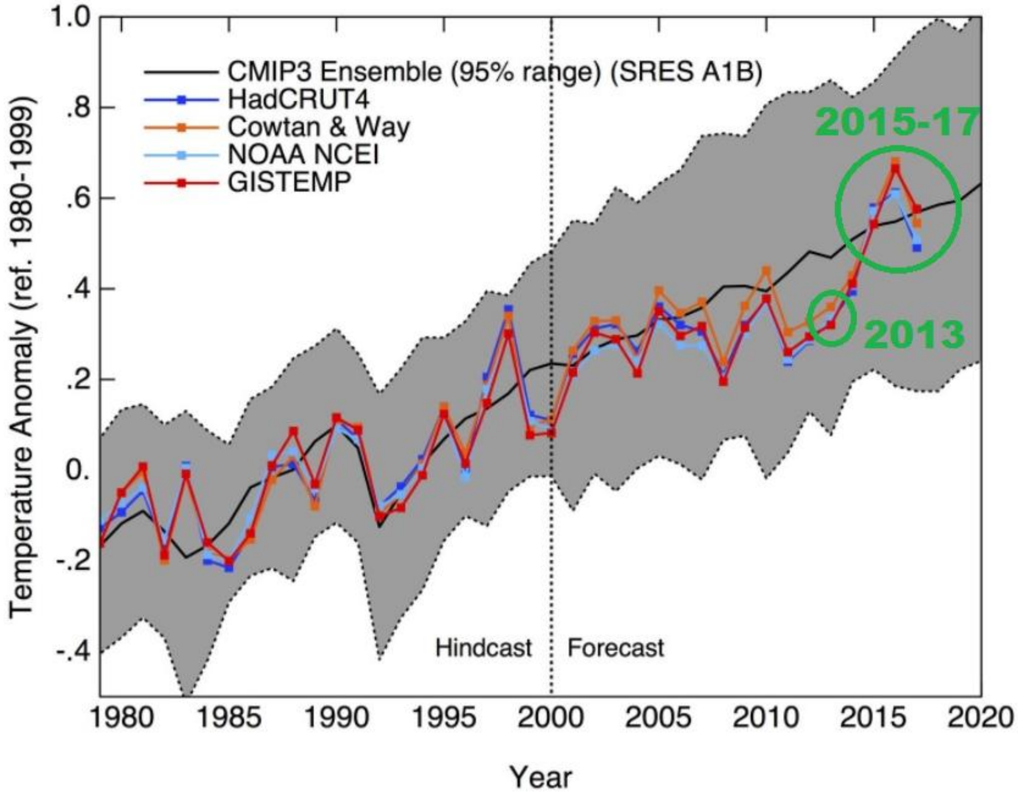
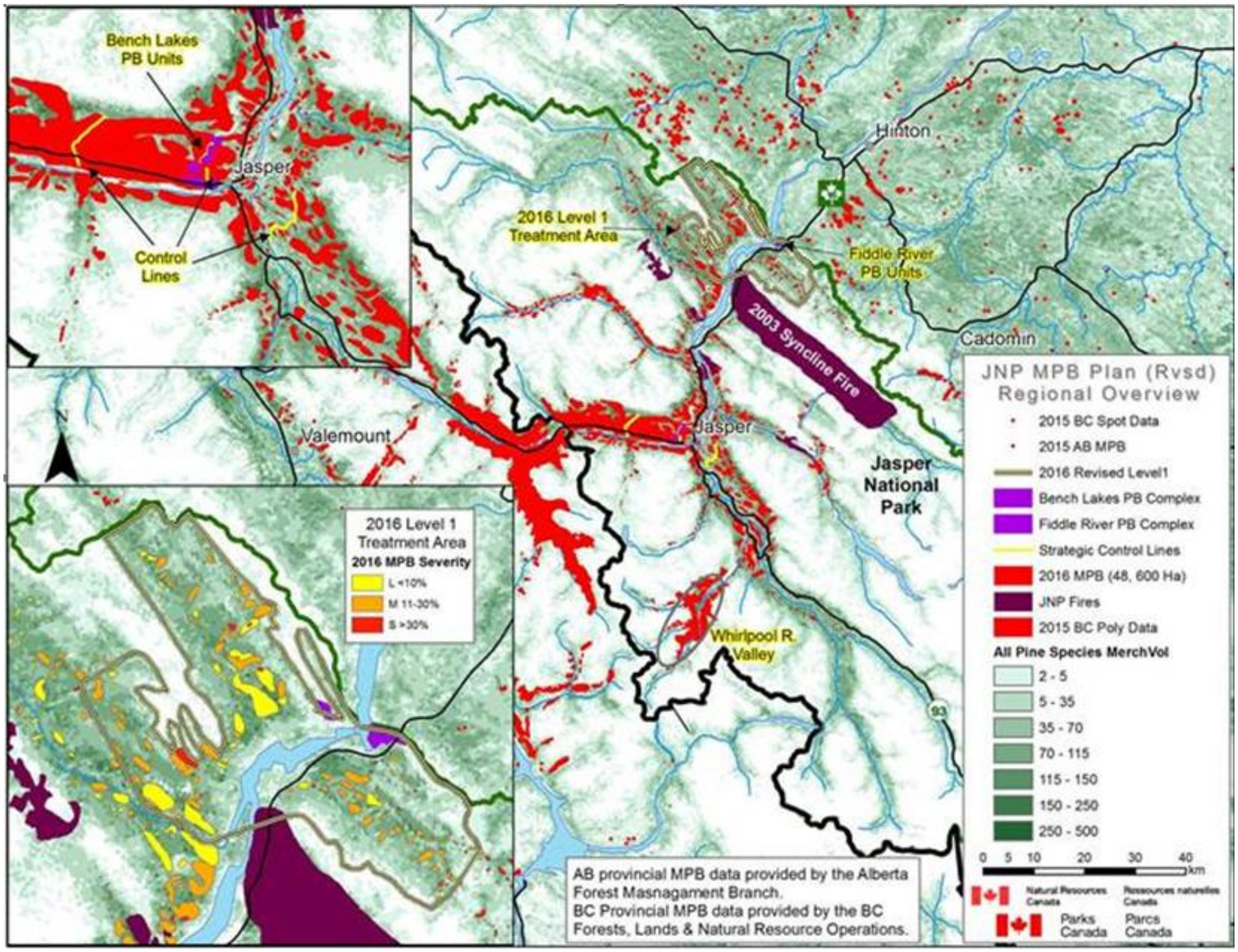


Figure 1. Rising global mean temperature. The warm pulse in 2015-17 put the observations back on track within the envelope of GCM predictions (grey) after a decade of relative stability and some climate skepticism. (Adapted from NOAA, G. Schmidt)



AB provincial MPB data provided by the Alberta Forest Masnagament Branch.
BC Provincial MPB data provided by the BC Forests, Lands & Natural Resource Operations.

Figure 2. MPB in Jasper National Park and Hinton district prior to the 2017 field season.

Managing MPB in the long-run requires stand management, including both the endemic and epidemic niches. Where removing commercial pine in the over-storey is an economically viable option in heavily infested Rocky Mountains regions (e.g., British Columbia and the Foothills region of Alberta), it is not an option in the frontal regions (Lac La Biche-FortMcMurray-Cold Lake) where pine has little commercial value. Here, direct control is very expensive due to poor access and stand management options are extremely limited. Optimal control requires a judicious deployment of limited control resources (no more than $1-2M per year). To date no one has examined the tradeoffs between direct control and stand management in the invasive context. Direct control of populations might be effective in the short-run, but stand management might be more effective in the long-run. It is not clear what mix of approaches is best suited to slowing the long-term rate of spread.

In a homogeneous forest environment, frontal attack on newly establishing populations is the surest way to the slow the spread of invasive organisms, as has been demonstrated with gypsy moth in the eastern US, where a frontal attack strategy has cut the rate of spread of GM from 20km/y to 10km/y. However for organisms invading a very patchy environment – which is the case for MPB in jack pine – or invading along a gradient of host availability – which is the case across the larger scale of Alberta-Saskatchewan – it may be judicious to use a hybrid approach of frontal attack on short-range migrants in combination with a hindflank attack on long-range migrants (for example, emanating from the Slave Lake region). The balance between these two approaches should depend on assumptions on the number and range of migrants from each of the two zones. The only way to derive the optimal rate of spread reduction in such a hybrid situation is through spatially explicit simulation using a validated model of eruption, and its relation to forest structure.

Development of a comprehensive large-scale spread management model to evaluate the fast and slow dynamics of invasive eruption in the long-run (over the next 100 years or more), has been fraught with multiple scientific and technical challenges. Previous attempts were unable to satisfactorily integrate multiple ecological and geophysical interactions (e.g.,vegetation succession, fire disturbance, and climate) in part because of limitations in the availability of suitable geospatial data, the difficulty of getting models from multiple disciplines to talk to one another, and the computational limitations of the time. The most comprehensive process simulation model of MPB developed to date, the Safranyik et al. (1999) stand-level model, developed for BC, has never been validated, and has never been subject to formal analysis. Thus it is not clear what this model implies regarding the role of drought and host defenses in promoting eruptive behaviour – which we believe to be critical. That this stand-level model has not been formally studied provides little assurance that spatialized versions (such as MPB-SELES) exhibit realistic eruptive, dispersal, and spread behaviour. Worse, the documented equations in the 1999 publication are known to deviate significantly from the actual Fortran code, such that a formal analysis would require the compilation and execution of thirty-year old code. Meanwhile, the SELES platform (Fall and Fall 2001) is no longer actively supported. Clearly, alternative simulation tools are desirable.

Due to the integrated nature of this problem (inputs from climate, vegetation, insects, fire, etc.) a flexible simulation framework is required. One that supports interoperability of system components (i.e., modularity), one that is agnostic to the modeling paradigm used (Individual/agent based modeling, population modeling, landscape modeling, GIS/raster-based models, statistical models, etc.), and one that allows multiple modeling paradigms to be used concurrently and interact. This type of modular simulation framework would facilitate the reusability of model components to produce new models. Additionally, predictive models and their outputs also need to be accessible (usable) by a wide range of different end users, each of which may possess vastly different skills with regard to programming, modeling, and policy expertise.

Recent aspatial modeling results from Cooke & Carroll (2017) suggest that the most critical factors affecting invasive dynamics are positive feedback processes occurring at low density, including dispersal, host-finding, congregational host mass-attack, and egg and early larval survival. We are spatializing this model of MPB nonlinear eruptive dynamics by including two key components: (1) a dispersal component, and (2) spatial variation in validated models of climatic suitability. Building on this previous work, we developed a long-range MPB spread simulation model using the SpaDES simulation platform (Chubaty and McIntire 2017a; http://spades.predictiveecology.org/). As an open source platform implemented in R, SpaDES is freely available, and the MPB module code will be equally accessible to all members of the public. This model consists of several modules comprising data import and processing (e.g., map cropping and reprojection) as well as the simulated population and dispersal dynamics. We use this model to explore MPB eruptive dynamics in lodgepole pine and jack pine habitats, and to evaluate several management scenarios to answer questions critical to eastern concerns. In particular, we examine how stand dynamics influence eruptive potential of MPB populations, and explore the impacts of several management strategies on spread mitigation.

Methods

Study area

Our study area spans a region of north-central Alberta (AB) and into Saskatchewan (SK), Canada (Figure 3; 114.9704°W – 108.4075°W; 54.42415°N – 56.35593°N).This area was selected because it coincides closely with the Cold Lake Air Weapons Range, which represents the east-most extension of MPB in boreal jack pine. The west end extends to Lesser Slave Lake, so there are light populations persistently established since 2010, which yields enough data to test model growth and spread predictions.

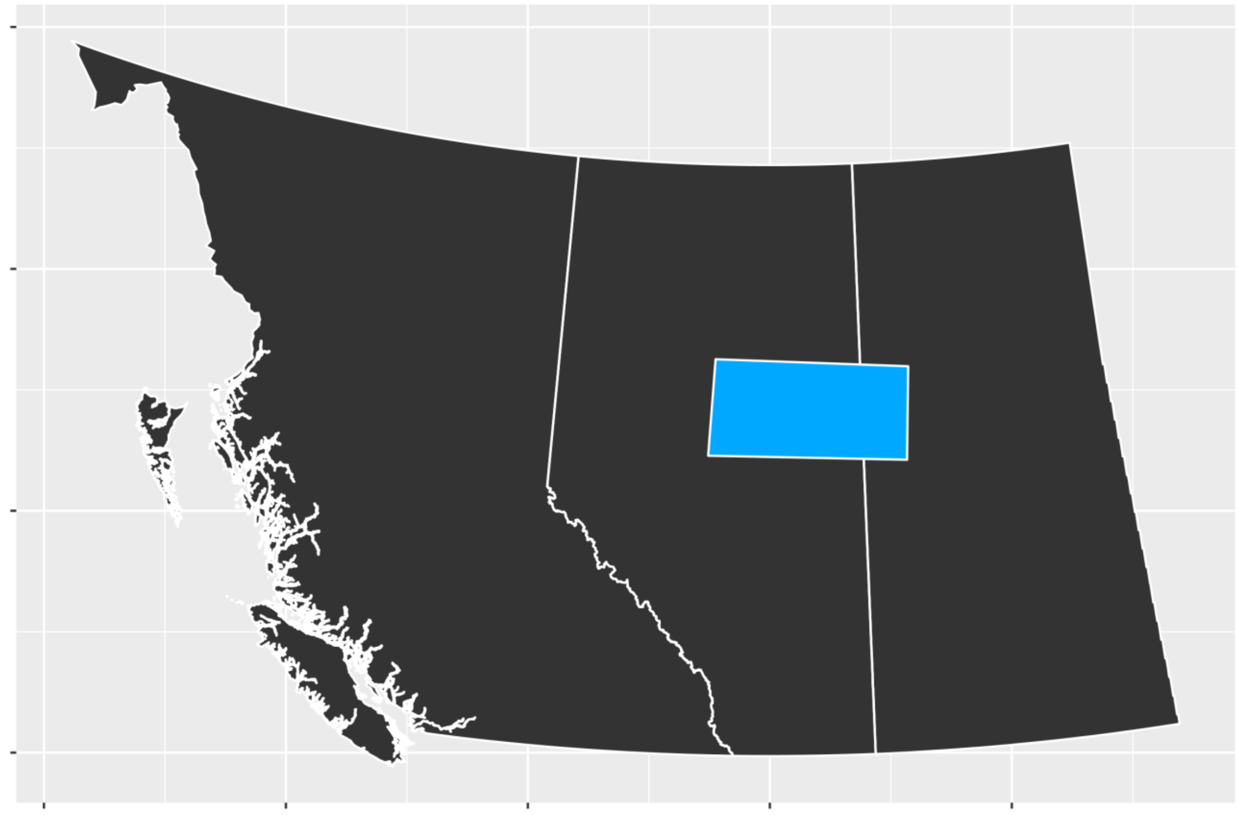


Figure 3. Map of project study area in north-central Alberta, extending eastward from Lesser Slave Lake into the western part of Saskatchewan.

Simulation and data analyses

The *SpaDES* (Spatial Discrete Event Simulator) package for R (Chubaty and McIntire 2017a) is the collection of tools that allows model integration across disciplines. This modularity will allow several quasi-independent modules to interact effectively with minimal coordination, enforced through simple protocols. The modules that combine to build such system must have certain general features (Reynolds and Acock, 1997):

1. A module is an integrated set of coded procedures for transforming data; for example statistical parameter estimation, geospatial data operations, simulating a time step of a discretized physical process like MPB, and methods for summaries or visualizations;
2. Modules have metadata that define their inputs, outputs and other elements. In addition, internally, they can schedule calls to any procedure within any other module to occur at some future time. Models are not created as complex, nested loops.
3. No modules ever depends directly on another. Module dependencies arise only through shared data objects (Figure 4). This allows modules to be added, removed or replaced without changing anything else. This is the critical engineering idea that allows modelling project of the magnitude we propose to be attempted with the expectation of success.

SpaDES is an alternative to the normal tendency for modelling groups to build increasingly complex models of everything. This never works. Because *SpaDES* is an R package, its ability to run complex models for large problem instances in *SpaDES* depends on R's ability to do so. In recent years, R has proved to have this ability R's compiled, optimized base code is as fast as C for vectorized calculations, and the language has become the world leader in data sciences, for both "normal" data and Big Data, far outstripping the designers original purpose. In addition to having many easily accessible high performance tools (e.g., packages *Rcpp*, *parallel*, *OpenMP*, *data.table*, *raster*, *rgdal*). Specialised tools optimised in high performance languages can be also accessed (e.g., C, C++, FORTRAN). R is no barrier to high performance computing. It is also useful and usable by the intended user and scientific community, and is one of the most widely used within the ecological sciences and will remain so for decades to come. R is also the means whereby novel statistical and analytical tools are developed and made accessible to the scientific community. The enables another enormously powerful feature of *SpaDES*, namely the close integration of statistical modeling, parameter estimation, and simulation.

Reproducible workflow

In an effort to maximize long-term maintainability of the modules' code, and to facilitate collaborative development and module reuse, we have adopted an open source model that follows most of the suggested best practices for scientific computing (Wilson et al. 2014; Croucher et al. 2017). In the acquisition and preprocessing of data, the development of the modules, and the development of the simulation model framework, we have automated as many steps in the workflow as possible (e.g., through the use of scripts) to avoid steps that may require manual intervention. Several parts of this workflow make use of automated tests, which are automatically run following any changes to the codebase.

All code is modularized as much as possible (e.g., through the use of R functions and using the modular simulation platform *SpaDES*), and is stored in a [currently private] GitHub repository under version control. We plan to make the code base public as part of the publication process. We also make use GitHub's issue tracking and other collaborative coding features to maximize future collaborative potential. We have strived to ensure that all code is well documented using a literate programming approach (Knuth 1984), which describes "the what" of each code chunk (what it does), as well as "the why" (why a particular approach or subequation is used).

To ensure reliable execution on multiple machines and cross-platform, we cache deterministic data preprocessing and simulation steps (e.g., GIS operations) to speed up subsequent calls to those functions. We also make use of reproducible R package management solutions via the *packrat* R package (Ushey et al. 2016).

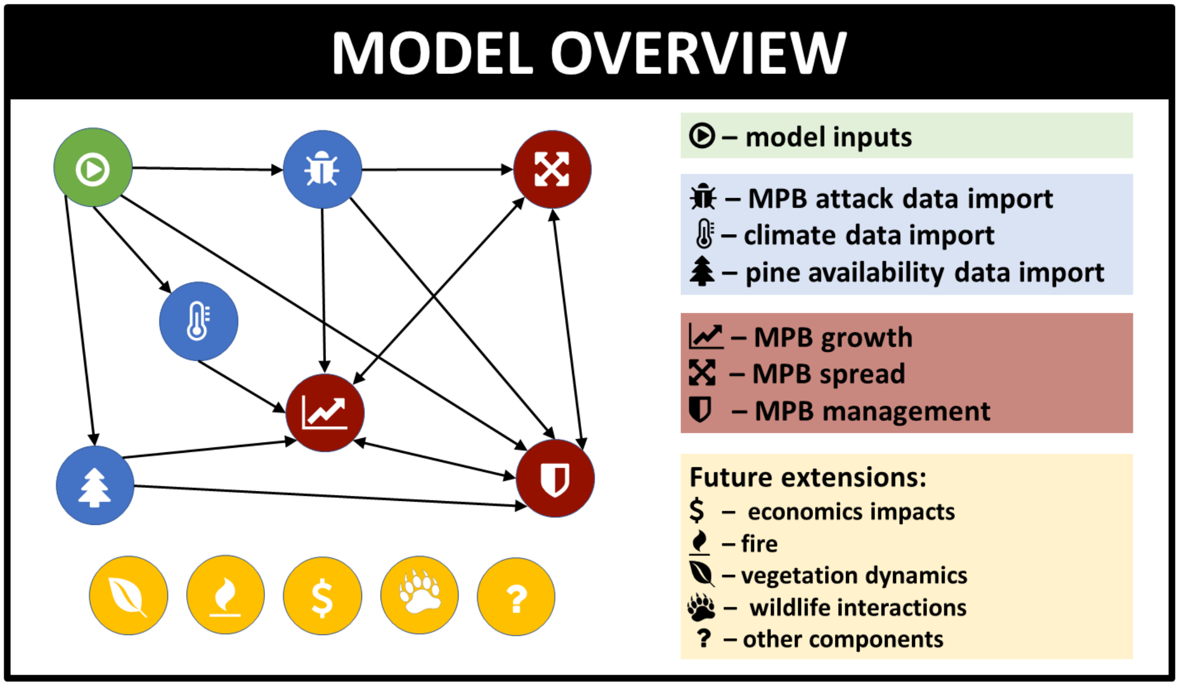


Figure 4. An overview of the MPB spread model, showing interchangeable simulation components (modules). Arrows indicate shared data dependencies. Modules highlighted in blue are responsible for import and preprocessing of data; those highlighted in red provide the core simulation components; and those in yellow indicate future extensions of the model.

Pine availability distributions

Remote sensed data from Canada's National Forest Inventory (NFI) provide the basis for the pine layers used in this module. These data were used by Beaudoin et al. (2014) to map coverage of several forest species using a k-nearest (kNN) method to generate national 250m x 250m pixel resolution maps. Here we use the estimated percentage cover pine per species for Jack pine (*Pinus banksiana*) and Lodgepole pine (*P. contorta* var *latifolia*), reprojected to use an Albers equal-area conic projection within the simulation. All data downloads and preprocessing were scripted for reproducibility from raw, original sources.

In the current model, we calculate the number of available jack pine and lodgepole pine trees from the proportion pine for each species, by multiplying by the average stem density of 1125 mature stems per hectare, which is typical for beetle-prone lodgepole pine stands (Whitehead and Russo, 2005). A more sophisticated estimate of pine stem density for each species is currently under development for future versions of this module. As mentioned above, a key benefit of the SpaDES platform is modularity, and future updates (or wholesale replacement of this module) are made easier using this modular simulation approach.

MPB red attack

This module imports the raw MPB red-top attack data from AB and BC over several years (2001-2016), following the methodology of Cooke and Carroll (2017). MPB attack data for AB (derived from spatial points) and BC (derived from spatial polygons) were reprojected to use an Albers equal-area conic projection and rasterized using a common resolution of 250 m x 250 m (matching the pine raster data) and merged into a single raster layer for each year. BC raster cell were calculated from the proportion of a polygon within that cell, again assuming an average stem density of 1125 stems/ha (per Whitehead and Russo, 2005). Raster cells for AB were calculated by summing the point values within each 6.25 ha raster cell. Where possible, all data downloads and preprocessing were scripted for reproducibility from raw, original sources.

Because of the long processing time and high memory requirements of the GIS operations performed during data preprocessing, with use a combination of caching approaches – implemented in the *reproducible* R package (McIntire & Chubaty, 2018) – to speed up subsequent loading and use of these data. All preprocessed data are made available in a private Google Drive folder, and subsequently downloaded from there when used by a SpaDES module.

Climate suitability models

Over the last 20 years, and using four indices of MPB climatic suitability, there appears to be a long-term trend in enhanced MPB survival throughout BC and Alberta (Cooke & Carroll 2017), as illustrated in Figure 5, fuelled by accelerating warmth through the last 20 years. The effects of temperature on seasonal biology in the US Rocky Mountains have been modelled by Logan et al. (1992). The Logan suitability index (L) is based on summer temperatures (Logan, Régnière, and Powell 2003). The effects of temperature on winter mortality in the US Rocky Mountains have been modelled by Régnière & Bentz (2007). In the Canadian context, the many effects of temperature on MPB recruitment in British Columbia have been modelled by Safranyik et al. (1975). The Safranyik index (S) was subsequently extended to Alberta by Carroll et al. (2004). Once the MPB had come into Alberta in large numbers in 2006 the Régnière index (R) was field validated in Alberta (Cooke 2009). The North America-wide outputs of these models under standard climate change scenarios were first presented in Nealis and Pater (2008), and later received peer-review validation through Bentz et al. (2010) and Safranyik et al. (2010). A composite SLR index (G, the default) takes the geometric mean of these three models. These are described in further detail in Nealis and Peter (2008) and Nealis and Cooke (2014).

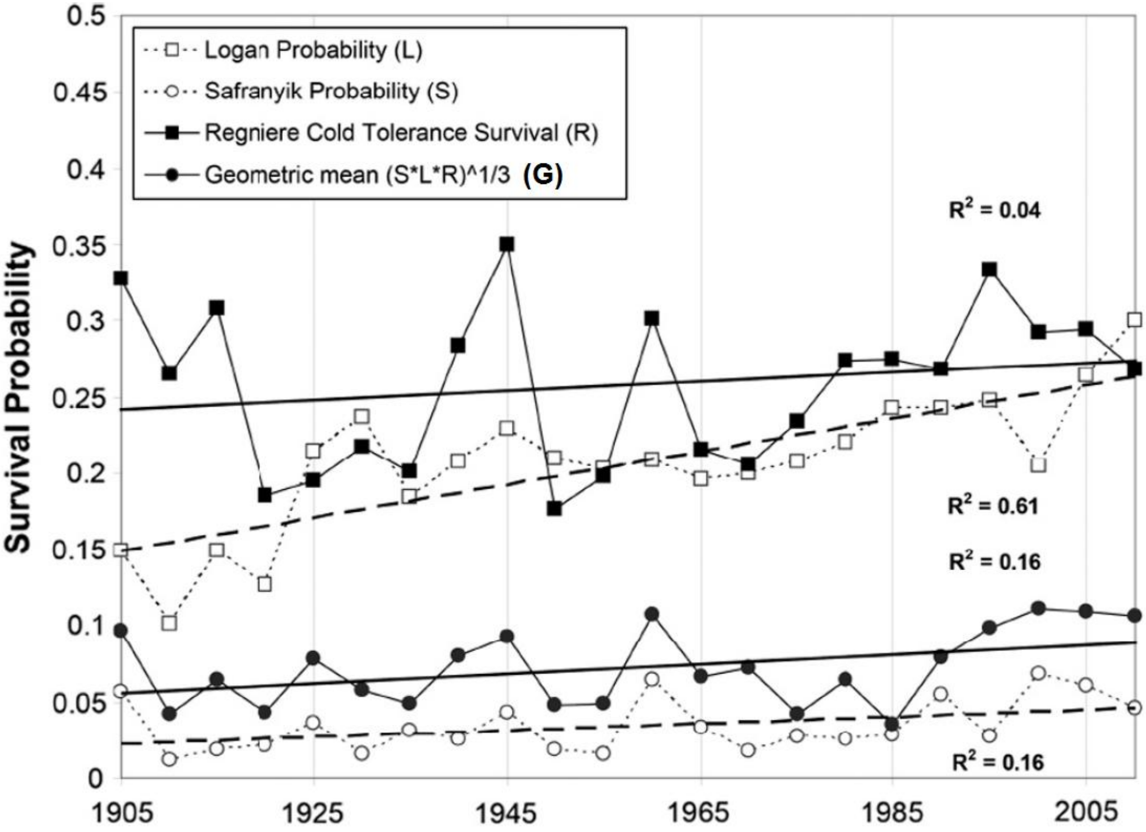


Figure 5. In western Canada, rising global mean temperature leads to rising MPB survival. Note the positive trend in "G" - the variable used to drive the MPB growth model. Survival probability G was anomalously high for four pentads in a row (1991-95, 1996-2000, 2001-05, 2006-10 = 20 years), but had stabilized through the shorter interval 2001-2010. However GCMs (see Figure 1) predicted this stabilization would not last. Adapted from Cooke & Carroll (2017).

Our simulation models explore 7 MPB climate scenarios (using each of the four indices: S, L, R, G) used as model drivers. BioSim version 10 (Régnière et al. 1995) was used to generate the climate suitability maps (see Bentz et al. 2010; Logan, Régnière, and Powell 2003; Safranyik et al. 2010). The values of each of these indices are bound between 0 and 1, and this value is used to scale the vertical shift of the red-top recruitment curve (described below). For each of the four indices, there are 1981-2010 normals plus the projections from two RCP scenarios (either 4.5 or 8.5), covering a span of 120 years, split into 30-year frames. All climate maps are projected using a Lambert Conformal Conic projection and cover all of Canada. Where possible, all data downloads and preprocessing were scripted for reproducibility from raw, original sources.

Red-top recruitment

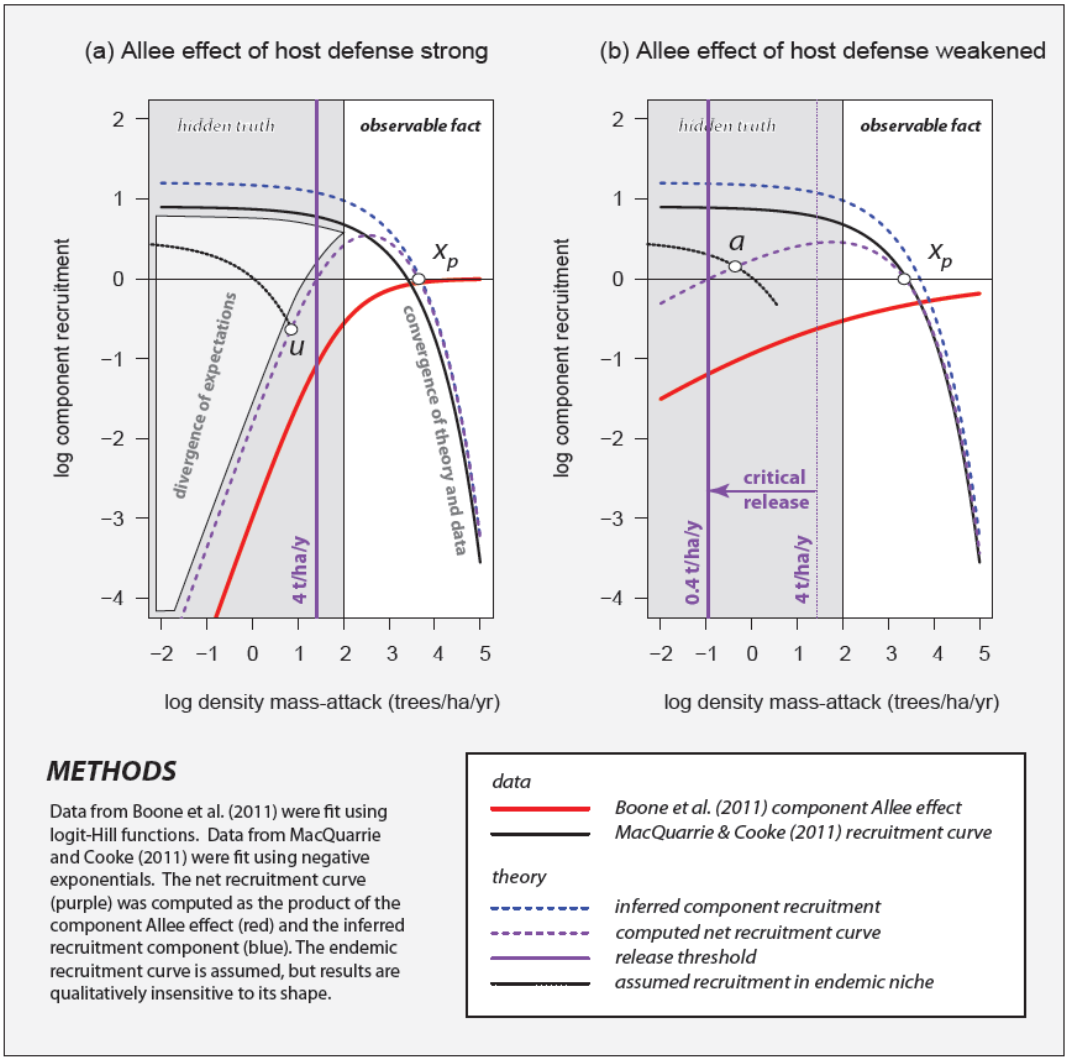
Recruitment is an annual process, with the inter-annual recruitment function taking a variety of possible forms, depending who one takes as an authority on the question. Berryman (1979) was an early pioneer who inspired much work in the area, and there are several possible interpretations of the data he used from Glacier and Yellowstone National Parks in Montana (MT) and Wyoming (WY) the 1960s. Consequently, the module offers the option of using several possible interpretations (Berryman 1979 fit, Berryman 1979 forced, Boone et al. 2011 [current default]). Additional options are forthcoming at the time of writing.

Raffa and Berryman (1983) provided definitive laboratory proof of Berryman's (1979) hypothesis that the recruitment curve for mountain pine beetle took the form of an inverted parabola. There is only one Canadian field data set that captures the rising phase of recruitment, the so-called "cooperation curve" (Boone et al. 2011). Meanwhile, Cooke & Carroll (2017) showed how this cooperation curve could be "hybridized" with the down-sloping "competition curve" of MacQuarrie and Cooke (2011) which was also derived from Rocky Mountain region field data. If the two curves are wholly orthogonal, the parameterized up-sloping co-operation curve can be multiplied by the parameterized down-sloping competition curve, to yield a generation recruitment curve.

Box 1 is drawn from Cooke (2013) and depicts the derivation of the generation recruitment curve used as the default population growth function in our simulation model. The base case for a well-defended pine tree is shown in panel (a) [This is the curve form reproduced in Figure 6]. In panel (a) the co-operation curve is steep, because the tree is well-equipped to pitch out beetles when they are attacking at low density. When they are attacking at high density, the attack is successful and net recruitment is positive. The red curve comes from Boone et al. (2011) and relates to probability of successful colonization in early life stages post oviposition. The black curve comes from MacQuarrie & Cooke (2011), and relates to the competition between late larval stages, predation during pupation, and adult female fecundity. Panel (b) exhibits the effect of moisture stress on tree defenses, which leads to higher recruitment for low-density attacks. This rapid transition between well-defended (a) and poorly-defended (b) pine tree is the essential seed for sudden eruption of an endemic population that subsequently rushes toward the outbreak equilibrium point x_p.

The dynamics of host-defense "relaxation" release are not observable in operational Aerial Detection Survey (aka "red-top") data because they occur at very low attack densities (on the order of 0.4 mass-attacked trees per hectare per year). Hence the grayed portion of either panel, a, b. Typically, operational survey programs are initiated only after attack densities have surpassed 5 trees per hectare. The right-hand side of the graphs contrast that which is observable in operational data, versus the left-hand-side of the graphs (shaded), where "careful observation" is required. This is why "Allee effects" (positive response to rising density at low density) are never observed in operational field data, as noted by Trczinski & Reid (2009), and it underscores the importance of detailed observations during the endemic stage (Boone et al. 2011). In panel (a) host defenses are strong, leading to heavy pitch-out of adult beetles and heavy resinosis of eggs and young larvae (red curve). As attack densities rise, the component eect of aggregative cooperation leads to higher component recruitment. As attack densities rise further, beyond peak, overall recruitment (dashed purple) is dominated by competitive effects that arise in the late larval recruitment component (dashed blue). In the absence of mass-immigration crossing the 4 trees/ha/y threshold, no outbreak is possible because the epidemic niche (purple) is unavailable ("u") to MPB existing in the endemic niche (dashed black). In panel (b), one year later, host defenses have been compromised by drought stress, the component

Allee effect of host defenses weakens, recruitment rises, and the critical threshold for outbreak drops from 4 trees/ha/y to 0.4 trees/ha/year. Now, populations in the endemic niche that were formerly incapable of mass-attacking at a rate of one tree per hectare per year may explode to a new equilibrium level, x_p, as the epidemic niche is now effectively available (”a”). The process of recruitment curve intersection point “u” moving from position $R<0$ to position “a”, where $R>0$, is called a “saddle-node bifurcation” and is the formal mathematical definition of “outbreak”.



METHODS

Data from Boone et al. (2011) were fit using logit-Hill functions. Data from MacQuarrie and Cooke (2011) were fit using negative exponentials. The net recruitment curve (purple) was computed as the product of the component Allee effect (red) and the inferred recruitment component (blue). The endemic recruitment curve is assumed, but results are qualitatively insensitive to its shape.

Box 1: The hidden role of host-defense relaxation in MPB release.

The purple dashed parabola in Box 1 panel (a) is the generation recruitment curve for MPB on a well-defended pine tree. The effect of climate warming in the cool climate of Alberta and Saskatchewan is to raise recruitment rates in a manner that is independent of attack density. This results in a vertical shift of the generation recruitment curve (Figure 3).

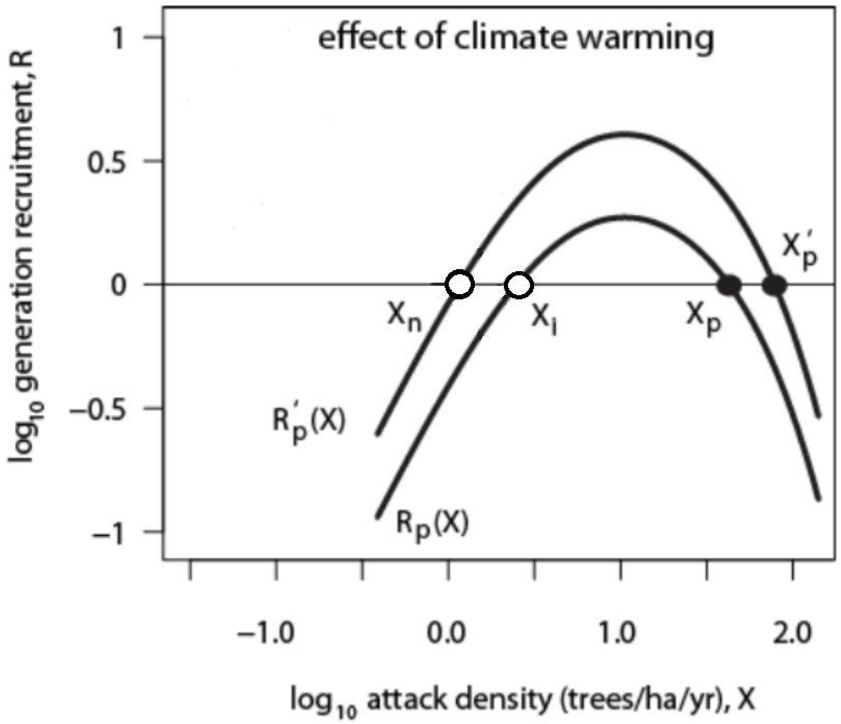


Figure 6. The effect of attack density on eruptive potential, and the influence of rising temperature in a cool climate. (Adapted from Cooke & Carroll 2017.)

The amount of vertical shift in MPB recruitment response to climate change is given by models of MPB climatic suitability, such that lower climatic suitability results in a downward shift of the curve, and thus a decrease in eruptive potential.

In summary, the base Boone-MacQuarrie red-top recruitment function's behaviour is regulated by the fast variables of cooperation, competition, and sudden changes in soil moisture affecting host-tree defenses. Weather is a fast stochastic variable affecting beetle winter and summer survival survival; however these same processes will respond slowly to deterministic changes in climate caused by greenhouse gas concentrations.

Red-top spread

Spread is modeled as an asymmetric percolation process, based on the availability of pine and distance to beetle sources. Infested pixels disperse "propagules", which follow a dispersal kernel and settle in pixels based on the proportion pine in the receiving pixel, such that cells with more pine accept more propagules. Pattern Oriented Modeling (POM; *e.g.*, Grimm 2005), a heuristic optimization approach, is used to select the dispersal kernel and its parameters, as well as the parameters associated with asymmetric spread, based on the MPB attack data. The POM-fit parameterization is then used for projecting MPB spread into the future. Currently, the module implements a negative exponential dispersal kernel (others will be implemented) and we use use the *spread2* function from the *SpaDES.tools* package (Chubaty and McIntire, 2017b) to disperse propagules.

The final implementation of the spread model is currently being tested. Results using this model will be included in an updated version of this report.

MPB management

We employ a simple module of MPB management based on the detection probability of newly infested pixels. Our initial explorations (currently in progress) assume new attacks are detected with 60% probability, and infested trees removed with 100% efficacy. This closely matches the current AB management policy implementation. We are evaluating the impacts of these two management parameters on MPB spread using several metrics, including changes in spread rates. Future simulations may examine more direct economic impacts such as volume loss of merchantable timber, and thus be integrated with economic models (modules).

The final implementation of the management scenario analyses are being tested. Results using this model will be included in an updated version of this report.

Results

Data import and preprocessing

All data import modules use original data sources and are scripted to automatically extract, crop, and reproject the geospatial data into the formats suitable for use within the *SpaDES* simulation model. Data are downloaded from web sources where possible, and file checksums verified to ensure data download integrity. Most of the GIS operations are cached to disk to speed up future invocations of the model (i.e., once these operations have been done for a particular study region they do not need to be rerun in subsequent calls). If any of the datasets should change (e.g., by updating the MPB attack data to include 2017 and beyond), the modules detect these changes and can automatically incorporate the updated data into the simulations. Thus, the model structure enables (indeed, expects) ongoing updates to data inputs.

Climate suitability models

We have assembled data for 7 MPB climate scenarios using each of the four MPB climatic suitability indices under two climate change scenarios (RCP 4.5 and 8.5) and developed a *SpaDES* module to import these data into our simulations (see Figure 7). At time of writing, a comparison of model results with respect to the choice of climate index and climate change scenario used has not been completed. These will be included in an updated version of this report.

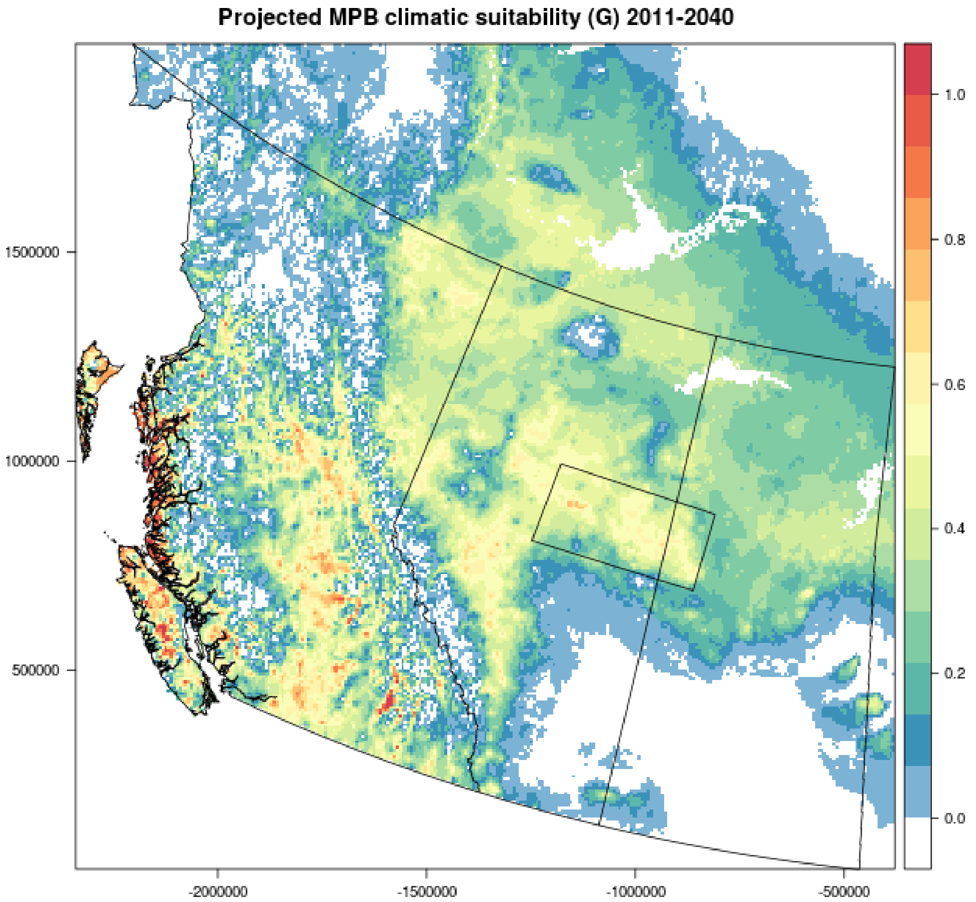


Figure 7. Projected MPB climatic suitability index (G) 2011-2040, with Canadian provincial boundaries and study area overlaid.

MPB red attack

Having assembled the red attack data for MPB in BC and AB, we have developed a *SpaDES* module that imports these data into the simulation as inputs for estimating MPB population growth and spread (see Figure 8).

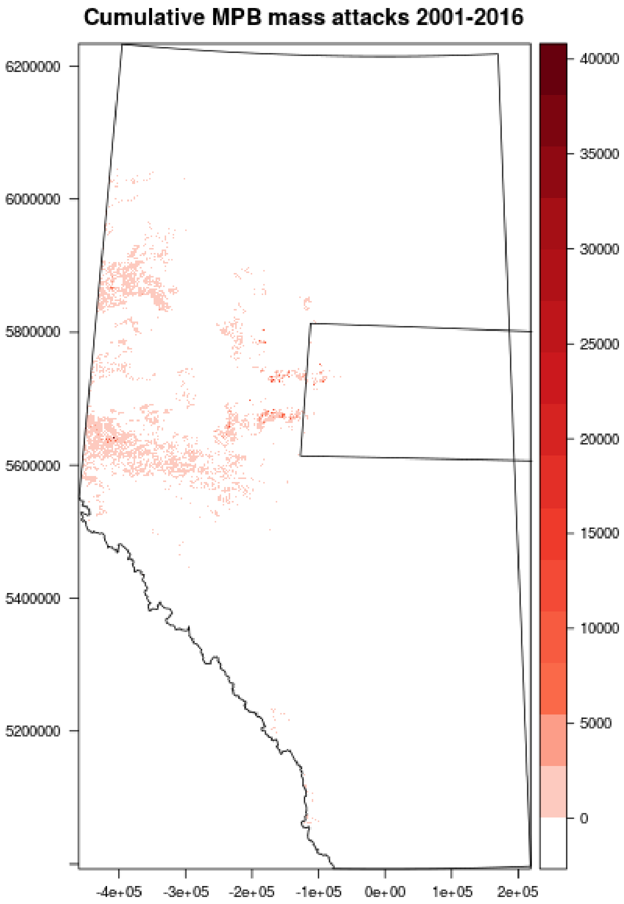


Figure 8. Cumulative MPB red attack data for Alberta from 2001-2016. Note that the values in AB are not nearly as large as those in BC, and thus appear in lighter shades of red.

Pine abundance

Pine availability layers for lodgepole pine and jack pine are imported for use as inputs to the MPB growth and spread modules see Figure 9). The model can be parameterized differently for each pine species. At time of writing, we have not completed this two-species parameterization. We will include this in an forthcoming update to this report.

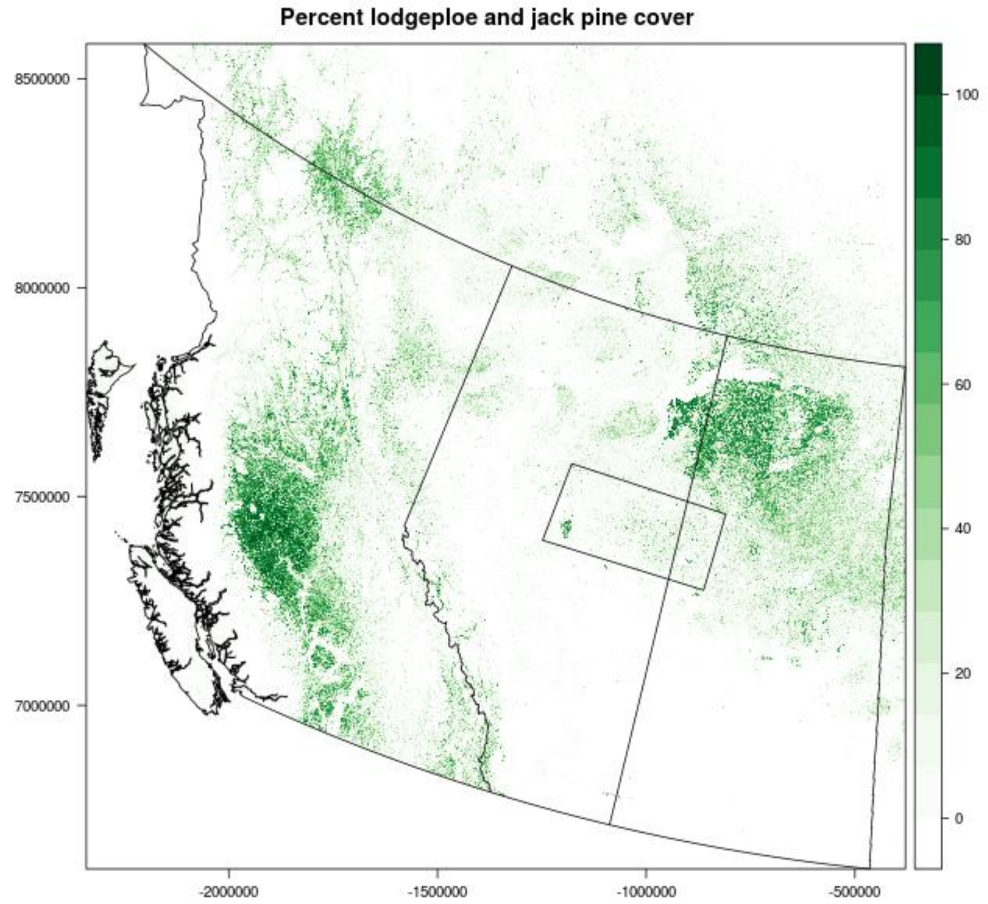


Figure 9. Percent lodgepole and jack pine cover (data from Beaudoin et al. 2014). Separate data layers are used for each species in the model.

Climate modeling and MPB population growth forecasting

With warm temperatures continuing through this winter of 2017-2018 there is no relief predicted for the hindflank region of the foothills, where temperatures have failed to drop below -30°C. Survival rates in Jasper and Hinton are predicted to exceed 50%, leading to a critical change in MPB population status in the foothills (Figure 10), much as was warned in Cooke et al. (2013) and Cooke & Carroll (2017):

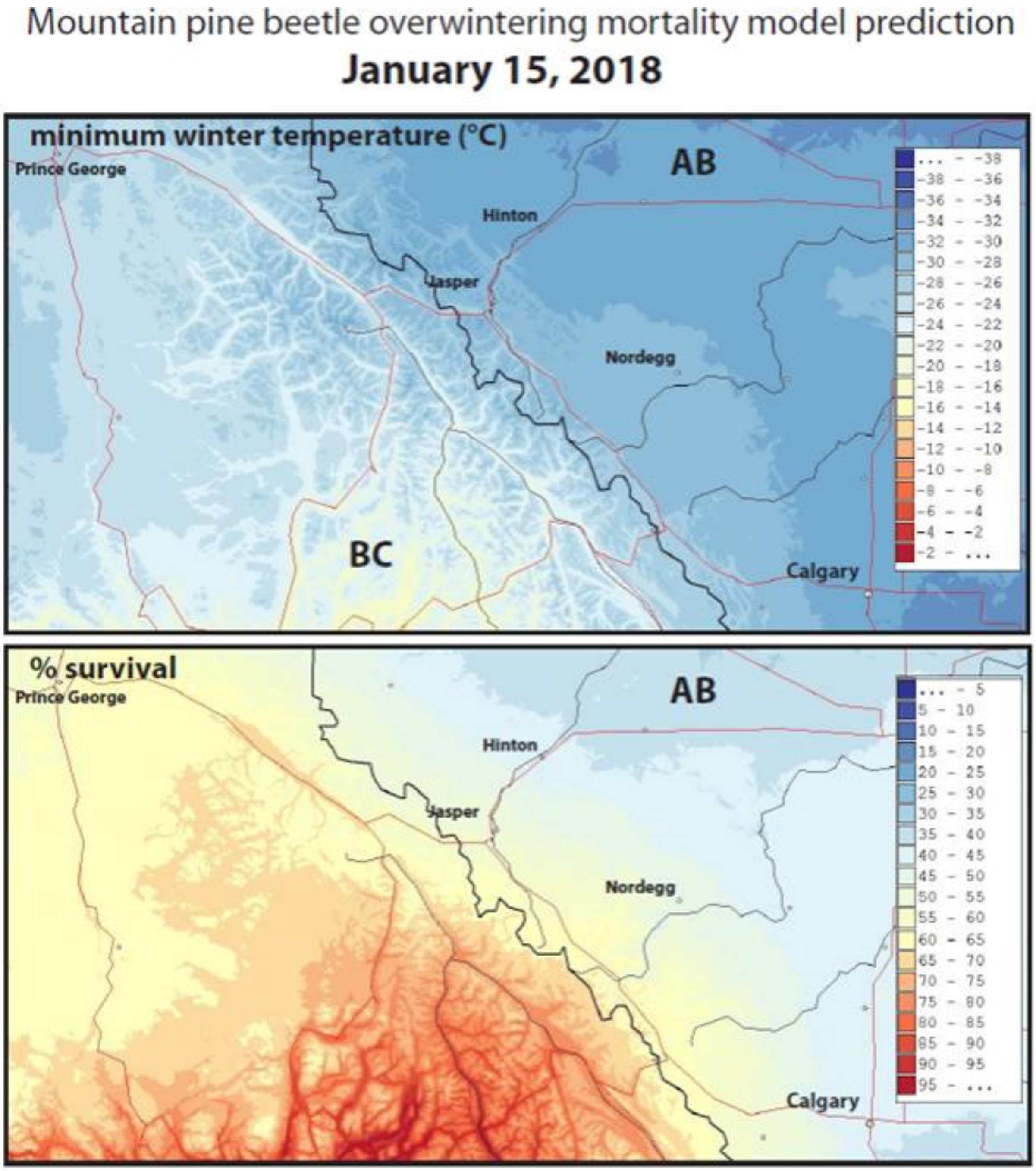


Figure 10. Observed temperatures and predicted MPB overwintering mortality in Jasper National Park and Hinton district prior to the 2017 field season. Survival rates as high as 50% will only serve to accelerate MPB growth through the Yellowhead/Foothills region.

Meanwhile, MPB on the leading edge of the invasion front, in the Alberta-Saskatchewan border region, have taken both steps forward (2014-2016) and steps back (2016-2017). MPB had been captured at baited sites, on jack pine, in 2015 and 2016, with the number of sites doubling from 3 to 6. However in 2017 this worrisome trend abated, with no captures on the CLAWR (Figure 11).

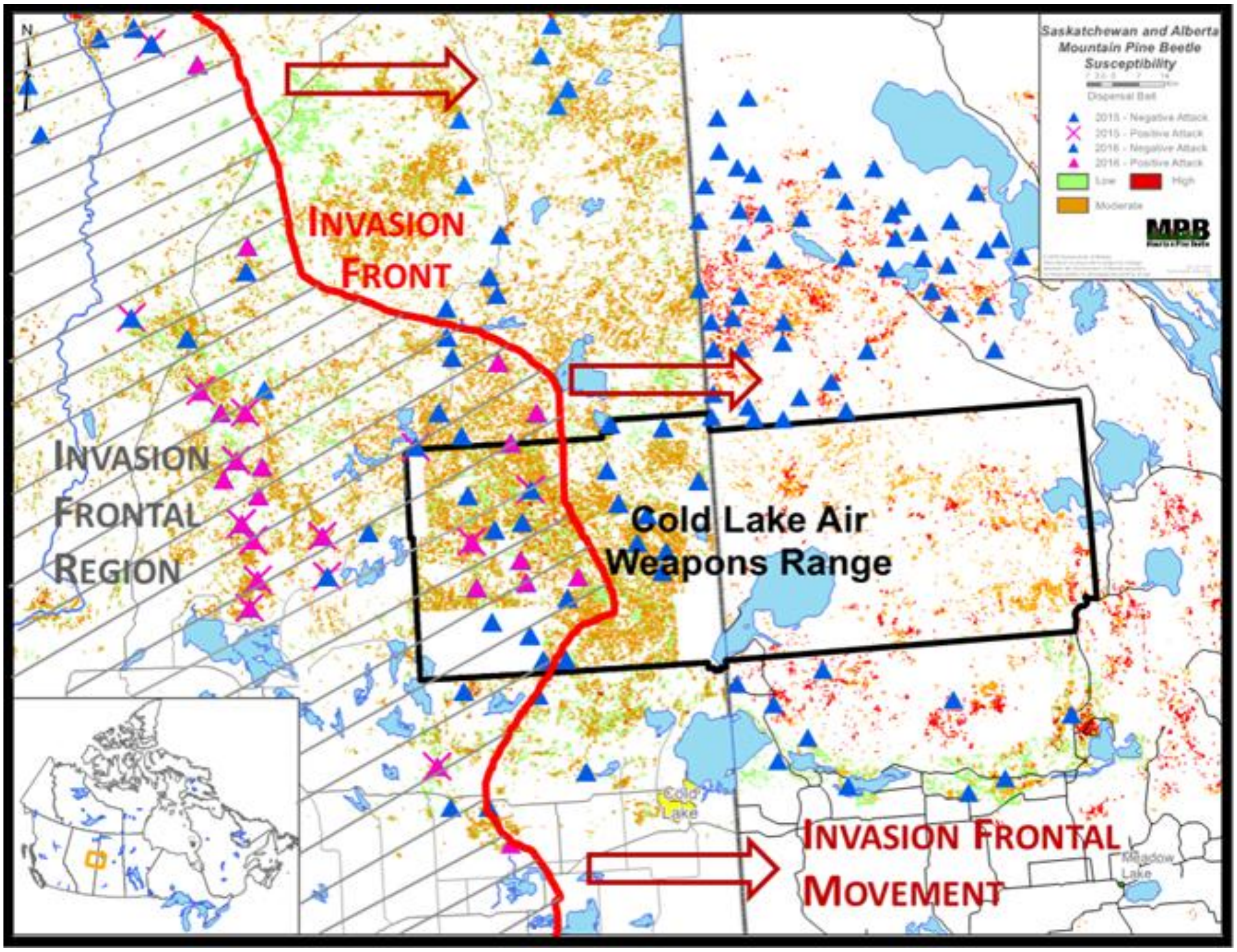


Figure 11. MPB status on the leading edge of the invasion front near Cold Lake, Alberta.

This is consistent with AAF observations that baited and natural attacks on jack pine in the Lac La Biche area are not leading to eruptive establishment. Populations appear to be stably endemic or incipient, not having changed since they first arrived in the summer of 2009. This failure to erupt in jack pine is occurring despite the warm conditions favourable for winter survival and summer development. Our surmise is that this is the effect of low numbers of attackers, as argued in Cooke & Carroll (2017), which trumps the positive effect of climate warming. This is, at least in some part, also due to the efforts of the Spread Management Action Collaborative (SMAC) in reducing living MPB populations along the leading edge zone.

The regional-scale forecasts for MPB overwintering survival current to 15 January 2018 show a familiar gradient of much lower survival rates to the North and East of the BC-Alberta Rocky Mountains and Foothills region (Figure 12). Note that existing populations of MPB in Cypress Hills (south end of the AB-SK border) are predicted to have pockets of >60% survival.

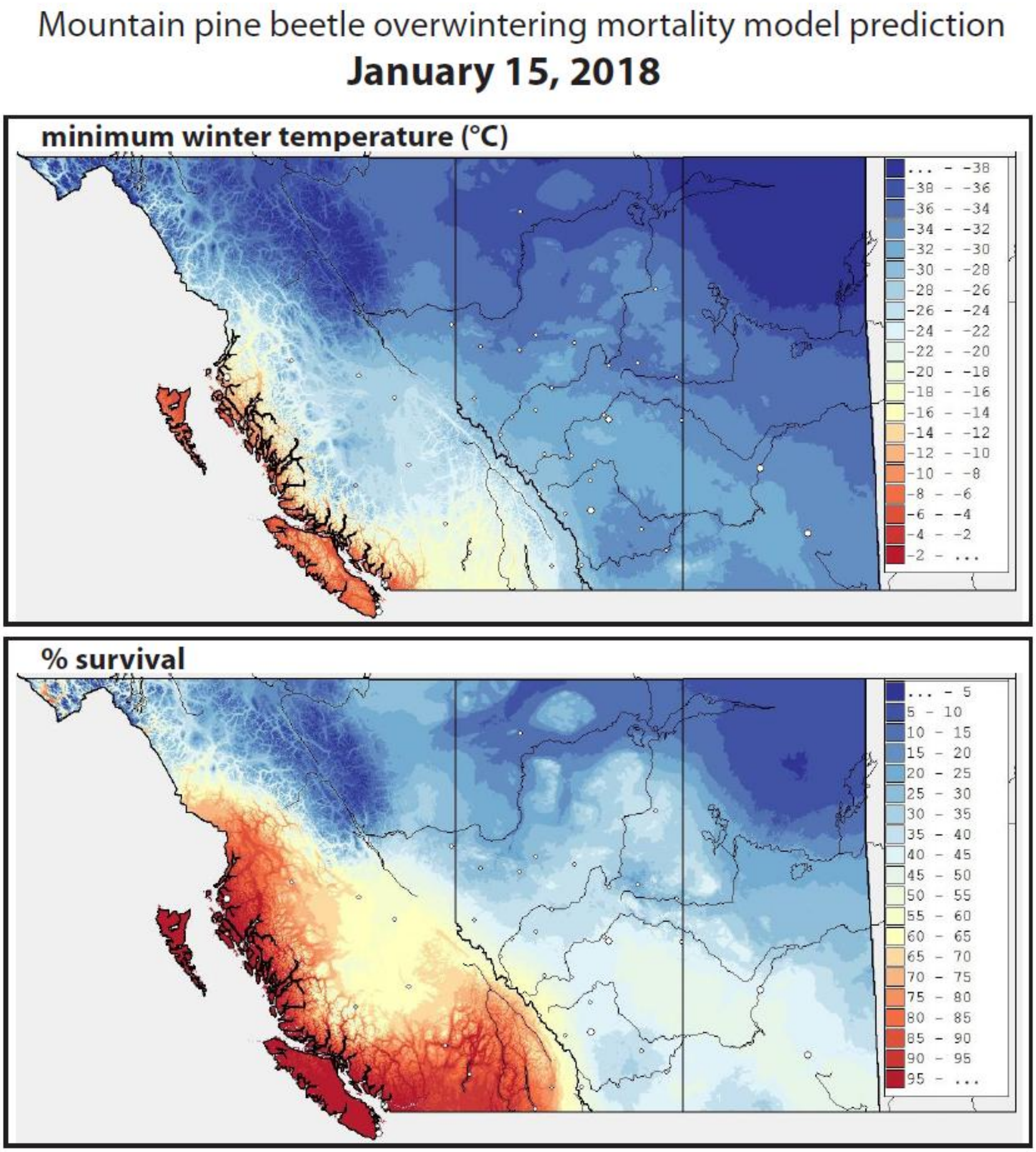


Figure 12. A regional perspective on the mild winter of 2017-2018.

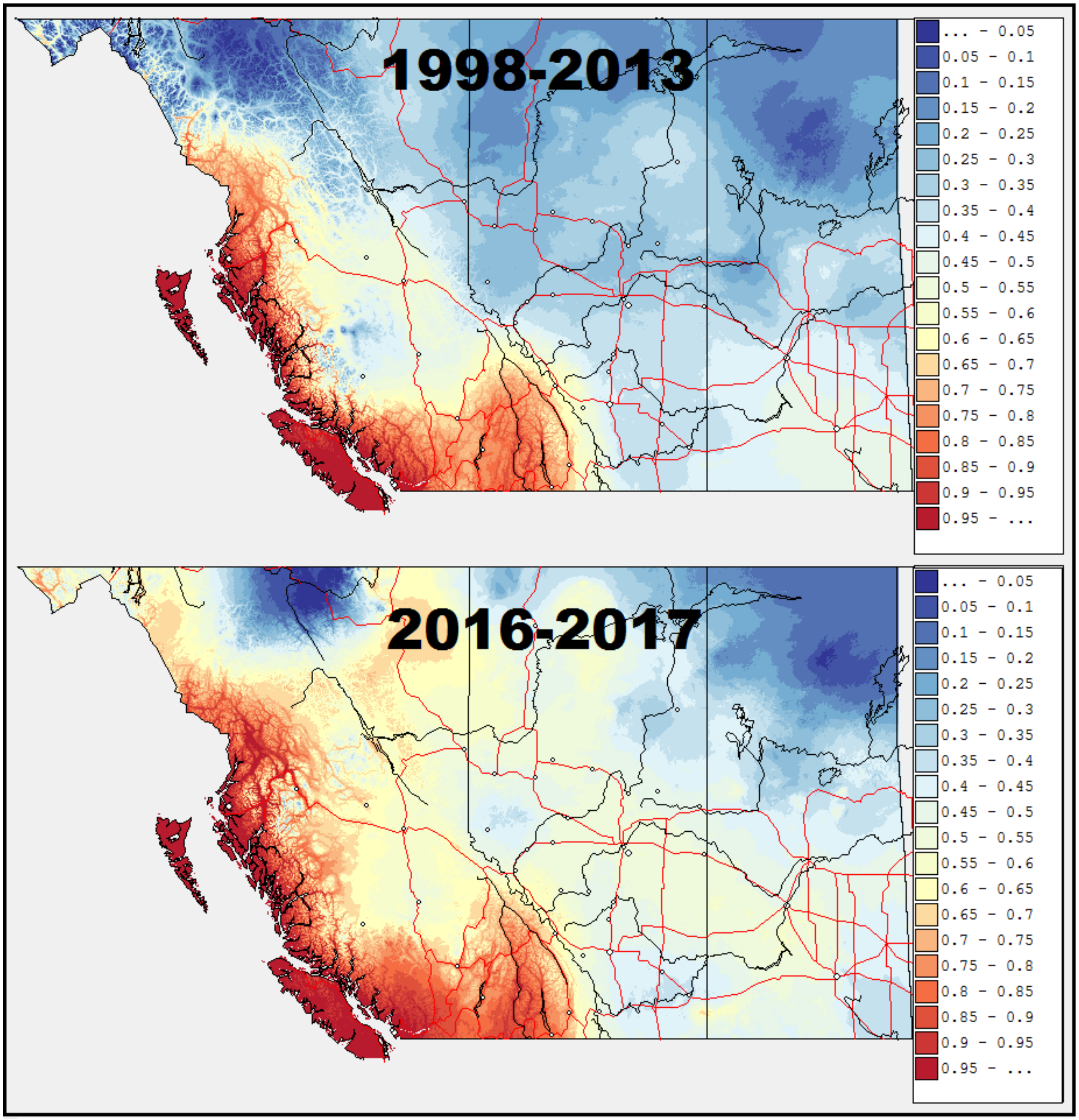


Figure 13. A comparison between predicted winter survival between for the cool temperatures 1998-2013 relative to the record-breaking warm winter of 2016-2017 (see Figure 1 for global mean temperatures in these periods). In fact, all of the last four winters 2014-18 have all been considerably warmer than the previous decade, leading to the unprecedented outbreak of MPB in Jasper and Hinton district. Note that the collapse and retreat of MPB in the AB-SK border region is occurring despite warmer temperatures there.

Figure 13 illustrates the sharp contrast between the cool weather of 1998-2013, where global mean temperatures hardly changed, versus the warm weather of the last three years. Beyond winter temperature, and beyond the past 4-15 years, there appears to be a long-term trend in enhanced MPB survival throughout BC and Alberta (Cooke & Carroll 2017), as illustrated in Figure 12; a trend that is fuelled by accelerating warmth through the last 20 years.

MPB population spread modelling

Depending on the parameterization of our the asymmetric spread algorithm, we can generate ellipsoidal and conical spread from infested pixels within a heterogeneous pine landscape (Figure 14). The final implementation of the spread model is currently being tested. Results using this model will be included in an updated version of this report.

As beetles deplete trees from an attacked stand, this will result in medium-speed changes in recruitment that are not currently known, but may be guessed at. (We have made some preliminary guess based on a simple re-interpretation of MacQuarrie & Cooke (2001), but these are not shown in this report at this time.) As the depleted stand breaks up the slow process of regeneration and succession come into play, and these will affect the long-term prognosis for eruptive growth and spread. These processes are also uncertain, and are left as future work to accomplish. Presently, forest conditions are taken as fixed in the short-run, and we are concerned primarily with the probability of fast eruptive spread in the short-run, and how this prognosis changes under climate change.

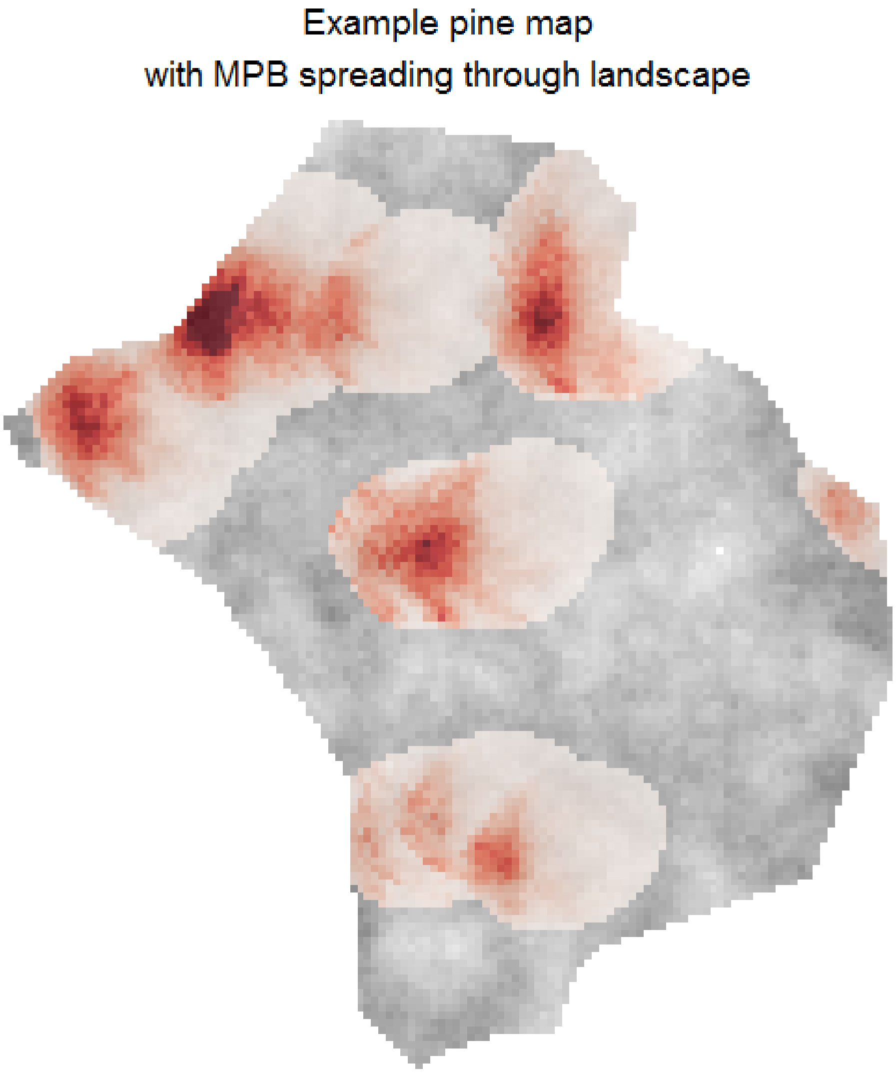


Figure 14. Sample outputs of red-top spread algorithm using negative exponential dispersal kernel and eastward asymmetry. Even with a strong eastward bias, there is backfilling of stands to the west of the original outbreaks.

Project milestones

Informatic and technical milestones

- Development of a reproducible data and simulation workflow;
- Ongoing development and testing of SpaDES-related R packages;
- Submission of SpaDES-related R packages to CRAN;
- Data acquisition, preprocessing, and workflow integration.

Model development milestones

- Development of data import modules (refinement ongoing);
- Development of MPB growth module (refinement ongoing);
- Development of MPB spread module (refinement ongoing);
- Development of MPB management module (refinement ongoing).

Publication and communication milestones

- Cooke and Carroll (2017) formalize the aspatial theoretical growth model;
- Submission of the MPB STS national strategy draft to the NFPS MPB task force;
- Strategic contributions to the National MPB Summit meeting Feb 8-9, 2018;
- Publication of the SpaDES platform (in progress)
- Publication of the MPB spread model (in progress)

Discussion

Workflow

The current implementation of our model benefits from a development process centered around reproducibility and that follows best practices for scientific software development. In particular, the use of version control and collaborative programming via GitHub facilitates frequent incremental updates that are frequently tested. Ongoing and future validation, updates, and extension of this model are simplified by the modular reproducible workflow enabled by the *SpaDES* platform.

Model components can be developed and run independently while also being interoperable with others. This permits addition or removal of model components, allowing *reusability* and *integration* of models across domains. In particular, the data import modules will be of interest to other MPB researchers, and can easily be adapted by others to facilitate data import into other *SpaDES*-based projects. Modules and data can easily be shared and improved by the research community.

A reproducible workflow, facilitated by *SpaDES*, promotes model transparency and accountability through reproducible workflow and enables continuous updates to models as data (or the models themselves) are updated by their experts. Thus, an insect ecologist modeling pest outbreak can make direct use of forest vegetation dynamics, climate suitability, and fire spread modules implemented by experts in those fields, rather than implementing simple “stub” versions of those components themselves. This approach respects the diversity of expert opinion by enabling multiple model comparisons (e.g., testing different dispersal kernels or recruitment curves).

Climate modelling and MPB population growth forecasting

To date we have begun to simulate MPB population growth using the composite (G) index of MPB climatic suitability, and have not yet explored the differences in outcomes when using the other indices. Likewise, we have only considered the Boone 2011 population growth curves, though we are currently building in additional ones. A key consideration of forecasting MPB populations into the future under uncertain climate is the ability to explore a range of climatic scenarios and growth curves, using a model comparison approach. Our integrated modelling approach using *SpaDES* facilitates these sorts of analyses, as well as making it easy to include future additions to this model.

MPB population spread modelling

Our current implementation of MPB spread uses a negative exponential dispersal kernel, which is the simplest kernel for estimating long distance dispersal. Obviously, the addition of other kernels, and a model comparison approach will be invaluable in determining the spread parameters that best fit the available data.

Updates to our model that take these additional considerations into account will be included in a forthcoming updated report.

Management implications

Previous climate modeling results support our model framework and presumption, including the characterization of the problem in the draft national strategy for MPB spread control, which was submitted to the NFPS MPB task force in March of 2017. Specifically, we posit that once the primary necessary condition of high density of attackers has been met, then the secondary consideration of climatic suitability comes into play, and that this will determine whether it takes low or high levels of pest management investment and effort to stabilize and extinguish MPB (Figure 15).

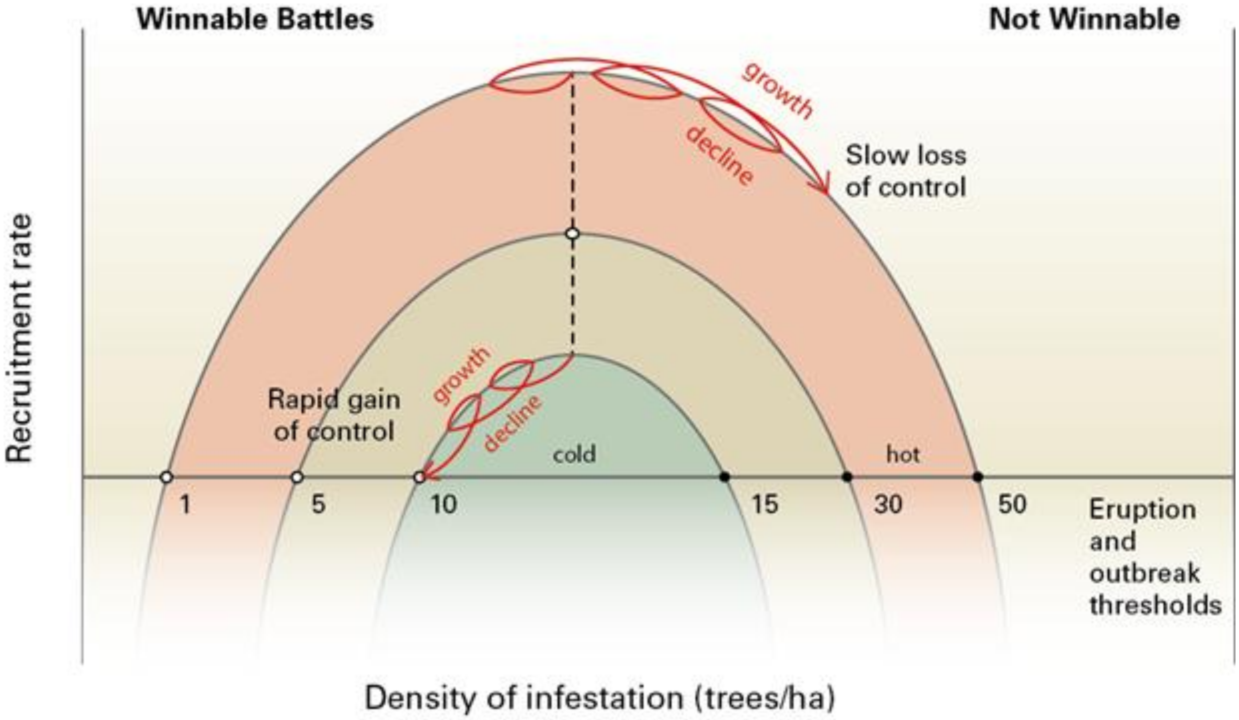


Figure 15. The ability to control MPB given three climate regimes (cold, normal, hot), and a fixed suppression rate of 2/3 of a population. [This rate is realistic for low density MPB on the very leading edge of the invasion front as this rate is ~44% in western Alberta.]

For each year that the population grows (red arrow: rightward jump above recruitment curve), pest managers have the opportunity to remove some fraction of that population (red arrow: leftward jump below recruitment curve), depending on the amount of funding available. The higher the vertical profile of the recruitment curve the more likely it is that annual population growth rates will exceed institutional capacity to remove that growth. Populations following trajectories positioned below the green envelope are readily manageable, leading to “rapid gain of control”. This is because annual removals can exceed annual recruitment. Populations above the gold envelope are not manageable, leading inevitably to “slow loss of control” through the painful process of investing heavily to no avail.

We surmise that as of 2018 Jasper and Hinton have switched from manageable/winnable to unmanageable/not winnable. In contrast, despite the warm weather 2015-2018, leading edge populations in jack pine - not just on CLAWR, but also as far West as Lac La Biche - continue to struggle to establish at high-enough densities to erupt and cause significant tree mortality. This aspatial analysis is what is being implemented spatially, in order to formalize our logic. Our spatially explicit simulations will guide strategic pest management investment with specific spread management scenarios. The working hypothesis still in play is that the prognosis for pest management on the leading edge is attractive, because it represents an opportunity to work with nature, instead of against it.

Future extensions

Our current implementation of boreal MPB spread is an important step in the development of a comprehensive model of eruptive dynamics and forest management. Beyond the current questions we explore here, we have begun validation and testing of the current model and plan to further explore sources of model uncertainty and make model comparisons using different dispersal kernels, growth curves, climate models, and alternative parameterizations. This model, with its modular implementation, can further be extended to include additional drivers such as fire and vegetation dynamics, and consider additional outcomes on wildlife, economics, etc. (see Figure 4).

To facilitate use of the model by others (including non-R users and non-scientists) for scenario analysis, and decision support, we have also begun to develop interactive web apps based on the model and its outputs using the *shiny* (Chang et al. 2017) and *SpaDES.shiny* (Chubaty et al. 2018) R packages. Details of the MPB-SpaDES web app are forthcoming.

Acknowledgements

Data sources

Mountain pine beetle red attack (aerial survey) data provided by BC Ministry of Forests, Lands, Natural Resource Operations & Rural Development and Alberta Agriculture and Forestry.
All other data sources were freely available via hosted internet repositories.

Funding

		Fiscal year 2016-2017	**Fiscal year 2017-2018**
fRI Research:	Mountain Pine Beetle Ecology Program	$65,000.00	$65,000.00
SERG-I:	Saskatchewan MoE	$8,000.00	$8,000.00
	Alberta-AAF	$8,000.00	$8,000.00
	Manitoba-SD	$2,000.00	$2,000.00
	Ontario MNRF	$7,000.00	$7,000.00
TOTAL FUNDING:		**$90,000.00**	**$90,000.000**

fRI Research
Mountain Pine Beetle
Ecology Program

SERG
International

References

Beaudoin, A, P Y Bernier, L Guindon, P Villemaire, X J Guo, G Stinson, T Bergeron, S Magnussen, and R J Hall. 2014. “Mapping attributes of Canada’s forests at moderate resolution through kNN and MODIS imagery.” *Canadian Journal of Forest Research* 44: 521–32. doi:10.1139/cjfr-2013-0401.

Bentz, B J, J Régnière, C J Fettig, E M Hansen, J L Hayes, J A Hicke, R. G. Kelsey, J. F. Negrón, and S. J. Seybold. 2010. “Climate Change and Bark Beetles of the Western United States and Canada: Direct and Indirect Effects.”*BioScience* 60 (8): 602–13. doi:10.1525/bio.2010.60.8.6.

Berryman, Alan A. 1979. “Dynamics of bark beetle populations: analysis of dispersal and redistribution.” *Bulletin of the Swiss Entomological Society* 52: 227–34.

Berryman, Alan A. 1999. *Principles of Population Dynamics and Their Application*. 1st ed. Taylor & Francis.

Carroll, A.L., Taylor S.W., Régnière J. and Safranyik, L. 2004. Effects of climate and climate change on the mountain pine beetle. Pages 221-230 in Shore, T.L., J.E. Brooks and J.E. Stone (eds) Challenges and Solutions: Proceedings of the Mountain Pine Beetle Symposium. Kelowna, British Columbia, Canada October 30 - 31, 2003. Canadian Forest Service, Pacific Forestry Centre, Information Report BC-X-399. 287 p.

Chang, Winston, Joe Cheng, J J Allaire, Yihui Xie and Jonathan McPherson (2017). shiny: Web Application Framework for R. R package version 1.0.5. https://CRAN.R-project.org/package=shiny.

Chubaty, Alex M and Eliot J B McIntire (2017a). SpaDES: Develop and Run Spatially Explicit Discrete Event Simulation Models. R package version 2.0.0. https://CRAN.R-project.org/package=SpaDES.

Chubaty, Alex M and Eliot J B McIntire (2017b). SpaDES.tools: Additional Tools for Developing Spatially Explicit Discrete Event Simulation (SpaDES) Models. https://cran.r-project.org/package=SpaDES.tools.

Chubaty, Alex M, Eliot J B McIntire, Damian Rodziewicz and Mateusz Wyszynski (2018). SpaDES.shiny: Utilities for building 'shiny' apps for 'SpaDES' simulations. http://spades-shiny.predictiveecology.org/.

Cooke, B.J. 2009. Forecasting mountain pine beetle-overwintering mortality in a variable environment. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, BC. Mountain Pine Beetle Working Paper 2009-03. 25 p.

Cooke, B.J. 2013. The hidden role of host defense relaxation in MPB release: a lesson in real-world, low-density nonlinear dynamics. Foothills Research Institute Research Forum. Poster Presentation, Sep 30-Oct 1, Edmonton, AB.

Cooke, B.J., Lux, D., Samis, E., McIntosh, R. 2013. IBFRA Poster Presentation. SMAC: The art and science of mountain pine beetle spread control. Oct 8-10, Edmonton, AB.

Cooke, Barry J, and Allan L Carroll. 2017. “Predicting the risk of mountain pine beetle spread to eastern pine forests: Considering uncertainty in uncertain times.” *Forest Ecology and Management* 396: 11–25.

Croucher, M., Graham, L., James, T., Krystalli, A., & Michonneau, F. 2017. A Guide to Reproducible Code in Ecology and Evolution. In N. Cooper & P.-Y. Hsing (Eds.), BES Guides to Better Science. British Ecological Society.

Evans, M R, Bithell, M, Cornell, S J, Dall, S R X, Diaz, S, Emmott, S, Benton, T G (2013). Predictive systems ecology. Proceedings of the Royal Society B: Biological Sciences, 280(1771), 9. doi:10.1098/rspb.2013.1452.

Fall, A., & Fall, J. (2001). A domain-specific language for models of landscape dynamics. Ecological Modelling, 141(1–3), 1–18. doi: 10.1016/S0304-3800(01)00334-9.

Grimm, V., & Railsback, S. F. (2012). Pattern-oriented modelling: a “multi-scope” for predictive systems ecology. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 367(1586), 298–310. doi:10.1098/rstb.2011.0180.

Knuth, D. E. (1984). Literate Programming. The Computer Journal, 27(2), 97–111 doi: 10.1093/comjnl/27.2.97.

Logan, Jesse A, Jacques Régnière, and James A Powell. 2003. “Assessing the impacts of global warming on forest pest dynamics.” *Frontiers in Ecology and the Environment* 1 (3): 130–37. doi:10.1890/1540-9295(2003)001[0130:ATIOGW]2.0.CO;2.

McIntire, Eliot J B and Alex M Chubaty (2017). reproducible: A Set of Tools that Enhance Reproducibility Beyond Package Management. https://cran.r-project.org/package=reproducible.

MacQuarrie, C.J.K. and Barry J. Cooke, 2011. Density-dependent population dynamics of mountain pine beetle in thinned and unthinned stands. Canadian Journal of Forest Research 41: 1031–1046.

Nealis, Vince G, and Barry J Cooke. 2014. “Risk assessment of the threat of mountain pine beetle to Canada’s boreal and eastern pine forests.” Ottawa, ON: Canadian Council of Forest Ministers.http://cfs.nrcan.gc.ca/publications?id=35406.

Nealis, Vince G, and Brian Peter. 2008. “Risk assessment of the threat of mountain pine beetle to Canada’s boreal and eastern pine forests.” Information Report. Victoria, BC: Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre.

Polasky, S., Carpenter, S. R., Folke, C., & Keeler, B. (2011). Decision-making under great uncertainty: environmental management in an era of global change. Trends in Ecology and Evolution, 26(8), 1–7. doi:10.1016/j.tree.2011.04.007.

Raffa, K. F., and A. A. Berryman. 1983. The role of host plant resistance in the colonization behavior and ecology of bark beetles (Coleoptera: Scolytidae). Ecological Monographs 53:27-50.

Régnière, Jacques, and Barbara Bentz. 2007. “Modeling cold tolerance in the mountain pine beetle, Dendroctonus ponderosae.” *Journal of Insect Physiology* 53 (6): 559–72. doi:10.1016/j.jinsphys.2007.02.007.

Régnière, J.; Cooke, B.J.; Bergeron, V. 1995. BioSIM: a computer-based decision support tool for seasonal planning of pest management activities. User's manual. Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Sainte-Foy, Quebec. Information Report LAU-X-116. 67 p.

Reynolds, J. F. and Acock, B. (1997). Modularity and genericness in plant and ecosystem models. Ecological Modelling, 94(1), 7–16. http://doi.org/10.1016/S0304-3800(96)01924-2.

Safranyik, Les, Allan L Carroll, Jacques Régnière, David W Langor, William G Riel, Terry L Shore, Brian Peter, Barry J Cooke, Vince G Nealis, and Stephen W Taylor. 2010. “Potential for range expansion of mountain pine beetle into the boreal forest of North America.” *The Canadian Entomologist* 142 (5): 415–42. doi:10.4039/n08-CPA01.

Safranyik, L.; Shrimpton, D.M.; Whitney, H.S. 1975. “An interpretation of the interaction between lodgepole pine, the mountain pine beetle, and its associated blue stain fungi in western Canada.” In *Management of Lodgepole Pine Ecosystems Symposium Proceedings*, edited by D M Baumgartner, 406–28. Pullman, WA: Washington State University Coop. Extension Service. http://wfiwc.org/sites/default/files/Safranyik_Shrimpton_Whitney_1975.pdf.

Trzcinski, M.K. and Reid, M.L. 2009. Intrinsic and extrinsic determinants of mountain pine beetle population growth. Agricultural and Forest Entomology 11: 185-196.

Ushey, Kevin, Jonathan McPherson, Joe Cheng, Aron Atkins and J J Allaire (2016). packrat: A Dependency Management System for Projects and their R Package Dependencies. R package version 0.4.8-1. https://CRAN.R-project.org/package=packrat.

Whitehead, R. J. & G. L. Russo. 2005. "Beetle-proofed" lodgepole pine stands in interior British Columbia have less damage from mountain pine beetle. Information Report BC-X-402. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria BC.

Wilson, G;, Aruliah, D.A.; Brown, C.T.; Chue Hong, N.P.; Davis, M.; Guy, R.T.; Haddock, S.H.D.; Huff, K.D.; Mitchell, I.M.; Plumbley, M.D.; Waugh, B.; White, E.P.; Wilson, P. (2014). Best practices for scientific computing. *PLoS Biology*, *12*(1), e1001745. doi:10.1371/journal.pbio.1001745.

Effect of trap height, trap color, and trap lure on efficacy of detecting species of bark- and wood boring beetles (Cerambycidae, Buprestidae, Scolytinae) – SERG Progress Report, January 2018

Jon Sweeney[1], Peter Silk[1], Peter Mayo[1], Gaetan Leclair[1], Cory Hughes[1], Kate Van Rooyen[1], Jerzy M. Gutowski[3], Tomasz Mokrzycki[4], Dan Miller[5], Meng Qingfan[6], Li Yan[6], & Joe Francese[7]

[1]NRCan-Canadian Forest Service, Fredericton, NB
[2]NRCan-Canadian Forest Service, Sault Ste. Marie, ON
[3]Forest Research Institute (IBL), Białowieża, Poland
[4]University of Warsaw, Poland
[5]USDA Forest Service, Athens Georgia
[6]Beihua University, Jilin, China
[7]USDA APHIS PPQ, Buzzards Bay, MA

Abstract. Improved tools and methods for surveillance of potentially invasive bark- and wood boring beetles are critical needs of regulatory agencies because the earlier an invasive species is detected, the more feasible and less costly it is to eradicate, contain, or manage. We ran several field trapping experiments in 2017 designed to test the effects of lure, trap color, trap design, and trap height on the efficacy of detecting species of Cerambycidae, Buprestidae and Scolytinae. One of our main goals was to screen recently discovered longhorn beetle pheromones [10-methyldodecanal, racemic 2-hydroxyoctan-3-one, *syn*-octanediols, and 1-(1H-pyrrol-2-yl)-1,2-propanedione (hereafter referred to as pyrrole)] for their detection efficacy in the field in northeast China and Poland. 10-Methyldodecanal was weakly but significantly attractive to *Stictoleptura maculicornis* in Poland but attracted no species in Jilin. 2-Hydroxyoctan-3-one attracted two longhorn species Poland (*Plagionotus detritus, Xylotrechus antilope*) and *Plagionotus pulcher* in Jilin. The pyrrole was not significantly attractive to any species but boosted catches of four longhorn species when combined with ethanol or 3-hydroxyoctan-2-one plus ethanol. Baiting traps with *syn*-octanediols significantly increased the proportion of traps positive for *Rhaphuma acutivittis* in Jilin. Another goal of ours is to determine the optimal combination of lures, trap color, and trap placement that detects the most species of bark- and wood boring beetles at a given site, and to determine how that may vary among different forest types. Results from the factorial experiment conducted in Italy in 2016 clearly indicated that green traps in the canopy detect more buprestid species and more target species (longhorns and jewel beetles) overall than other color-height combinations and that the multicomponent pheromone lure detected more target species than did traps baited with ethanol alone. We also determined release rates and field longevity of new release devices for fuscumol and found that grey halobutyl septa soaked in 10% fuscumol may be equally efficacious and less expensive than the standard top-loaded red septa lure.

Résumé. Il est essentiel pour les organismes de réglementation d'améliorer leurs méthodes et outils de surveillance des coléoptères perceurs du bois potentiellement envahissants, car plus une espèce envahissante est détectée tôt, plus il est faisable et économique de l'éradiquer, de la contenir ou de la gérer. Nous avons mené plusieurs expériences de piégeage sur le terrain en 2017 pour tester les effets de l'appât utilisé, de la couleur du piège, du design du piège et de la hauteur du piège sur l'efficacité de la détection d'espèces de Cerambycidae, de Buprestidae et de Scolytinae. L'un des quatre principaux objectifs était d'étudier des phéromones du longicorne récemment découvertes [10-methyldodecanal, 2-hydroxyoctan-3-one racémique, *syn*-octanediols, et 1-(1H-pyrrol-2-yl)-1,2-propanedione (ci-après appelé pyrrole)] pour connaître leur efficacité de détection sur le terrain dans le nord-est de la Chine et en Pologne. La substance 10-Methyldodecanal a eu un pouvoir attractif faible mais significatif sur *Stictoleptura maculicornis* en Pologne, mais n'a attiré aucune espèce dans la province de Jilin, alors que 2-Hydroxyoctan-3-one a attiré deux espèces de longicornes en Pologne (*Plagionotus detritus, Xylotrechus antilope*) et *Plagionotus pulcher* à Jilin. Le pyrrole n'a attiré aucune espèce de manière importante, mais a quand même accru les prises de quatre espèces de longicornes lorsque combiné à de l'éthanol ou à 3-hydroxyoctan-2-one plus de l'éthanol. Des pièges appâtés avec *syn*-octanediols ont accru de manière importante la proportion de pièges ayant attrapé l'espèce *Rhaphuma acutivittis* dans la province de Jilin. Un autre de nos objectifs était de déterminer la combinaison optimale d'appât, de couleur de piège et de positionnement du piège pour détecter le plus d'espèces de coléoptères perceurs du bois à un site donné, et de voir comment les différentes combinaisons peuvent donner des résultats qui varient selon le type de forêt. Les résultats de l'expérience factorielle menée en Italie en 2016 montrent clairement que dans l'ensemble, les pièges verts dans le couvert forestier détectent plus d'espèces de buprestidés et plus d'espèces cibles (longicornes et buprestes) que les autres combinaisons de couleurs et de hauteurs, et que les leurres imbibés de plusieurs composants de phéromone détectent plus d'espèces cibles que les pièges appâtés uniquement avec de l'éthanol. Nous avons également déterminé les taux de libération et la longévité sur le terrain de nouveaux dispositifs de relâchement du fuscumol et constaté qu'un septum d'halobutyle gris trempé dans une solution de 10 % de fuscumol est peut-être tout aussi efficace comme appât qu'un septum rouge chargé par le haut.

Introduction

Exotic bark and wood boring beetles, particularly species in the families of Cerambycidae, Buprestidae, and Curculionidae, include some of the most damaging forest pests. In spite of international phytosanitary regulatory policies like ISPM 15, bark- and woodboring insects continue to be intercepted in solid wood packaging in North America and abroad. Surveillance for non-native forest insects in North America relies partly on surveys using black funnel traps and plant volatile lures (ethanol, α-pinene) that attract a broad range of bark and wood boring species. In recent years the Canadian Food Inspection Agency (CFIA) has baited their surveillance traps with multi-lure combinations of longhorn beetle pheromones and host volatiles that have been shown to synergize attraction of a number of species that might otherwise go undetected in traps baited with host volatiles alone. Many more opportunities exist for improving the efficacy of invasive woodborer trapping surveys. For example, green and/or purple traps are much more effective than black traps at detecting buprestids, especially *Agrilus* spp. (Crook et al. 2009, Francese et al. 2005, Rhainds et al. 2017), and traps in the tree canopy often detect species missed in the understory (Dodds 2014, Graham et al. 2010).

Our research has focused on learning the effects of multi-lure combinations, trap designs, trap height, and trap colors on the species richness and abundance of target taxa (i.e., cerambycids, buprestids, scolytines, siricids moved in solid wood packaging) in traps. Factorial experiments allow us to determine positive and negative interactions among various factors and the trapping methods that deliver the greatest detection efficacy per trapping effort. By replicating field experiments in Europe and China as well as North America, we directly determine the efficacy of new vs. currently operational survey methods for detecting non-native species at risk of becoming invasive pests in Canada and the US. The outcomes of this research are tools, knowledge, and methods that can be directly applied in operational surveillance programs to improve early detection of non-native and potentially invasive bark- and wood boring beetles. This research has been well supported by SERG-I partners ($25K, $66.3K, $65K, $20K, $12K, and $9K in in 2012–13, 2013–14, 2014–15, 2015–16, 2016–17, and 2017–18, respectively) as well as by NRCan-CFS, CFIA, the Atlantic Innovation Fund for pheromone commercialization (ACOA) and USDA APHIS PPQ.

Methods

We conducted eight trapping experiments in 2017. We tested the trapping efficacy of four recently identified cerambycid pheromones, alone and in combination with ethanol, in four separate experiments in China, Poland, and New Brunswick (NB), Canada in 2017: 10-methyldodecanal, racemic 2-hydroxyoctan-3-one, *syn*-octanediols, and 1-(1H-pyrrol-2-yl)-1,2-propanedione (hereafter referred to as pyrrole). We also tested the effect of trap color (green vs. purple vs. green&purple 2-tone traps) and trap height (understory, mid-upper canopy) on detection of cerambycids and buprestids in both NB and Massachusetts. We repeated an experiment comparing detection efficacy of ketol and hexanediol lures from different commercial suppliers (Synergy, Chemtica, Contech), and we compared the efficacy of Synergy's new "multi-trap" design funnel traps *vs.* Lindgren 12-funnel traps.

We also developed a more efficient method for impregnating rubber septa with fuscumol (by soaking septa in 10% or 30% solutions of fuscumol in solvent, rather than top loading individual septa by pipette) and tested grey halobutyl rubber septa (West Pharmaceutical Services, Exton, PA) as a cheaper alternative to red rubber septa (Wheaton, Millville, NJ) for release of fuscumol. We quantified their release rates at constant temperatures in the lab as well as at fluctuating temperatures outdoors, and determined their efficacy and field longevity for detection of *Tetropium* spp. and other longhorn species.

All trapping experiments were replicated in randomized complete blocks with at least 20–30 m spacing between traps and blocks. Fluon-coated Lindgren 12-funnel traps (Allison et al. 2011; Graham et al. 2010) were used at all sites. All collecting cups contained either a solution of ethylene glycol and water (Poland) or saturated salt solution. Specimens were collected every 2 weeks and specimens of Cerambycidae, Buprestidae and Scolytinae identified to species wherever possible. Voucher specimens have been retained in the collections of: Atlantic Forestry Centre; USDA APHIS PPQ, Buzzards Bay; Forest Research Institute of Białowieża, Poland; Beihua University, Jilin, China; and University of Padua, Italy.

Data analysis. Count data (e.g., total number of specimens of a given species per trap for the duration of the experiment, or number of different species of a target taxa (e.g., buprestids) captured per trap) were analyzed with generalized linear mixed models (PROC GLIMMIX) using SAS/STAT software (SAS/STAT, Version 9.2 of the SAS System for Windows. Copyright © 2002–2008). Trap color, lure treatment, and trap height were designated as fixed effects and blocks were designated as random effects. Models were run using Gaussian, Poisson and negative binomial distributions (log link) with the Laplace method of estimating model parameters, as recommended for count means that are sometimes <5 (Bolker et al. 2008). Results are reported from the model with the best fit (i.e., lowest AICc value, this was usually either the Poisson or the negative binomial). For species trapped in low numbers (e.g., <20 specimens) we used Cochran's Q chi square test to compare the detection rate (proportion of traps positive) among the various trap treatments. EstimateS version 9 software (Colwell 2013) was used to generate Coleman rarefaction curves that show the mean number of species detected per sample size (number of traps per site) for each trap treatment (e.g., green traps placed in the canopy) or combination of different trap treatments (e.g., green traps in the canopy plus purple traps in the understory).

Results

All longhorn beetles as well as the buprestids from Canada and Poland from 2017 trapping experiments have been identified to species and counted. Scolytinae ID is in progress.

Efficacy of new semiochemicals for detecting bark- and wood boring beetles in Jilin, Poland, and Canada. Species previously shown to be attracted to 3-hydroxyoctan-2-one in Poland (*Plagionotus detritus, Xylotrechus antilope*) and China (*Plagionotus pulcher*) were also attracted to 2-hydroxyoctan-3-one (Tables 1, 2, Figs 1, 2). There was a significant interaction between pheromone lure and ethanol on catch of *Agrilus laticornis* in Poland, in which the addition of ethanol increased catch in traps baited with 2-hydroxyoctan-3-one but decreased catch in traps baited with *syn*-2,3-octanediols. A similar but weaker trend was apparent for *Agrilus sulcicollis* and *Agrilus bigutattus* in which the addition of ethanol reduced catches for all pheromone treatments except 2-hydroxyoctan-3-one. Overall, ethanol caused a marginally significant (P = 0.08) decrease in number of buprestid species detected per trap (Fig. 1). This experiment bears repeating with a larger sample size to confirm results.

The pyrrole was not significantly attractive to any species in Jilin but boosted catches of four longhorn species when combined with ethanol or 3-hydroxyoctan-2-one plus ethanol (Table 2, Fig. 2). 10-methydodecanal was weakly but significantly attractive to the lepturine, *Stictoleptura maculicornis,* in Poland, detecting it in 3 of 6 trap blocks (Cochran's Q = 16, P < 0.05) but attracted no species in Jilin. Baiting traps with *syn*-octanediols significantly increased the proportion of traps positive for *Rhaphuma acutivittis* in Da Shi, Jilin, China (Table 3, Fig. 3). Note: males of *R. acutivittis* have the typical pore fields on their pronotum associated with pheromone glands (Li et al. 2013) but this was the first time we tested octanediols in Jilin and the first time we trapped significant numbers of *R. acutivittis*. In Poland, ethanol significantly enhanced attraction of *Leiopus linnei* (Fig. 1) but decreased catches of several *Agrilus* species (Table 1). In China, ethanol significantly enhanced catches of the longhorn species *P. pulcher, Neocerambyx raddei,* and *Mesosa myops* (Table 2, Fig. 2).

Optimal lure combinations, trap color, and trap placement for maximum number of species of bark- and wood boring beetle species detected.

Results from the 2 x 2 x 2 (trap color x trap height x lure) factorial experiment conducted in broadleaf plantations and natural woodlands in Italy in 2016 clearly indicated that green traps in the canopy detect more buprestid species and more target species (longhorns and jewel beetles) overall than other color-height combinations (Figs. 4, 5). Furthermore, the multicomponent lure that included hydroxyketones, hexanediols, fuscumol, and fuscumol acetate and ethanol detected more target species than did traps baited with ethanol alone (Figs, 4, 6).

Alternate release devices of fuscumol

Release rates. Initial release rates measured indoors showed an exponential decline in release rate of fuscumol over time for both grey and red rubber septa, starting at 9-12mg/day for the red 30%, 6-8mg/day for the red 10%, 5-6mg/day for the grey 30% and 2-3mg/day for the grey 10%. By day 73, daily release rates had dropped to 2mg, 1mg, 1mg and 0.5mg respectively. In contrast to the exponential decline in release rates from septa measured indoors, the daily release rate of lures measured outdoors remained constant over the first 50 days and dipped only slightly after 63 days. Rates were 4.2mg/day for 30% red, 2.2mg/day for 10% red, 1.4mg/day for grey 30% and 0.5mg/day for grey 10%.

Efficacy at detecting Tetropium fuscum. All rubber septa (grey 10%, grey 30%, red 10% red 30% fuscumol) as well as red septa top-loaded with 50 mg fuscumol (standard Sylvar lure) captured significantly more *T. fuscum* than did traps baited with only UHR ethanol and UHR BSLB kairomone lures, but there were no differences in mean catch among the different septa treatments ($P > 0.05$). Results suggest that the grey halobutyl septa soaked in 10% fuscumol may be equally efficacious and less expensive than the current red septa top-loaded lure. Additional tests will be conducted in 2018 but with *E/Z*-fuscumol and *E/Z*-fuscumol acetate lures, targeting efficacy at detection of Laminae species.

Reporting of results (Presenter's name underlined)

Presentations at Workshops and Conferences:

Allison, J.D., Bouwer, M., Strom, B. and J. Sweeney. Factors Affecting Trap Performance for Longhorned Beetles. ORAL presentation at the Joint meeting of IUFRO Working Parties 7.03.05 and 7.03.10. 11-15 September, 2017 in Thessaloniki, Greece.

Flaherty, L., Pohl, G., Gutowski, J., Mayo, P., Mokrzycki, T., Silk, P., Webster, R. and Sweeney, J. Use pheromone-enhanced lures & place traps in both the forest canopy and understory to improve exotic bark & wood borer detection. (ORAL presentation by Leah Flaherty). Joint Annual Meeting of the Entomological Society of Canada & Entomological Society of Manitoba, 22–25 October 2017, Winnipeg, MB.

Miller, D., Dodds, K. and Sweeney, J. Factors affecting catches of hardwood borers: Need for changes in detection programs for exotic species. (ORAL presentation by Dan Miller). Western Forest Insect Work Conference, 1–5 May, 2017, Jackson, WY.

Rassati, D., Faccoli, M., Marini, L., Rapuzzi, P., Poloni, P., Battisti, A., and Sweeney, J. The effect of forest landscape, trap height, trap color and lure on longhorn beetle communities detected in survey traps. (ORAL presentation by Davide Rassati). IUFRO 125th Anniversary Congress, 18-22 September 2017, Freiburg, Germany.

Sweeney, J., P. Silk, P. Mayo, R.P. Webster, D.R. Miller, C. Hughes, C. Crowe, K. Ryall, J.M. Gutowski, T. Mokrzycki, J. Francese, Q. Meng, Y. Li, and T. Kimoto. Detection of bark- and wood boring beetles is increased by using a diversity of trap colors and heights and is decreased by *Z*-3-hexenol. POSTER presented at the 28th U.S. Department of Agriculture Interagency research forum on Invasive species, 10–13 January 2017, Annapolis, MD (displayed by David Gray) and the Atlantic Canada Forest Health Workshop, 17–18 January 2017, Charlottetown, PEI.

Sweeney, J., P. Silk, P. Mayo, C. Hughes, K. Van Rooyen, J.M. Gutowski, T. Mokrzycki, Improving surveillance of potentially invasive wood boring beetles: results from recent field trials. ORAL presentation made at the Atlantic Canada Forest Health Workshop, 17–18 January 2017, Charlottetown, PEI.

Sweeney, J., Silk, P., Webster, R., Flaherty, L., Pohl, G., Hughes, C., Cab Rooyen, K., Mayo, P., Gutowski, J.M., Mokrzycki, T., Miller, D., Ryall, K., Meng, QQ., Yan, L., and Francese, J. Using a diversity of trap heights, lures, and colors increases species richness in survey traps for wood boring beetles. (ORAL) IUFRO Joint Working Parties 7.03.05 and 7.03.10, 11–17 September 2017, Thessaloniki, Greece.

Sweeney, J., Silk, P., Webster, R., Hughes, C., Mayo, P., Gutowski, J.M., Mokrzycki, T., Miller, D., Ryall, K., Meng, QQ., Yan, L., and Francese, J. Recent improvements in trapping methods for surveillance of bark and wood boring beetles. (ORAL) IUFRO 125th Anniversary Congress, 18-22 September 2017, Freiburg, Germany.

Sweeney, J., Silk, P., Mayo, P., Webster, R., Miller, D., Hughes, C., Gutowski, J.M., Ryall, K., Mokrzycki, T., Meng, QQ., Yan, L., T Francese, J., Flaherty, L., Pohl, G., and Rassati, D. What combinations of semiochemicals, trap placements and trap colors have the greatest efficacy for detecting cerambycids and other target taxa in exotic woodborer surveys? (ORAL) Annual meeting of the Entomological Society of America, 5–8 November 2017, Denver, CO.

Van Rooyen, K., C. Hughes, V. Webster, C. Alderson, and J. Sweeney. Gotcha! The effect of trap color on catch in a mixed wood forest in Halifax, Nova Scotia. ORAL presentation made at the Atlantic Canada Forest Health Workshop, 17–18 January 2017, Charlottetown, PEI.

Publications:

Yan Li, Q. Meng, P. Silk, W. Gao, P. Mayo, and J.D. Sweeney. 2017. Effect of semiochemicals and trap height on catch of *Neocerambyx raddei* (Coleoptera: Cerambycidae) in Jilin province, China. Entomologia experimentalis et Applicata 164: 94–101.

Miller, D.R., J. Allison, C. Crowe, D. Dickinson, A. Eglitis, R. Hofstetter, S. Munson, T. Poland, L. Reid, B. Steed, and J. Sweeney. 2016. Pine sawyers (Coleoptera:

Cerambycidae) attracted to α-pinene, monochamol and ipsenol in North America. J. Econ. Entomol. 109: 1205–1214.

Miller, D.R., C.M. Crowe, P.D. Mayo, L.S. Reid, P.J. Silk, and J.D. Sweeney. 2017. Interactions between ethanol, syn-2,3-hexanediol, 3-hydroxyhexan-2-one, and 3-hydroxyoctan-2-one lures on trap catches of hardwood longhorn beetles in Southeastern United States. Journal of Economic Entomology 110: 2119–2128. (doi: 10.1093/jee/tox188).

Monitoring of exotic insect pests at home and abroad. Impact Note. No. 62. Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre – Making a Difference. Cat. No. Fo103-3/62-2016E-PDF (Online).

We also completed analyses of data from several trapping experiments conducted from 2014 to 2016, on effects of trap height, lure combinations, and trap color on woodborer detection and have two manuscripts that will be submitted on 31 January 2018 for a special issue of the Journal of Pest Science.

Acknowledgements

We thank SERG International, USDA APHIS PPQ, Natural Resources Canada -Canadian Forest Service, the Atlantic Innovation Fund & Acadia University, Ontario Ministry of Natural Resources, Forest Protection Limited, and the Nova Scotia Department of Natural Resources for funding and generous support. We also thank K. Strong and the Acadia Research Forest, NBDNR, Don Murray and the City of Fredericton for permission to trap in Odell Park, The Meduxnekeag Nature Reserve for permission to trap in the Bell Forest, Sara Weatherby and the Maritime College of Forest Technology for permission to trap in the Kingsclear Forest, the Bialowieza National Park, Poland, and the Jiaohe Experimental Area Administration Bureau of Jilin Province, China for permission to conduct experiments on their land. Finally, we thank Chantelle Alderson, Jeremy Allison, J.P. Astorino, Feng Li Chao, Dave Davies, David Dutkievicz, Hugh Evans, Meganne Harrison, Natasha Higgins, Cory Hughes, Lisa Leachman, Isabelle Ochoa, Gina Penny, Krista Ryall, Taylor Scarr, Lena van Seggelen, Wang Chong Shu, Krzysztof Sućko, Kalle Wainio, Vincent Webster, Li Lu Yao, Chen Yibao, and Liu Yiu for logistical support and/or technical assistance.

References

Allison JG, Johnson CW, Meeker JR, Strom BL, Butler SM. 2011. Effect of aerosol surface lubricants on the abundance and richness of selected forest insects captured in multiple-funnel and panel traps. Journal of Economic Entomology 104:1258-1264.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHM, White J-SS (2008) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24: 127–135.

Crook DJ, Francese JA, Zylstra KE, Fraser I, Sawyer AJ, Bartels DW, Lance DR, Mastro VC (2009) Laboratory and field response of the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae) to selected wavelength regions of the visible spectrum. Journal of Economic Entomology 102: 2160–2169.

Dodds K (2014) Effects of trap height on captures of arboreal insects in pine stands of northeastern United States of America. The Canadian Entomologist 146: 80–89.

Francese JA, Mastro VC, Oliver JB, Lance DR, Youssef N, Lavallee SG (2005) Evaluation of colors for trapping *Agrilus planipennis* (Coleoptera: Buprestidae). Journal of Entomological Science. 40: 93–95.

Graham EE, Mitchell RF, Reagel PF, Barbour JD, Millar JG, Hanks LM (2010) Treating panel traps with a fluoropolymer enhances their efficiency in capturing cerambycid beetles. Journal of Economic Entomology, 103: 641–647.

Graham EE, Poland TM, McCullough DM, Millar JG (2012) A comparison of trap type and height for capturing Cerambycid beetles (Coleoptera). Journal of Economic Entomology 105:837–846.

Hughes C, Johns R, and Sweeney J (2014) A technical guide to installing beetle traps in the upper tree canopy. Journal of the Acadian Entomological Society 10: 12–18.

Rhainds M, Kimoto T, Galko J. Nikolev C, Ryall K, Brodersen G, Webster V (2017) Survey tools and demographic parameters of Slovakian *Agrilus* associated with beech and poplar. Entomologia Experimentalis et Applicata 162: 328-335.

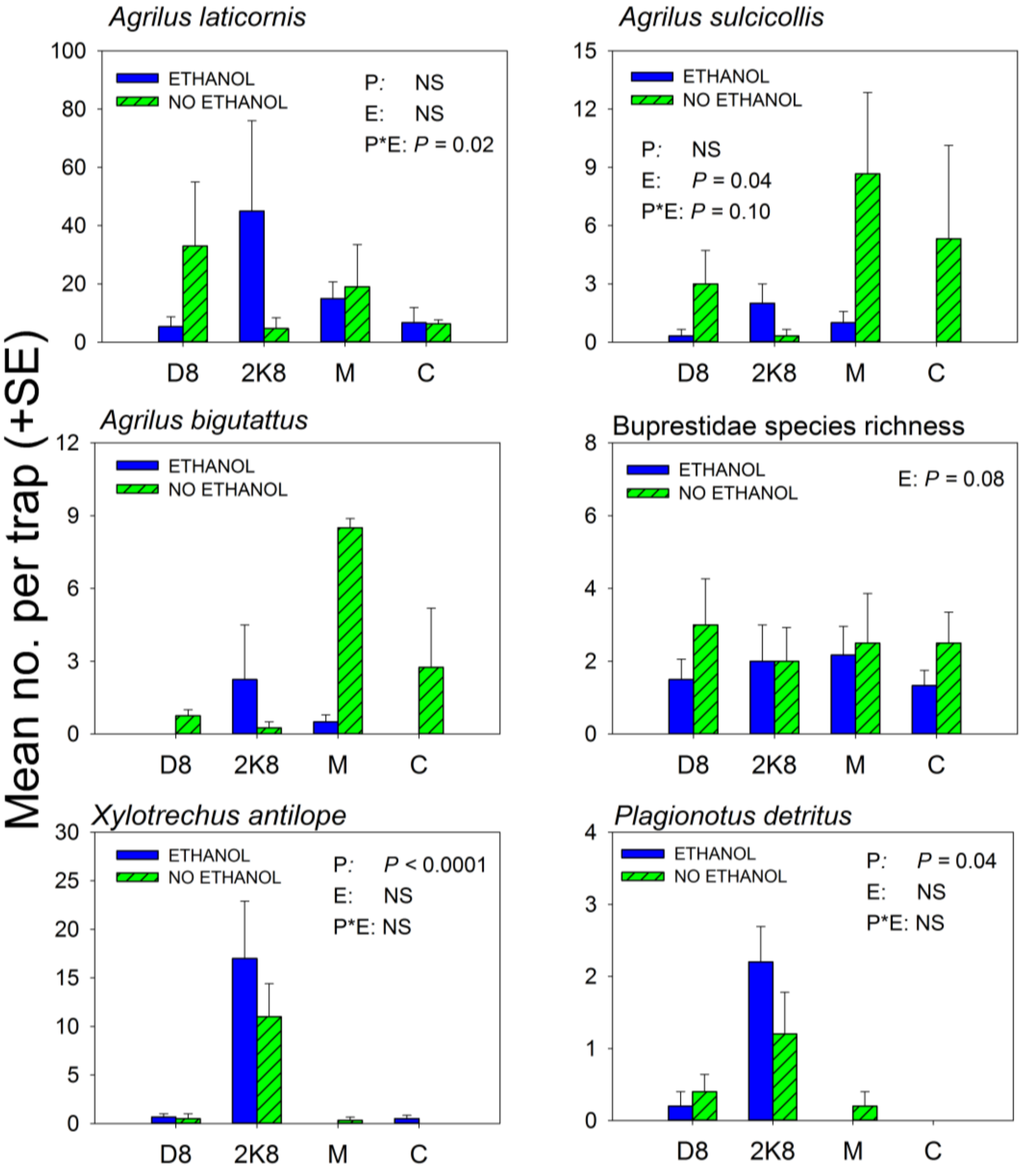


Fig. 1 Mean catch per trap of species of buprestids and cerambycids in funnel traps baited with longhorn beetle pheromones, with vs. without UHR ethanol, in a mixed broadleaf-coniferous forest in Białowieża, Poland, from 23 June to 18 August 2017. D8 = *syn*-2,3-octanediols, 2K8 = racemic 2-hydroxyoctan-3-one, M = 10-methyldodecanal; C = Control. *P* values from generalized linear mixed model in SAS GLIMMIX.

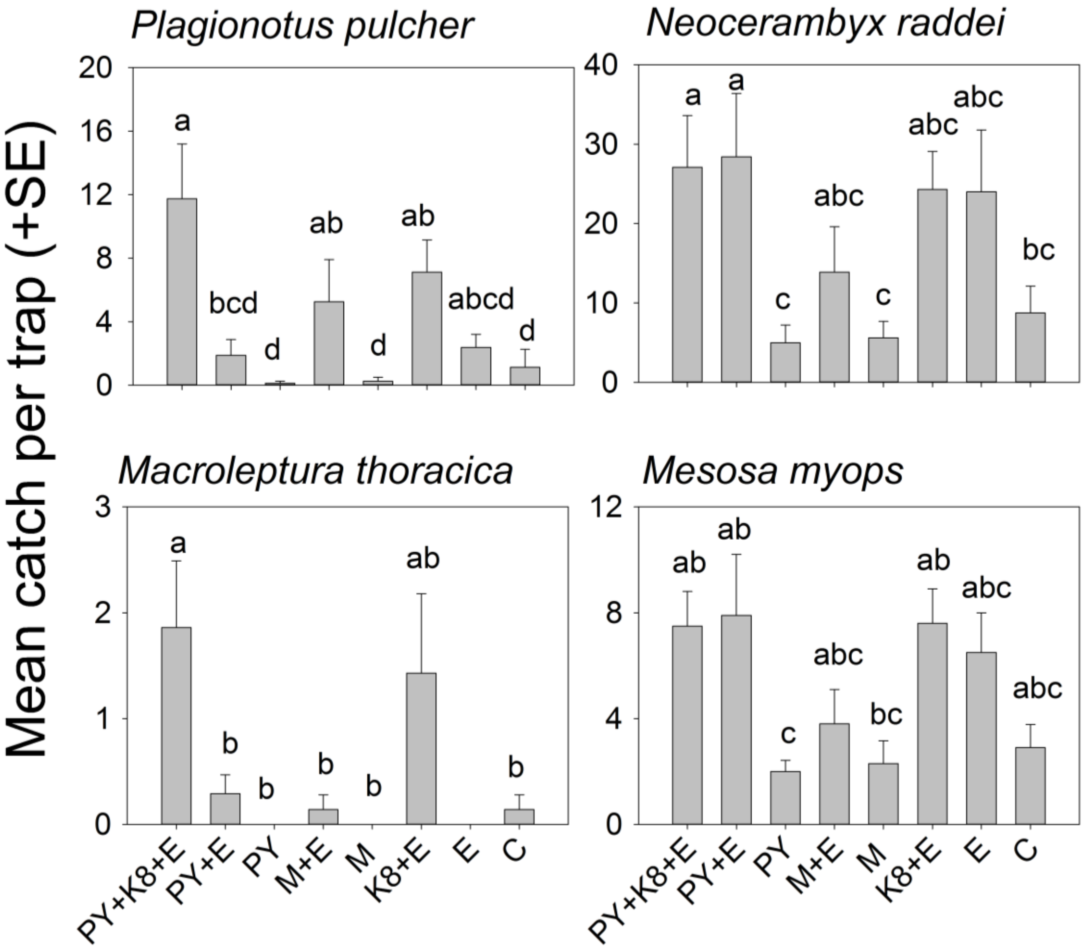


Fig. 2 Mean catch per trap of longhorn beetles in funnel traps baited with various combinations of pheromone lures and ethanol in an oak (*Quercus mongolica*) dominated forest near Da Shi, Jilin province, China, June–September 2017. PY = pyrrole, K8 = racemic 3-hydroxyoctan-2-one, M = 10-methyldodecanal; E = UHR ethanol. Means with different letters significantly different (Generalized linear mixed model in SAS GLIMMIX, followed by Tukey –Kramer comparison of least squares means adjusted for multiple comparisons, $P < 0.05$).

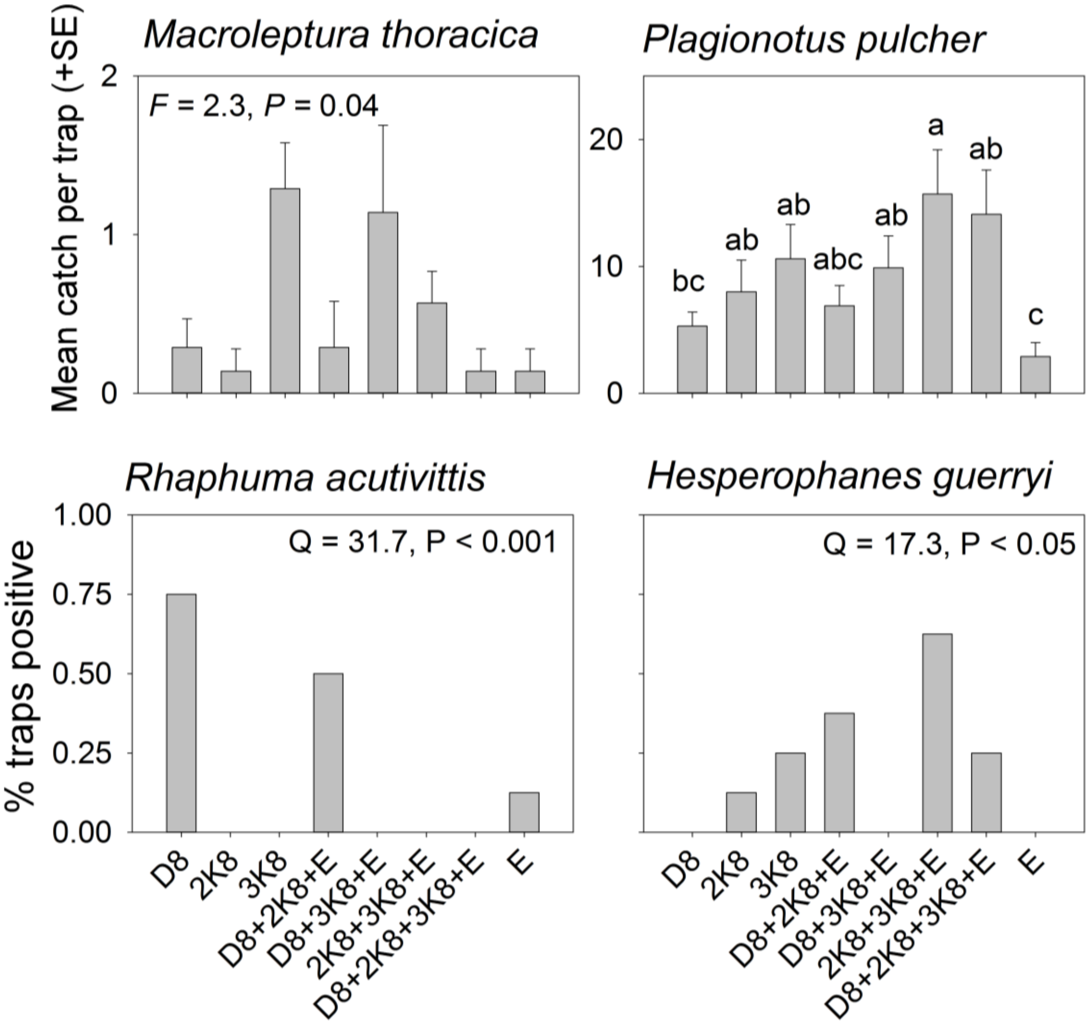


Fig. 3. Response of longhorn beetles to 12-funnel traps baited with various combinations of pheromones and ethanol in an oak (*Quercus mongolica*) dominated forest near Da Shi, Jilin province, China, June–September 2017. D8 = *syn*-2,3-octanediols; 2K8 = racemic 2-hydroxyoctan-3-one; 3K8 = racemic 3-hydroxyoctan-2-one, E = UHR ethanol. Top graphs: mean catch per trap (+SE); means with different letters significantly different (Generalized linear mixed model in SAS GLIMMIX, followed by Tukey –Kramer comparison of least squares means adjusted for multiple comparisons, $P < 0.05$). Bottom graphs: percentage of traps that detected the species (Cochran's Q test).

.

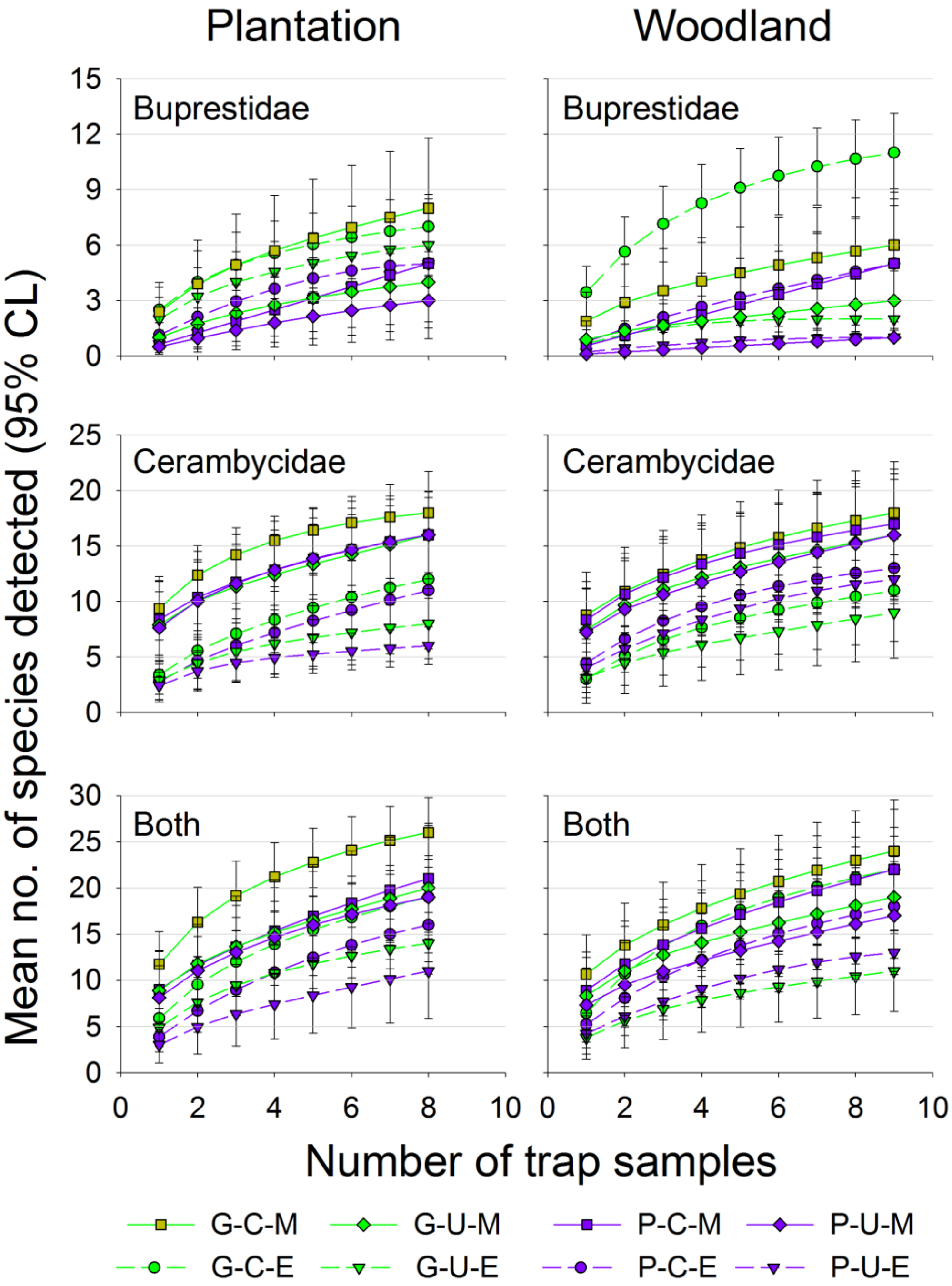


Fig. 4. Rarefaction curves showing the average number of species detected per number of trap samples in hardwood plantations and natural forests in Northern Italy in 2016. Lindgren 12-funnel traps were either green (G) or purple (P), placed in the canopy (C) or understory (U), and baited either with UHR ethanol (E) or a multicomponent lure (M) consisting of UHR ethanol plus racemic 3-hydroxyhexan2-one, racemic 3-hydroxyoctan-2-one, *syn*-2,3 hexanediols, *E,Z*-fuscumol and *E.Z.* fuscumol acetate.

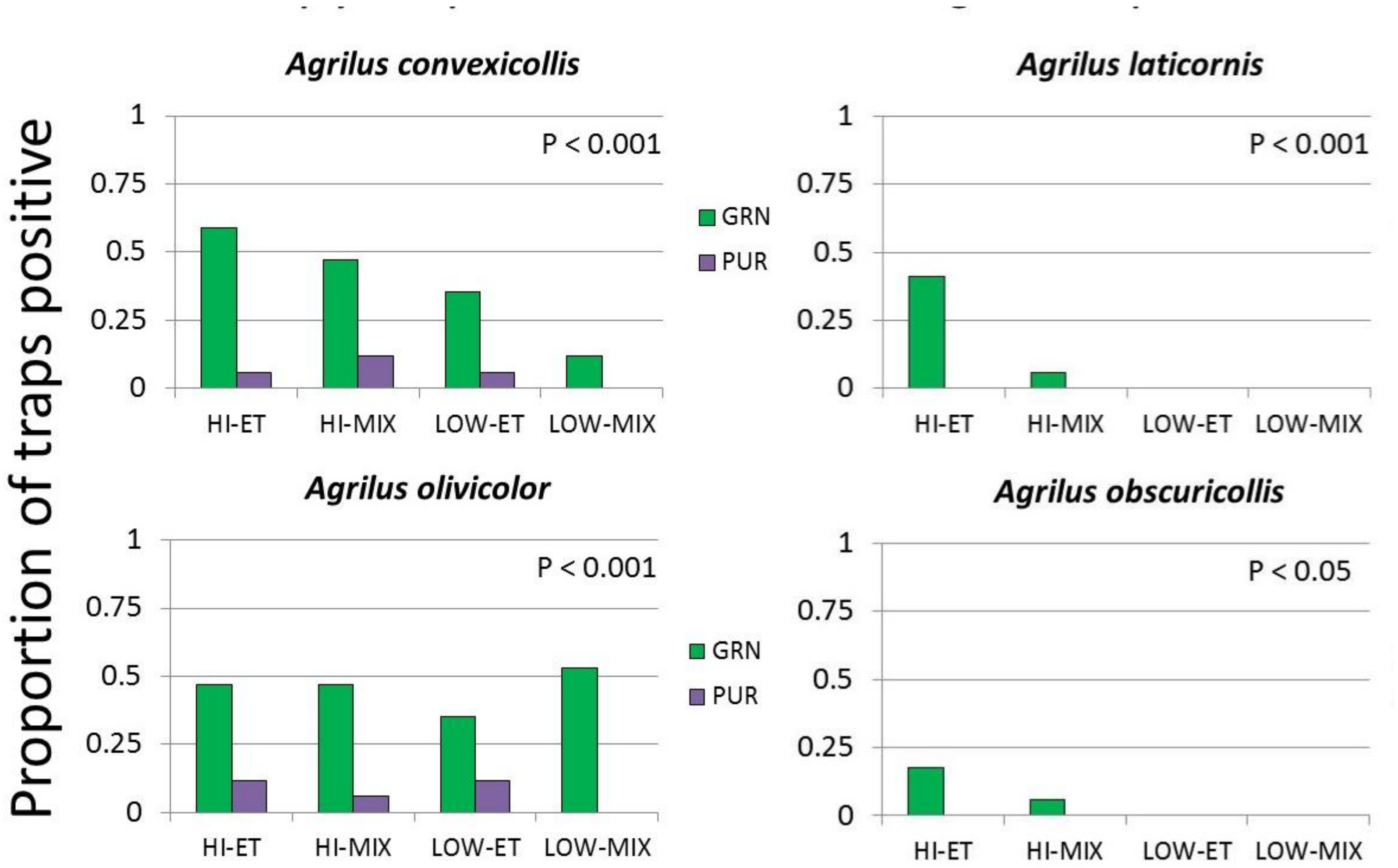


Fig 5. Detection efficacy of *Agrilus* species (Buprestidae) in green and purple 12-funnel traps, placed in the canopy (HI) or understory (LOW) of hardwood forests and plantation in Northern Italy in 2016, and baited with ethanol (ET) or a multicomponent lure (MIX).

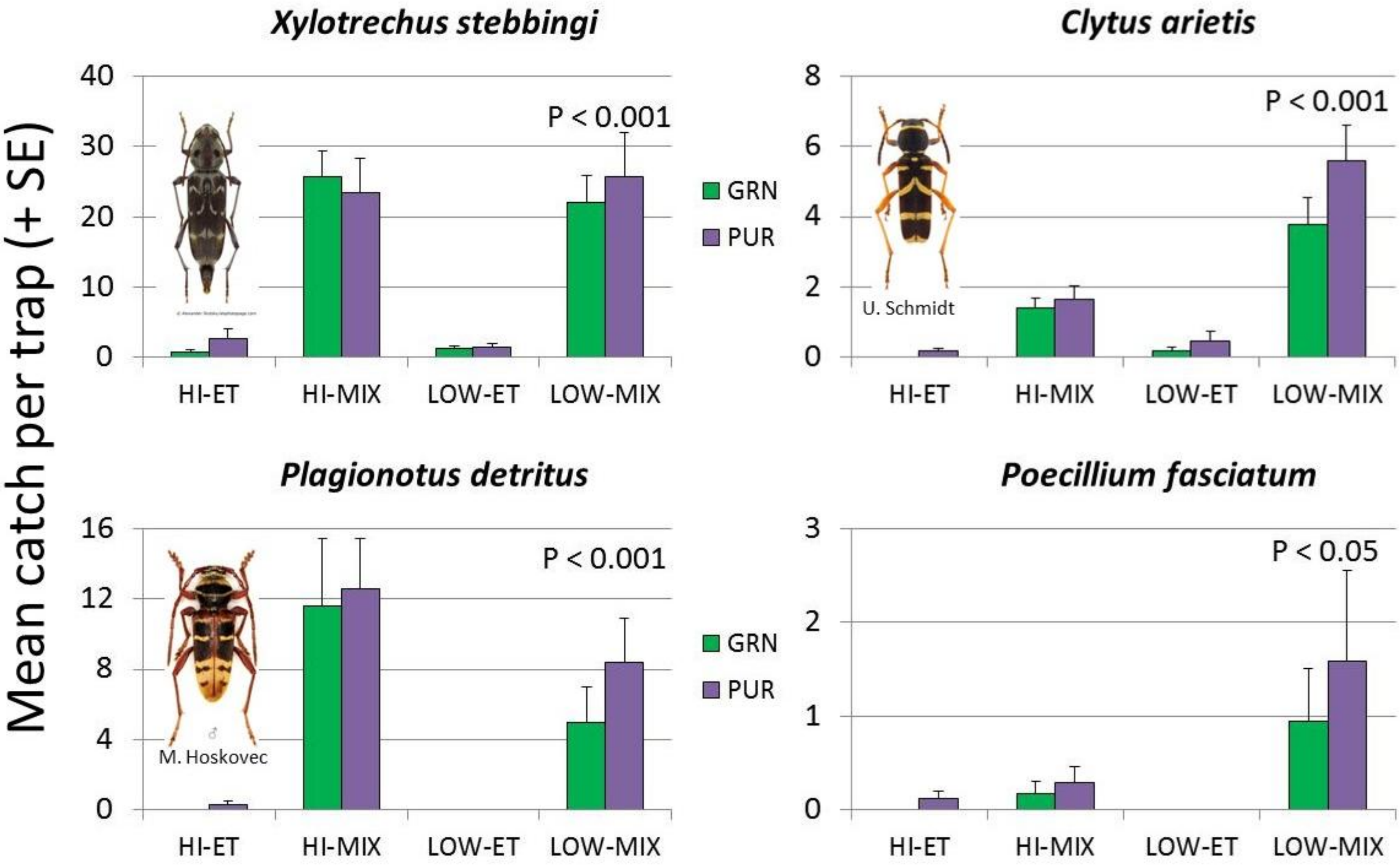


Fig. 6. Mean catch of various longhorn beetles in green and purple 12-funnel traps, placed in the canopy (HI) or understory (LOW) of hardwood forests and plantation in Northern Italy in 2016, and baited with ethanol (ET) or a multicomponent lure (MIX).

Table 1. Total numbers of longhorn beetles captured in Experiment 1, Da Shi, Jilin province, China, 2017. Lure abbreviations: 2,3K8 = racemic 2-hydroxyoctan-3-one; racemic 3,2K8 = 3-hydroxyoctan-2-one; *syn*D8 = *syn*-octanediols; E= ethanol. All traps were black, 12-funnel Lindgrens placed in the mid-canopy. Total of 35 longhorn species and 4226 specimens.

			Lure treatment									Detection efficacy		mean catch per trap		Notes
Subfamily	Tribe	Species	2,3K8	3,2K8	*syn* D8	E	2,3K8 +3,2K 8+E	2,3K8 +*syn* D 8+E	3,2K8 +*syn* D 8+E	2,3K8 +3,2K 8+*syn* D8+E	Total catch	Cochran's Q	*P*	*F*	*P*	
Cerambycinae	Cerambycini	*Neocerambyx (Massicus) raddei*	190	241	154	144	222	261	196	205	1613	-	-	0.89	0.52	
Cerambycinae	Clytini	*Chlorophorus diadema*	0	2	0	0	0	1	0	0	3	-	-	-	-	
Cerambycinae	Clytini	*Chlorophorus motschulskyi*	1	0	0	0	0	0	0	0	1	-	-	-	-	
Cerambycinae	Clytini	*Cyrtoclytus capra*	6	4	1	1	2	3	2	1	20	6.3	NS	-	-	
Cerambycinae	Clytini	*Plagionotus pulcher*	56	74	37	20	110	48	69	99	513	-	-	5.54	<0.0001	**3-hydroxyoctan-2-one boosts trap atch significantly**
Cerambycinae	Clytini	*Rhaphuma acutivittis*	0	0	20	1	0	11	0	0	32	31.6	<0.001	-	-	**Traps baited with *syn-* octanediols detected this species in 6 of 7 blocks. The C8-ketol may deter catch**
Cerambycinae	Clytini	*Teratoclytus plavilstshikovi*	0	0	0	0	0	1	0	1	2	-	-	-	-	
Cerambycinae	Clytini	*Xylotrechus clarinus*	0	1	0	0	0	0	1	0	2	-	-	-	-	
Cerambycinae	Clytini	*Xylotrechus cuneipennis*	2	2	7	1	2	5	5	0	24	10.9	NS	-	-	
Cerambycinae	Clytini	*Xylotrechus magnicollis*	0	0	0	0	1	0	0	0	1	-	-	-	-	
Cerambycinae	Clytini	*Xylotrechus sp.*	0	1	0	0	0	0	1	0	2	-	-	-	-	
Cerambycinae	Hesperophanini	*Hesperophanes (Trichoferrus) guerryi*	1	3	0	0	7	0	2	3	16	17.3	<0.05	-	-	**traps baited with the hydroxyoctanone + ethanol detected the most; the octanediols appear to deter attraction**
Cerambycinae	Purpuricenini	*Purpuricenus sideriger*	0	1	0	0	0	0	0	0	1	-	-	-	-	
Lamiinae	Acanthocinini	*Leiopus stillatus*	0	0	0	0	0	0	0	1	1	-	-	-	-	
Lamiinae	Acanthocinini	*Neacanista tuberculipennis*	0	3	0	1	1	1	1	1	8	3.6	NS	-	-	
Lamiinae	Hecyrini	*Moechotypa diphysis*	109	148	75	240	169	248	155	178	1322	-	-	1.45	0.21	
Lamiinae	Lamiini	*Lamiomimus gottschei*	0	0	0	1	0	1	0	0	2	-	-	-	-	
Lamiinae	Mesosini	*Mesosa myops*	48	89	53	66	89	89	74	93	601	-	-	0.95	0.48	
Lamiinae	Monochamini	*Anoplophora glabripennis*	0	0	0	1	0	0	0	0	1	-	-	-	-	
Lamiinae	Monochamini	*Monochamus saltuarius*	0	0	0	1	1	0	0	1	3	-	-	-	-	
Lamiinae	Pogonocherini	*Exocentrus fisheri*	0	0	0	0	0	0	1	0	1	-	-	-	-	
Lamiinae	Pteropliini	*Pterolophia granulata*	0	0	0	0	1	0	0	0	1	-	-	-	-	
Lamiinae	Saperdini	*Eutetrapha metallescens*	0	0	1	0	0	0	0	0	1	-	-	-	-	
Lamiinae	Saperdini	*Saperda octomaculata*	0	0	0	0	0	1	0	0	1	-	-	-	-	
Lepturinae	Lepturini	*Anoploderomorpha cyanea*	0	0	1	2	0	0	0	0	3	-	-	-	-	
Lepturinae	Lepturini	*Corymbia succedanea*	0	0	0	1	0	0	0	0	1	-	-	-	-	
Lepturinae	Lepturini	*Macroleptura thoracica*	1	9	2	1	4	2	8	1	28	15.5	<0.05	2.31	0.041	**Highest catch in traps baited with 3-hydroxyoctan-2-one, but means not separated by Tukey-Kramer**
Lepturinae	Lepturini	*Pedostrangalia femoralis*	1	0	0	1	0	1	2	0	5	-	-	-	-	
Lepturinae	Lepturini	*Pseudalosterna misella*	0	1	0	0	0	0	0	0	1	-	-	-	-	
Lepturinae	Rhagiini	*Gaurotes ussuriensis*	0	1	0	0	0	2	0	0	3	-	-	-	-	
Lepturinae	Rhagiini	*Macropidonia ruficollis*	1	1	0	0	0	0	0	0	2	-	-	-	-	
Lepturinae	Rhagiini	*Pidonia debilis*	0	0	0	0	0	0	1	0	1	-	-	-	-	
Lepturinae	Rhagiini	*Rhagium rugipenne*	0	0	0	0	1	0	0	0	1	-	-	-	-	
Necydalinae	Necydalini	*Necydalis pennata*	0	0	1	0	0	0	0	0	1	-	-	-	-	
Prioninae	Prionini	*Prionus insularis*	1	0	1	1	1	1	2	1	8	1.1	NS	-	-	

Table 2. Total numbers of longhorn beetles captured in Experiment 2, Da Shi, Jilin province, China, 4 June – 13 September 2017. Lure abbreviations: Py = pyrrole; 3,2K8 = 3-hydroxyoctan-2-one;10-Me = 10-methyldodecanal; E= ethanol. All traps were black, 12-funnel Lindgrens placed in the mid-canopy. Total of 30 longhorn species and 2468 specimens collected.

Subfamily	**Tribe**	**Species**	**Pyrrole**	**Py + 3,2K8 + E**	**Py + E**	**3,2K8 + E**	**10-Me**	**10-Me + E**	**Control**	**E**	**Total catch**	**Cochran's Q**	***P***	***F***	***P***	**Notes**
Cerambycinae	Anaglyptini	*Anaglyptus colobotheoides*	0	0	0	0	0	0	0	1	1	-	-	-	-	
Cerambycinae	Callidiini	*Callidium violaceum*	1	0	1	0	0	0	0	0	2	-	-	-	-	
Cerambycinae	Cerambycini	*Neocerambyx raddei*	40	217	227	194	45	111	70	192	1096	10.6	NS	6.6	<0.0001	Only two treatments had significantly greater catch thaan unbaited controls: Py+K8+E, and Py+E
Cerambycinae	Clytini	*Chlorophorus motschulskyi*	0	0	0	0	1	0	0	0	1	-	-	-	-	
Cerambycinae	Clytini	*Cyrtoclytus capra*	0	0	2	0	0	1	0	1	4	-	-	-	-	
Cerambycinae	Clytini	*Plagionotus pulcher*	1	94	15	57	2	42	9	19	239	33.4	<0.0001	6.8	<0.0001	3-hydroxyoctan-2-one significantly boosts catch when added to pyrrole + ethanol
Cerambycinae	Clytini	*Rhaphuma acutivittis*	0	1	0	1	0	0	0	0	2	-	-	-	-	
Cerambycinae	Clytini	*Xylotrechus clarinus*	0	0	0	1	0	0	0	2	3	-	-	-	-	
Cerambycinae	Clytini	*Xylotrechus cuneipennis*	0	6	2	7	2	1	0	2	20	7	NS	-	-	
Cerambycinae	Clytini	*Xylotrechus pyrrhoderus*	0	0	1	0	0	0	0	0	1	-	-	-	-	
Cerambycinae	Clytini	*Xylotrechus sp.*	0	2	0	0	0	1	0	0	3	-	-	-	-	
Cerambycinae	Hesperophanini	*Hesperophanes* (*Trichoferrus*) *guerryi*	0	0	1	2	0	0	2	0	5	-	-	-	-	
Cerambycinae	Lepturini	*Leptura ochraceofasciata*	0	0	0	0	0	1	0	0	1	-	-	-	-	
Cerambycinae	Saperdini	*Eumecocera impustulata*	0	1	2	0	0	0	3	0	6	9.4	NS	-	-	
Lamiinae	Acanthocinini	*Neacanista tuberculipennis*	0	2	0	5	0	2	1	1	11	7	NS	-	-	
Lamiinae	Acanthoderini	*Oplosia suvorovi*	0	0	4	0	0	1	0	0	5	-	-	-	-	
Lamiinae	Hecyrini	*Moechotypa diphysis*	81	88	136	44	48	84	61	128	670	7	NS	1.37	0.24	
Lamiinae	mesocini	*Mesosa myops*	16	60	63	61	18	30	23	52	323	7.6	NS	4.5	<0.001	Ethanol significaantly increased catch when added to traps baited with pyrrole
Lamiinae	Mesocini	*Setomesosa rondoni*	0	1	0	0	0	0	0	0	1	-	-	-	-	
Lamiinae	Monochamini	*Monochamus guttulatus*	0	0	1	0	0	0	0	0	1	-	-	-	-	
Lamiinae	Monochamini	*Monochamus saltuarius*	0	0	1	1	0	7	0	0	9	-	-	-	-	
Lamiinae	Pteroplini	*Pterolophia granulata*	0	0	0	2	0	0	0	0	2	-	-	-	-	
Lamiinae	Saperdini	*Eutetrapha metallecens*	0	0	1	0	0	1	1	1	4	-	-	-	-	
Lamiinae	Saperdini	*Saperda octomaculata*	0	0	0	0	0	0	0	1	1	-	-	-	-	
Lepturinae	Lepturini	*Anastrangalia sequensi*	0	0	1	0	0	0	0	0	1	-	-	-	-	
Lepturinae	Lepturini	*Anoploderomorpha cyanea*	0	0	0	0	0	0	2	0	2	-	-	-	-	
Lepturinae	Lepturini	*Leptura duodecimguttata*	1	0	0	0	0	1	0	0	2	-	-	-	-	
Lepturinae	Rhagini	*Gaurotes ussuriensis*	2	4	5	1	0	0	1	5	18	6.8	NS	-	-	
Lepturinae	Rhagini	*Macroleptura thoracica*	0	13	2	10	0	1	1	0	27	22.9	<0.01	-	-	pyrrole + K8+E detected this species in 75% of traps
Prioninae	Prionini	*Prionus insularis*	0	1	1	2	1	0	0	2	7	-	-	-	-	

Formulations of Emamectin Benzoate (TREE-äge®) and Propiconazole (Propizol®) for Protecting Engelmann Spruce from Mortality Attributed to Spruce Beetle and Associated Fungi

Christopher J. Fettig[1], Darren C. Blackford[2], Donald M. Grosman[3], and A. Steven Munson[2]

[1]Pacific Southwest Research Station, USDA Forest Service, 1731 Research Park Drive,
Davis, California 95618, cfettig@fs.fed.us.
[2]Forest Health Protection, USDA Forest Service, 4746 South 1900 East, Ogden, Utah 84403.
[3]Arborjet Inc., 99 Blueberry Hill Road, Woburn, Massachusetts 01801.

Abstract

Spruce beetle is the most significant mortality agent of mature spruce in North America, and many forests in the Rocky Mountains are experiencing outbreaks. In this study, we determine the efficacy of TREE-äge® (TREE-äge® G4 and TREE-äge® 9.5; Arborjet Inc., Woburn, MA) combined with and without the fungicide propiconazole (Propizol®; Arborjet Inc.) for protecting Engelmann spruce from mortality attributed to spruce beetle and associated fungi.

Résumé

Le dendroctone de l'épinette est le plus important agent de mortalité de l'épinette mature en Amérique du Nord, et de nombreuses forêts dans les Rocheuses sont actuellement aux prises avec des infestations. Dans cette étude, nous déterminons l'efficacité de TREE-äge® (TREE-äge® G4 et TREE-äge® 9.5 - Arborjet Inc., Woburn, MA) utilisé avec et sans fongicide propiconazole (Propizol® - Arborjet Inc.) pour protéger l'épinette d'Engelmann contre la mortalité attribuable au dendroctone de l'épinette et aux champignons qui y sont associés.

Project Details

In the western U.S., protection of individual conifers from bark beetles often involves liquid formulations of insecticides applied to the tree bole. Researchers attempting to find safer, more portable, and longer-lasting alternatives have evaluated injecting systemic insecticides directly into the tree. Early research indicated that several methods, active ingredients and formulations were largely ineffective, but more recently phloem-mobile active ingredients injected with pressurized systems have shown promise (reviewed by Fettig et al. 2013). For example, an experimental formulation of emamectin benzoate protected ponderosa pine, *Pinus ponderosa*, from mortality attributed to western pine beetle, *Dendroctonus brevicomis*, for three field seasons in California (Grosman et al. 2010). This and other research led to registration of a commercial formulation of emamectin benzoate for tree protection (TREE-äge®; Arborjet Inc., Woburn, MA). Later, TREE-äge was demonstrated effective for protecting lodgepole pine, *P. contorta*, from mortality attributed to mountain pine beetle, *D. ponderosae*, but only when injected in the fall (September) before being challenged by beetles the following year (Fettig et al. 2014).

While injections can be applied at any time of year when trees are actively transpiring, sufficient time is needed for adequate distribution of the active ingredient (e.g., emamectin benzoate) to the target tissue (i.e., the phloem where most bark beetles feed) prior to colonization. This appears to be of significant importance in high-elevation (>2,438 m) forests where cold temperatures retard transport within the tree. For example, research has shown that a root-zone threshold temperature of 8−12°C is required for normal physiological function in Engelmann spruce, *Picea engelmannii*, and occurs for only a short period (~3.5 months) each year in high-elevation forests of the Rocky Mountains (Fettig et al. 2014) where Engelmann spruce is dominant.

Spruce beetle, *Dendroctonus rufipennis*, is the most significant mortality agent of mature spruce in North America (Jenkins et al. 2014). Grosman et al. (2010) evaluated the efficacy of an experimental formulation of emamectin benzoate for protection of Engelmann spruce from mortality attributed to spruce beetle in Utah. Despite injections being applied in fall of the year prior to treatments being challenged by spruce beetle, a lack of efficacy was observed. Here we explore an alternative timing of TREE-äge (G4 and 9.5) for protecting Engelmann spruce from mortality attributed to spruce beetle, and combining TREE-äge with a fungicide to limit the spread and distribution of blue stain associates within the tree.

Accomplishments (to date)

Treatments were implemented the week of 16 July 2017 on the Bridger-Teton National Forest, Wyoming, and included:

(1) TREE-äge® G4 injected at 7.5 ml/2.54 cm dbh (1.37 m in height)
(2) TREE-age® G4 injected at 7.5 ml/2.54 cm dbh + Propizol® injected at 6 ml/2.54 cm dbh mixed with 6 ml/2.54 cm dbh of distilled water
(3) TREE-äge® 9.5 injected at 3.2 ml/2.54 cm dbh*
(4) TREE-äge® 9.5 injected at 3.2 ml/2.54 cm dbh + Propizol® injected at 6 ml/2.54 cm dbh mixed with 6 ml/2.54 cm dbh of distilled water
(5) Untreated control (2018)
(6) Untreated control (2019)

Treatments 1–4 were injected using Tree IV™ microinfusion systems (Arborjet Inc.) at a narrow spacing (7.6 cm between plugs) to 30 randomly-assigned trees. All trees in treatments 1-5 will be baited in 2018 and tree mortality will be estimated initially based on spruce beetle attack density and patterns in September 2018. However, final assessments of tree mortality will be based on the presence or absence of crown fade in September 2019 (for the first field season) and September 2020 (for the second field season, following baiting of surviving trees in treatments 1–4 and treatment 6 in 2019). The only criterion used to determine the effectiveness of treatments will be whether or not individual trees succumb to attack by spruce beetle (Shea et al. 1984).

Deliverables in CY2017: Presentations were made at two scientific meetings. A manuscript sharing preliminary results from a related study was published in the *Journal of Entomological Science* (Fettig et al. 2017).

Acknowledgments

We thank J. Doccola (Arborjet Inc.) for loaning equipment and donating insecticides. This ongoing research is supported by SERG-International ($9,499 received in CY17 and $10,000 expected in CY18), Arborjet Inc. ($4,000 in CY17), and the USDA Forest Service ($10,000 in CY17). This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides in the United States must be registered by appropriate State and/or Federal agencies before they can be recommended. This article was written and prepared by US Government employees on official time and it is, therefore, in the public domain and not subject to copyright.

Literature Cited

Fettig, C.J., D.C. Blackford, D.M. Grosman, and A.S. Munson. 2017. Injections of emamectin benzoate protect Engelmann spruce from mortality attributed to spruce beetle for two years. *Journal of Entomological Science* 52:193–196.

Fettig, C.J., D.M. Grosman, and A.S. Munson. 2013. Advances in insecticide tools and tactics for protecting conifers from bark beetle attack in the western United States. In Trdan, S., ed. *Insecticides - Development of Safer and More Effective Technologies*. InTech, Rijeka, Croatia. p. 472–492.

Fettig, C.J., A.S. Munson, D.M. Grosman, and P.B. Bush. 2014. Evaluations of emamectin benzoate and propiconazole for protecting individual *Pinus contorta* from mortality attributed to colonization by *Dendroctonus ponderosae* and associated fungi. *Pest Management Science* 70:771–778.

Grosman, D.M., C.J. Fettig, C.L. Jorgensen, and A.S. Munson. 2010. Effectiveness of two systemic insecticides for protecting western conifers from mortality due to bark beetle attack. *Western Journal of Applied Forestry* 25:181–185.

Jenkins, M.J., E.G. Hebertson, and A.S. Munson. 2014. Spruce beetle biology, ecology and management in the Rocky Mountains: An addendum to spruce beetle in the Rockies. *Forests* 5:21–71.

Shea, P.J., M.I. Haverty, and R.W. Hall. 1984. Effectiveness of fenitrothion and permethrin for protecting ponderosa pine from attack by western pine beetle. *Journal of the Georgia Entomological Society* 19:427−433.

SERG-I#128: Interim Progress Update, January 19, 2018

Temperature Regulation of Spruce Beetle's Life Cycle and its Potential Role in Outbreaks

Régulation de la température du cycle de vie du dendroctone de l'épinette et rôle potentiel dans les infestations

K. Bleiker, Canadian Forest Service, Natural Resources Canada
V. Rezendes, D. Huber, University of Northern British Columbia
J. Robert, BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development

Abstract

Spruce beetle has a two-year life cycle throughout much of its range. However, one generation may be produced per year in areas or years with warmer growing-seasons potentially doubling the rate of population increase. In fact, there may be a mix of semivoltine (two-year life cycle) and univoltine (one-year life cycle) insects in the same stand and even within the same tree. Voltinism is controlled by inner bark temperatures, although genetic variation in spruce beetle diapause has not been studied. The few studies that have investigated the impact of temperature on the life cycle of spruce beetle have had variable and somewhat contradictory results, including whether there is a true diapause and the life stages sensitive to the environmental cue. This study examines the interaction between temperature and development in the spruce beetle. Our ultimate goal is to improve predictions of spruce beetle populations trends associated with shifts in voltinism in current forests and under climate change. Here, we report on progress in year one of the three-year study.

Résumé

Le cycle de vie du dendroctone de l'épinette est de deux ans presque partout dans son aire de répartition. Toutefois, une génération par année peut être produite dans certaines régions, et des années de saisons de croissance plus chaudes peuvent éventuellement doubler le taux d'augmentation. En fait, il pourrait y avoir un mélange d'insectes semivoltins (cycle de vie de plus d'un an) et univoltins (cycle de vie d'un an) dans le même peuplement, voire dans le même arbre. La température interne de l'écorce a une influence sur le voltinisme, bien que les variations génétiques dans la diapause du dendroctone de l'épinette n'aient pas été étudiées. Les quelques études qui ont exploré les répercussions de la température sur le cycle de vie du dendroctone de l'épinette ont obtenu des résultats variables et quelque peu contradictoires, y compris à savoir s'il se produit ou nom une véritable diapause et les stades de vie qui sont sensibles à un signal environnemental. La présente étude examine l'interaction entre la température et le voltinisme chez le dendroctone de l'épinette. Notre objectif ultime est de mieux prédire les tendances des populations de dendroctone de l'épinette associées aux changements dans le voltinisme dans les forêts actuelles et en regard des changements climatiques. Nous faisons ici rapport sur les progrès réalisés durant la première année de cette étude de trois ans.

Definitions of terms:

Voltinism - number of generations in a year. Univoltine: 1 generation per year; semivoltine: 1 generation every two years
Diapause - a type of dormancy, which is initiated and terminated by specific stimuli or conditions

Main intended outcome of research

Improve predictions of spruce beetle populations trends associated with shifts in voltinism in current forests and under climate change.

Introduction

Spruce beetle (*Dendroctonus rufipennis*) is native to spruce forests across North America. Beetles preferentially attack large-diameter, freshly downed or stressed spruce trees, which have reduced defensive capacity. Outbreaks, such as the Bowron outbreak in the 1970s in central British Columbia, are often triggered by discrete stress events that provide a sudden increase in ideal hosts (i.e. wind throw). Beetle populations can increase rapidly in downed trees and then attack standing susceptible trees once the preferred host material has been exhausted. Climatic stress, such as drought, may also contribute to outbreaks by increasing the susceptibility of standing trees (Schmid and Frye 1977).

Voltinism has implications for population growth rates and the potential for outbreaks. A two-year life cycle is common throughout much of spruce beetle's range. However, one generation may be produced per year in areas or years with warmer growing-seasons potentially doubling the rate of increase. In fact, there may be a mix of semivoltine (two-year life cycle) and univoltine (one-year life cycle) insects in the same stand and even within the same tree. Voltinism is thought to be controlled by inner bark temperatures, although genetic variation in spruce beetle diapause has not been studied. Spruce beetle adults typically disperse in late May or June and attack new host trees. Female beetles lay eggs in the inner bark where the larvae mine, passing through four instars before pupating and eclosing to new adult beetles. In the typical two-year life cycle, insects overwinter as larvae in year one and as brood adults in year two. In the one-year life cycle, insects overwinter as brood adults. It is generally accepted that brood adults must overwinter to become sexually mature and emerge: that is adult diapause is obligate so new adults must overwinter to complete development. Temperature conditions during insect development determines whether larvae also overwinter meaning larval diapause is facultative and only occurs under certain conditions or in response to a specific cue. A shift from semi- to uni-voltinism can facilitate population increases and the potential for spruce beetle outbreaks increases as the length of the life cycle shifts from two years to one year.

Studies attempting to identify conditions resulting in either a one-year or a two-year life cycle have had variable and somewhat contradictory results with respect to the temperature conditions triggering diapause and the larval instar(s) sensitive to the induction cue (Dyer 1969; Dyer 1970; Dyer and Hall 1977; Hansen et al. 2001a, 2001b; Hansen et al. 2011). Interestingly, these studies were conducted on two geographically distant populations (Dyer worked in British Columbia and Hansen worked in Utah). Phylogenetic work has revealed substantial geographic variation in the genetic population structure of spruce beetle. Insects from Utah and southern British Columbia cluster together in a Rocky Mountain clade being distinct from beetles from two other clades that span northern Canada; however, Utah and British Columbia beetles group separately from each other within the Rocky Mountain clade (Maroja et al. 2007). Populations could adapt to regional climate over time so any predictive system should account for both geographic variation and the potential impact of climate change on spruce beetle population dynamics (Jenkins et al. 2014).

This project examines the impact of temperature on spruce beetle population dynamics by examining the role of temperature in controlling the length of the life cycle through larval diapause. Our ultimate goal is to provide the biological parameters necessary to improve predictions of spruce beetle population trends now, and in the future under climate change.

Specific Objectives

1. Elucidate temperature conditions influencing one- and two-year life cycle in spruce beetles (i.e., non-diapausing versus diapausing larvae);
2. Identify the larval instar(s) sensitive to the thermal conditions that induce diapause; and
3. Analyze the historical associations between temperature and spruce beetle outbreaks in western Canada

Progress to Date

This project was started in May 2017; M.Sc.student, Victoria Rezendes, was identified and hired. Our focus in the first 2 years of the project will be on objectives 1 and 2. Objective 3 will be addressed in years 2 and 3 of the project.

Year 1 Experiments

Naturally infested wood from several trees was collected from each of three field sites in the spring and early summer of 2017. The three sites were: Prince George (north-central British Columbia), Duffy Lake (southern British Columbia) and Whitecourt (north-central Alberta). Healthy trees were felled prior to the 2017 flight at each site to provide susceptible host material to attract dispersing beetles (such trees are also known as "trap trees"). We endeavoured to time the collection of infested material at each site to coincide with, or immediately follow, the main flight period so that female beetles were extending galleries and laying eggs in these new host trees when collected. Logs were cut from the infested portion of the lower bole of trees and transported to the Pacific Forestry Centre in Victoria, BC where the ends were sealed with hot paraffin wax to reduce desiccation. The infested wood was immediately assigned to treatments in one of two experiments upon its arrival at the laboratory (see below). Due to a shortage of infested material for some populations, not all populations were included in all treatments.

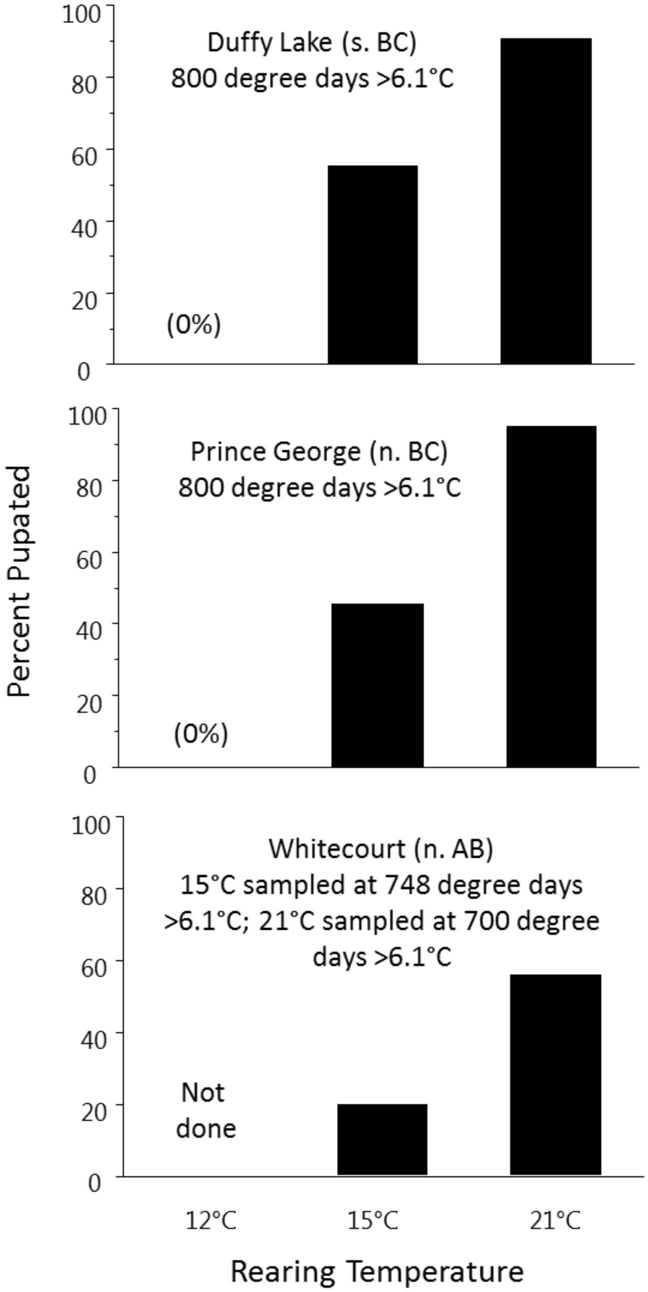
Experiment 1: Rearing at constant temperatures

To test for a temperature threshold associated with diapause induction in larvae, we reared insects in walk-in growth chambers at 12, 15 or 21°C. Insect development was sampled on day 0, when infested material was placed at the target rearing temperatures; most brood were eggs at this time and females were still ovipositing. Insect development was also sampled periodically over time based on degree days above 6.1°C.

Experiment 2: Exposure of larval instars to variable cool temperatures

To test for the larval instar sensitive to a diapause-inducing cue, and further elucidate the conditions triggering diapause, we reared insects to primarily LII, LIII, or LIV at 21°C and then subjected them to 14 or 45 days at 9, 12 or 15°C before returning them to 21°C. Treatments were selected based on previous work (Dyer and Hall 1977; Hansen et al. 2001a). Insect development was sampled pre- and post-cold treatments as well as periodically over time at 21°C.

Preliminary Results from Experiment 1



Our preliminary results from one sample time in experiment 1 indicate a significant developmental delay of all individuals at 12°C indicative of a diapause, while only a proportion of the population were developmentally delayed at 15°C. There was little evidence of a diapause in insects reared at 21°C. Comparisons among populations should be made with caution: although most insects were in the egg stage when the experiment was started, the eggs may have been of different ages since the material used was collected in the field. Also note that the population from Whitecourt was sampled after fewer degree days than the other populations (see headings on individual graphs). The experiment is on-going with the last sample time planned for March 2018.

Figure 1: Percent of insects that had pupated after a target number of degree days above 6.1°C (see sub-titles on graphs). Naturally infested wood was collected from three sites following the main dispersal flight when female beetles were ovipositing. The infested wood was transported to the laboratory and placed at 12, 15 or 21°C (Duffy Lake and Prince George only).

Next Steps

Sampling for these two experiments will be completed in March 2018 and the data examined by mid-April 2018 to plan experiments for the 2018 season. We will use the results of the preliminary experiments conducted to 2017, which included a large number of treatments, to focus our treatments in 2018 experiments and further elucidate key temperature and time thresholds differentiating one- and two-year life cycles.

Acknowledgements

We thank Lorraine MacLauchlan, Colton Briggs, Caroline Whitehouse, Jeanne Robert and Alena Charlston for assistance locating field sites and obtaining permits. We also thank G. Smith, E. MacLeod, B. Wilmot, V. Waring, B. Tomm, and G. Thandi for help collecting field material or laboratory assistance.

References

Dyer EDA. 1969. Influence of temperature inversion on development of spruce beetle, *Dendroctonus obesus* (Mannerheim) (Coleoptera: Scolytidae). J. Ent. Soc. BC. 66: 41-45.

Dyer EDA. 1970. Larval diapause in *Dendroctonus obesus* (Mannerheim) (Coleoptera: Scolytidae). J. Ent. Soc. BC. 67: 18-21.

Dyer EDA, Hall PM. 1977. Factors affecting larval diapause in *Dendroctonus rufipennis* (Mannerheim) (Coleoptera: Scolytidae). Can. Ent. 109: 1485-1490.

Hansen EM, Bentz BJ, Turner DL. 2001a. Physiological basis for flexible voltinism in the spruce beetle (Coleoptera: Scolytidae). Can. Ent. 133: 805-817.

Hansen EM, Bentz BJ, Turner DL. 2001b. Temperature-based model for predicting univoltine brood proportions in spruce beetle (Coleoptera: Scolytidae). Can. Ent. 133: 827-841.

Hansen EM, Bentz BJ, Powell JA, Gray DR, Vandygriff JC. 2011. Prepual diapause and instar IV developmental rates of the spruce beetle, *Dendroctonus rufipennis* (Coleoptera: Curculionidae, Scolytinae). J. Insect Phys. 57: 1347-1357.

Jenkins MJ, Hebertson EG, Munson AS. 2014. Spruce beetle biology, ecology and management in the Rocky Mountains: an addendum to spruce beetle in the Rockies. Forests. 5: 21-71.

Schmid JM, Frye RH. 1977. Spruce beetle in the Rockies. GTR RM-49, USDA Forest Service: Fort Collins, CO, p. 38.

B.C. Report for SERG-I AGM February, 2018

This report summarizes the results of the 2017 Provincial Aerial Overview Survey (AOS). The full summary will be available in the annual publication 2017 *Summary of Forest Health Conditions in British Columbia* (BC) that will be available on-line in April 2018.

Approximately 84% of the province was flown between July 4th and October 14th 2017 by 25 surveyors with ten aircraft companies. Total flight time was 701 hours. A total of 7 million hectares (ha) of damage caused by at least forty-five damaging agents was mapped on many different commercial tree species of various ages. This only included damage visible at the time and height that the AOS is flown, which is known to under-represent some damaging agents, particularly some diseases.

Bark beetles continued to damage the most area, with 4.4 million hectares of mortality detected. Mainly trace to light intensity western balsam bark beetle infestations affected 3.7 million hectares, with the largest disturbances located in northern BC. Spruce beetle damage rose to half a million hectares, with the majority of the attack continuing to occur in Omineca Region. Douglas-fir beetle damage rose to 119,096 ha, with the most of the mortality occurring in central BC. For the eighth consecutive year, mountain pine beetle attack declined to 119,089 ha, which was primarily located in localized infestations at higher elevations.

Abiotic damage increased more than five-fold since 2016 to 1.4 million hectares. A record wildfire year was responsible for this increase with 1.2 million hectares burned, mainly in the southern interior. Post-wildfire damage diminished to 26,183 ha, primarily located in northern BC. Yellow-cedar decline damage remained stable with 56,563 ha delineated along coastal BC. Flooding damage, cheifly in Northeast Region, increased to 42,075 ha. Drought damage that resulted in mortality affected mainly western redcedar on 4,045 ha, though excessive needle shedding suspected to be from drought affected 3,571 ha of lodgepole pine and 3,800 ha of western redcedar.

Defoliation damage decreased to just under 1 million hectares of damage provincially. Deciduous trees (in particular trembling aspen) continued to be the most affected. Aspen leaf miner damage declined but still impacted 428,986 ha, primarily in the northern BC interior. Satin moth defoliated a record 160,085 ha, also mainly in the northern BC interior. Bruce spanworm infestations affected 4,456 ha in Dawson Creek TSA of Northeast Region. Two-year-cycle budworm was the most significant conifer defoliator with 376,100 ha attacked in the BC interior. Pine needle sheathminer damage decreased to 2,944 ha, all located in Cariboo Region.

Damage caused by diseases that were visible during the AOS continued to increase to 191,230 ha. Young lodgepole pine stands were most affected with 90,232 ha of

Lophodermella needle cast and 69,243 ha of Dothistroma needle blight, with an additional 4,015 ha of damage most likely caused by one of these two agents. Venturia blight damage declined to 12,067 ha, observed primarily in Skeena Region. Septoria leaf spot damage in Great Bear Rainforest North TSA rose to 9,197 ha. Low intensity white pine blister rust damaged a record 3,752 ha and larch needle blight affected 2,169 ha.

Localized damage due to agents such as bear, slides, birch leaf miner and root disease were observed in small, scattered disturbances across the province as well.

The full report and other information related to B.C.'s Forest Health program is available at:

https://www2.qa.gov.bc.ca/gov/content/industry/forestry/managing-our-forest-resources/forest-health

Summary of Forest Pest Conditions in Alberta, 2017

Introduction

In Alberta, forest health monitoring is the responsibility Alberta Agriculture and Forestry (AAF) and is conducted on forested land under AAF management[1]. To this end, AAF annually conducts aerial surveys (overview (AOS) and heli-GPS surveys) in addition to ground surveys. This report is a summary of major forest damage agents (excluding wildfire disturbance) detected in 2017 during aerial and ground surveys.

Bark beetles

Mountain pine beetle (*Dendroctonus ponderosae*)

Population forecast surveys are conducted each spring to assess the relative overwintering success of MPB and the potential for the infestation to spread. Approximately 323 trees at 80 sites were surveyed in 2017 (Fig. 1). Over the majority of its core range, success varied from low to high in 2017 which is indicative of decreasing, static, and increasing populations. Overall population success was predicted to be lower in 2017 compared to 2016. Of the sites sampled, 14% showed low success, 46% showed moderate success, and 19% predicted high success. Extremely high MPB success was noted in only one site south of Grande Prairie. Surveys were not conducted in northwestern Alberta due to the lack of ground accessible infested sites.

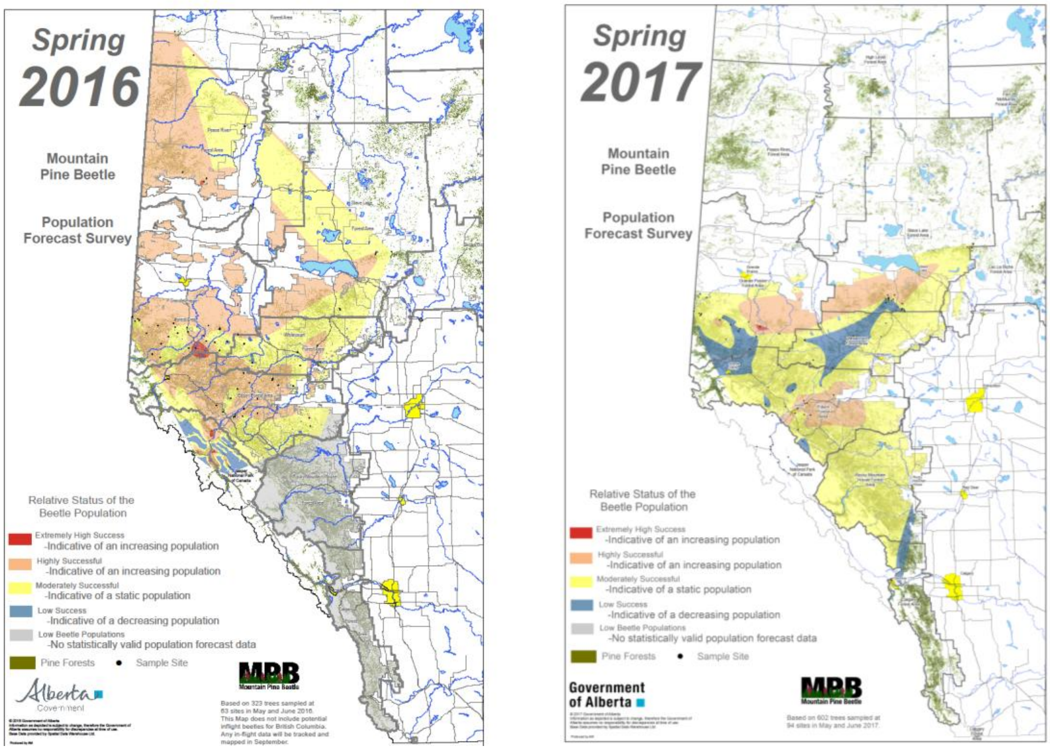


Figure 1. Mountain pine beetle population forecast surveys, 2015 and 2016.

---

[1] Defined as forested public land that is managed for timber production, watershed, fish and wildlife, recreation, energy development, and other uses. Agricultural use is limited to grazing when compatible with other uses. In general, this is public land outside the parkland and prairie regions, or roughly in the northern half of the province and within a strip running along the Rocky Mountains and foothills.

Mountain pine beetle aggregation pheromones are used to monitor the presence or absence of this insect along the eastern slopes of the Rocky Mountains and along the Saskatchewan border. Sites are ranked as MPB being absent (zero attacked trees), present (at least one tree with less than 40 attack starts), or mass-attacked (at least one tree with more than 40 attack starts). In 2017, 248 sites were monitored (Fig. 2). In northeastern Alberta, MPB was absent from most sites (79%) and zero sites were mass-attacked which was similar to the pattern observed in 2016. In the central region of the Rocky Mountains, MPB mass-attacked fewer sites in 2017 compared to 2016 (68% versus 87%) but was present at larger number of sites (25% in 2017 and 9% in 2016). In southern Alberta, MPB continued to be largely absent from the majority of sites (81%), present at 11% of sites and mass-attacking trees at 7% of sites. Provincially, attack intensities were similar to those observed in 2016 with the exception of the increase in presence in central Alberta Rocky Mountains.

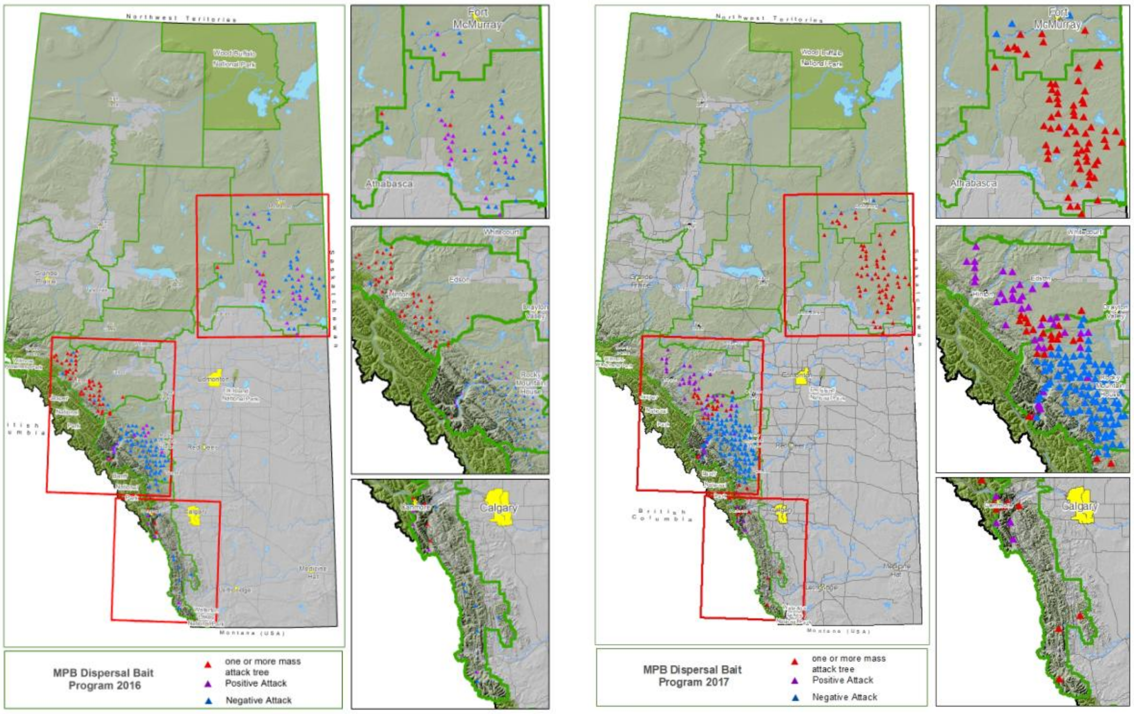


Figure 2. Mountain pine beetle long-distance dispersal baiting survey 2016 and 2017.

Aerial surveys are conducted annually in late summer and early fall to determine the number of red-crowned pine symptomatic of MPB infestations. Generally, groups of three or more pine with red crowns are recorded using sketch mapping and heli-GPS techniques. These surveys span the regions that are a priority for control activity that year. The region of the province prioritized for control activity has been similar since 2014, therefore aerial surveys were conducted over comparable areas. 2017 surveys detected 89,520 red trees spread over 17,677 sites which was an increase of 23% compared to 2016 (72,571 trees at 16,317 sites) (Fig. 3). The greatest increase in MPB populations occurred in the Edson Forest Area; a four-fold increase in the number of red trees and red tree sites compared to 2016. The number of red trees and red tree sites decreased in the Grande Prairie and Whitecourt Forest Areas, and remained mostly unchanged in the Slave Lake Forest Area.

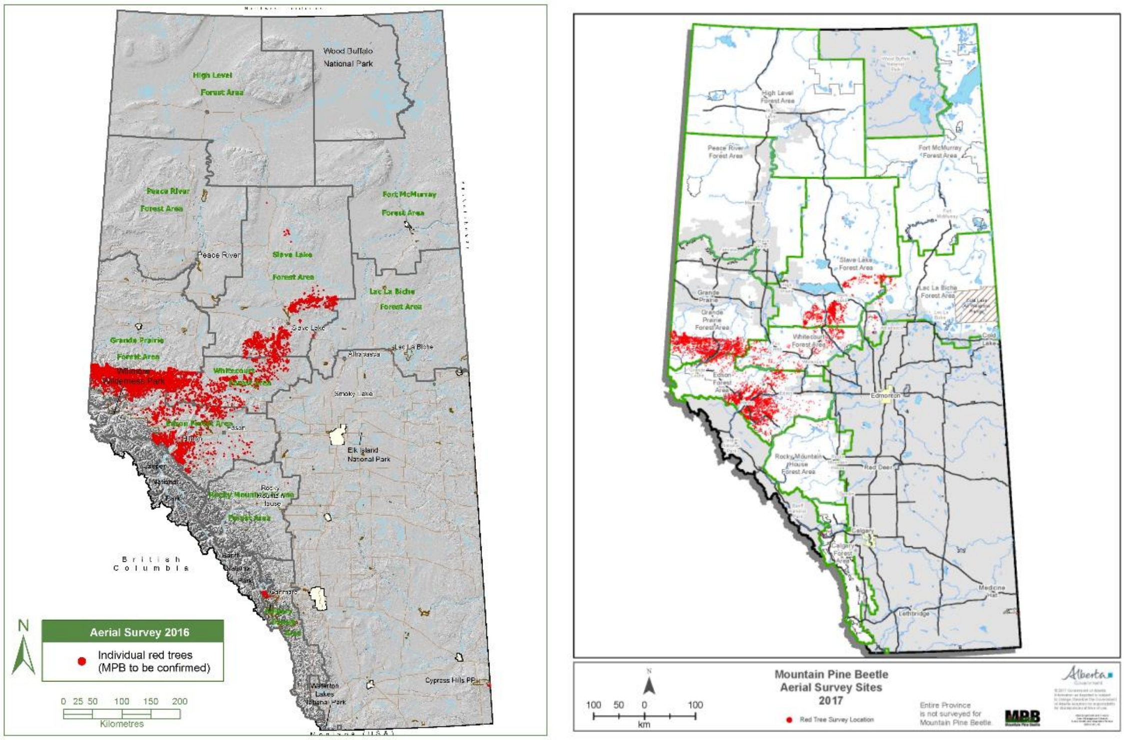


Figure 3. Locations of red-crowned pines detected during aerial surveys 2016 and 2017.

Green to red ratio surveys are conducted each fall to assess the relative success of MPB and potential for their spread the following summer. These surveys are based on a ratio of green attack (trees with current year attacks, retaining green crowns) to red attack (trees with red crowns, attacked the previous year) trees calculated by site. Surveys were carried out at 468 plots in 2017 (Fig. 4). The majority of plots predicted low population growth in 2017 (36%) while the number of plots that predicted high population expansion remained stable between 2016 at 2017 at 29%.

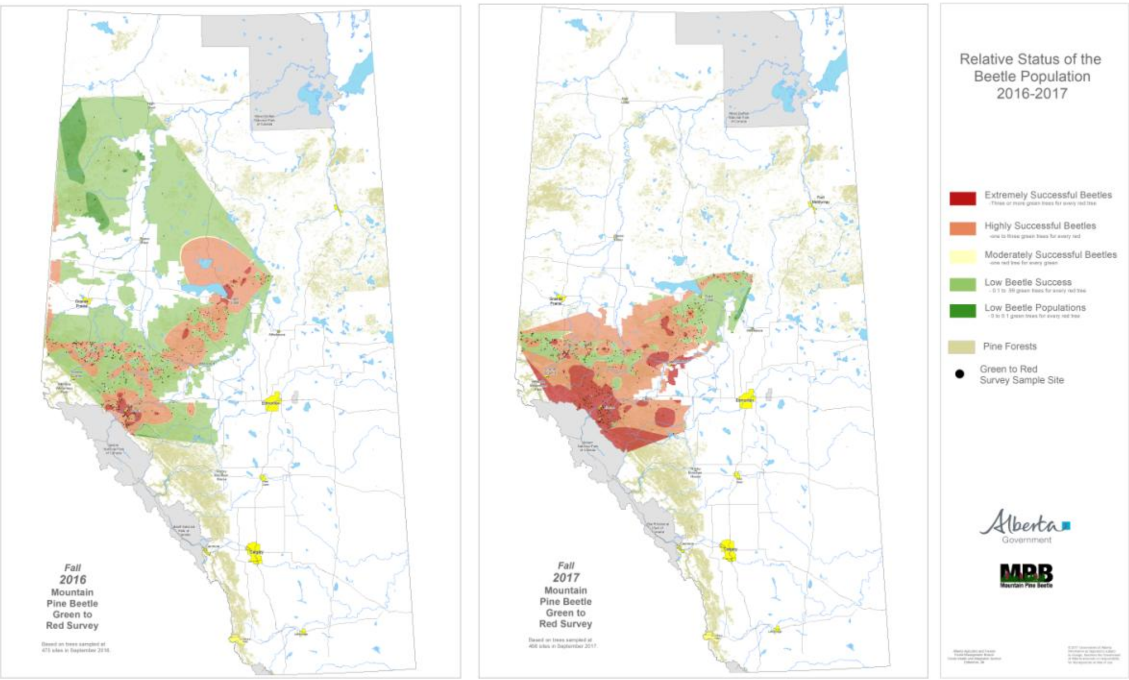


Figure 4. Green to red ratio results from 2016 and 2017 surveys.

Concentric ground surveys to assess trees for management occur each year in late fall and early winter. The majority of these concentric surveys were conducted by external contractors though some of the work was performed in-house. The number of trees flagged for control decreased slightly between 2016 (91,997) and 2017 (current estimate: 83,000). These trees are removed from the landscape during single tree cut-and-burn control operations conducted in the winter. Between 2006 and 2016, AAF has controlled approximately 1.4 million MPB-infested pine trees.

Spruce beetle (*Dendroctonus rufipennis*)

In 2017, 3,139 ha of spruce-beetle infested stands were scattered thoughout the province (Fig. 5). Spruce beetle activity has remained at levels expected from an endemic population. Note that there was a substantial increase in the area affected by spruce beetle between 2015 and 2016 which was primarily due to different mapping practices between the years (Table 1). In 2016 AAF mapped cumulative spruce mortality in order to create a baseline from which to track population expansion.

Eastern larch beetle (*Dendroctonus simplex*)

Eastern larch beetle infestations were identified in the Rocky Mountain House, Whitecourt, Lac La Biche and Fort McMurray FAs in 2017. A total of 3,139 ha of infested stands were mapped and infestation severity varied widely across all sites (Fig. 5).

Defoliators

Spruce budworm (*Choristoneura fumiferana*)

Aerial surveys conducted in the summer of 2016 detected 17,337 ha of visible defoliation due to spruce budworm, a decrease of 10% over 2016 (Fig. 5, Table 1). Provincially defoliation activity by spruce budworm has continued to decline since the population peak in 2010 and subsequent collapse the following year.

Aspen and willow defoliators

Aspen defoliators were responsible for 48% of the damage observed during the surveys (Fig. 5, Table 1). Almost half of the provincial defoliation was attributed to forest tent caterpillar even though populations have been decreasing since 2015. Large aspen tortrix populations have been on the rise in southern Alberta since 2015, while aspen two-leaf tier defoliation dropped from 18,786 ha in 2016 to zero in 2017. Willow leafblotch miner activity has been observed in the northern reaches of the province since 2013, though 2017 was the first year that defoliation was formally reported.

Alberta Agriculture and Forestry cooperates in the annual province-wide survey to detect both sub-species of gypsy moth. The survey is led by the Canadian Food Inspection Agency (CFIA). In 2016, AAF deployed 87 pheromone-baited delta traps in AAF-managed stands and neither sub-species were trapped. CFIA has conducted intensive delimitation surveys around the 2014 and 2015 positive trap sites in the Fort McMurray FA, and they have not discovered established populations of gypsy moth near these sites.

Other Damage Agents

Occurrences of other damage agents such as needle disease and abiotic damage are also mapped during aerial overview surveys (Fig. 5, Table 1). The prevalence of pine needle cast increased dramatically in 2017, which can be expected in the years following moist summer weather. Much of the observed dieback occurred in aspen stands. Dieback has become easier to detect as the defoliation by forest tent caterpillar has decreased and is likely a result of the additive effects of drought combined with repeated defoliation events. Note that AAF does map tree mortality but only when evidence suggest that the mortality is due to something other than natural tree senescence.

Acknowledgements

This report was compiled by Caroline Whitehouse, Forest Health Specialist. The data reported were collected by Forest Health Officers (Colton Briggs, Allison Brown, Megan Evans, Ryan Hermanutz, Devin Letourneau, Jennifer MacCormick, Bart McAnally, Fraser McKee, Pam Melnick, and Andrea Sharpe) and Forest Health Technicians (Devin Abraham, Caroline Charbonneau, Crystal Ionson, Marian Jones, Clint McCrea, and Jarrett Totton). Aaron McGill and Brad Tyssen prepared the maps included in this report.

For further details on forest pest conditions in Alberta, please visit the Forest Health website.

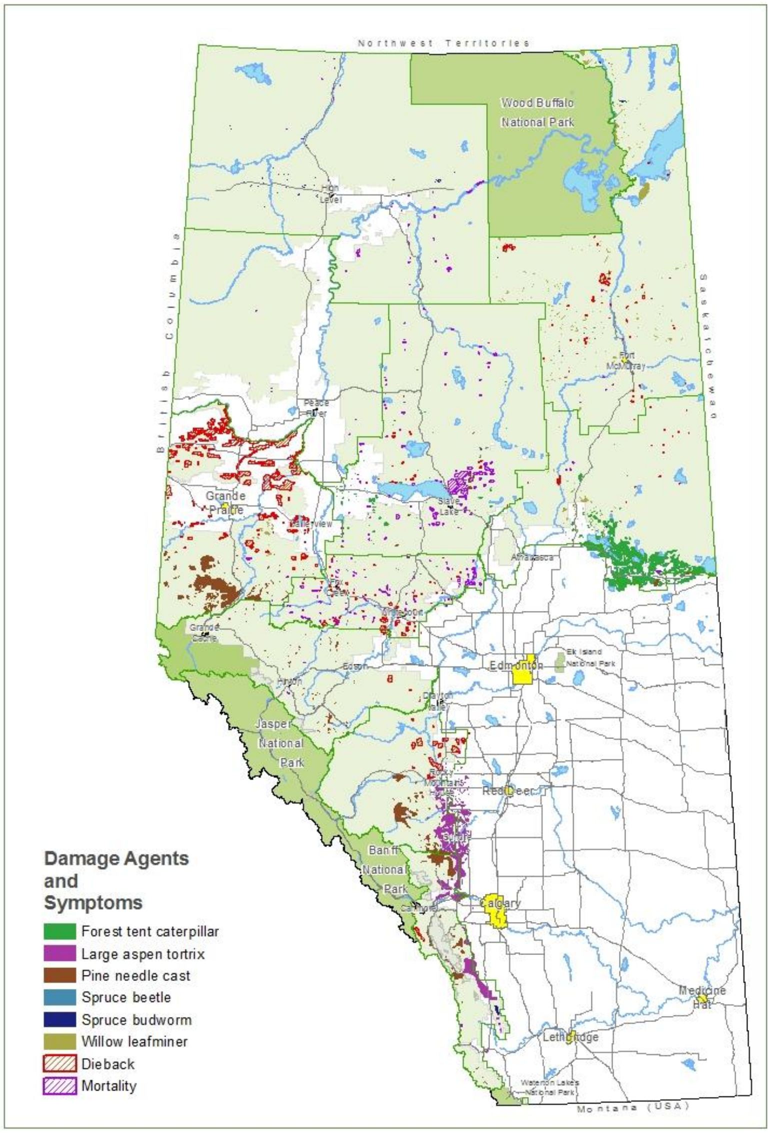


Figure 5. Distribution of select damage agents mapped during 2017 aerial overview surveys.

Table 1. Summary (in hectares) of Alberta forest disturbance agents mapped during aerial overview surveys.

	2015	2016	2017
Bark beetles			
Eastern Larch Beetle	918	6,583	2,927
Spruce beetle	1,405	10,465	3,139
Total bark beetles	**2,323**	**17,048**	**6,066**
Defoliators			
Aspen serpentine leafminer	--*	--*	1,277
Aspen two-leaf tier	536	18,786	--
Bruce spanworm	3,564	--	--
Forest tent caterpillar	1,586,486	525,135	394,286
Large aspen tortrix	54,444	213,316	294,123
Linden looper	--	--	25,504
Spearmarked black moth	--	--	710
Spruce budworm	51,750	19,265	17,337
Unknown	--	859	8,321
Willow leafblotch miner	--*	--*	118,539
Total Defoliators	**1,696,780**	**777,361**	**860,097**
Diseases			
Armillaria root disease	--*	--*	11,665
Lodgepole pine dwarf mistletoe	--*	--*	7,195
Pine needle cast	20	36,097	354,898
Other	--	--	3,224
Total diseases	**20**	**36,097**	**376,982**
Other			
Dieback	23,657	115,728	350,158
Flooding	5,457	2,415	9,075
Foliar damage	--*	34,000	38,640
Hail	1,419	1,050	11,416
Mechanical - unknown	--	--	1,869
Mortality	--*	144,693	130,631
Windthrow/blowdown	1,204	1,338	2,376
Winter desiccation	15,341	7,766	--
Total Other	**47,078**	**306,990**	**544,165**
Total Disturbance	**1,746,201**	**1,137,496**	**1,787,310**

*Observed on the ground but not formally assessed from the air.

Summary of Forest Pest Conditions in Saskatchewan 2017

Rory McIntosh, Brian Poniatowski and Colin Arndt.
Saskatchewan Ministry of Environment, Forest Service Branch
Prince Albert, Saskatchewan

INSECTS

Softwood Defoliators

Spruce budworm *Choristoneura fumiferana*

- In 2017 again no spruce budworm defoliation was detected during annual aerial surveys.
- No overwintering larval (L2) surveys were conducted.
- Pheromone trap monitoring network of 50 plots

Jack pine budworm *Choristoneura pinus pinus*

- Last outbreak on record ran from 1984 to 1988 reaching a peak in 1986. For the first time since the 1980's severe defoliation has been detected during aerial surveys. In 2016, 2,012 net ha of moderate and severe defoliation was mapped in the Torch Island Forest. This area increased to 11,068 ha in 2017 including areas within the fringe forest area (Figure 1).
- Overwintering Larval (L2) surveys were conducted in 32 sites throughout the Boreal Lowland Ecoregion. Results will be used to predict defoliation for 2018.
- In 2017 the ministry continued to monitor using a network of pheromone monitoring sites (35) across the provincial forest.

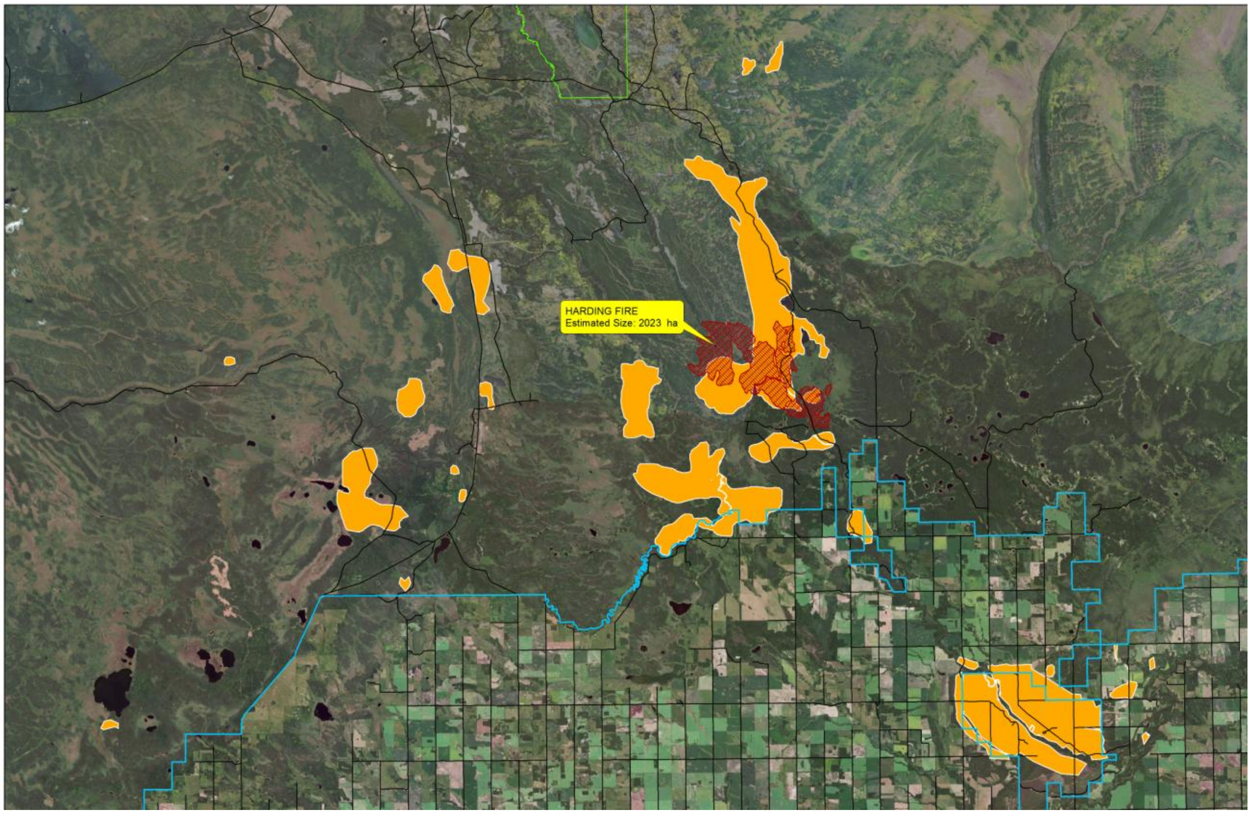


Figure 1. Distribution of Jack pine budworm defoliation in the Torch Island Forest and forest fringe in, 2017. Note – some of the defoliated area was burned in 2017.

Forest Tent Caterpillar *Malacosoma disstria* and Large Aspen Tortrix *Choristoneura conflictana*

- The annual area of hardwood defoliation has been approximately doubling since 2010. In 2014, the net area tripled to 365,953 ha and in 2015 almost doubled again to451,033 ha. Based on observations, it is estimated that approximately an additional 500,000 ha was actually defoliated – resulting in double if not triple the 2014 area of severe defoliation (See graph inset in Figure 2).
- In 2016, 576,738 hectares of severe defoliation were mapped. In 2017, the net area was 650,121 ha – this was almost exclusively Forest Tent caterpillar. The outbreak continued to be severe in the western part of the province and in Greenwater and duck Mountain Provincial parks in the south east.
- Forest Tent caterpillar continues to defoliate areas throughout the Aspen Parkland and Prairie Grassland Ecozones and in urban areas to the south of the forest fringe.
- SK MoE continues to monitor populations using pheromone monitoring traps at 40 sites across the provincial forest.

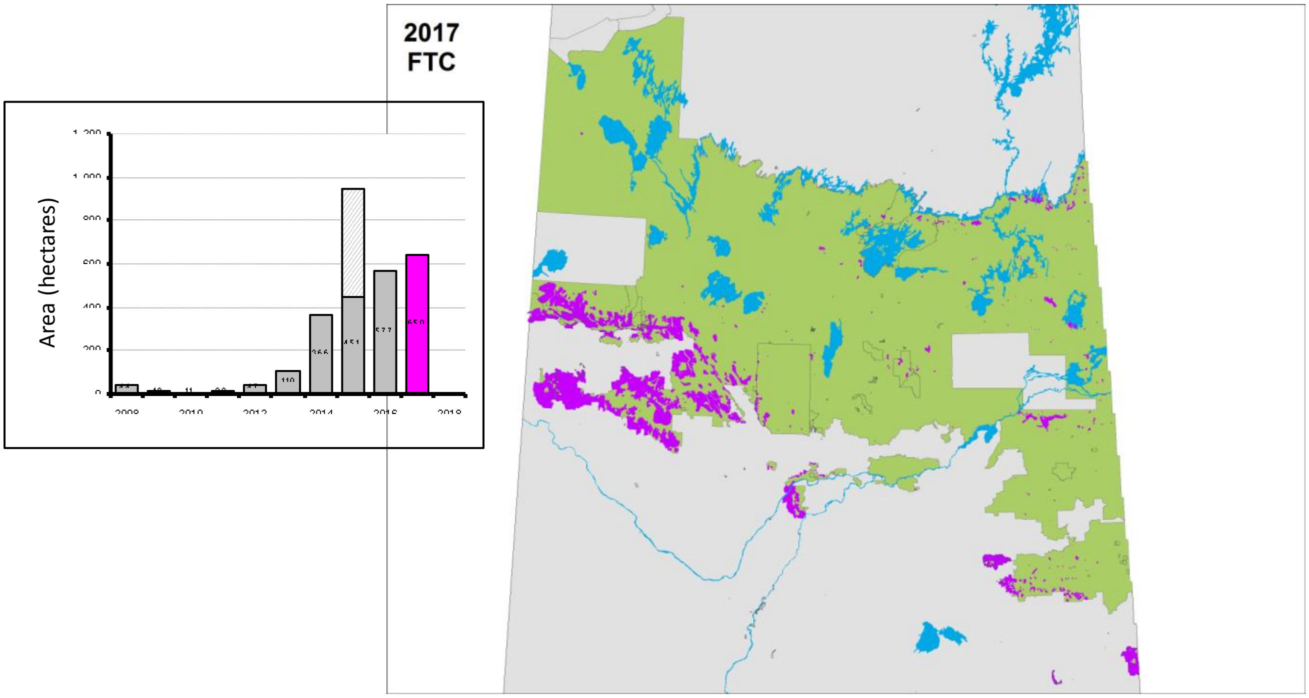


Figure 2 Distribution of severe defoliation caused by Forest Tent caterpillar, in 2017. Note in the inset graph, the stacked bar in 2015 reflects an estimate of severe defoliation. It is estimated that a significantly greater area was actually defoliated - possibly double the area observed because aerial surveys were constrained by Active fires over much of the commercial forest survey zone in 2015.

Other Insects

Eastern Larch Beetle *Dendroctonus simplex*

- Aerial surveys continue to detect small areas of trees attacked by the Eastern Larch beetle. The damage is broadly scattered throughout the forest including areas around Nipawin, Cumberland House, Candle Lake, Torch River and the Nisbet and Fort a La Corne island forests.

Yellow Headed spruce sawfly *Pikonema alaskensis*

- Yellow headed spruce sawfly (YHSS) is still occurring in 2017, but the number of enquiries from the public is declining.

Mountain Pine Beetle *Dendroctonus ponderosae*

- Mountain Pine beetle remains the highest priority pest threat to Saskatchewan forests.
- The governments of Alberta and Saskatchewan have worked together and shared forest insect and disease information for many years. In January 2012, the government of Saskatchewan signed a multi-year memorandum of agreement with the government of Alberta to implement a collaborative coordinated control program to slow the spread of MPB in the Marten and Swan Hills areas of east central Alberta.
- This partnership agreement has been renewed twice to date and the renewal of the agreement in 2017-2018 will be for an additional three year term.
- MPB is currently invading pure Jack pine stands in east Central AB.
- A network of pheromone baited trees has been established in Alberta to monitor leading edge and detect eastward spread of MPB. This detection system has also been established on the SK side of the border (Figure 3).
- In 2017, Alberta Forest Health Officers found MPB in one baited tree at one of the baiting sites inside the Cold Lake Air Weapons Range approximately 27 km west of the Alberta-Saskatchewan border.
- In 2017, SK deployed tree baits at 57 locations in the northwest, including seven locations (three other sites were denied) inside the weapons range in SK (Figure 3).

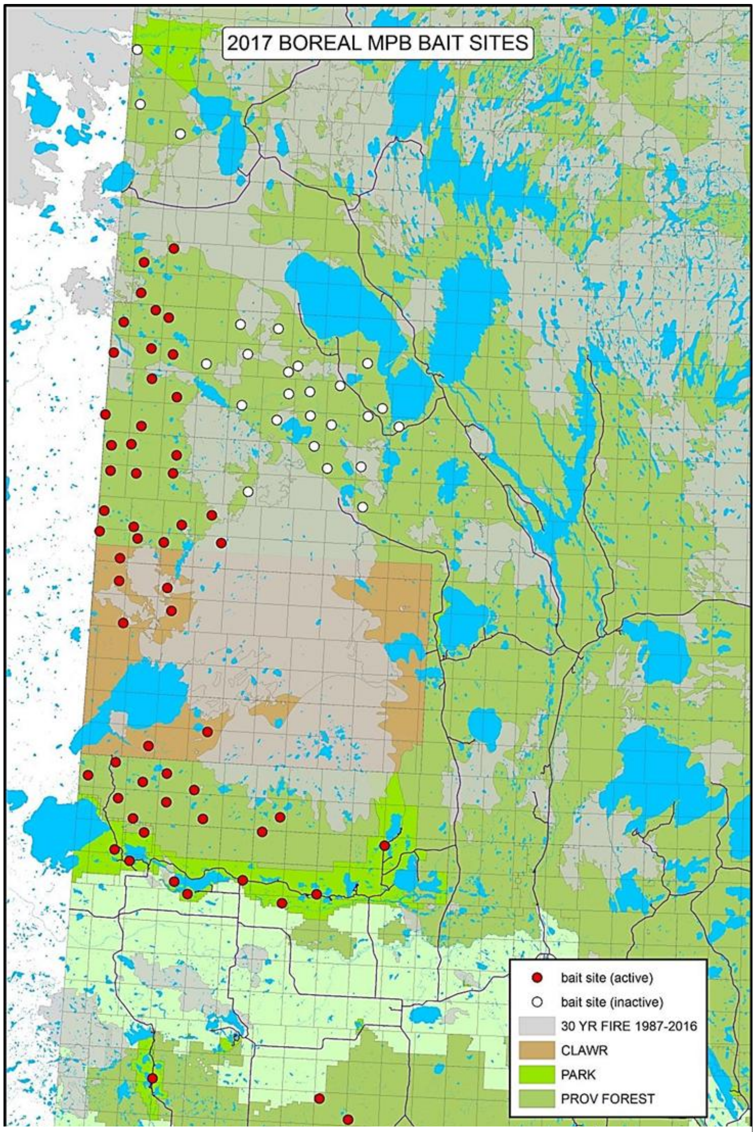


Figure 3 Map shows the distribution of tree bait network deployed in susceptible pine sites In western SK. 2017 baited trees were deployed in 57 townships (red dots). White dots represent currently inactive sites. Note baited sites deployed inside CLAWR (orange polygon) in 2017.

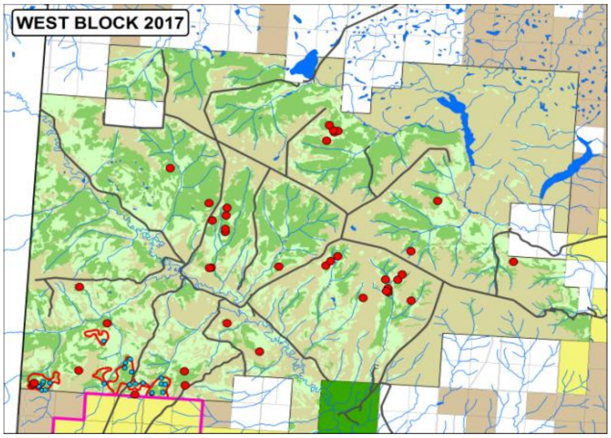


Figure 4. Total number of trees controlled in the Cypress Hills Interprovincial Park 2006 to 2017.

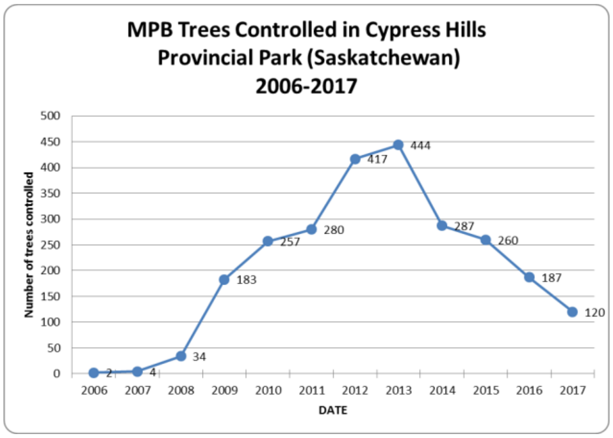


Figure 5. 2017 Location of trees marked for control in the West Block of cypress Hills Interprovincial Park (CHIPP)

- SK MoE continued to conduct systematic surveys monitoring forests in northwestern Saskatchewan and in the Cypress Hills Inter-provincial Park (CHIPP) (Figure 4) under a Memorandum of Agreement signed with the Ministry of Parks Culture and Sport (PCS).
- The outbreak is on the decline in the CHIPP. In 2010 there were 257 trees controlled; in 2011 this number increased to 280, and again to 417 in 2012, reaching a peak 444 in 2013. In 2014, the total number of trees declined to 287 and again to 260 in 2015 and 187 in 2016. In 2017, the total number of infested trees marked for control was **DOWN** again for a fourth year in a row to 120 trees suggesting provincial control efforts in the Cypress Hills are having a positive effect (Figure 5).
- Restriction/Regulation on import transport and storage of pine forest products with bark attached was implemented in 2002. The order, amended in 2008, remains in place today.
- SK MoE implements an early detection strategy followed by rapid aggressive response to remove all MPB infested trees detected on crown lands.
- SK MoE continues to work with other jurisdictions through the National Forest Pest Strategy.
- **TO DATE NO MOUNTAIN PINE BEETLES HAVE BEEN FOUND IN THE BOREAL FOREST IN SK.**

DISEASES

Lodgepole pine dwarf mistletoe *Arceuthobium americanum*

In 2016, SK initiated a project to re-survey Dwarf mistletoe (DMT). The most comprehensive systematic survey was conducted in 1998 by Canadian Forestry Service[1]. An additional survey was conducted by BioForest in 2005. However, since DMT grows and spreads very slowly there is no need to conduct an annual survey. DMT affected area will be surveyed over the course of 2-3 years to provide a provincial overview of severe DMT extent which is sufficient for provincial purposes. In 2017, 66,520 net hectares of severe DMT were mapped. The total area will decline over time because areas are burned and it is controlled through harvesting and sanitation activities. Changes in area can be revised using area depletions reported by Wildfire Management Branch and forest industry.

EXOTICS/ALIEN INVASIVES

Dutch elm disease *Ophiostoma novo ulmi*

- DED is spreading rapidly in parts of Saskatchewan.
- According to the provincial crop protection laboratory, in 2017 there were six communities with their first confirmed cases of DED, including the cities of Melfort & Yorkton. This is up significantly from 2016, where only one new community saw its first infection.
- The number of infected trees removed in the ministry's rural management (buffer) zones in 2017 (253) decreased slightly 2016 (278).
- The number of trees removed from provincial parks in 2017 increased. In 2017 a total of 117trees were removed as compared to 89 in 2016.
- Provincially, the affected rural area is expected to continue to expand. For now, the west side of the province remains DED free.

CFIA Monitoring Activities [2]

- The Canadian Food Inspection Agency (CFIA) continued ongoing monitoring European Gypsy moth *Lymantria dispar* in SK. In 2017 500 Tréce Delta II Green Traps baited with Disparlure Flex lure were deployed. The City of Regina deployed an additional 24 traps for a total of 472 traps**. .**
- CFIA continued Emerald Ash Borer *Agrilus planipennis* trapping and visual surveillance. In 2017, 10 green sticky prism panel traps baited with (z)-3 Hexanol were deployed. Visual surveys were conducted in mid-July and late September, and pedestrian visual surveys during trap collection.

---

[1] Brandt, J.P., R.D Brett, K.R. Knowles, and A Sproule. 1998. Distribution of Severe dwarf mistletoe damage in west central Canada. Nat. Resour. Can., For. Serv. North For. Cent., Special Report 13, Edmonton Alberta.

[2] CFIA Data provided by Caley Letkeman, Acting senior Agrologist, Operations Branch, Saskatchewan Section.

- In the fall, CFIA conducted visual surveys for Asian long horned beetle *Anoplophora glabripennis* (ALB) at 10 sites (5 in the city of Regina and 5 in city of Saskatoon). No signs of ALB were found.
- **IN 2017, NO GYPSY MOTH DETECTED IN SASKATCHEWAN.**
- **IN 2017 NO EMERALD ASH BORERS OR ASIAN LONGHORNED BEETLES WERE FOUND IN ANY OF THE TRAPS OR SURVEYS IN SASKATCHEWAN**.

ABIOTIC

In 2017, a net area of 14,472 hectares was affected by wind damage and a further 4,887 hectares flooded (over and above previous years flood).

Forest Pests in Manitoba

2017

Prepared by: Fiona Ross, Forestry and Peatlands Management Branch, Manitoba Sustainable Development, 200 Saulteaux Crescent, Winnipeg, Manitoba, R3J 3W3

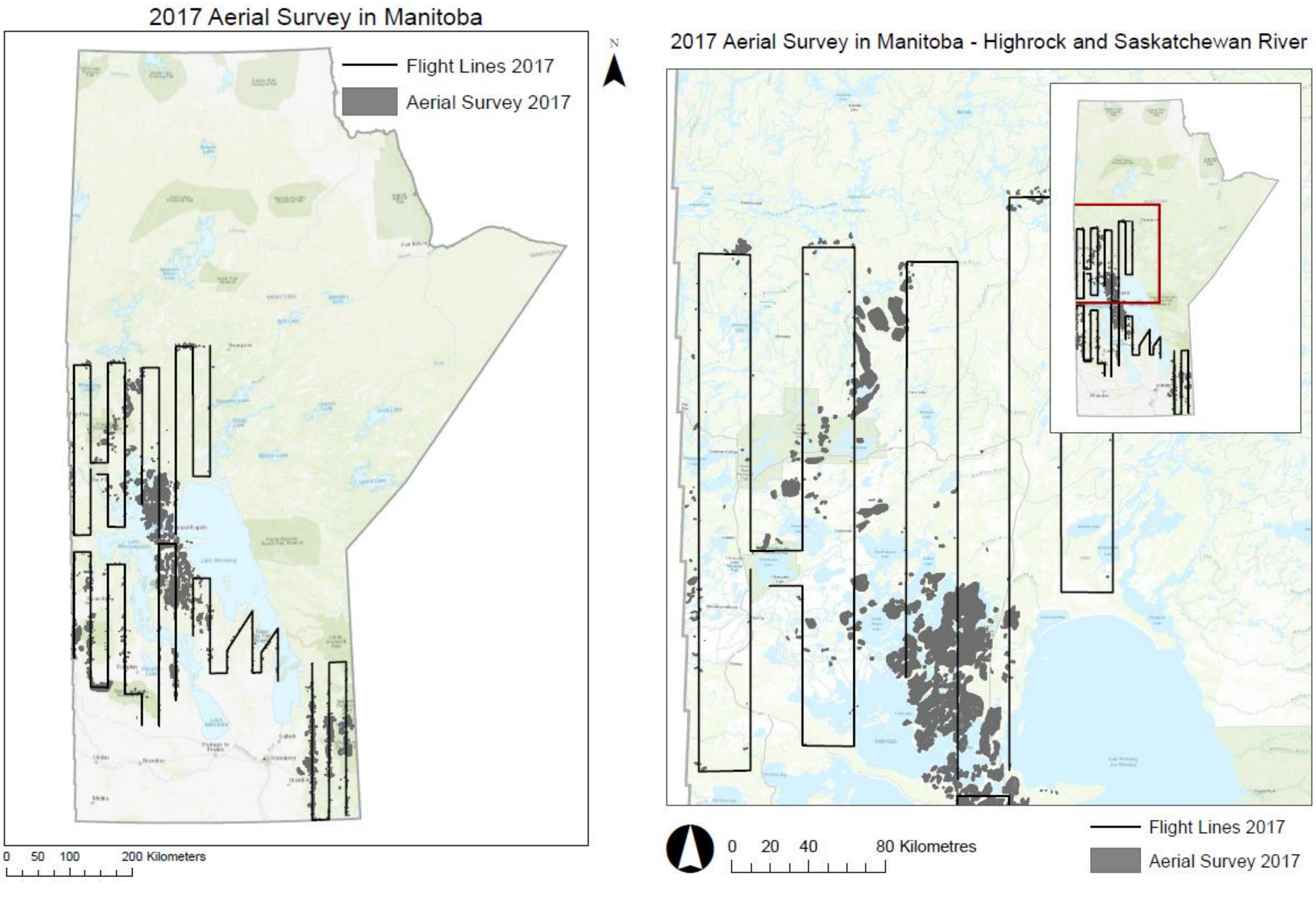
Aerial Surveys

In 2017, Manitoba Sustainable Development continued with a systematic aerial survey of managed crown land. This survey provides an overall picture of potential forest health issues and an estimate of forest defoliation and damage.

The annual baseline survey utilizes flights at 27 kilometre line spacing (figure 1) over commercially important forests. In addition, intensive surveys (3 kilometre flight line spacing) are added when potential damage is observed. Results from the intensive surveys are ground-truthed, so additional branch samples can be collected to determine the presence and density of the suspected pest or causal agent.

Intensive surveys were completed in the Saskatchewan River and Interlake forest sections in north central Manitoba.

Figure 1: Map of aerial survey conducted in Manitoba in 2017 and close up of Highrock and Saskatchewan River forest sections.



Forest Tent Caterpillar - *Malacosoma disstria*

The population of forest tent caterpillar increased in the province in 2017. Much of southern Manitoba experienced moderate to severe defoliation from forest tent caterpillar. Similar defoliation is expected for 2018.

Spruce Budworm - *Choristoneura fumiferana*

Spruce budworm pheromone traps were placed at 40 locations throughout the province. Three MULTIPHER® insect traps containing spruce budworm pheromone (PVC lure containing 0.3% by weight of a 95:5 blend of (E)- and (Z)-11-tetradecenal) are placed 40 metres apart at each plot location in a triangular configuration.

In August and September, branch samples and pheromone traps are collected from the annual monitoring plots. Branches were processed to assess current defoliation levels and egg mass densities while the Moths found in traps were counted. Data collected is used to predict the next year’s defoliation.

Spruce Budworm - Survey Results

Based on 2016 defoliation sampling and predictions, no operational spruce budworm suppression program was implemented in 2017.

Spruce budworm defoliation for 2017, based on egg masses and defoliation, is predicted to be light to moderate in Manitoba (Table 1). However, there are notable increases in moth captures in several forest sections including Saskatchewan River and Highrock that may suggest a building spruce budworm population.

Table 1: 2017 Spruce Budworm Defoliation, Egg Mass and Predictions for 2018

Location by Forest Section	2017 Defoliation*	Average 2016 Egg Mass/10m^2	2018 Defoliation Prediction
Aspen Parkland	Light	0	Nil
Mountain	Light	0	Light
Pineland	Nil	0	Light
Lake Winnipeg East	Light	0	Nil
Interlake	Light	0	Nil
Saskatchewan River	Light	17.7	Light
Highrock	Light	0	Light
Churchill	N/A	N/A	N/A
Nelson River	Light	94.7	Moderate
Hayes River	N/A	N/A	N/A

*****Defoliation classes are as follows:

Nil - 0% defoliations of current shoots
-0 egg masses per 10 m^2 of branch area
Light - 1% to 35% defoliation of current shoots
- based on <40 egg masses per 10 m^2 of branch area
Moderate - 36% to 70% defoliation of current shoots
- based on 40 to 185 egg masses per 10 m^2 of branch area
Severe - greater than 70% defoliation of current shoots and possible feeding on old foliage
- based on >185 egg masses per 10 m^2 of branch area

Jack Pine Budworm - *Choristoneura pinus*

Jack pine budworm pheromone traps were placed at 37 locations throughout the province. Three MULTIPHER® insect traps containing jack budworm pheromone were placed 40 metres apart at each plot location in a triangular configuration. This trapping method is being evaluated as an early warning method for outbreaks and a supplemental technique to defoliation predictions by egg mass density surveys.

In August and September, branch samples and pheromone traps were collected from the annual monitoring plots. An additional 63 plots were established to collect additional branch and L2 samples within the outbreak area. Branches were processed to assess current defoliation levels and egg mass densities while the Moths found in traps were counted. Data collected is used to predict the next year's defoliation (Table 2).

Jack Pine Budworm – Survey Results

A small pocket of jack pine budworm defoliation was observed in the Interlake Forest Section in 2015. The outbreak area expanded in 2016, and has further expanded 2017. In 2017, 635,813 ha of defoliated jack pine budworm forest was observed across the Province (Figure 2).

Figure 2: Map of jack pine budworm defoliation observed by aerial survey in Manitoba

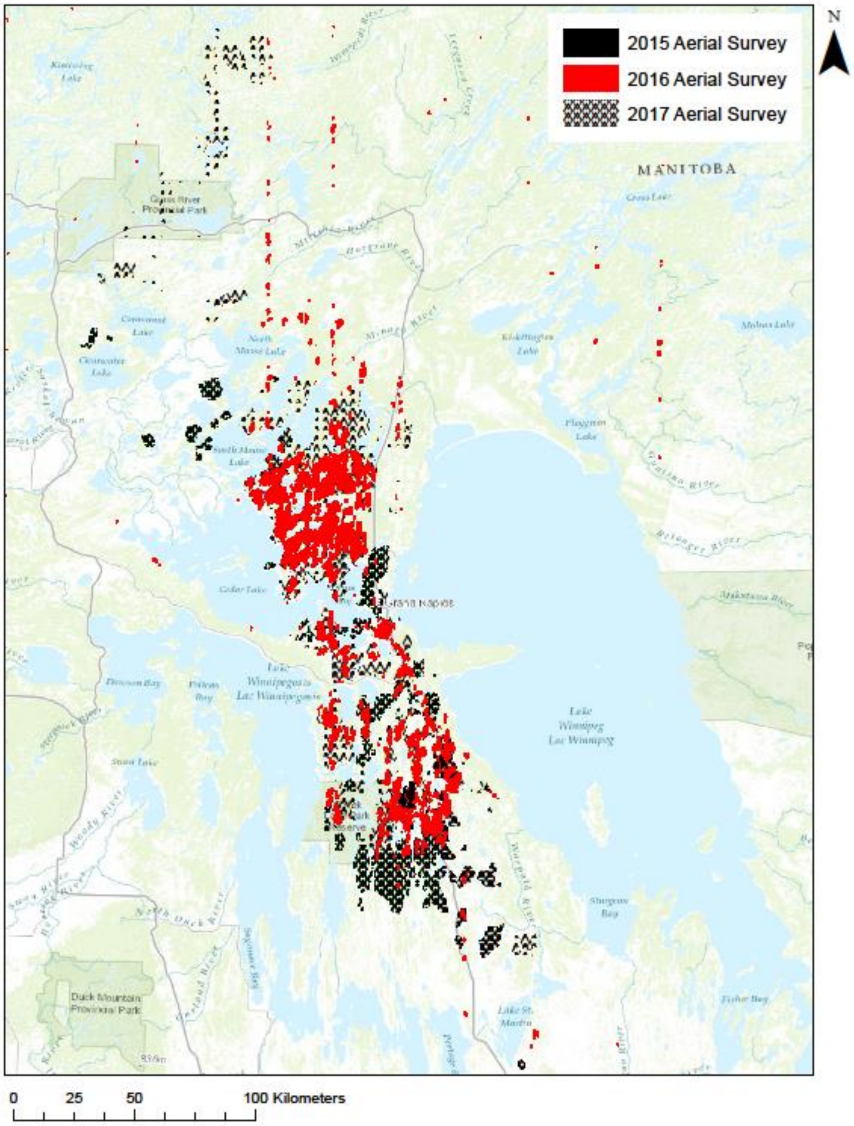


Table 2: Jack Pine Budworm Defoliation and Predictions for 2018

Location	2017 Defoliation*	Average 2017 Egg Mass/10m^2	2018 Defoliation Prediction
Aspen Parkland	Nil	0	Light
Mountain	Nil	0	Light
Pineland	Nil	0	Light
Lake Winnipeg East	Nil	0	Light
Interlake	Severe	2.7	Moderate
Saskatchewan River	Severe	2.5	Severe
Highrock	Nil	0	Light
Churchill	N/A	N/A	N/A
Nelson River	Light	2	Light
Hayes River	N/A	N/A	N/A

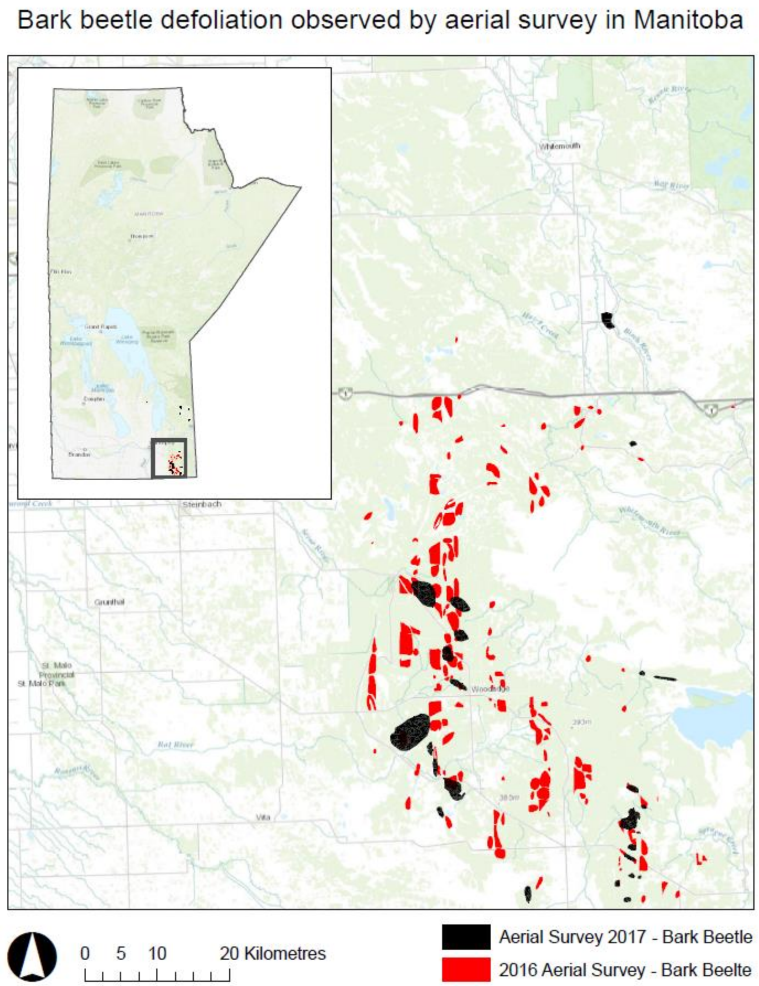
*****Defoliation classes are as follows:

Nil - 0% defoliations of current shoots
Light - up to 35% defoliation of current shoots
Moderate - 36% to 70% defoliation of current shoots
Severe - greater than 70% defoliation of current shoots and possible feeding on old foliage

Bark beetle/sawyer beetle damage - *Ips pini, Ips grandicollis, Monochamus* spp

A small area (4000 ha) observed in 2014 through the aerial survey that was thought to be jack pine budworm defoliation has since been identified as bark beetle and sawyer beetle damage. These insect populations have developed in high numbers due to large amounts of woody debris and standing dead trees caused by a severe winter storm in 2012. This damage expanded in 2015, and could be seen throughout the Pineland Forest Section totalling 11,868 hectares. The infestation continued in 2016, as can be seen in figure 3. The bark beetles were identified as two native Ips species: *Ips pini and Ips grandicollis.*

Figure 3: Map of bark beetle damage observed by aerial survey in Manitoba



Dutch Elm Disease - *Ophiostoma novo-ulmi*

Manitoba’s integrated, community-based Dutch Elm Disease (DED) Management Program partners with 38 participating communities throughout the province. With financial support provided by the province, communities are responsible for tree removals and implementing preventative measures such as basal spraying. The Manitoba government continues to coordinate the delivery of the program, provide technical support, and conduct annual detection surveys. This partnership approach results in increased community participation and enhanced protection efforts for Manitoba’s urban forests.

Provincial survey crews marked a total of 5,052 infected trees in 2017. The City of Winnipeg, which has its own independent program, marked 8,095 elms. The total number of DED infected elm trees marked in 2017 in Manitoba was 13,147.

European Gypsy Moth - *Lymantria dispar*

In the fall of 2014, Manitoba Sustainable Development assisted the Canadian Food Inspection Agency (CFIA) in conducting a survey for European gypsy moth egg masses within the Rural Municipality of Lac du Bonnet. For two consecutive years' pheromone traps in this small area captured an increasing number of moths. The ground survey resulted in positive finds of egg masses, pupal casings, dead larva, and a dead adult moth. An eradication program was conducted by the Manitoba government in 2015.

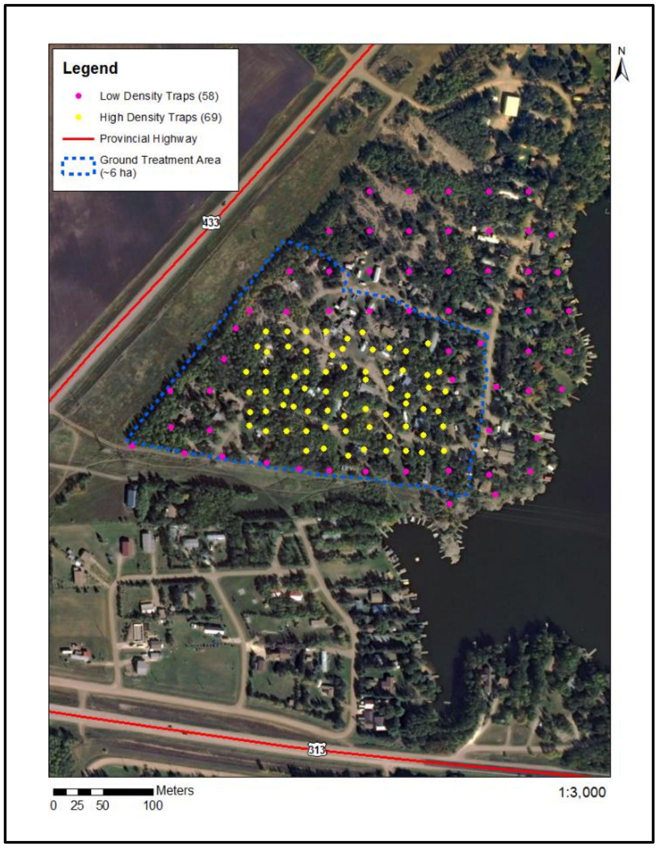
Three aerial applications of Foray 48B were applied to coincide with L1, L2 and L3 larval development as predicted by BioSIM over the 230 hectare treatment area. Follow-up surveys and trapping detected a small residual population of gypsy moth in the area.

In cooperation with the CFIA, an intensive trapping program was implemented in 2016 to eradicate the remaining population. The program involved setting up 164 pheromone baited traps in a low-intensity and high-intensity area. Adult male moths were captured in 10 traps (most within the intensive trap area). A survey for additional gypsy moth life stages was undertaken in mid-October, and 20 non-viable egg masses (likely from 2015) were found.

In 2017, a pesticide and intensives trapping program was implemented to eradicate European gypsy moth. A 6 ha ground application of Foray 48B was applied to coincide with L1, L2 and L3 larval development as predicted by BioSIM and 127 pheromone baited traps were placed in a low-intensity and high intensity area (figure 4).

No moth were caught in any traps, nor were any other life stages observed following treatment. The CFIA will continue with monitoring the area in 2018.

Figure 4: Map of European gypsy moth management area in 2017



Invasive Forest Pests and Movement of Firewood

Manitoba is concerned about the spread of invasive forest insects and diseases through the movement of firewood. In 2008, four wood collection bins were placed on major highways at provincial boundaries: two along the TransCanada Highway and one each at Highways 5 and 16. In 2017 the woodbine along Highway 5 was moved to a new location at the Emmerson Boarder along Highway 75. New signs were placed at each of the 4 bin locations (figure 5).

In 2017, travelers deposited pine, ash, oak and other tree species, in both bins along Highway 1 and in bins along Highways 16. A total of 539 pieces of firewood were deposited by the public. Sustainable Development staff inspect the firewood for signs of insect activity before being burned. Minimal insect activity was found in the inspected wood.

Figure 5: New signage on Woodbins in Manitoba

Emerald Ash Borer - *Agrilus planipennis*

Ash species (*Fraxinus spp.)* are a cornerstone species in riparian and urban forests in Manitoba. As such, planning and preparation has been ongoing for emerald ash borer (EAB) since its discovery in Eastern Canada and the US. To aid in EAB detection, Manitoba Sustainable Development deployed 39 green prism traps at high risk locations throughout the province. The City of Winnipeg, in cooperation with Trees Winnipeg and the City of Brandon also set up EAB traps. All traps in Manitoba were found to be negative for the presence of EAB.

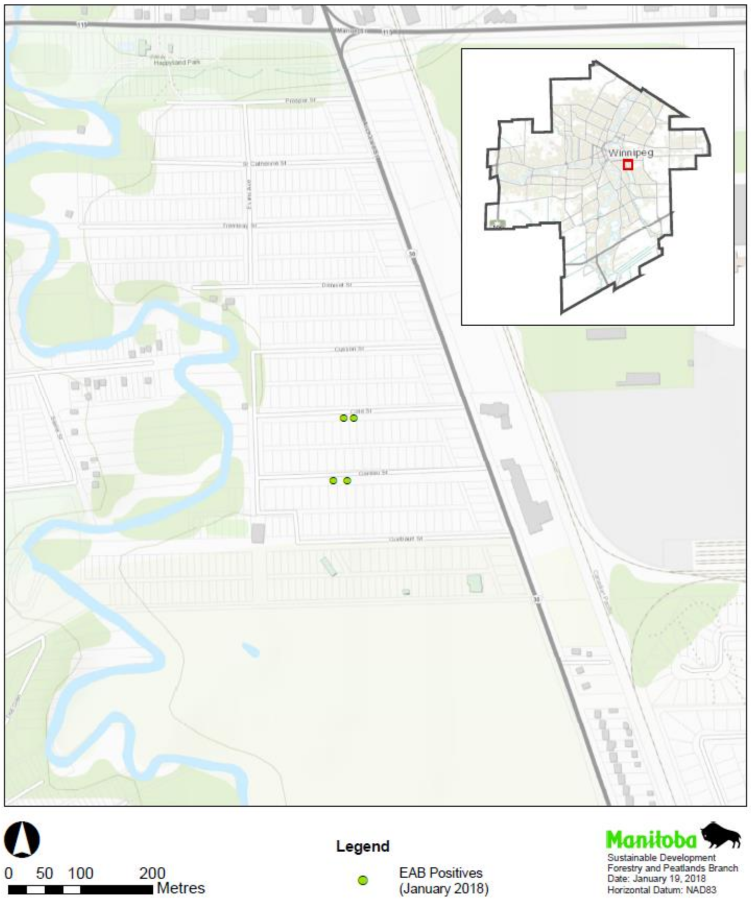
In November the city of Winnipeg received notification from a member of the public that a boulevard tree was not looking health. Suspect larval samples were taken from the tree and sent for diagnostic testing at a CFIA laboratory facility. **Emerald ash borer was confirmed in the city on Winnipeg on November 30, 2017.**

Visual surveys were conducted within the city of Winnipeg and in some neighbouring communities. As of January 22, 2018 two additional ash trees in the city of Winnipeg were identified as having positive EAB symptom (galleries). Based on visual surveys EAB has not been found outside of the city of Winnipeg (figure 6).

Branch sampling in the city of Winnipeg is being conducted to help delimitate the outbreak area. The city of Winnipeg is leading the survey with support provided by the Province.

On January 18th 2018, the city of Winnipeg became the first city in Manitoba to be regulation by the CFIA for EAB. The Province and CFIA will continue to monitor for EAB outside of the regulated area.

Figure 6: Map of confirmed emerald ash borer trees in Manitoba as of January 2018



Public Education and Outreach

Increasing public knowledge and understanding of forest health issues, including the risk associated with firewood movement and invasive species management, is important to the Manitoba. In 2017, Manitoba Sustainable Development answered online inquires from its "*Got a Sick Tree*" questionnaire, and forest health staff participated in trade shows, provided presentations and training to special interest groups/communities/industry, and responded to public inquiries.

Forest Health Monitoring

Interim Update

2017

Introduction/Summary

Currently the forest health monitoring program is continuing to compile field data collected during the 2017 core field season. This interim report will provide an update to the accompanying 2017 summary report and will be documented in a more formalized document *(2017 Forest Health Conditions Report)* later in the fall which will include areas and forecasts.

This season the forest health monitoring program within the biodiversity and monitoring section (BAMS) consisted of twelve field personal and one program lead coordinator. The 2017 field program consisted of the evaluation of a permanent sample plot network to monitor the health of jack pine and spruce fir mixed stands in the province. Pheromone trapping for jack pine and spruce budworm was also conducted and served as an early detection tool and as well was part of a DNA collection research program. Trapping for the invasive walnut twig beetle, the known vector for thousand canker disease was carried out in southern region at a number of predetermined locations. Surveys for the invasive emerald ash borer continued throughout the northern reaches of the province where the pest currently is not known to exist. Ontario continues to be concerned about the impact of beech bark disease. In 2017 an earlier established network of beech health sample plots are in the process of being evaluated for the both tree vigor and as well the presence or absence of beech bark disease. Last but certainly not least aerial surveys were carried out to delineate major forest disturbances. In 2017 forest health staff logged approximately 150 aircraft hours and delineated in excess of 1,000,000 hectares of damaged forest. The bulk of that area made up of the ongoing forest tent caterpillar outbreak in the north and now southern regions. Once again the forest health monitoring section within BAMS was supported by entomology and pathology diagnostic support that ensures our data is both verified and credible. The summary of major forest disturbances mentioned in the following information is by no means an exhaustive list but will serve as an update to the condition of the Ontario landscape in 2017.

Climatic Events

2017 had a wet spring and summer across the province, which caused high water in most areas and flooding in some. Blowdown was mapped around northern Ontario in 2017, but was less than mapped in 2016. A hail event that occurred northwest of Fort Frances, in the Boffin Lake area, in 2016 had mortality in 2017 within the most damaged areas of red and jack pine. Another area of hail damage, from the same event as in 2016, was aerially mapped in 2017 southwest of the Town of Mine Centre at the junction of Barber and Bliss Lake roads, where a total of 46 ha of severe damage and mortality, to mostly young jack pine, was recorded at this location in 2017. The effects of extreme drought conditions in the southern part of the province in 2016 were still visible in 2017. A small area of scorch was mapped on the eastern most edge of Peterborough District in 2017.

Blowdown 2017

Ontario
Areas-within-which blowndown caused damage

Moderate-to-severe = 2,439 ha

🟥 Area of moderate-to-severe damage

Hail Damage
2017

Northwest Region
Areas-within-which hail caused damage

Moderate-to-severe = 75 ha

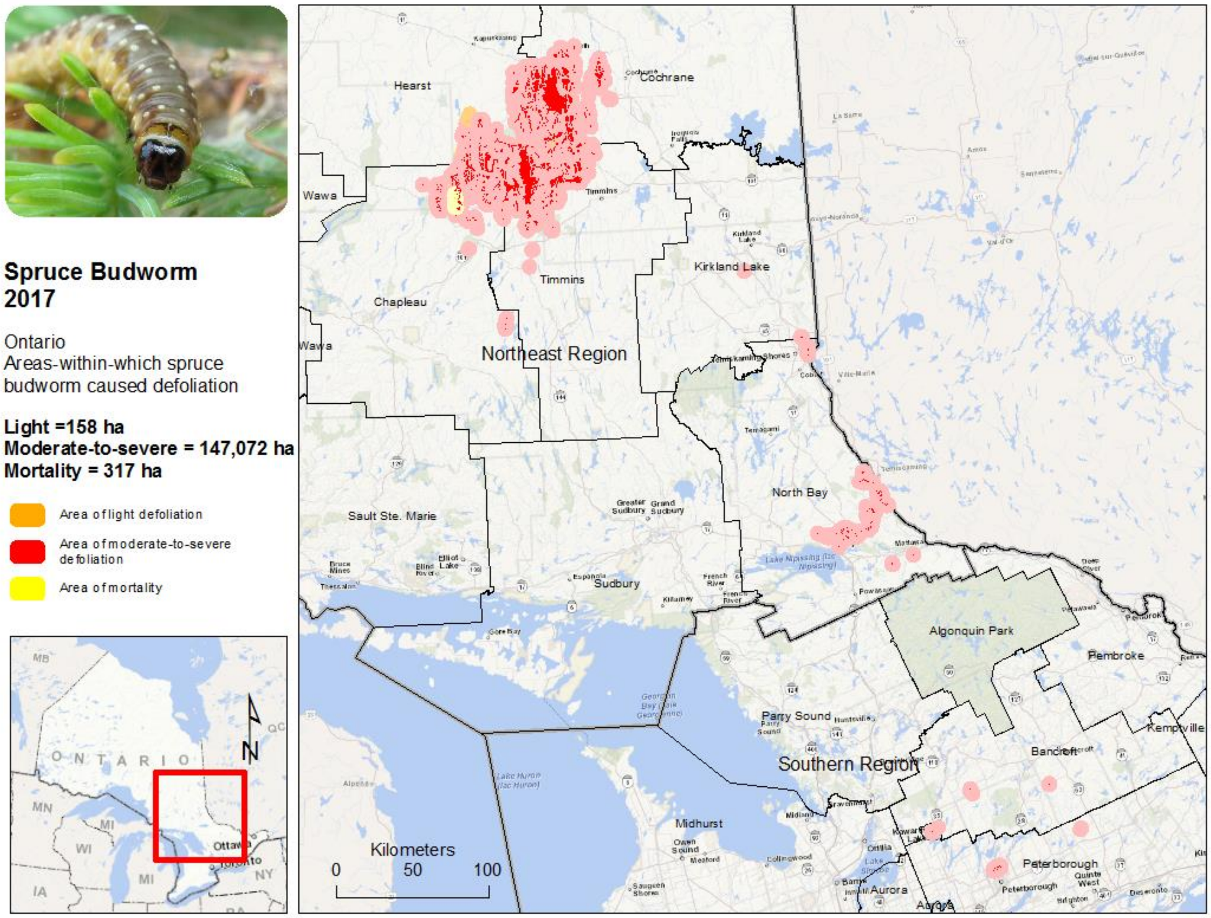
🟥 Area of moderate-to-severe damage

Forest Tent Caterpillar

The province of Ontario continues to work its way through the cyclic outbreak of forest tent caterpillar in 2017. This season there was an expansion of hardwood defoliation in the Northeast Region from Sault Ste. Marie, north of North Bay to the eastern border of the province. 2017 is the first year for significant defoliation to be detected in the southern areas of the province. Preliminary numbers show provincial defoliation area to exceed 1,000,000 ha. Although expanding in the Northeast and Southern regions, the Northwest Region saw a decline of forest tent caterpillar, from 940,399 ha mapped in 2016 to just over a 10th of that number, 91,218 ha in 2017.

Spruce budworm

Spruce budworm continues to persist across the landscape of Ontario. The main area of infestation was located in the Northeast Region in 2017, where there was an increase in size of area and overall intensity in the northwest corner of Chapleau District, northeast to Cochrane District, and in the area of the town of North Bay, northeast to the Ontario border. Other areas of spruce budworm infestation in 2016 had decreased in 2017. Mortality was mapped in the Chapleau District, within the core area of damage. Area had also increased in the Southern Region in 2017, from a small area in Balsam Lake Provincial Park, east to a couple of areas in the southern portion of Bancroft district and northern end of Peterborough District. Overall, pheromone trap collections have increased in approximately half of the trap locations in the Northeast Region, and approximately a quarter of the trap sights in the Southern Region, north of Carlton Place.



Jackpine budworm

There was an outbreak of this pest in northwestern Ontario, in the Whitefeather Forest in 2017. Discussion is ongoing for response and further monitoring of this area with the district office. Pheromone trapping continued for this pest in 2017 and results were generally down in the Northeast Region. Higher counts were encountered in Kenora and Dryden districts in Northwest Region and Pembroke District in Southern Region.

Balsam fir sawfly

In 2017 moderate-to-severe balsam fir sawfly defoliation was aerially mapped in the southeast corner of Pembroke District (135 ha). In Pembroke District, balsam fir sawfly defoliation was aerially mapped south of Stewartville and along Robertson Line, south of the Madawaska River in McNab Twp. This area was neighbouring a small area mapped in 2016, where light defoliation was also observed again in 2017 but not aerially mapped. Balsam fir sawfly defoliation was also observed early in the season on Hwy 20 east of Waba near the border of Kemptville district. Red crowns and damage to old foliage was observed.

Balsam fir sawfly
2017

Southern Region
Areas-within-which balsam
fir sawfly caused defoliation

Moderate-to-severe = 135 ha

🟥 Area of moderate-to-severe defoliation

Emerald ash borer

Monitoring the emerald ash borer infestation was not done aerially in 2017, but continued by setting out a series of traps across the province. Aerial surveys will continue on a bi-annual basis for this pest in the future. Monitoring continued in the Thunder Bay area, with Thunder Bay District staff this year. Rutter Urban Forestry monitored the City of Thunder Bay and their forester, confirmed that it has spread this year, with 12-15 positive findings on the traps in the south end of the City.

Gypsy moth

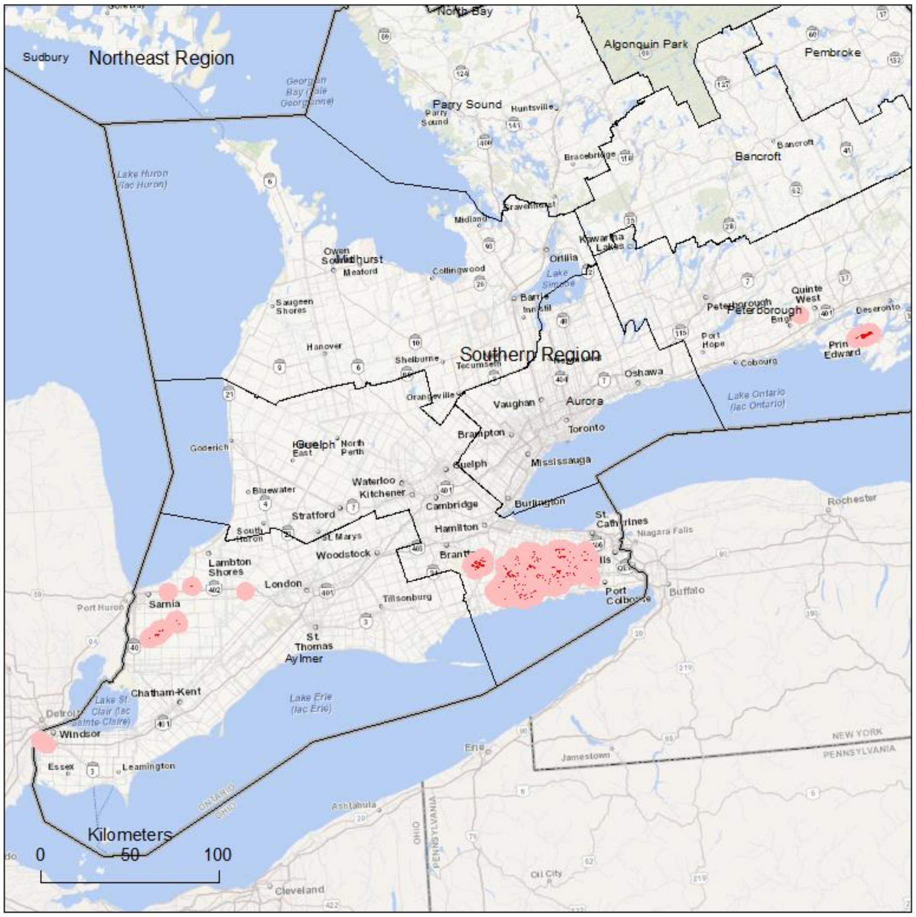
Gypsy moth outbreaks occurred in a few areas of Southern Region in 2017. One of the largest areas was eastern Guelph District where 8,768 ha of moderate-to-severe defoliation were aerially mapped. Defoliation was mapped from Brampford, south and then east to Port Colburn, north to St. Catherines. 1461 ha of moderate-to-severe defoliation was aerially mapped in Peterborough District, in areas around Trenton in Quinte West, and Prince Edward County, and 625 ha of moderate-to-severe defoliation in Aylmer District, south and east of Sarnia and a small patch in the city of Windsor, where >50 mature black oak *(Quercus velutina* Lam*.)* trees was observed along Ojibway Parkway, in City of Windsor. Defoliation in these areas was found on a variety of hardwood species, including, oak, maple, hickory, basswood, elm, cherry, black walnut, hop hornbeam, ash, witch hazel, blue beech and American beech, on all canopy and age classes. It was quite often found feeding with fall cankerworm larvae. In Wooler, Murray Twp, gypsy moth was seen feeding with willow leaf beetle, causing moderate-to-severe defoliation in that area. Other areas of defoliation caused by gypsy moth were observed in Aurora District, in areas from Burlington east to Oakville, Mississauga, Etobicoke and Toronto, eastern Bancroft District, Kemptville District, Pembroke District, in areas around Renfrew, Midhurst District, in Simcoe County.

Gypsy Moth 2017

Southern Region
Areas-within-which gypsy
moth caused defoliation

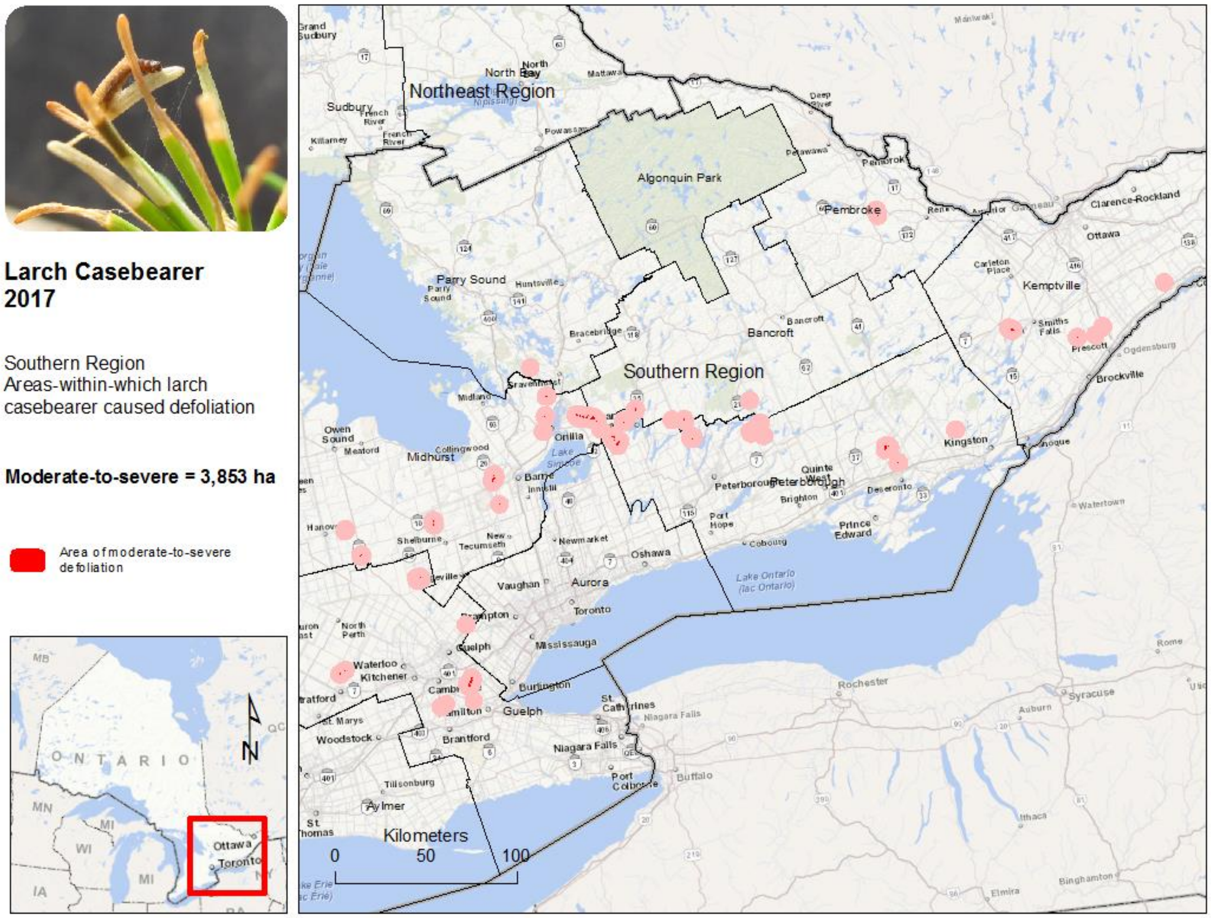
Moderate-to-severe = 10,856 ha

🟥 Area of moderate-to-severe defoliation



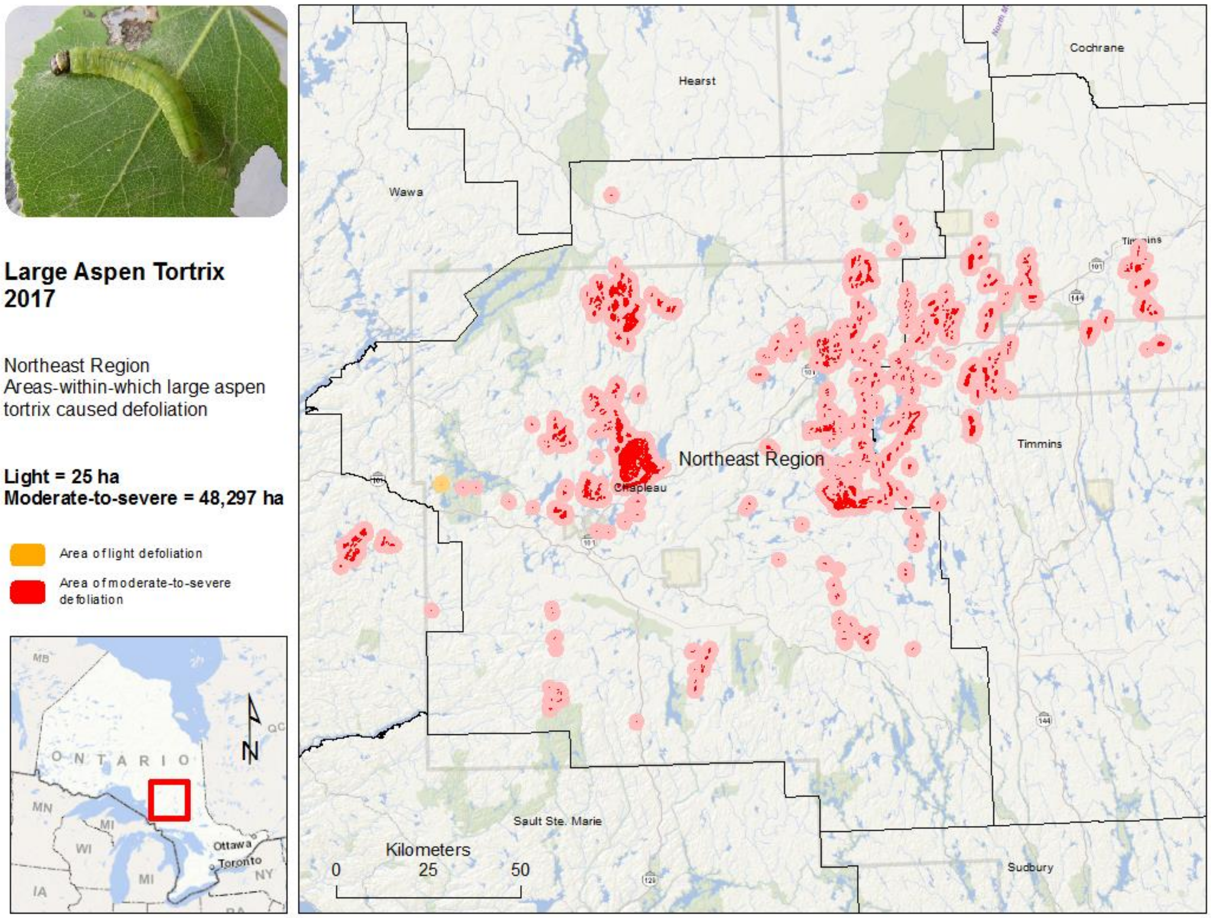
Larch Casebearer

Overall the area of defoliation caused by larch case bearer in the Southern Region increased slightly from 2016. From aerial mapping results, Guelph, Peterborough, Kemptville, Bancroft, Midhurst and Parry Sound districts increased and Pembroke District experienced a decrease in disturbance.



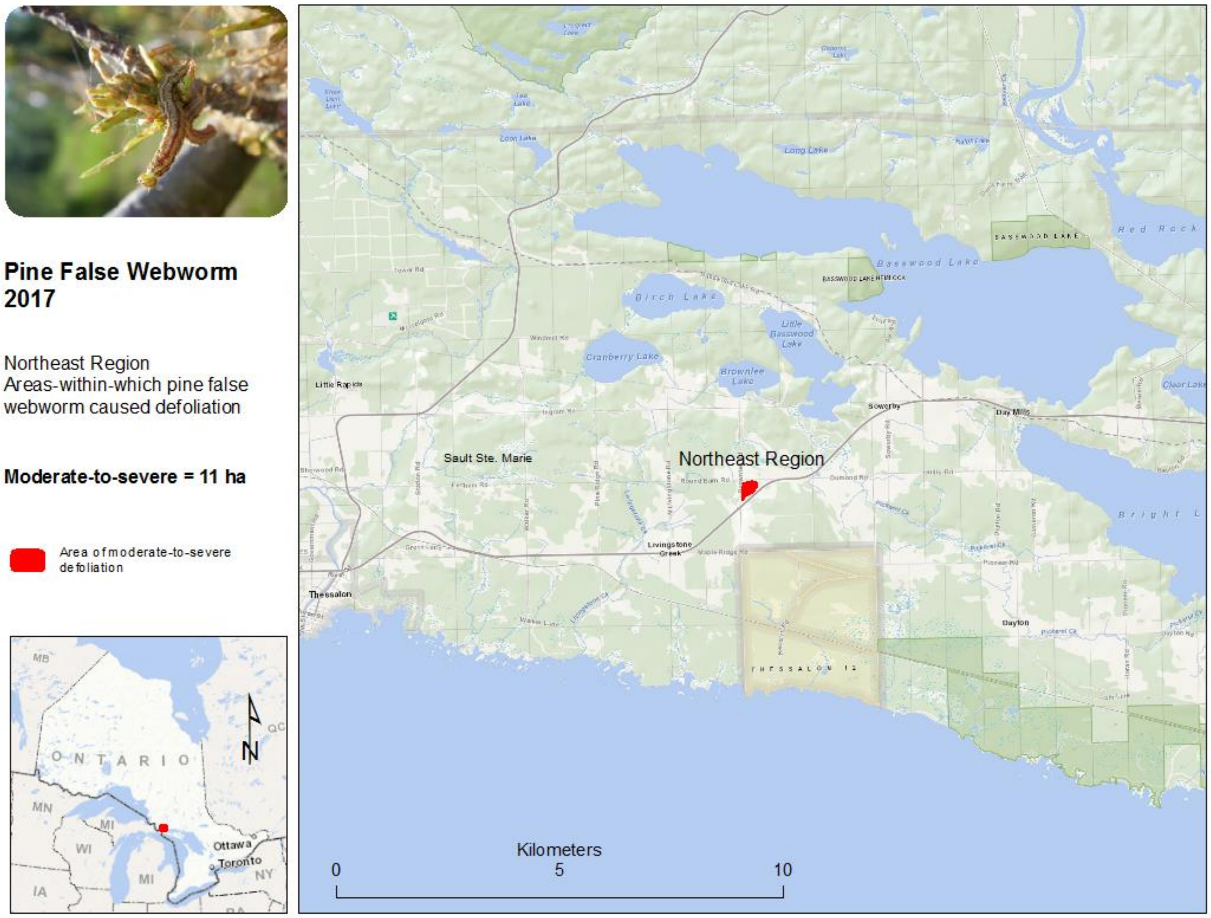
Large aspen tortrix

Large aspen tortrix defoliation increased more than two times from 2016 in the Northeast Region. Moderate-to-severe large aspen tortrix defoliation was aerially mapped in Northeast Region, increasing over two times in total area compared to 2016. The majority was aerially mapped in Chapleau District in 2017, over 34,000 ha compared to almost 20,000 ha in 2016. In Timmins District 12,355 ha of moderate-to- severe defoliation was aerially mapped in 2017, a significant increase from 2,980 ha in 2016. In the southeast corner of Wawa District a new infestation of large aspen tortrix caused a total of 1,858 ha of moderate-to-severe defoliation in 2017. Within the areas affected, the incidence of affected trees was 80-100% with 70-90% defoliation.



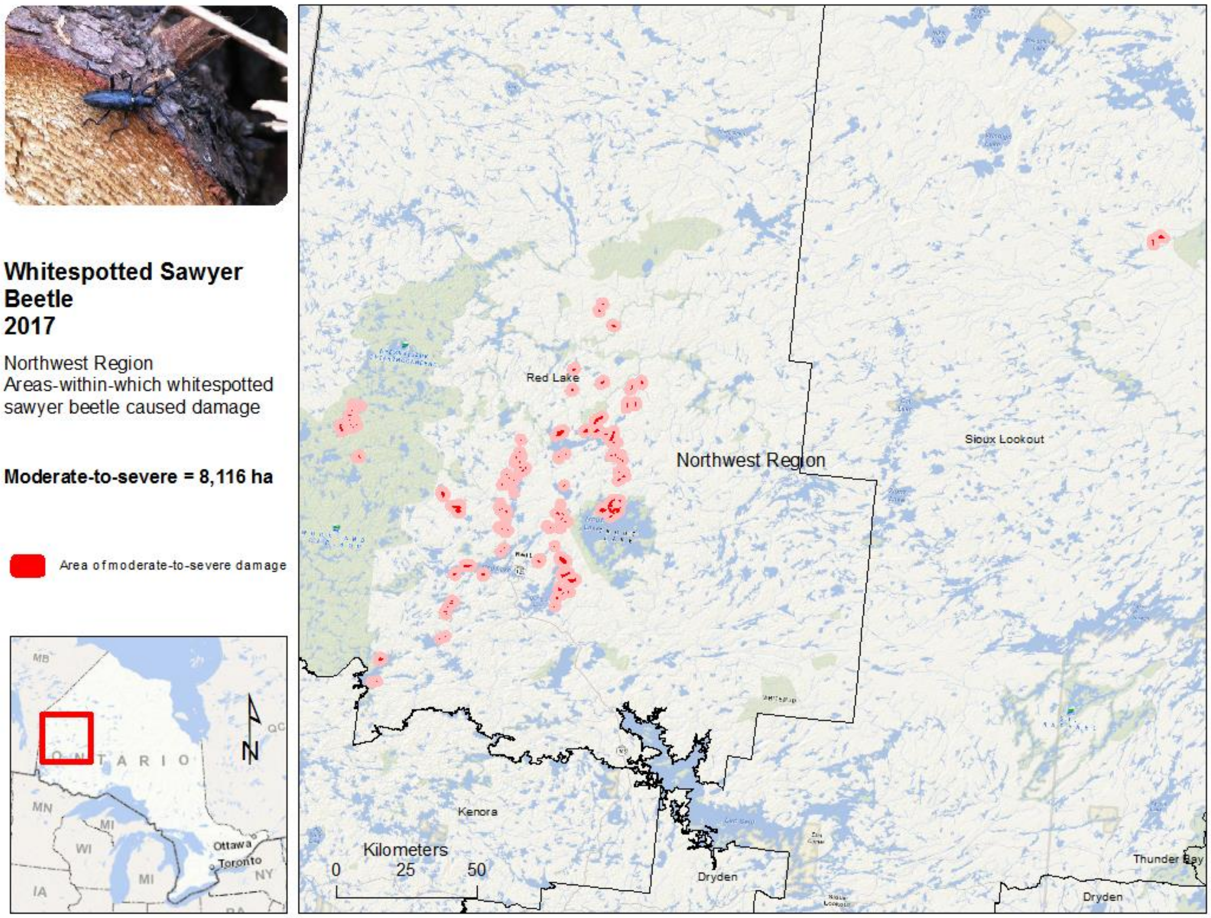
Pine false webworm

In southern Sault Ste. Marie District a red pine plantation east of Thessalon (NE corner of Thessalon Twp) in Maple Ridge Gravel Pit was heavily infested with pine false webworm for the third consecutive year. A total of 11 ha of moderate-to-severe defoliation were recorded in this area. The most severe defoliation was seen in the heart of the plantation. Being a young plantation (15 years old) the rigorous growing trees have not sustained any mortality as of yet. Pine false webworm defoliation was also recorded during an extension call in Iron Bridge. Several overmature white pine trees had light defoliation on lower branches. Upon further investigation a younger, smaller white pine tree in the neighbor's yard had moderate defoliation. This area was not aerially mapped.



Whitespotted sawyer beetle

In Red Lake district moderate-to-severe damage by whitespotted sawyer beetle decrease over half in 2017. Of the 40,697 ha that were aerially mapped in 2016, only 8,116 ha were mapped in 2017. This is residual outbreak from an event that occurred 2012, where there was a large snow event that resulted in uprooted and downed trees. Along with the snow damage, Red Lake has been subject to subsequent blowdown and forest fires that provided more material for the pest to feed on. Outbreaks occurred in the same general areas as snow damage, blowdown and forest fire events that provided abundant food for the sawyer beetles.



Beech bark disease

Evaluation of a beech health plot network was carried out again in 2017. New locations of beech bark disease were added to an existing database of confirmed infestations in Southern Region. In Aylmer and Guelph districts, beech bark disease continued to spread westward through the range of American beech. In Pembroke District, beech bark disease was reported by MNRF Pembroke District staff in McKay Twp, off Barron Canyon Road, which is the most northern report of the disease in the district.

Ink spot of Aspen

618 ha of moderate-to-severe damage by ink spot of aspen were aerially mapped in the Northeast Region in 2017. Damage was spread throughout four districts, southeast of Hearst District, south of Cochrane District, north of Timmins District and northeast of Chapleau District. Cochrane District had the greatest amounts of ink spot of aspen, with 427 ha of moderate-to-severe damage being aerially mapped. Heavy damage was encountered east of the Town of Cochrane, north of Lake Abitibi, and south of Little Abitibi Lake along Hwy 652. Heavily infested mature aspen trees had a distinct reddish hue. Damage ranged between 10-70%.

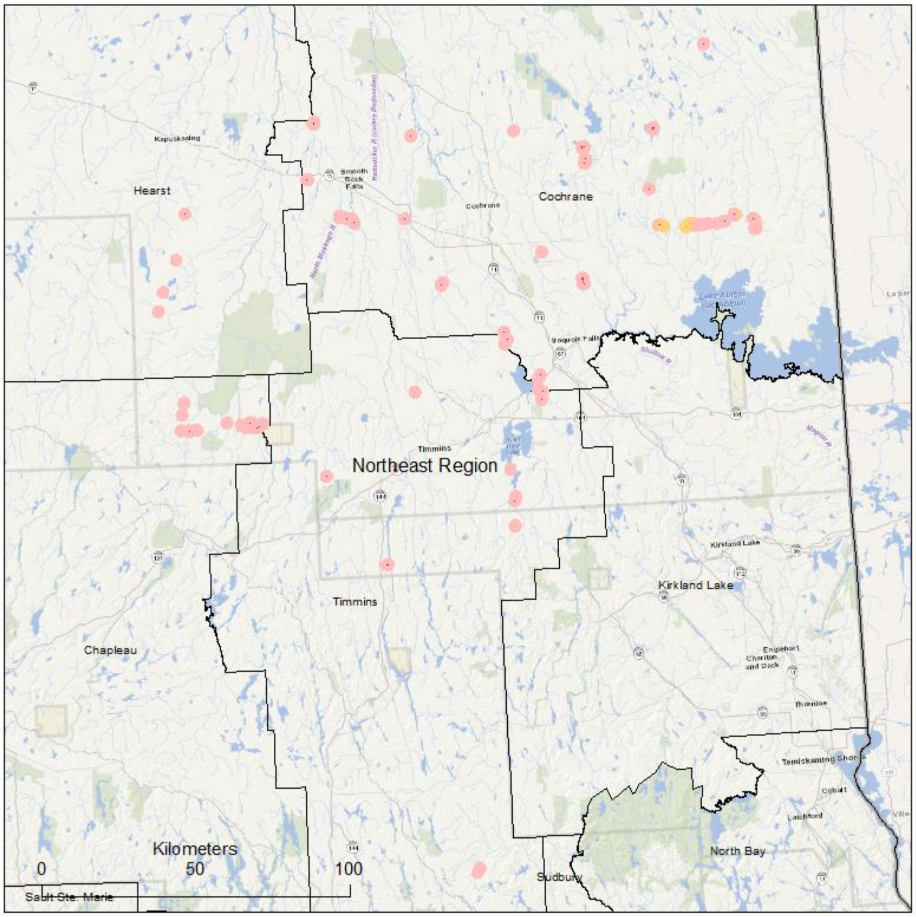
Ink Spot of Aspen 2017

Northeast Region
Areas-within-which ink spot of aspen caused defoliation

Light = 92 ha
Moderate-to-severe = 618 ha

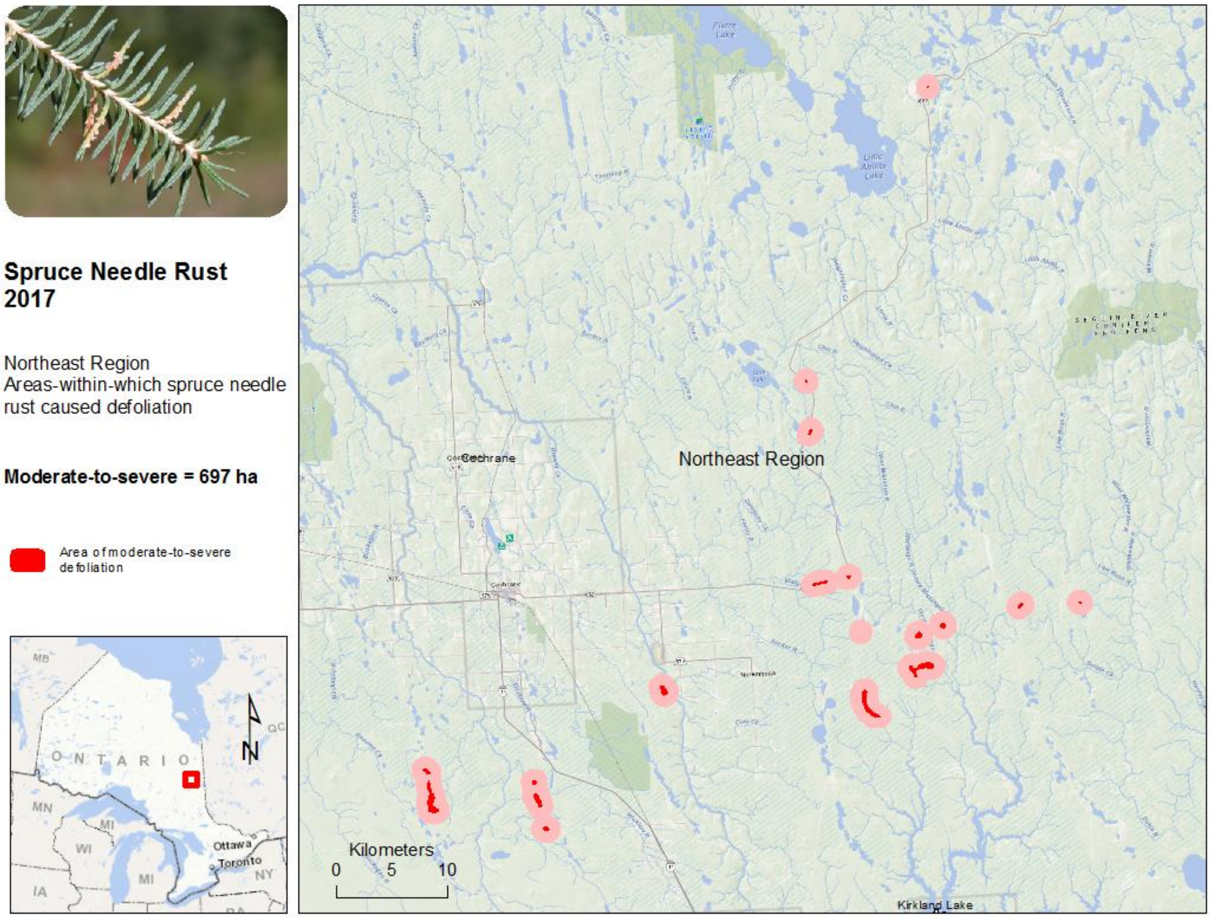
🟧 Area of light defoliation

🟥 Area of moderate-to-severe defoliation



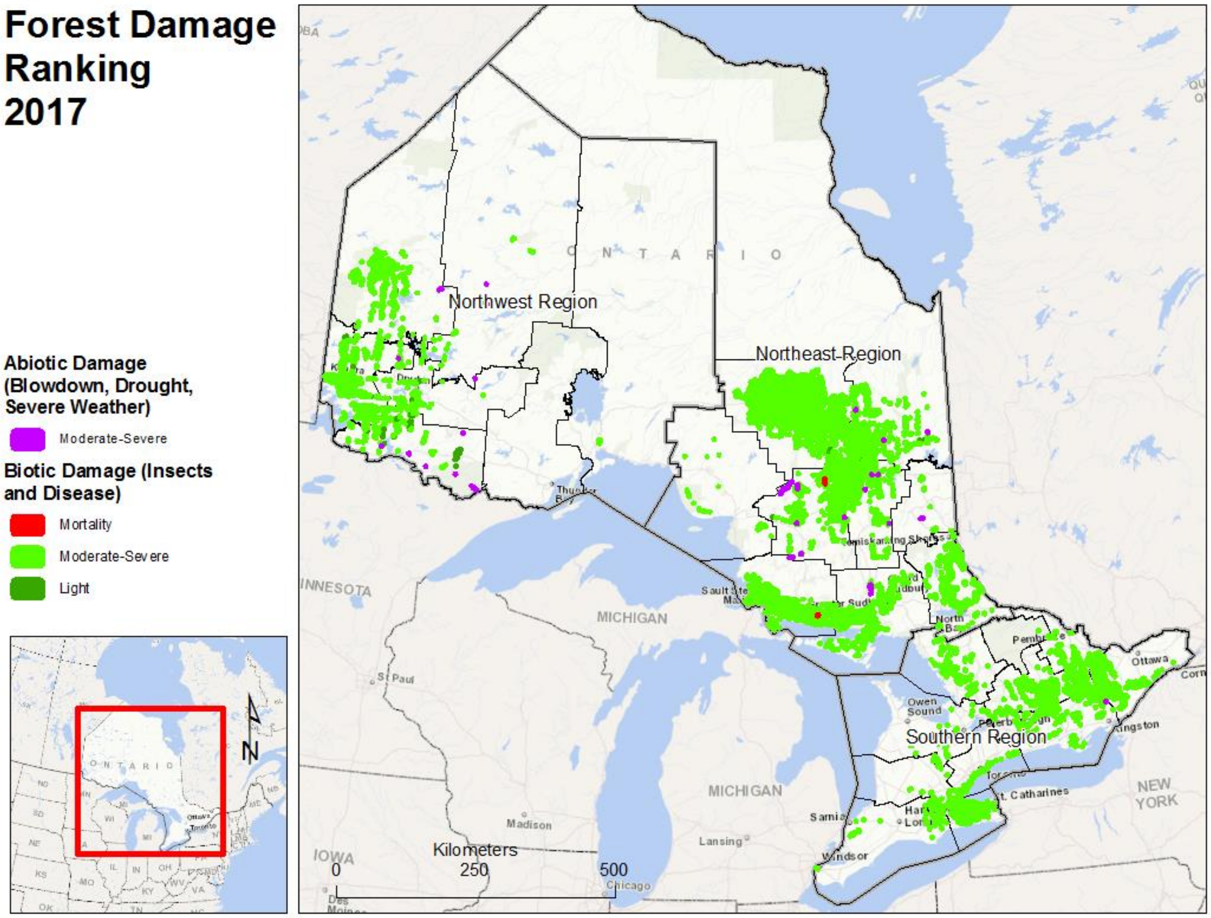
Spruce needle rust

Spruce needle rust was found in the Northeast and Northwest regions in 2017. Hearst, Timmins, Cochrane, Chapleau and Wawa districts all had reports of spruce needle rust, occurring in greater quantities later in the season, August to September. Cochrane District had the greatest amounts of rust, with 697 ha of damage aerially mapped at the end of July south and east of the Town of Cochrane. In the northeast, spruce needle rust was observed on co-dominant to dominant mature white and black spruce during surveys. During the fall of 2017 two far north sightings of spruce needle rust were observed, the first was in Polar Bear Provincial Park at the end of August, and then again along the coast of Hudson Bay between Moosonee and Polar Bear Provincial Park at the end of September. Spruce needle rust damage became visible early August in Red Lake, Kenora, Dryden and Sioux Lookout districts. Damage in those districts varied from 5 – 20%, with Red Lake having moderate levels of damage to young understory black spruce. Spruce needle rust in the Dryden and Sioux Lookout districts was mainly found while carrying out jack pine health plot surveys, on understory black and white spruce, in overmature jack pine stands.



Aerial Surveys (Major disturbance delineation)

Aerial surveys were carried out across all regions in 2017. Approximately 150 aircraft hours were logged by staff to delineate in excess of 1,500,000 hectares of damaged forest. Upon completion of the aerial surveys ground verification was carried out to confirm the disturbances observed from the air. Shape files have been submitted to our GIS support staff and final products along with disturbance areas have been completed for all disturbances.



Summary of Forest Pest Conditions in New Brunswick in 2017

Prepared by

New Brunswick Department of Energy and
Resources Development
Forest Planning & Stewardship
Branch

January 2017

Forest Pest Management Group

Director: Chris Norfolk
Forester / Analyst: Jeremy Gullison
Entomologist: Drew Carleton
Forest Technician: Ross Farquhar (retired)
Forest Technician: Eric Knopf
Forest Technician: Doug Winter
Forest Technician: Justin Chase

Field & Lab Technicians
David Hansen
Merle Lewis
Elaine Moreau
Dave Niblett
Kayla Risteen
Jared DiCarlo
Brett Crain
Kyle Macarthur
Kenda Cooper (FPL)
Anthony Sulpizo (FPL)

List of Figures

Supplementary Material

Overview

This report provides an overview of the status of forest insect and pest conditions and diseases in New Brunswick in 2017; it highlights many of the activities of the NB Department of Energy and Resource Development (here after “the department”), Forest Pest Management Section, but it is not intended to itemize all details for each survey conducted. Where required, the reader may contact FPMS for further information.

This report includes information on the status on several invasive alien species that have been detected or close to NB which have the potential to cause significant impacts to forest values.

Monitoring for insect and disease pests occurs in many ways and numerous reports are received each year identifying potential pest concerns. This report covers only those pest considered to be a risk to NB forest values, timber or otherwise. A full list of incidental reports can be found in **Appendix A**.

Introduction

The Forest Pest Management Section (FPMS) has the mandate of protecting New Brunswick's forests from native insect and disease pests. For regulated, non-native pests, the department works with the Canadian Food Inspection Agency which is responsible, under the Federal Plant Protection Act, for preventing the introduction into Canada, spread within Canada, and spread from Canada, of non-native pests.

Annual monitoring and forecast surveys are conducted for only a limited number of targeted pests. An effective forest pest management program requires both efficient monitoring the ability to accurately forecast future conditions of both forest and pest. Numerous techniques may be employed to accomplish these tasks. Both technique and survey intensity are reflective of the Department’s objectives and assessed level of risk associated with the particular pest at that time. For example, some insects known historically to cause high levels of economic loss to forest values, such as spruce budworm or hemlock looper, receive more intensive survey and monitoring efforts than insects that are less likely to cause significant damage in our region, such as jack pine budworm or yellow-headed spruce sawfly. For some pests, methodologies are well established and repeated annually with only changes in intensity or technology incorporated from time to time; for others (e.g. novel pests), techniques are typically unrefined and conducted at a coarser level (i.e. aerial surveys, public reports and so on). General surveillance of forest conditions is done through collaboration with the Department’s Regional Rangers and Wardens, members of forest industry, and through inquiries of the general public.

This report provides an overview of the status of forest insect and pest conditions in New Brunswick in, and highlights many of the pest management activities of FPMS.

Pathogens of Softwoods

In general, few reports of pathogens were submitted in 2017 compared to 2016, wide spread reports of pathogens affecting balsam fir in 2016 appear to have dissipated in 2017.

***Sirococcus* Shoot Blight on Red Pine** (*Sirococcus conigenus* [Dc.] Cannon & Minter): *S. conigenus* is a fungal disease, affecting primarily red pine, *Pinus resinosa* Sol. ex Alton. Beyond contributing to the native biodiversity of NB forests, red pine provides the raw material for the creation of utility poles for electric power transmission, which are high value forest products. Wet weather in May and June often results in intensification of disease symptoms (branch dieback and, after successive attacks, tree mortality). The FPMS has conducted red pine stand assessments for many years as a result of concerns for timber losses and as a means to establish proper rating of stand decline. Ultimately, industry members looking to utilize crown royalty reduction rates based on assessed stand decline require the approval by regional foresters who have been trained on this rating system. In 2017, FPMS discontinued annual assessment of red pine stands, focusing on assessing only those stands reported via inquiry.

Insect Pests of Softwoods

Hemlock Looper (*Lambdina fiscellaria* [Guenée]): This insect is a pest primarily of hemlock and balsam fir. It can kill trees within a single year due to its wasteful feeding habits. Besides consuming entire needles, it also partially eats many needles causing desiccation and death. Severe outbreaks are common in Newfoundland & Labrador and Québec. One reported outbreak of hemlock looper in NB occurred from 1989-1993.

A pheromone trapping network of 100 traps distributed throughout the province continues to forecast endemic looper populations in 2018 (**Figure 1**). 2017 represents the third year of testing Sylvar lures against the traditional PVC lure. Results indicate similar trends as were reported in 2015 & 2016 and we anticipate a switch to exclusive Sylvar lure use in 2018.

Spruce Budworm (*Choristoneura fumiferana* [Clemens]): Spruce budworm (SBW) is the most devastating insect pest in NB forests. Its primary host species are balsam fir and native spruce. The SBW goes through cyclical periods of outbreak that last 10-15 years followed by prolonged endemic phase lasting +30 years. In 2007 an outbreak of SBW began in Baie Comeau, Québec and has grown in both intensity and scale in each successive year. The most recent report from Québec indicates an estimated >7.2 million ha of Québec forests have some level of defoliation. With the largest increases in defoliation coming from the lower St. Lawrence area, closest to NB borders. As a result of this growing concern, the FPMS significantly increased its monitoring effort for SBW in 2012, and each year thereafter.

In 2014, as a result of the anticipated SBW disturbance, a four year, Atlantic

Canadian Opportunities Agency (ACOA) research program aimed at testing early intervention strategies to prevent or impede an outbreak was developed. The Healthy Forest Partnership comprised of Federal and Provincial governments, industry partners and educational institutes was formed to address these research issues. The FPMS has had a large role in this program, which is schedule to end in April of 2018. This section highlights some of the efforts the FPMS has made to this program in 2017.

Aerial surveillance is conducted at low level with the assistance of aircraft contracted from Forest Protection Limited. FPMS staff detected patchy SBW defoliation located sporadically in north-west NB near the Québec border **(Figure 2)**. Ground surveys, conducted at the same time as the L2 survey, confirmed the presence of patchy SBW defoliation throughout much of northern NB (**Figure 3)**.

Pheromone trap monitoring occurred at 100 locations in 2017 (**Figure 4**). In each site, 3 traps were places at ~40m apart. This marked the second year in which a 3 trap array, similar to that used in Quebec, was used in NB. Results suggested that both local populations are rising as indicated by the general trend across the northern sites, as well, that local and long distance dispersal from Quebec populations are likely occurring, as suggested by sites where high moth counts were recorded but no other indication of budworm population establishment was available, such as defoliation or overwintering populations.

The most reliable method used to estimate population trends, is the second instar larvae survey, (hereafter L2 survey). This annual survey has increased in intensity from 102 plots sampled in 2010 to 1976 plots sampled in 2017 (**Figure 5**). Since the inception of the ACOA project the survey has relied upon industry partners (J.D. Irving Limited, AV Cell Inc., Fornebu Lumber Company Inc., Acadian Timber, and Twin Rivers Paper Co.), FPL staff and ERD Regional staff to aid in the collection of samples that are subsequently processed by the FPMS lab in Fredericton.

In 2017, average SBW counts at or above the treatment threshold level of 7 L2/branch/site were reported in 83 plots (5%) from first stage sampling and in an additional 29 (9%) second stage sample plots. Both represent increases from the 2016 in which 57 (4%) and 22 (12%) plots were reported at or above threshold in the initial and secondary surveys respectively. Highest populations were reported in north eastern NB from Campbellton to Miramichi, (**Figure 5**). It has been suggested by J. Regniere (CFS-Laurentien), that based on these data, a treatment program of similar intensity to the 2016 program, could be in the range of 150,000 – 250,000 hectares in 2018. At this time, funding is uncertain so multiple scenarios based on potential budgets are being developed in anticipation for the 2018 season. For further details on the research studies conducted over the 4 year ACOA program and future status, please visit www.healthyforestpartnership.com

Brown Spruce Longhorn Beetle (*Tetropium fuscum* [Fabricius]): This wood boring beetle is an alien invasive species that first made entry to Canada through the ports of Nova Scotia. Although its presence was only detected in the spring of 2000, it was subsequently discovered through pinned specimens that it had been present since at least 1990, but had mistakenly been misidentified as a native congener *Tetropium cinnamopterium* (Kirby). In its native range in Europe, the BSLB is known as a secondary establisher, attacking mainly weakened trees, however in NS, it has attacked seemingly healthy red spruce *P. rubens* Sarg. as well as white spruce *P. glauca* (Moench) Voss, black spruce *P. mariana* (Mill.) Britton, Sterns & Poggenburg and Norway spruce *P. abies* (L.) H. Karst. As an alien invasive species, it is the role of the Canadian Food Inspection Agency (CFIA) to manage this pest by reducing the risk of spread outside of the area it is currently established in by using strategies that regulate the movement of specified high-risk spruce materials along with annual surveys and research. In 2015, the CFIA moved to regulate all of NS.

In 2011, a single beetle was found in a trap in Kouchibouguac National Park (KNP), most likely transported to the park in a piece of firewood from NS. This prompted collaborative efforts by CFIA, CFS and KNP to eradicate the beetle from the park and to establish extensive monitoring efforts. The CFIA also established a Prohibition of Movement (POM) Order for KNP. FPMS began conducting visual assessments of spruce trees at locations throughout NB looking for any signs and symptoms of BSLB attack in conjunction with other operational surveys. In 2014, CFIA surveyed 209 sites and identified two that were positive for BSLB. The first site was again in KNP, while the second was in Memramcook. The property near Memramcook on which the beetle was caught was placed under a POM order in 2014. In 2015, 196 sites were monitored in NB, including additional traps in the area around the original 2014 find near Memramcook. Since 2014 no further beetles have been trapped in KNP, however ongoing detections have occurred in Memramcook, where the CFIA, CFS and ERD have actively been managing populations through a series of host material removals, POM's and education program with the local community, supported by the local wood marketing board. In 2017, 3 beetles were found in the Memramcook managed zone, all from previously reported locations, CFIA's decision regarding any new regulatory requirements has not been made public at the time of writing of this report.

Pine Shoot Beetle (*Tomicus piniperda* [L.]): First discovered in Ohio, US in 1992, this non-native insect has gradually spread into Québec and western Maine. In Ontario, it has been found in association with mortality in Scots, red, white and jack pines. It is considered a pest of quarantine significance and is regulated by the CFIA under the federal Plant Protection Act. The CFIA conducts monitoring for pine shoot beetle in New Brunswick. In 2015 two sites in north-western NB reported a single specimen each, marking the first report of this insect in NB. No additional reports were made in 2016, however in 2017 it was again discovered in Connors, NB, near Edmundston. It is likely that CFIA will recommend regulations for this area, however, what and when these will occur has yet to be determined.

Hemlock Wooly Adelgid *Adelges tsugae* (Annand): The first discovered entry of HWA in North American was in Virginia in 1951. This exotic forest pest regulated by the CFIA under the federal Plant Protection Act which attacks both eastern and carolina hemlock, has been making its way north throughout the range of hemlock since its establishment, assisted mainly via wind and bird movement. Currently there are no known locations with HWA in NB; however its 2017 discovery in southern NS has led to increased surveys by CFS and CFIA staff in NB in 2017 with plans for continued survey work in 2018.

Pests of Hardwoods

Asian and European Gypsy Moth (*Lymantria dispar* [L.]): The gypsy moth complex of moths are again exotic invasive species regulated by the CFIA. They are capable of feeding on several hundred different species of plants and shrubs ranging from ornamentals to forest trees. Many hardwoods, such as oak *Quercus*, poplar *Salicaceae* and birch *Betula* are favored hosts. Though AGM has not been discovered in NB, EGM has been present for many years and is regulated in most counties. No formal control program is in place, however the CFIA continue to monitor for EGM establishment in those counties where it has not yet been established.

Forest Tent Caterpillar (*Malacosoma disstria* [Hubner]): This insect generally defoliates poplar, but will attack numerous hardwood species during an outbreak. Severely defoliated trees can produce another crop of leaves within the same growing season, and therefore can withstand the infestation fairly well. Small areas of defoliation have been observed in the province each year since 2010. Aerial surveys have noted ~3000 hectares of defoliation since 2014 (**Figure 2**). Damage has been limited to north-eastern NB, in and around Bathurst and east of Miramichi.

Miscellaneous Observations

Several of the surveys conducted by FPMS, and most of the general surveillance activities conducted by FPMS, regional PDO's, forest industry partners and other collaborators are not incorporated into this report but are summarized in **Appendix A**. Other notable issues included the ice storm damage reported in 2016/2017 which resulted in damage to a limited number of stands in north eastern NB. This damage appeared to be limited to hardwood stands and poorly stocked Jack Pine plantations.

.

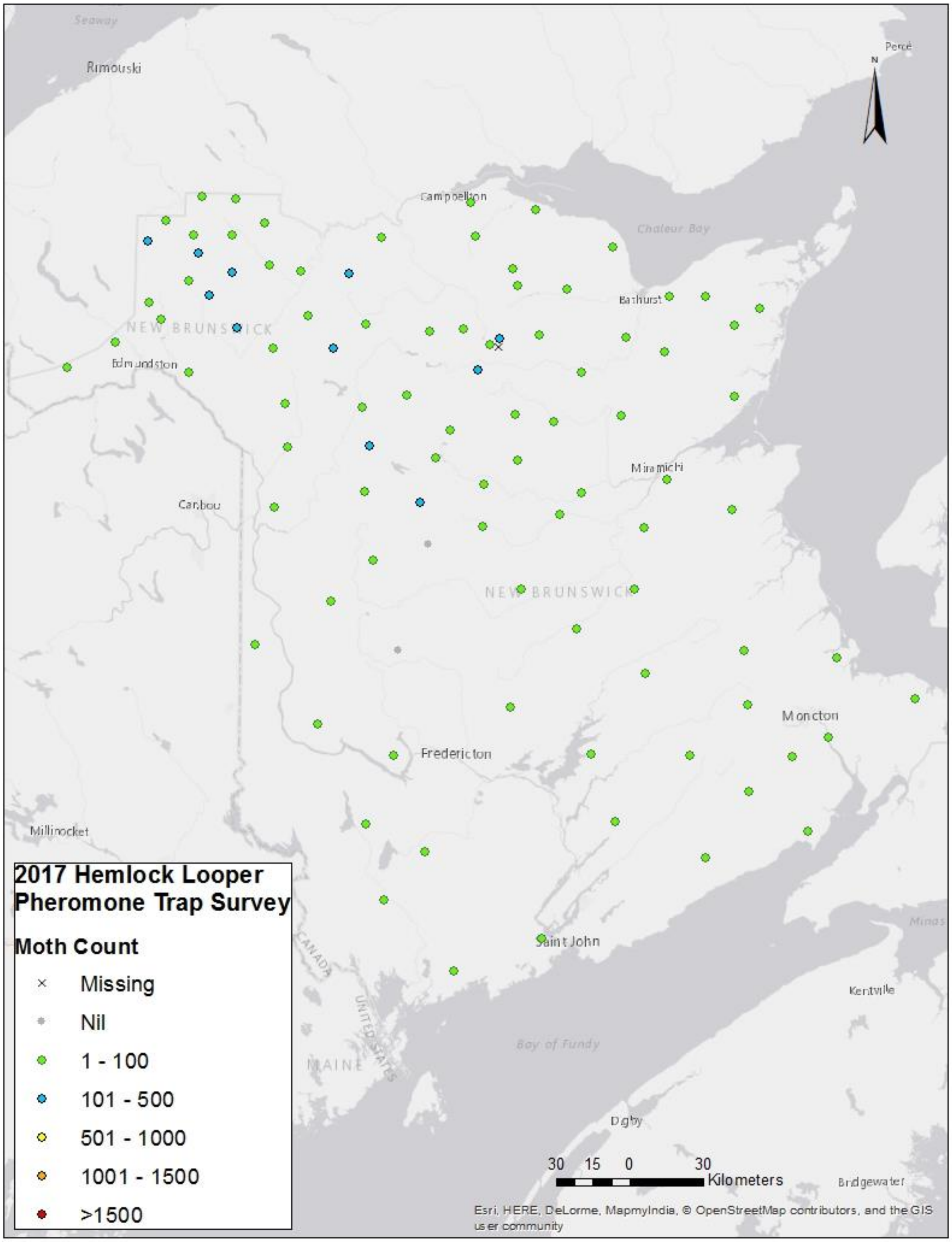


Figure 1. New Brunswick hemlock looper pheromone monitoring results from 2017. For display purposes, only the results of the Contech PVC flexlures are shown.

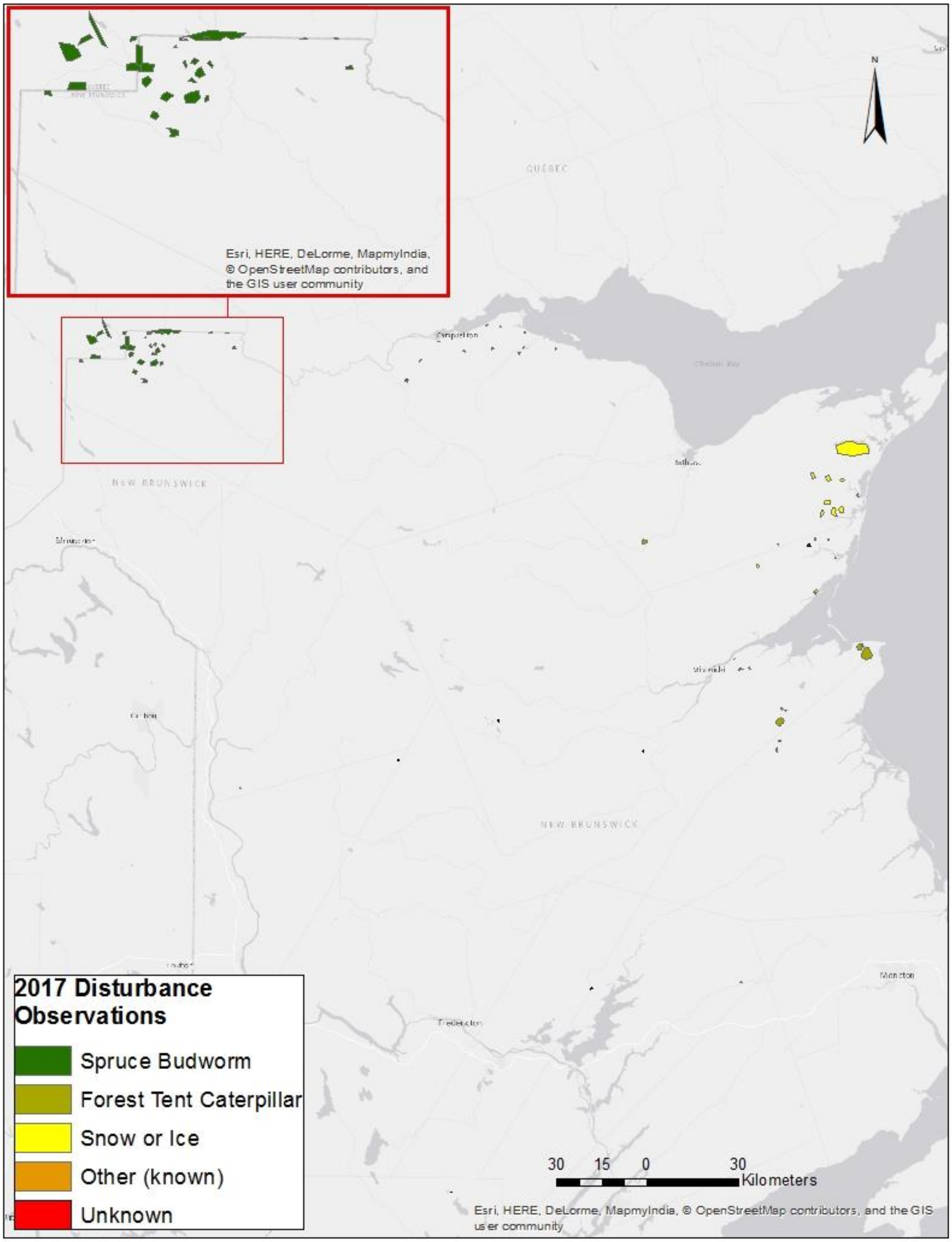
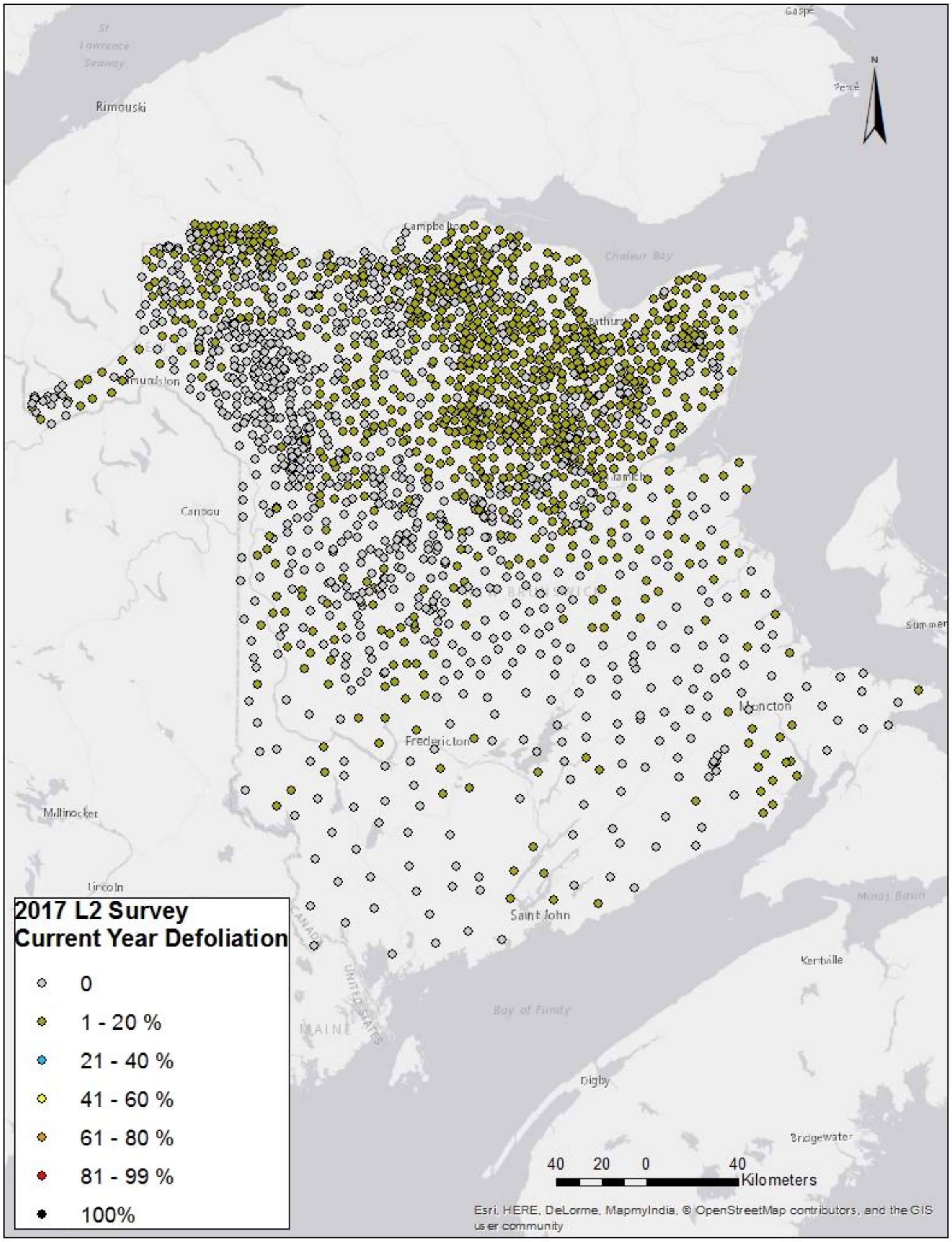


Figure 2. Results from the 2017 aerial survey for defoliation by all sources. Approximately 13,329 ha of damage by all disturbance agents was reported province-wide (spruce budworm defoliation ~5,392 ha).



Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community

Figure 3. Current year defoliation estimates at the branch level during the 2017 spruce budworm L2 survey by all sources. Points represent average defoliation of 3 branches sampled per site.

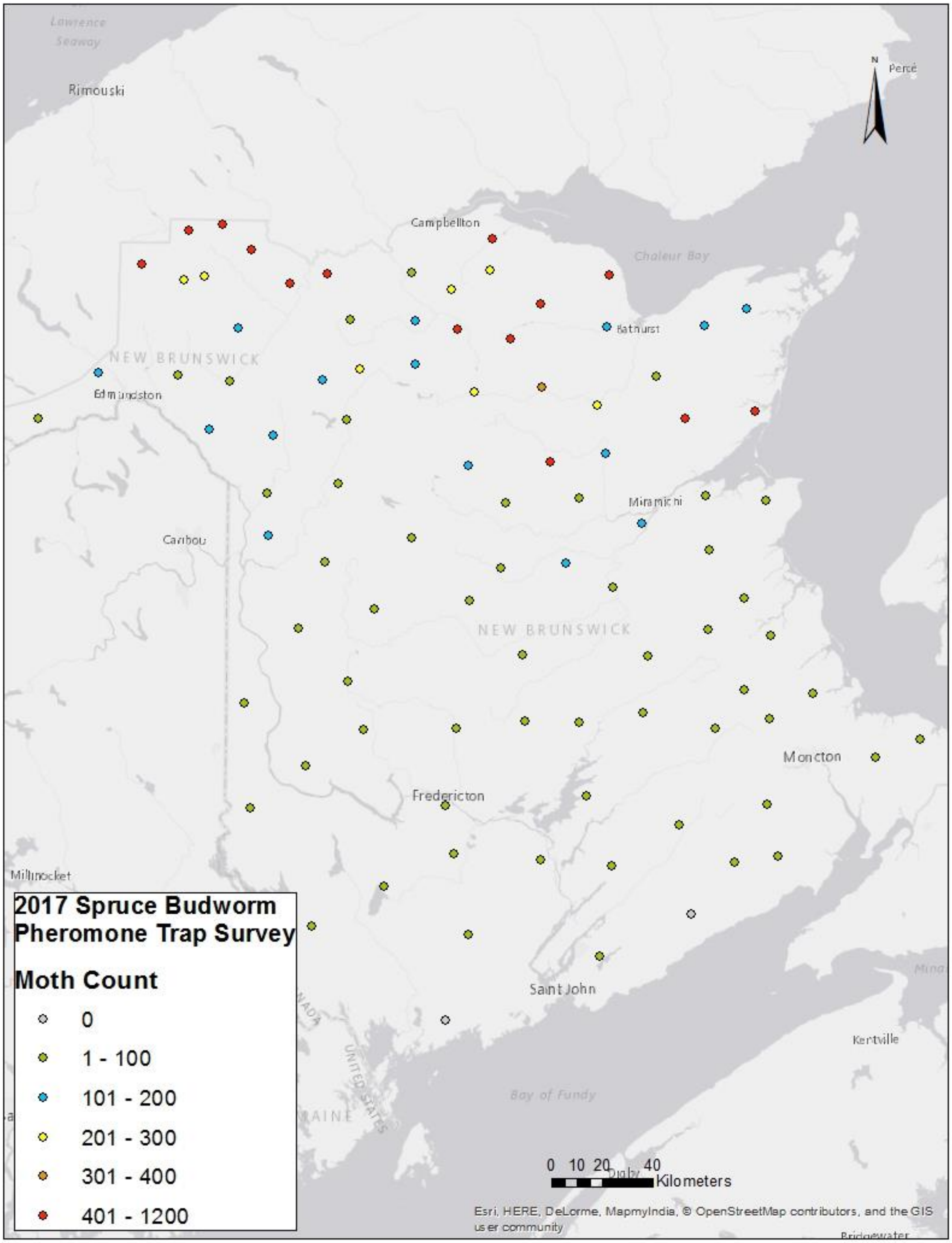
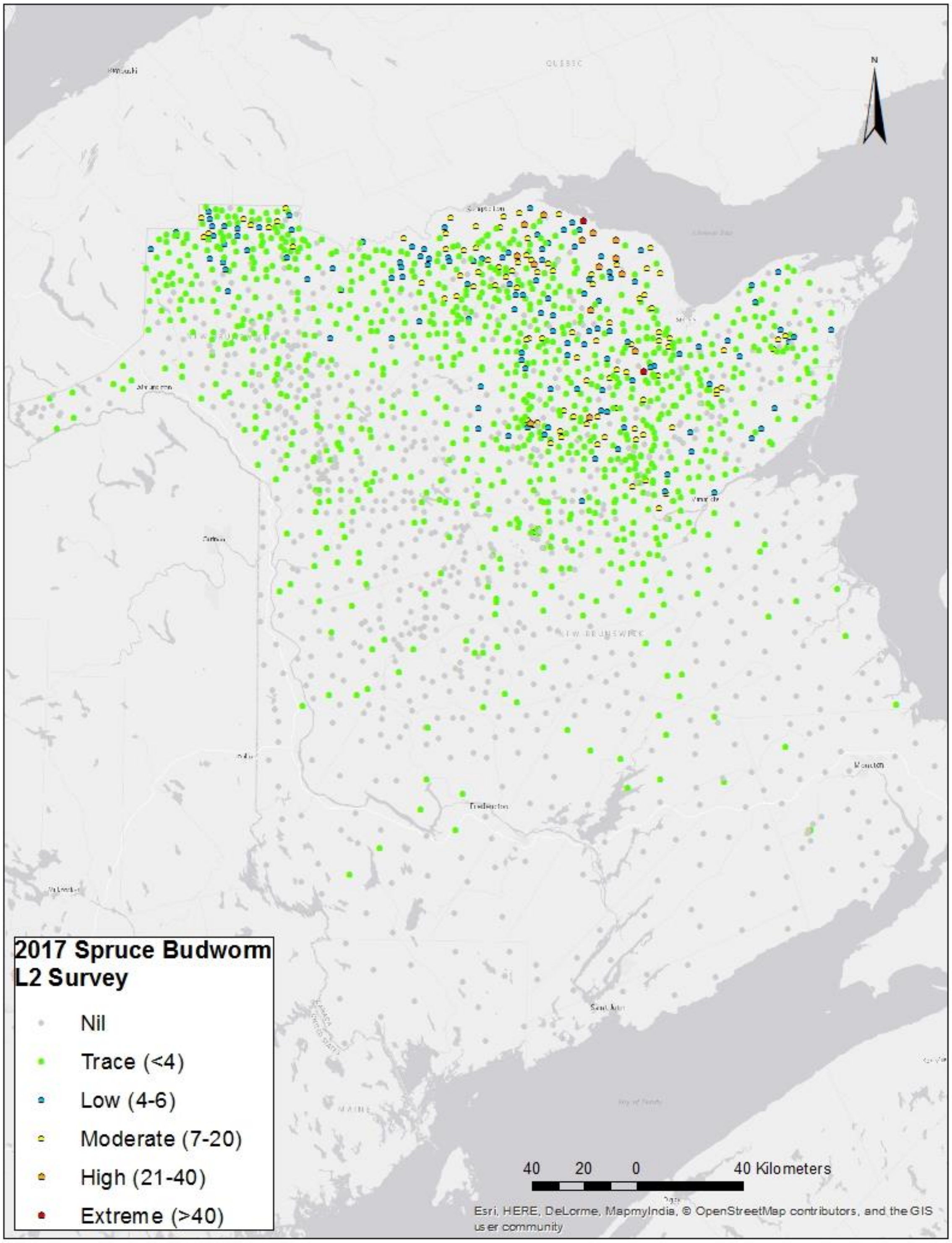


Figure 4. Results from the New Brunswick Forest Pest Management Group, spruce budworm pheromone trap monitoring program in 2017. Categories are based on the average count of three trap counts in each location.



Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community

Figure 5. Results of the 2017 survey of second instar spruce budworm larvae (L2). Each plot value (N= 1974) represents the average number of L2 found on three 75 cm long, mid-crown branches of either balsam fir or spruce species.

Appendix A – Summary of Pest Detection Reports from 2017

2017 saw the full integration of a new pest reporting system for both the public and for regional staff. This new system provided a simpler, faster method of reporting pest concerns through the online GNB website, as well, it added new feature, like the ability to select disturbance types based on images and categories, rather than asking the lay person to describe in details the nature of the report. An in-house program was also installed on the computer systems of all regional staff to allow reporting to occur in the field. This shift allowed for all regional staff to partake in pest reporting, a departure from the previous system that assigned a small number of staff in each region to provide a specific number of reports, based on stand content. Each region was then assigned a monthly target number of reports based on Districts (10 reports/ district/ month). The following tables represent the summarized raw data collected in the 2017 season.

Metric	2017
Total Reports	681
Total Officers Reporting	72
Industry Reports	2
Public Reports	2
REPORTS BY REGION	
Region 1	
East	34
North	41
West	59
TOTAL	**134**
Region 2	
North	56
South	29
West	63
TOTAL	**148**
Region 3	
East	82
North	52
South	50
West	107
TOTAL	**291**
Region 4	
North	52
South	56
TOTAL	**108**

Table A1. Number of pest reports conducted by regional staff, industry and public, using the new reporting form in 2017, by Region and District.

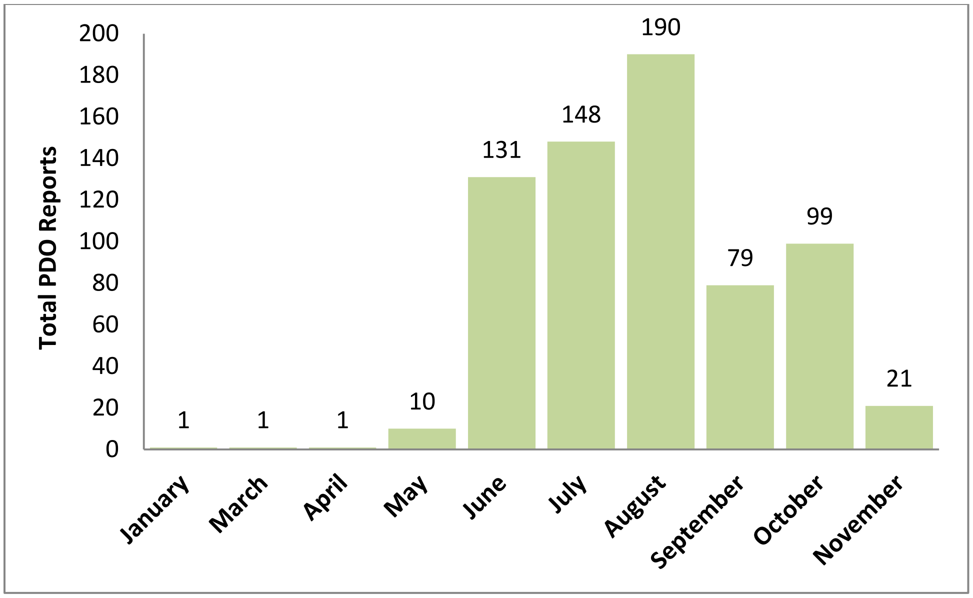


Figure A1. Number of pest reports conducted by regional staff, industry and public, using the new reporting form in 2017, by month.

AGENT	Severity					Total
	Trace	Light	Moderate	Severe	Mortality	
Healthy Stand						**476**
unknown	36	13	4	1	1	**55**
Vertebrate	23	5	6		6	**40**
Abiotic	11	12	8	1	2	**34**
White Pine Weevil	10	16	4	1		**31**
Spruce Budworm	13	11	1	3		**28**
Misc./Unidentified Insects	11	3	2			**16**
Discolouration	9	5				**14**
Gall/Broom/Needle Rusts	4	5	2	1		**12**
Sirococcus	4	3	2	1	1	**11**
Beech Bark Disease	1	4	2	2	1	**10**
Leaf Miners/Gallers/Fungi	4	4	2			**10**
Eastern Spruce Gall Adelgid	5	3	1			**9**
Human	2	3	1			**6**
Fall Webworm	2	1	1			**4**
Pitch Nodule Maker	2	2				**4**
Forest Tent Caterpillar	3		1			**4**
Black Knot	1		1	1		**3**
Spittlebug	2		1			**3**
Balsam Twig Aphid	2	1				**3**
Larch Sawfly	1			1		**2**
Birch Dieback (potential)					1	**1**
Hemlock Looper	1					**1**
Birch Sawfly			1			**1**
White Pine Blister Rust				1		**1**
Spruce Cone Worm	1					**1**
Balsam Wooly Adelgid			1			**1**
White-marked Tussock Moth	1					**1**
Introduced Pine Sawfly	1					**1**
GRAND TOTAL	**150**	**91**	**41**	**13**	**12**	**783**

Table A2. Summarized list of reported disturbances in 2017 as reported by regional staff, industry and public.

Forest Health Monitoring in Nova Scotia 2017

Prepared by Nova Scotia Department of Natural Resources, Forest Protection Division, Risk Services, P.O. Box 130, Shubenacadie, N.S. B0N 2H0

Native pests

The last **Eastern Blackheaded Budworm (*Acleris variana* (Fernie))** outbreak erupted in 2004; covering approximately 114,000 ha, in the Cape Breton Highlands. The following year a spray trial was conducted by the Canadian Forest Service (CFS) and in 2006, the population collapsed. In addition to our annual overwintering egg survey, Forest Health began using pheromone traps to monitor populations in 2013. In 2017, 40 traps were deployed throughout the Cape Breton Highlands and Nova Scotia Uplands ecoregions. The percentage of positive traps was 100%, up from 89.5% in 2016. The maximum trap catch was 316 moths, down from 720 in 2015.

The **Spruce Budworm (*Choristoneura fumiferana* (Clemens))** has caused more damage to Nova Scotian softwood forests than any other insect. The last spruce budworm outbreak in Nova Scotia began in the early 1970's and at its peak, caused moderate to severe defoliation over 1,220,000 hectares on Cape Breton Island and in pockets on northern mainland counties. Forest Health monitors eastern spruce budworm populations using a combination of pheromone traps and branch samples to collect overwintering second instar larvae (L2s). In 2017, 146 pheromone traps were deployed province-wide, of which 99.3% were positive, up from 93.1% in 2016. The maximum trap catch was 313 moths, up from 113 in 2016. In 2017, 318 sites were sampled for overwintering larvae and a total of 69 L2s were detected at 33 sites, down from 97 L2s at 41 sites in 2016. In 2013, the first L2s detected in Nova Scotia since 1994, when six L2s were detected at five sites in the Central Region.

Jack Pine Budworm (*Choristoneura pinus* (Freeman)) has been a pest of concern in Nova Scotia since 2005 when it was found feeding on mature white pine in the Western Region. Defoliation was last detected in this region 2009. Forest Health began using pheromone traps to monitor populations in 2006. In 2017, 48 traps were deployed in the Western and Central regions, of which 62.5% were positive. Maximum trap catch was 14 moths. There were no positive traps in 2016 and no overwintering L2 survey due to the zero moth catch.

Control programs for **Hemlock Looper (*Lambdina fiscellaria* (Guenée))** were conducted in the Cape Breton Highlands in 1996 and 1997. Defoliation was last detected in 2006 in the Cape Breton Highlands National Park. Since that time, hemlock looper numbers have remained at low levels. In 2017, 143 traps were deployed province-wide, of which 95.1% were positive, up from 87.0% in 2016. In 2017, maximum trap catch was 122, down from 216 moths.

The last **Whitemarked Tussock Moth (*Orgyia leucostigma* (J.E Smith))** outbreak occurred in 1998 covering 1.4 million hectares in Central and Northern Nova Scotia; 60,000 ha were treated at a cost of approximately six million dollars making it our largest treatment program to date. Since that time two mini population eruptions have occurred: Cape Breton in 2005 and Guysborough in 2007. We monitor whitemarked tussock moth populations using an overwintering egg mass survey. In 2017, 143 sites were sampled province-wide, of which 17.9% were positive, up from 7.8% in 2016. Trace to low populations were detected throughout the province.

The **balsam fir sawfly (*Neodiprion abietis* (Harris))** is was first recorded in North America in 1910 and is now found throughout Nova Scotia. Recorded outbreaks in Nova Scotia date back to 1942, and tend to last three to four years with a varying number of years in between. The principal host is balsam fir but it can also be found on white, black, and red spruce. Forest Health monitors balsam fir sawfly populations using an overwintering egg survey. In 2017, 116 sites were surveyed in the Eastern and Central regions, of which 17.2% were positive, up from 9.7% in 2016. Maximum egg niches / 100 cm branch was 2.2, down from 12.0 in 2016.

Balsam twig aphid (*Mindarus abietinus* (Koch)) in Nova Scotia feeds primarily on balsam fir but may also attack Fraser fir, white fir and spruces. It is a major pest of firs grown for Christmas trees but is considered a secondary forest pest as it does not cause tree mortality. Outbreaks are generally short in duration because populations tend to quickly build and fall. Balsam twig aphid populations are monitored in a general way by assessing their presence on balsam fir branch samples collected for the balsam fir sawfly survey. This survey is not predictive, but simply quantifies the damage that occurred the previous season. In 2017, 116 sites were sampled, of which 22.4% were positive, up from 7.1% in 2016.

Balsam gall midge *Paradiplosis tumifex* (Gagné) was first recorded in eastern Canada in 1938 and have since been reported regularly. It is not considered a significant forest pest in Nova Scotia, but is one of the most serious pests of balsam fir in Christmas tree stands. Infestations are generally of short duration, lasting approximately three years. Repeated severe infestations can cause tree growth loss but does not result in mortality. If infested Christmas trees are not to be harvested for another three to four years, they may outgrow the damage. Balsam gall midge populations are monitored in a general way by assessing their presence on balsam fir branch samples collected for the balsam fir sawfly survey. This survey is not predictive; it simply quantifies the damage that occurred the previous summer. In 2017, 116 sites were samples, of which 20.7% were positive, up from 5.3% in 2016.

Non-native pests

The **Balsam Woolly Adelgid (*Adelges piceae* (Ratzeburg))** was first reported in Canada in 1910, in southern Nova Scotia. More than 100 years after its introduction, symptoms of feeding on balsam fir can be found throughout the province. Forest Health monitors balsam wooly adelgid populations by inspecting the branches collected for the balsam fir sawfly survey for dormant overwintering nymphs in buds and balsam woolly adelgid damage, *i.e.* gouting. In 2017, branches were collected from 116 sites. The percentage of sites with overwintering nymphs was 10.6%, up from 1.9% in 2016. The percentage of sites with gouted branches was 9.5%, up from 2.7% in 2016. Forest Health also conducts routine monitoring in a series of permanent plots within the nine provincial ecoregions of Nova Scotia, assessing the annual change in the number of balsam woolly adelgid adults and gout assesses the levels found in each plot. In the spring of 2017, populations had increased at 7 plots, decreased at 3 sites, and 7 had no change. Three plots were added in 2017 raising the total of permanent plots to 20.

Gypsy Moth (*Lymantria dispar* (Linnaeus)) has been present in Nova Scotia since 1981. As a quarantine pest, the gypsy moth is under the jurisdiction of the Canadian Food Inspection Agency (CFIA). The regulated area currently includes the Western and Central regions of Nova Scotia where established populations have been identified. Cape Breton County was added to the regulated zone in 2017. Gypsy moth defoliation was last detected in Kentville in 2002. Forest Health uses a pheromone monitoring system to detect gypsy moth in Nova Scotia. This survey is conducted in two parts. Multipher traps are deployed at designated sites province-wide to monitor population trends, while delta traps are placed in towns throughout the non-regulated area to determine if the population is spreading into new areas. In 2017, 22 multipher traps were deployed, of which 81.8% were positive overall, up from 71.4% in 2016. The Average trap catch is up in the Western and Central regions were 502.1 and 422.6, respectively, down from 635 and 520, respectively, in 2016. In the Eastern region, 9 moths were captured in 2 traps, up from 0 in 2016. In 2017, 10 delta traps were deployed in each of nine towns outside of the CFIA regulated area. Since 2003, average trap catch in all of these non-regulated towns has been 1.6 moths per trap or less with the exception of Antigonish, which continues to increase with an average moth catch of 8.9 moths/trap, up from 4.5 in 2016. Sherbrooke also increased from 0.4 moths/trap in 2016 to 2.0 in 2017.

Hemlock Woolly Adelgid (*Adelges tsugae* (Annand)) is native to Asia and was first detected in Eastern North America in 1951. Since its initial introduction, it has quickly spread to over half of the native range of eastern hemlock in the United States, devastating hemlock forests throughout its range. It was detected in isolated locations in Ontario in 2012 and 2013. In 2017, the hemlock woolly adelgid was detected in Nova Scotia for the first time in Digby County. Intensive surveying in the Western region by the CFIA, and in cooperation with the NS DNR, resulted in the detection of hemlock wooly adelgid in 5 counties: Digby, Yarmouth, Shelburne, Annapolis, and Queens. The CFIA declared these counties regulated zones and a Ministerial Order was issued 15 December 2017 restricting movement of susceptible wood products, e.g. hemlock, yeddo spruce, tiger-tail spruce and all species of firewood, out of the regulated zone by unauthorized persons. As a quarantine pest, the hemlock woolly adelgid is under the jurisdiction of the CFIA. Forest Health staff also conducts annual visual detection surveys for hemlock woolly adelgid in 11 remote hemlock stands in the Western Region, targeting stands with greater than 40% hemlock. At each stand two lower crown branches from 15 trees were assessed for life stages or damage. No hemlock woolly adelgid were detected in these stands.

Emerald Ash Borer (***Agrilus planipennis*** **(Fairmaire)**): This beetle, native to Asia, was first discovered in North America in 2002. It has proven to be highly destructive in its new range, killing tens of millions of ash trees throughout Ontario, Quebec and the United States. It poses a significant economic and ecological threat to urban and forested areas and can be spread to new areas by the movement of firewood. It has not yet been detected in the Maritime Provinces. In cooperation with the CFIA and their 2017 survey efforts, Forest Health conducted detection surveys for emerald ash borer adults and deployed green prism intercept traps at 15 sites throughout the province. No adult beetles detected.

Forest Pest Update for Newfoundland and Labrador
Prepared for SERG International Workshop - February 2018

The following is a summary of the status of forest pests with results on monitoring and control activities conducted in the Province of Newfoundland and Labrador (NL) in 2017.

Please Note *– these are interim results - final results will be made available in the Provinces Annual Forest Pest Status report. This report will be available at http://www.faa.gov.nl.ca/forestry/idc/monitoring_control.html in April/May of 2018.*

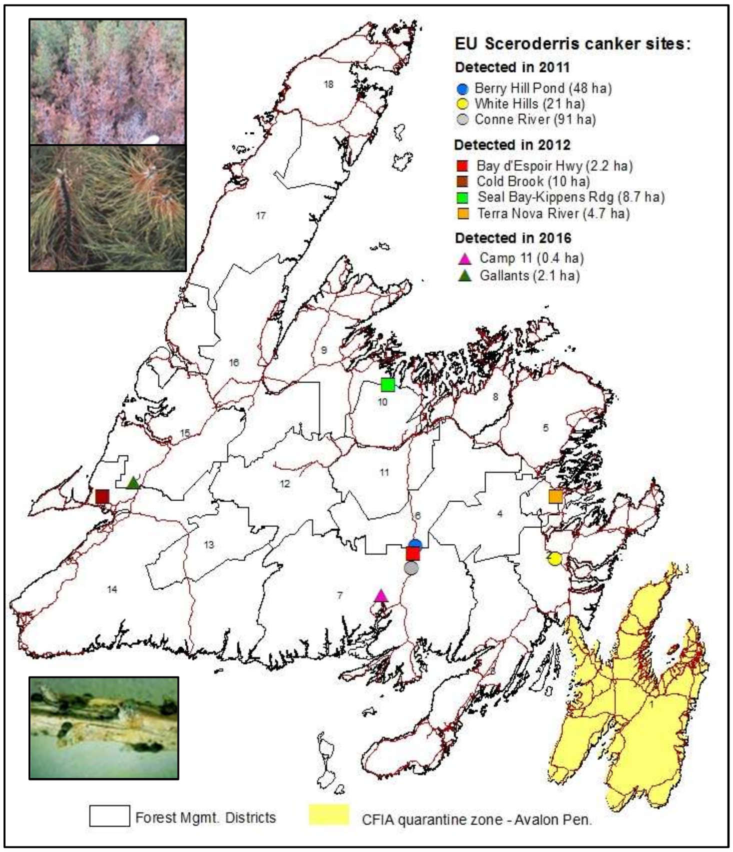
Invasive Forest Pests

In the Province of NL invasive forest pests are primarily monitored by the Canadian Food Inspection Agency (CFIA). Pests monitored by the CFIA in NL in 2018 included: gypsy moth, brown spruce longhorn beetle (BSLB), emerald ash borer, Asian long-horned beetle, Japanese beetle and other wood boring insects through the IAS forest pest survey program. Results of monitoring conducted for these invasive pests can be obtained from Ron Neville, Plant Health Survey Biologist, CFIA, Atlantic Canada (ron.neville@inspection.gc.ca).

Presently the Province only monitors European Scleroderris Canker and Balsam Woolly Adelgid - two invasive species that are well established in the Province.

European Scleroderris Canker

The European strain of Sclerroderris canker was first found in St. John's in 1979. This introduced disease threatens indigenous red pine of ecological significance, as well as, planted red pine. Following its discovery on the island, efforts were made to contain this disease through sanitation and the use of quarantine zones to restrict the movement of infected material. This was successful for approximately 25-years; however, in 2007 the disease was found 150-km outside the quarantine zone in the Berry Hill area. This site was sanitized in 2008. Despite these efforts, however, an additional three sites were detected in 2011. One of these sites was only 3 km north of the site detected in 2007. In 2012, survey efforts detected an additional four sites outside the quarantine area. Two more sites were detected in 2016. No new sites were found in 2017. Currently there are a total of nine sites outside the quarantine zone confirmed to have this disease (see map).

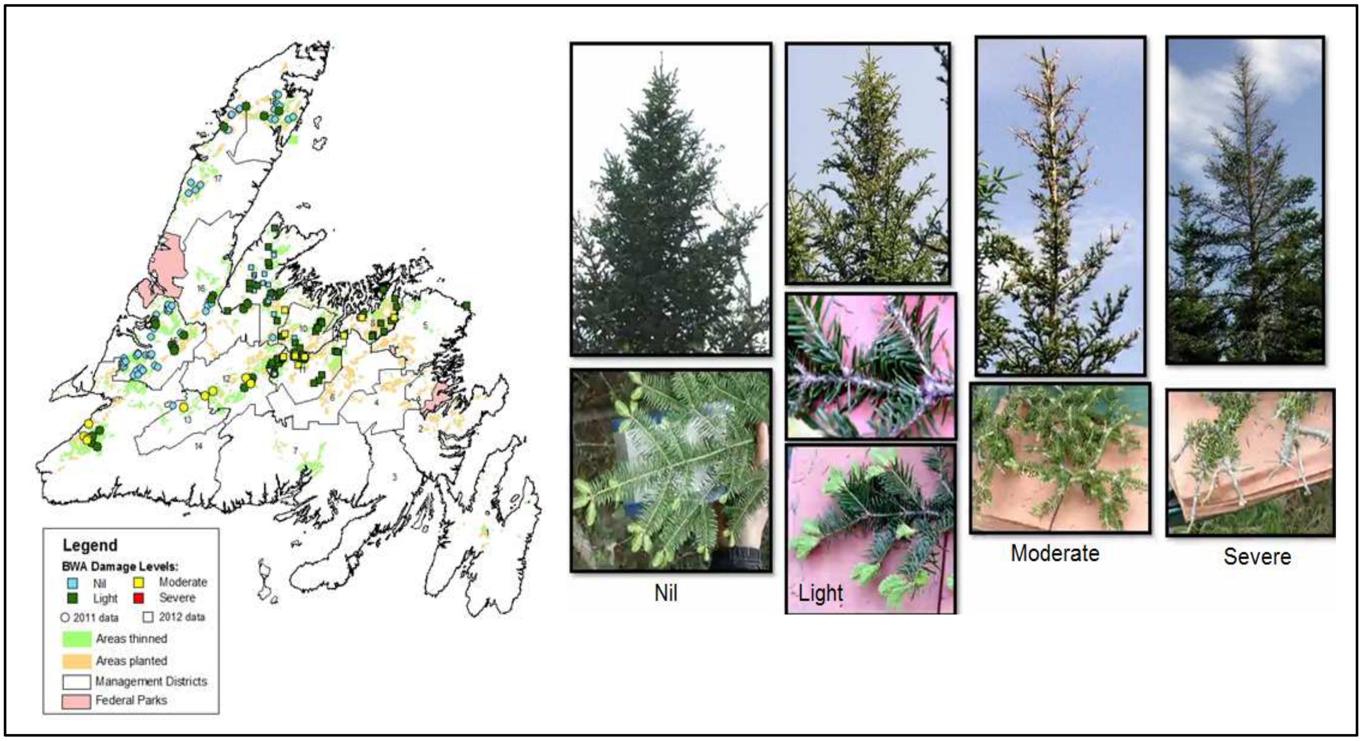


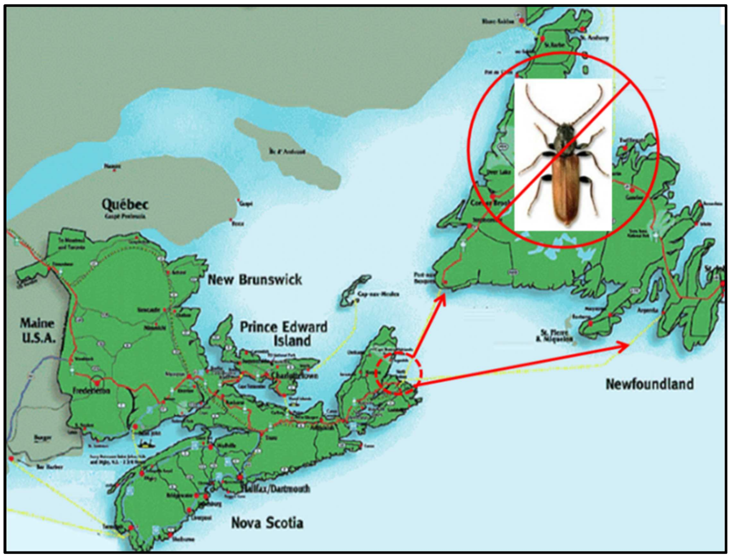
Prohibitions of movement are currently issued by the CFIA to restrict the movement of living pine within a 1 km radius of these sites. In 2014, an application to sanitize these sites was submitted and approved under the Provinces Environmental Protection Act. To date, only one of the seven sites (Cold Brook) has been sanitized. The high costs for sanitation have prohibited control efforts at the remaining sites. Over the last 5-6 years there has been a dramatic increase in the levels of infection and mortality observed at a number of these sites. Initially only scattered pockets of mortality were seen - now tree mortality is widespread in these plantations (see image).


European Scleroderris Canker mortality - Berry Hill Pond

Balsam Woolly Adelgid

Information on the incidence and levels of balsam woolly adelgid (BWA) damage within the Province was collected during surveillance of forest pests in silvicultural areas (i.e. plantations and thinnings). Information on observed damage from BWA is also provided by District silvicultural staff. To date, results indicate a higher incidence of BWA attack and damage in southwestern and central portions of Province (see map) with crown and twig attack (see images) the most common. Historically, the incidence and levels of damage found at higher latitudes and higher elevation sites has been low or absent – this is directly related to climate and the impact that colder winter temperatures have on the overwintering survival of this insect. In 2015, some reports of failed pre-commercial thinnings due to high levels of BWA damage were received from District staff. In the last several years District staff have also been reporting an increase in BWA incidence and damage at higher latitudes and elevations. There are concerns that damage from this pest will continue to increase in the future with climate change and warming temperatures





NOTE: Brown Spruce Longhorn Beetle

On the island over 85% of the growing stock is softwood with spruce representing ca. 35%. Spruce is an important species to both the sawmilling industry and in the pulping process utilized by Corner Brook Pulp and Paper (i.e. certain composition of spruce required).

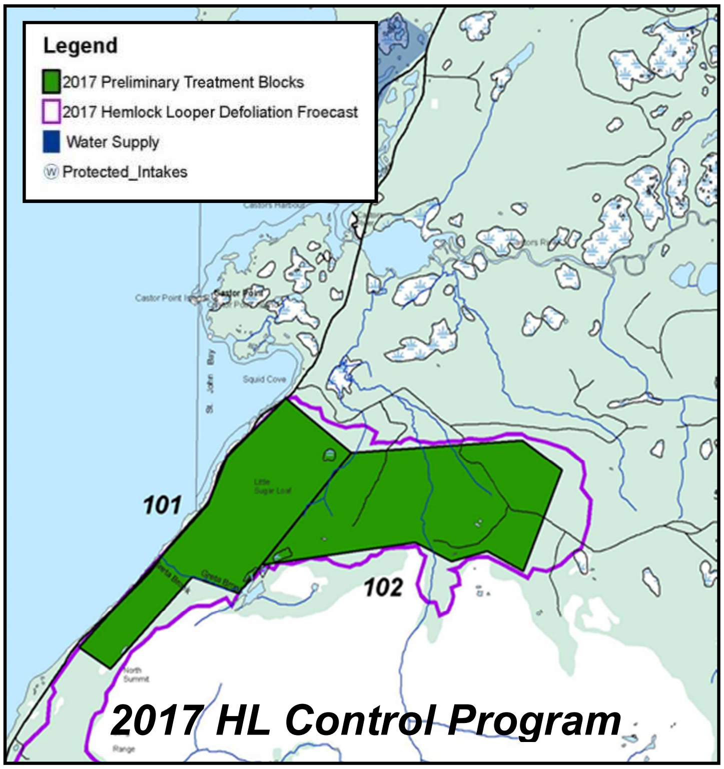
Concerns regarding the spread of BSLB to the island of Newfoundland were heightened in 2015 with the deregulation of BSLB in Nova Scotia (NS). Numerous discussions have been held with CFIA and NL Forestry officials regarding the use of measures at the Ferry Terminal in North Sydney to reduce the risk of spread of this IAS. This site has the potential to be a choke point to prevent entry of this pest into NL (see map). Discussions took place again in early 2017, but no reported measures were put in place in 2017.

With the detection and spread of BSLB into a number of locations (i.e. Kouchibouguac National Park, Memramcook) in southern New Brunswick (NB) over the last 5-6 years, and the potential link to the movement of firewood from known areas where this insect is established in NS – the Province of NL would like to see some measures taken to help prevent entry of this IAS into NL. Fortunately, CFIA trapping results for BSLB on the island, including traps in Gros Morne and Terra Nova National parks, were negative again in 2017.

Native Pests – Major Forest Insects

Major native forest insect pests are monitored annually by the Province. They include the eastern spruce budworm (SBW), eastern hemlock looper (HL), balsam fir sawfly (BFS), as well as, other minor pests. Aerial control programs are also conducted as needed to protect the forest resources of the Province.

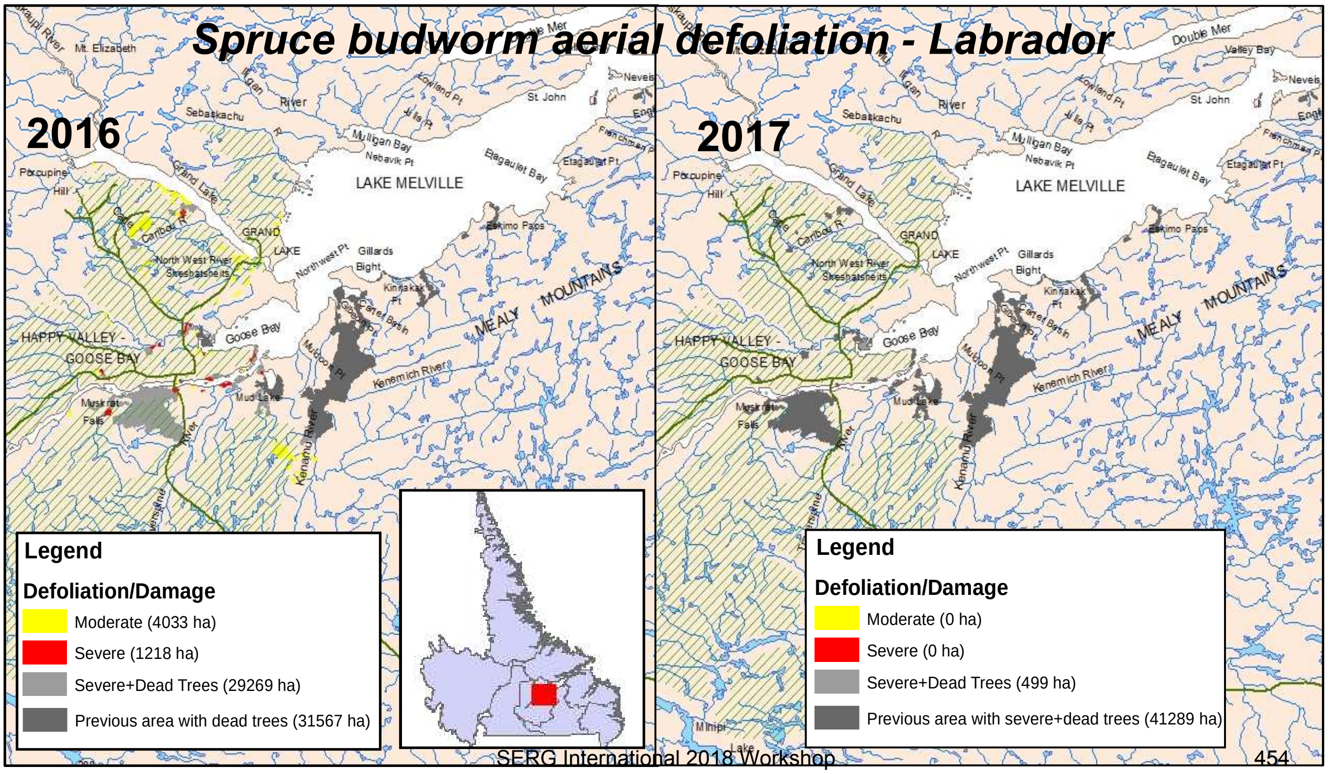
2017 Aerial Control Program



In the fall of 2016, 2,936 ha of forest land on the Northern Peninsula was found to have HL populations capable of causing moderate to severe (M-S) defoliation in 2017. A total of 111,500 cu m of forest volume was determined to be at risk including areas where investments in pre-commercial thinning were made. To mitigate the impacts expected from this pest a small aerial control program was conducted. Two blocks with a total area of 2,114 ha were treated with Btk, a biological insecticide. One Btk product, Foray 76B, was used and applied at a label rate of 2 L/ha using an M-18 Dromader aircraft equipped with Micronair AU5000 rotary atomizers, an AG NAV GPS navigational system, and Auto Cal Flow Meter and Auto Booms. A swath width of 76m was used and blocks were opened for treatment around peak 2nd instar. Western portions (327 ha) of block 101 and northern portions (418 ha) of block 102 also received a 2nd application. The program commenced on July 19 and was completed by July 27. Surveys conducted to assess treatment efficacy, indicated a 70% reduction in larval densities, a 98% reduction in egg densities, and current defoliation levels of less than 10 percent in the treatment blocks.

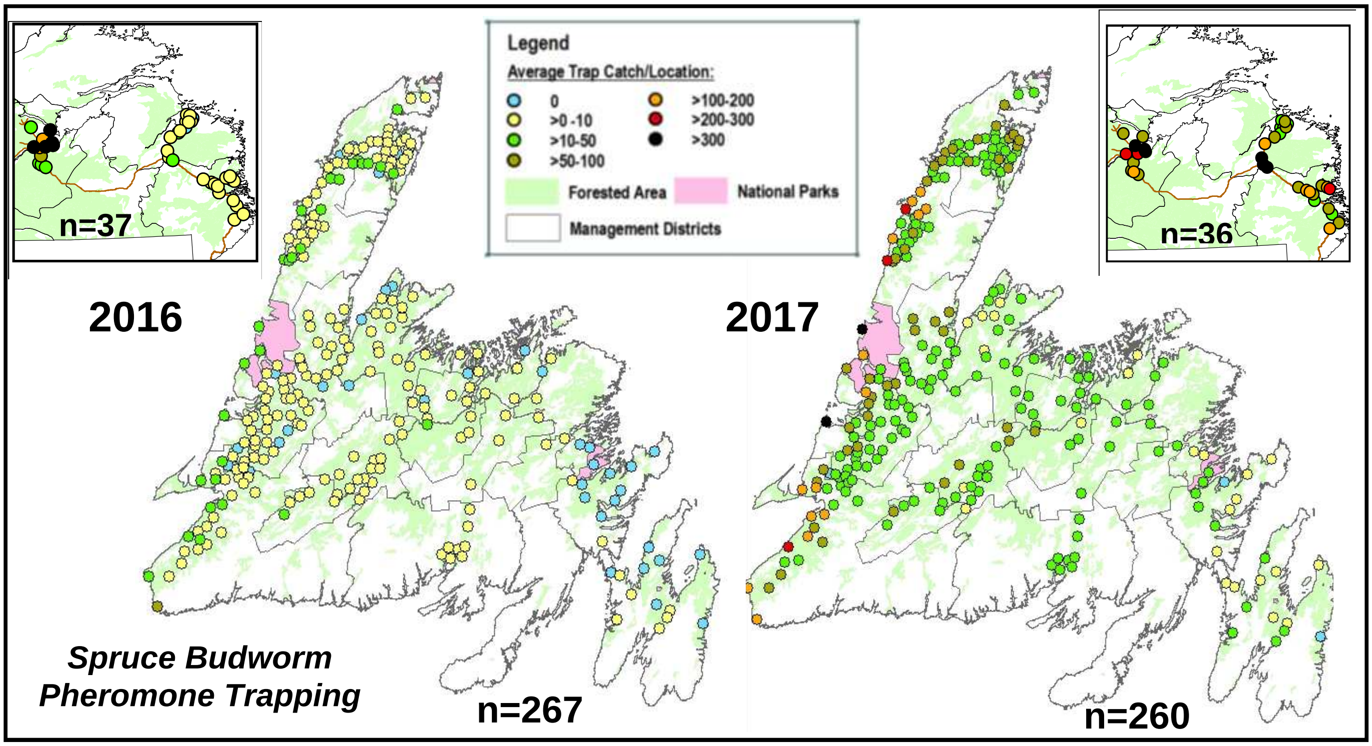
Eastern Spruce Budworm

Aerial Defoliation Survey Results – As expected, population and damage levels continued to decline in the Happy Valley Goose Bay area in 2017. The total area of moderate to severe defoliation decreased for a 4th consecutive year with only 499 ha of defoliation in 2017, compared to 34,520 ha in 2016, 36352 ha in 2015, 50767 ha in 2014, and 82231 ha in 2013. An area of ca. 41,289 ha with mortality from repeated defoliation in

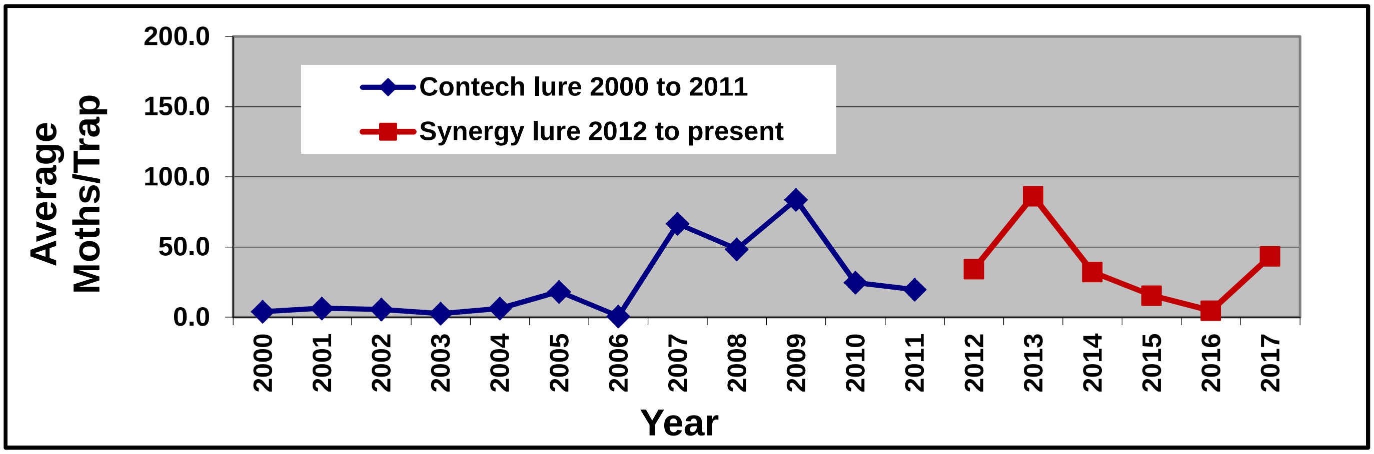


previous years was also very evident particularly along the Kenamu River and Carter Basin, and south of the Churchill River below Muskrat Falls. On the island of Newfoundland, no SBW defoliation was visible from air.

Pheromone Trapping Results – In response to rising SBW populations in the Province of Quebec, NL has been increasing its pheromone trapping network. From 2000 to 2011 there were only 50 trapping locations on the island. This trapping network has been increased to 100, 108, 110, 148, 267, and 260 locations on the island over the last six years. Since 2012, two Unitrap ® non-saturating traps have been placed 40 meters apart at each location. In each trap one Synergy 330 µg SBW flex lure and one Vaportape killing strip is placed. In 2017, an increase in the average number of moths per trap was observed on the island with highest trap catches along the west coast and northern peninsula. In Labrador, trap catches decreased in Goose Bay, but increased in eastern Labrador in the Cartwright and Port Hope Simpson areas.

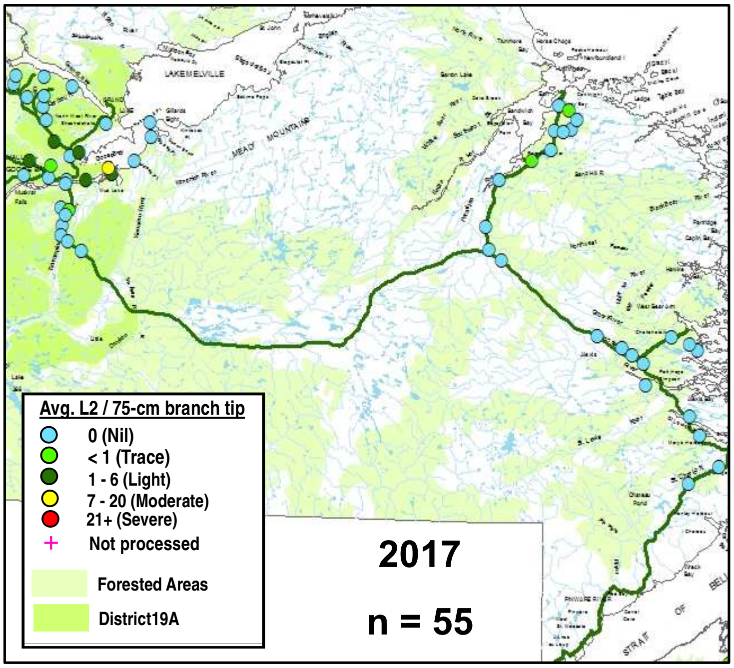


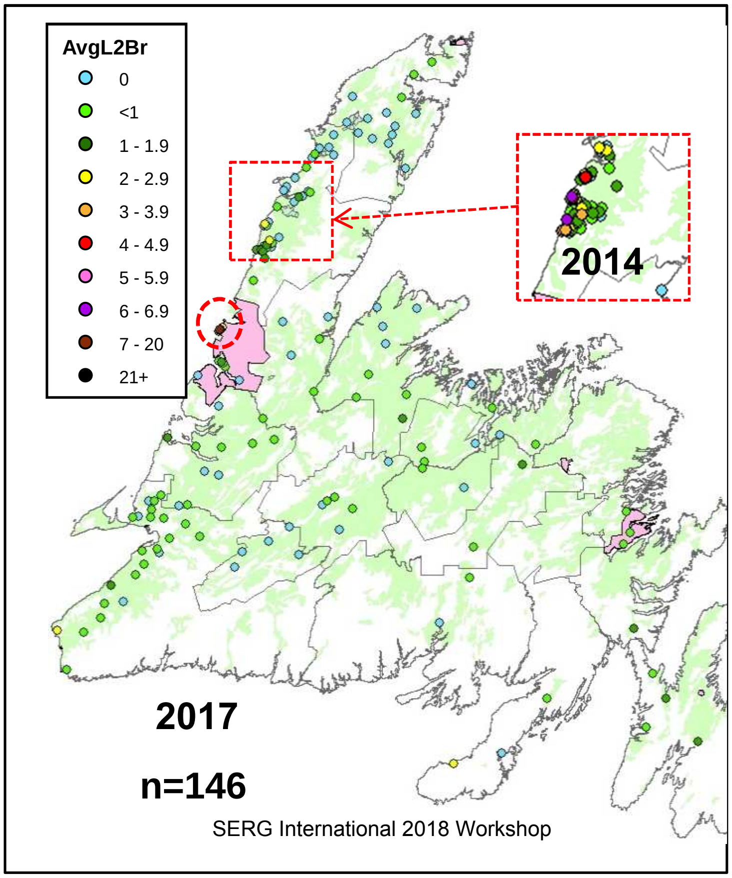
On the island the increase in trap catches observed in 2017 followed a three year period were the average number of moths per trap had declined (2013 – 86.3 moths/trap; 2014 – 32.2 moths/trap; 2015 – 15.4 moths/trap; 2016 – 4.6 moths/trap; 2017 – 43.3 moths/trap).



Light trapping conducted as part of a SERG-I research project by Dr. Marc Rhainds on the northern peninsula identified a potential immigration event at a location on the evening of July 26th. Using this location, a SBW dispersal tool (currently under development) was utilized to create backwards dispersal tracks to potential source areas. All backward tracks were westward towards Anticosti Island and the Gaspé. Once again it raises the question about the role that migration plays in the spread and increase in endemic SBW populations.

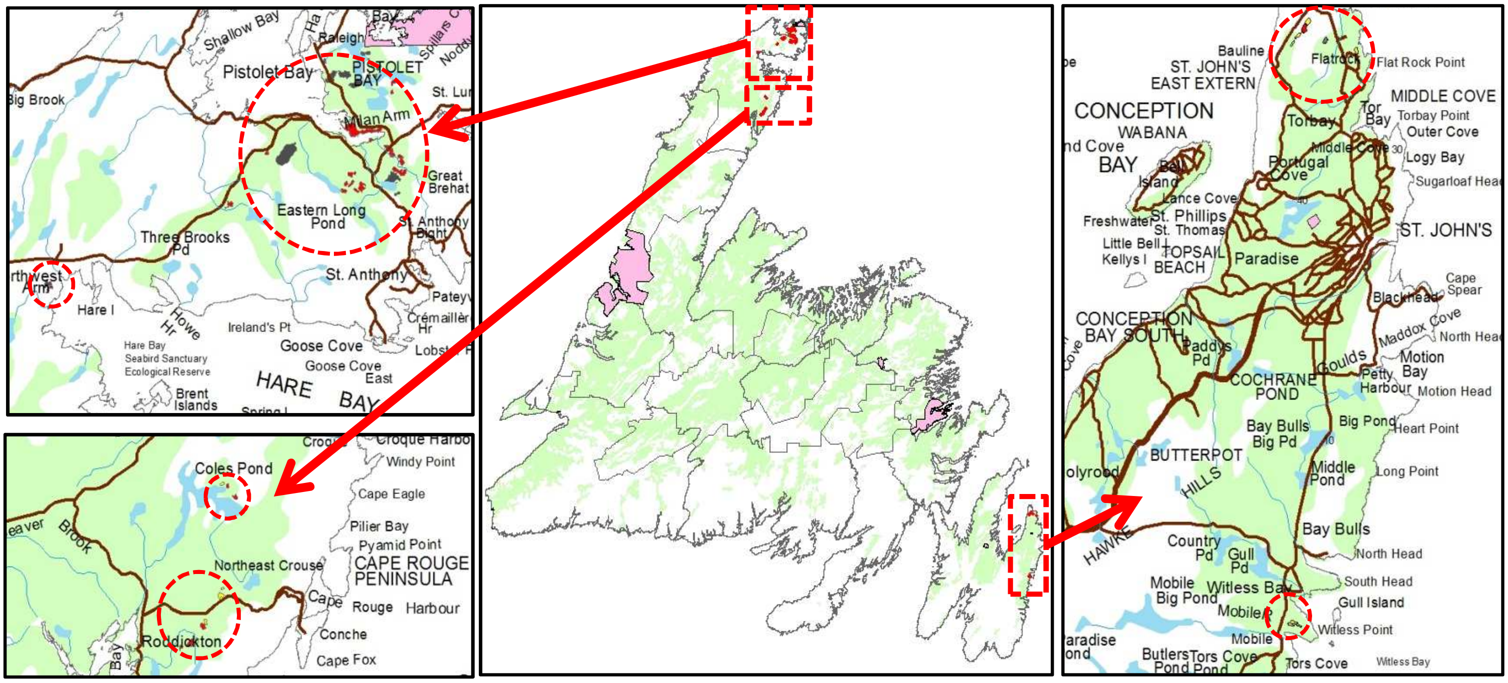
Forecast/Outlook for 2018 – To forecast SBW population and damage levels expected in 2018, branch samples were collected and processed for overwintering 2nd instar (L2) larvae. A total of 55 locations in Labrador and 146 locations in Newfoundland were assessed. In 2016, the Province began processing branch samples at its new forest insect and disease lab at the College of North Atlantic in Corner Brook. This lab now provides the capacity to process all branch samples collected to forecast major forest pest populations, including SBW. This was critical given the rise in SBW populations observed elsewhere in Eastern Canada. In 2018, results from the L2 survey indicate a continued decline in SBW populations and damage levels in the Happy Valley Goose Bay area in Labrador. Despite the higher pheromone trap catches observed in eastern Labrador in the Cartwright and Port Hope Simpson areas in 2017; L2 forecast results still indicate that SBW populations are very low (Nil, Trace) in these areas. In Newfoundland, SBW L2 population levels still remain at low levels over most of the island, including areas treated for SBW Early Intervention in 2015. Only three sites in a localized area on the northern peninsula north of Gros Morne National Park have SBW populations with 4 or more L2 per branch. This is in the same area where SBW immigration was potentially observed by Dr. Rhainds in 2017.



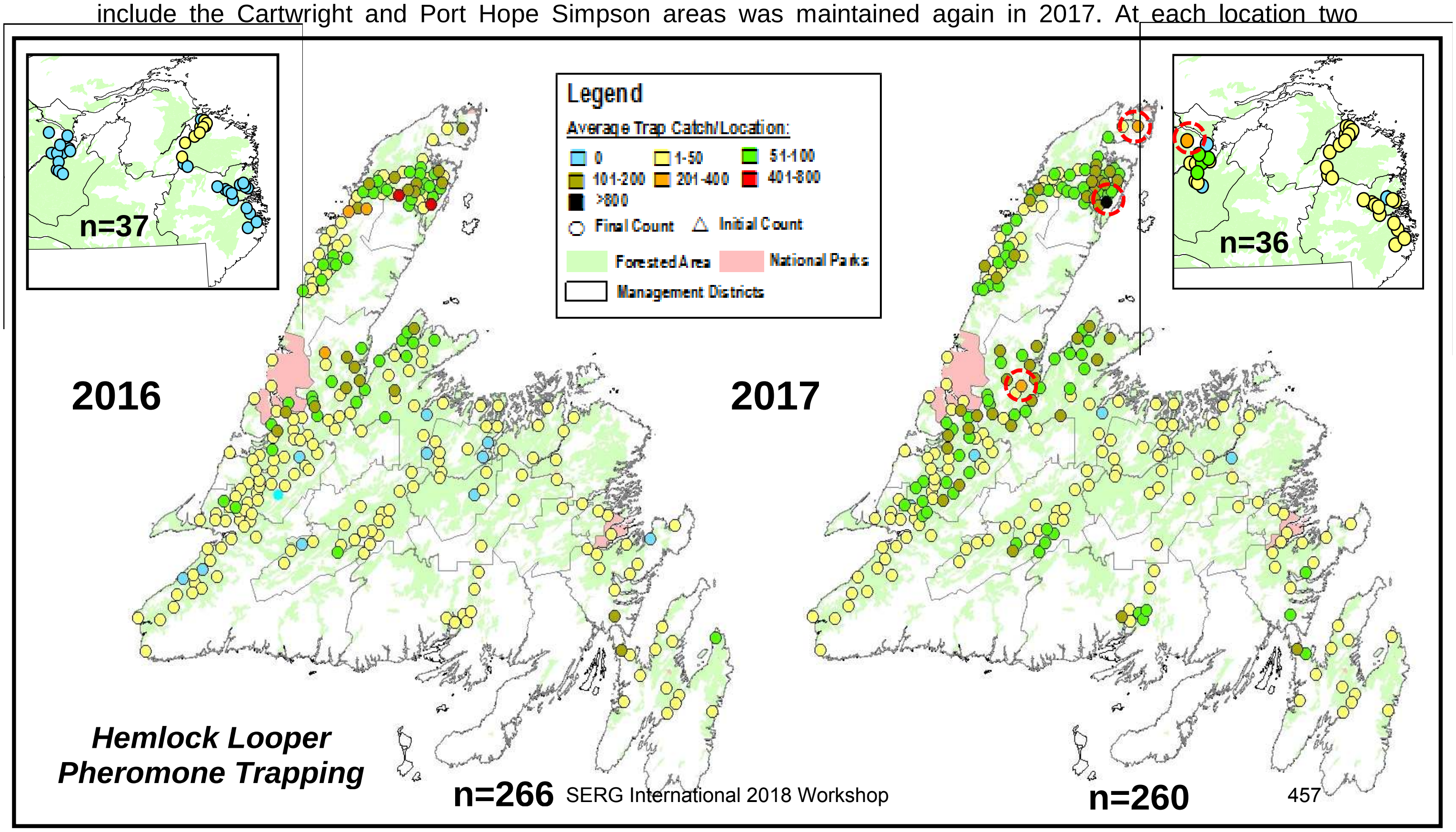


Eastern Hemlock Looper

Aerial Defoliation Survey Results – In 2017, a total of 1,309 ha of M-S defoliation caused by HL was mapped in localized areas on the Northern Peninsula. Areas included Pistolet Bay/Milan Arm/Eastern Long Pond (1166 ha), Northwest Arm (43 ha), Coles Pond (33 ha), and the Conche Rd. area (110 ha). There was also another 134 ha of M-S defoliation caused by HL in localized areas north (Marine Drive/Flat Rock Point -103 ha) and south (Witless Point – 31 ha) of St. John on the Avalon Peninsula. No defoliation from HL was observed in Labrador.



Pheromone Trapping Results – With the low HL populations observed over much of the Province over the last 6-years, a pheromone trapping network to monitor low density populations has been established to assess annual population changes. This network was established in 2011 and was increased in 2012 to ca. 100 locations, and increased again to 152 and 266 locations in 2015 and 2016, respectively. In 2017, 260 trapping locations were assessed on the island while the trapping network in Labrador which was expanded in 2014 to include the Cartwright and Port Hope Simpson areas was maintained again in 2017. At each location two



Unitrap ® non-saturating traps were placed 40 meters apart. In each trap, one 10 µg HL septa lure from Sylvar Technologies Inc. and one Vaportape killing strip were placed.

Average moths per trap were higher in 2017 than observed in 2016; however, trap catch results still indicate that HL populations remain relatively low over much of the island. Only three localized areas (Pistolet Bay, Roddickton, and Main River) had higher trap catches.

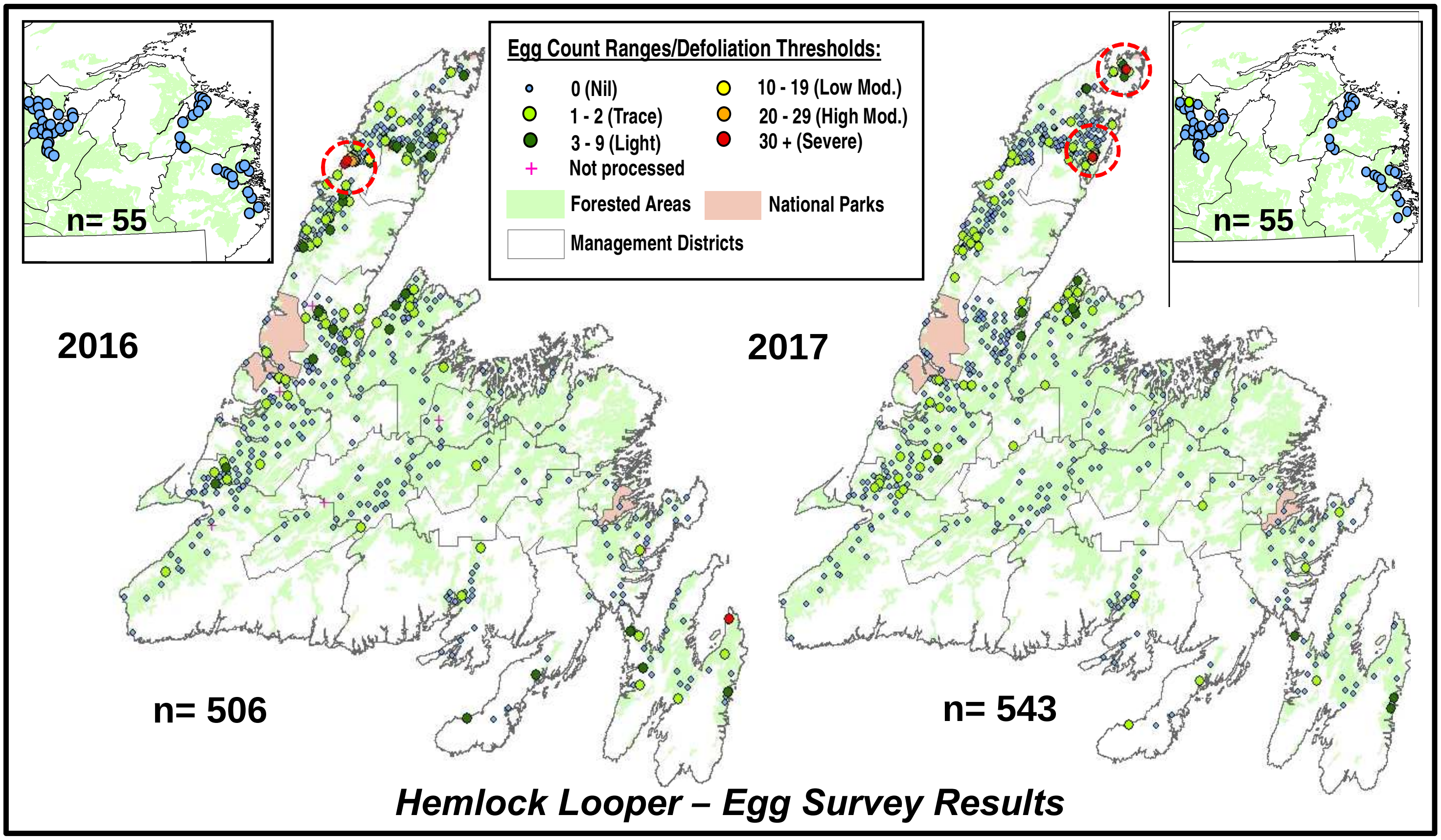
Forecast/Outlook for 2018 – To forecast HL populations and damage levels for 2018, branch samples were collected and processed for overwintering eggs. Aerial defoliation and pheromone trapping results were used to identify areas where higher levels of sampling should be conducted. Elsewhere sampling levels were reduced. A total of 543 locations were assessed on the island and 55 locations were assessed in Labrador.

On the island egg densities remain predominantly low. The Roddickton and Pistolet Bay areas on the Northern Peninsula are the only areas having locations with egg densities forecast to cause M-S defoliation in 2018. These are also the same areas where high HL moth counts were observed in 2017.

HL populations on the Avalon Peninsula forecast to cause M-S defoliation in 2016 and 2017 appear to have collapsed for 2018.

At the time of this report, no decisions have been made regarding whether a control program will be conducted in 2018 to protect forest areas on the northern peninsula from HL. An evaluation of the values at risk and an examination of the harvest schedule in the areas affected must be conducted before any decision is made.

In Labrador, HL populations remain very low. No HL eggs were found at 54 of the 55 locations sampled and only one egg was found at the remaining location.



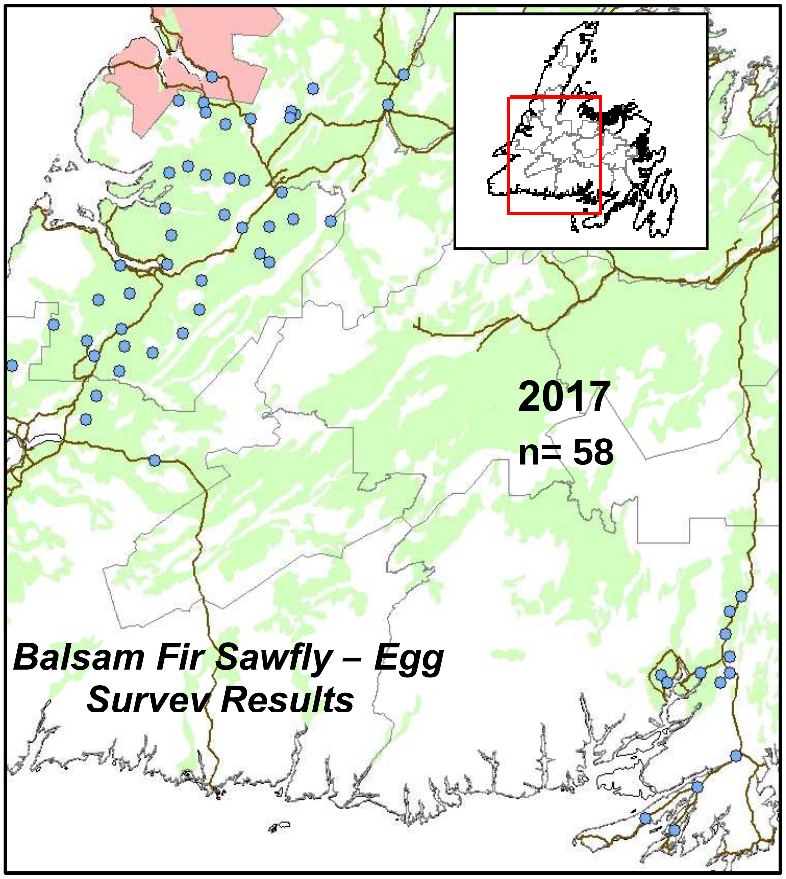
Hemlock Looper – Egg Survey Results

Balsam Fir Sawfly

Aerial Defoliation Survey Results – In 2017, no damage from BFS was detected – this is third consecutive year with no defoliation from this pest on the island.

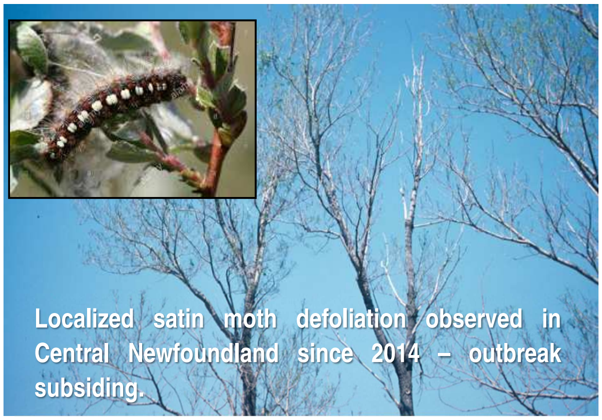
Pheromone Trapping Results – Despite efforts through SERG-I to try to develop a pheromone lure for monitoring BFS populations, no successful lure has been developed to date.

Forecast/Outlook for 2018 – To forecast BFS population and damage levels expected in 2018, collection and processing of branch samples for overwintering eggs was conducted at 58 locations. BFS populations remain at low levels - no eggs were found at any of the locations assessed in 2017. This is the second and fourth consecutive year that BFS eggs have not been found at fall forecast locations on the south and west coast, respectively.



Native Pests – Other Minor Forest Insects Observed in 2017

A few additional notes regarding damage observed from other minor pests in the Province of Newfoundland and Labrador in 2017.



Localized satin moth defoliation observed in Central Newfoundland since 2014 – outbreak subsiding.

Scattered rusty tussock moth defoliation in localized areas on norther peninsula in 2017.

Localized defoliation caused by fall webworm observed in Central Newfoundland in 2016 and 2017..

Dan Lavigne – Supervisor, Forest Insect & Disease and Wildland Fire Management
Forest Engineering & Industry Services Division
Department of Fisheries and Land Resources
January 19, 2018

SERG International

TERMS OF REFERENCE

Preamble

SERG International (SERG-I) is an association of forest management, regulatory and research agencies, and pesticide suppliers, interested in forest pest management. The primary aim of SERG-I is to improve application technology and pest management methods associated with the use of pest control products in the context of integrated forest pest management. SERG-I facilitates efficient use of resources for collaborative research to meet the needs and priorities of pest managers.

SERG-I is not a funding agency. Rather, SERG-I provides a means for the members to work cooperatively on research projects through the sharing of expertise, financial resources, in-kind resources, staff, and the results to achieve common goals in the areas of spray efficacy and pest management methods.

Through this co-ordinated approach, members of SERG-I are able to pool their resources, leverage other sources of funding, and work cooperatively to conduct research and share results amongst the members.

General Areas of Research that are coordinated by SERG-I

1. **Forest Pest Management Products:**
   Product efficacy - including conventionally applied products as well as the development of alternative products such as viruses, nematodes, pheromones and biological herbicides, etc.

2. **Improvement of Application Technology and Techniques:**
   Application technology - including aerial and ground based application techniques which provide improvements in environmental and efficiency goals.

3. **Environmental Impact and Benefit to Forest Pest Management:**
   Fate of applied products in terms of deposit, drift, impact and behaviour in the environment including impacts on biodiversity and ecological processes.

4. **Developing Forest Pest Management Strategies:**
   Develop working strategies for the management of forest pests.

5. **Technology and Information Transfer:**
   Transferring results of SERG-I supported research to forest managers through annual workshops, the SERG-I web site and stakeholders meetings.

STEERING COMMITTEE

The SERG International Steering Committee consists of Executive and Associate Committee members.

Membership:

Executive Members

Provincial representatives, along with the SERG-I Executive Director and a Director-level representative of CFS and the USDA-FS shall form the Executive. Membership fees for Executive members are set at $6000/year.

Executive Members presently include:

Province of Alberta (Alberta Sustainable Resource Development)
Province of British Columbia (Ministry of Forests, Lands and Natural Resource Operations)
Province of Manitoba (Department of Conservation and Water Stewardship)
Province of New Brunswick (Forest Protection Limited)
Province of Newfoundland and Labrador (Department of Natural Resources)
Province of Nova Scotia (Department of Natural Resources)
Province of Ontario (Ministry of Natural Resources)
Province of Quebec (SOPFIM [1])
Province of Quebec (MRNFQ [2])
Province of Saskatchewan (Saskatchewan Ministry of Environment)
Canadian Forest Service (Director)
USDA-Forest Service

[1] Société de protection des forêts contre les insectes et maladies
[2] Ministère des Ressources naturelles et de la Faune du Québec

Executive Members will contribute equally to the cost of maintaining the SERG-I Executive Director and the administrative expenses to run SERG-I. Executive Members have voting privileges on the Steering Committee. In addition, they set the strategic direction for SERG-I and participate in the annual prioritisation of work areas.

Associate Members

Associate Members of the Steering Committee shall include representatives of organisations able to conduct, benefit from or support pest management research and interested in co-ordinating their efforts. Associate Members neither have voting privileges, nor do they participate in establishing either the strategic direction or research priorities of SERG-I. They do, however, participate in the evaluation of SERG-I Proposals and may provide funds according to their individual interests.

Associate Members do not contribute to the cost of administering SERG-I but are encouraged to sponsor research projects

Associate Members presently include:

AEF Global
BioForest Technologies Inc.
Canadian Aerial Applicators Association
Canadian Food Inspection Agency
Canadian Forest Service – Atlantic Forestry Centre
Canadian Forest Service - Great Lakes Forestry Centre
Canadian Forest Service – Laurentian Forestry Centre
Canadian Forest Service - Northern Forestry Centre
Canadian Forest Service – Pacific Forestry Centre
Dow AgroSciences Canada Inc.
Micron Sprayers Limited
Monsanto Canada Inc.
Pest Management Regulatory Agency
Sylvar Technologies Inc.
USDA-ARS (College Station)
Valent BioSciences Corp.
Winfield Solutions

Other organisations may be invited by the Executive to participate as appropriate.

Steering Committee

The Executive Director of SERG-I will be the Chair of the Steering Committee.

SERG International activities are directed by a Steering Committee which will:

1. Develop and maintain the SERG-I Long-Term Plan.
2. Identify and prioritise research, development and technology transfer on an annual basis.
3. Identify and develop potential partnerships for SERG-I particularly in the areas of pest management, regulation, economic benefit, communications, new technology, and stakeholder needs (e.g. forest industry).
4. Request and review proposals (Appendix A,B) and funding for project priorities.
5. Co-ordinate application technology and efficacy research
6. Promote outreach activities such as workshops, a SERG-I website and distribution of research reports to interchange information amongst SERG-I, researchers, user groups and participating organisations.
7. Provide advice and recommendations to various organisations which either participate in or have an interest in SERG-I activities.

Each organisation shall have one representative (or its designated alternate) on the Steering Committee. Additional representatives may attend as observers and to provide expertise as needed. Additions can be made to the member organisations of the Steering

Committee subject to approval by the majority of the Executive. Additional individuals or organisations may be associated with SERG-I (without becoming Executive (voting) Members or Associate Members of the Steering Committee) by writing to the Executive Director with a request to be included on the SERG-I mailing list. All those included on the mailing list will be notified of the Annual Workshop and will receive the updated SERG-I publication list and the annually updated Long-Term Plan and Research Priorities.

Overhead and Administration Expenses:

Overhead costs for SERG-I, including the fees for the Executive Director, the Executive Director's travel expenses and administrative costs, will be shared equally by the Executive Members of the Steering Committee. The budget for the coming year will be established by the Executive previous to March 31 of the current year.

Disbursement of Surplus Funds:

1. A majority approval by the SERG-I Executive Committee members (see Note 3 for exception) is required for SERG-I surplus funds to be disbursed.
2. Any proposal by an Executive Committee member for use of SERG-I funds must be circulated to the Executive Committee and the Executive Director at least two weeks prior to the SERG-I meeting at which it is to be tabled. Members who will not be present at the meeting will have up to one week prior to the meeting to submit their votes to the Executive Director.
3. Any absentee Executive Committee member who does not submit a vote by the due date, will lose his/her voting right on the proposed motion. Majority approval will then be based on the remaining voting membership. In the event of a tie, the Executive Director may cast the tie-breaking vote.

Meetings:

Meetings of the Steering Committee shall be called by the Executive Director, after consultation with the Executive, and shall be held at least twice per year. Special Issue conference calls may be held at the request of an Executive Member.

1. Each year, $2500 of each Executive Member's annual SERG-I fee will be set aside for that Member to use for SERG-I travel to any Steering Committee meeting including project evaluation meetings. Travel to attend the SERG-I project evaluation meeting (Feb) shall take precedence over attending other meetings.
2. Each member’s travel funds will be carried to a maximum of 2 years to a total of $5,000.00. Any surplus will be transferred to SERG-I general revenues.

The agenda for meetings shall be determined by the Executive Director, in consultation with Steering Committee members.

Minutes shall be taken of all meetings and distributed by the Executive Director to all members of the Steering Committee.

Workshop:

A formal SERG-I Workshop will be held annually. The Annual Workshop will normally be scheduled in early February. Final or interim written reports will be submitted to the Executive Director prior to the Workshop (mid January). An oral presentation delivered in person preferably by the Principal Investigator on the current year's research will be made at the Workshop. Immediately following the Workshop, research proposals for the upcoming year will be evaluated for member's acceptance and support.

The Executive Committee will meet to update the SERG-I Long-Term Plan.

The location of the Workshop will rotate amongst the member provinces/and or agencies (i.e. the Executive). The host province or agency will co-ordinate the Workshop including the provision of secretarial and related services (such as registration desk and temporary custody of registration fees).

APPENDIX A

Proposal Format Specifications

Concept Proposal

A concept proposal should be no longer than 2 pages using font sizes no smaller than 10. Multi-year projects require a concept proposal each year.

General:
1. Title:
2. Principle Investigator or Agency:
3. Contact Person:
4. Telephone Number:
5. Address:

Executive Summary

- SERG-I general area of research addressed as stated in the Terms of Reference
- Objectives of study
- Completion date for study

Objective:

Clear and concise statement of objective (s).

Financial:

Budget requirements clearly identifying funding sought from SERG-I and other potential funding sources. Forecast budget requirements for future years (where applicable) including level of investment by all participants (to the extent possible).

Funding details should include potential funding sources and funding level
Provide details for Year 1 (and future years of multi-year proposals)*
Funding runs from April 1 to March 31

Proposed Funding Sources

	Year 1 (ie2010/11)	**Year 2 2011/12**	**etc**	**etc**
Identify all potential and secured funding sources including in-kind	level of funding			
and				
SERG-I	funding sought			
Total	-	-		

* *For new projects, Y1 starts the year when the full proposal is accepted.*

Final Proposal

Final Proposals should be no longer than 4 pages using font sizes no smaller than 10
Multi-year projects require a final proposal each year.

General: 1. Title:
2. Principle Investigator or Agency:
3. Contact Person:
4. Telephone Number:
5. Address:

Executive Summary

- SERG-I general area of research addressed as stated in the Terms of Reference
- Objectives of study
- Completion date for study
- Names of suggested reviewers from outside the proponent's agency

Objective:

Clear and concise statement of objective (s).

Financial:

Budget requirements (by year for multi-year projects) clearly identifying funding sought from SERG-I and other potential funding sources.

(a) Provide details of expected expenditures, i.e. casual/student salaries, related travel, materials, printing, etc. for current year using budget template below.

(b) Identify committed funding and in-kind contributions from all sources.

(c) Forecast budget requirements for future years (where applicable) including level of investment by each participant (to the extent possible).

Funding details should include potential funding sources and funding level
Provide details for Year 1 (and future years of multi-year proposals)*
Funding runs from April 1 to March 31

Proposed Funding Sources				
	Year 1 (ie2010/11)	**Year 2 2011/12**	**etc**	**etc**
Identify all potential and secured funding sources including in-kind	level of funding			
and				
SERG-I	funding sought			
Total	-	-		

* *For new projects, Y1 starts the year when the full proposal is accepted.*

Include funding details for Current Year

Funding Details	
Casual employee/ student salaries	
Field Trials	
Vehicles	
Field equipment supplies	
Travel, meals, lodging	
Lab	
Materials and Supplies	
etc	
etc	
Total	

Note: Full-time employee salaries/travel and administration fees will not be funded by SERG-I but can be noted as in-kind contributions.

Background:

Identify the problem/opportunity of concern and how it relates to SERG-I's Areas of Research in its Terms of Reference. Outline the magnitude of the impact of the project if successful and if not undertaken. Identify the specific solution required. Identify any previous funding received and progress to date.

Participants/Co-operators:

Identify the roles of researchers and user agencies involved in the project as well as the level of support and participation throughout the project.

Methodology:

Identify all relevant details including method of implementation, number of personnel involved, location, anticipated completion date, etc.

Deliverables including Timing and Reporting Milestones

Identify measurable outputs to be produced by which the success of the project may be evaluated and the manner in which these will be presented. **Note: Reporting requirements include a final SERG-I report due by mid-January of the year following acceptance of the proposal and an oral presentation preferably by the Principal Investigator at the annual SERG-I workshop.**

APPENDIX "B"
SERG International Proposal Review Procedures

The Executive will review the SERG-I Long-Term plan annually, update it as required, and set research priorities for the following year. Specific priorities, and the level of funding anticipated for each, will be identified whenever possible. The updated Long-Term Plan and Research Priorities will be distributed normally by early October, at the time of the call for SERG-I Concept Proposals. Specific agencies or individuals may be asked to submit proposals for certain projects or an open public call for proposals may be used for other work areas.

Call for Proposals

Concept and final proposals (in the formats detailed in Appendix A) will be accepted up to the date set by the Executive Director in his call letter. The research proponent may be invited at the discretion of the Executive to be available either in person or via telephone when a particular proposal is being reviewed.

Stage I Review

Members of the Steering Committee will review the executive summaries of the Concept Proposals to determine the proposal's compliance with the Long-Term Plan and Research Priorities, and to identify the funding potential for the proposed research. This review will be completed no later than the end of the National Forest Pest Management Forum week (usually first week in December). Only Concept Proposals approved at this stage will receive further consideration at Stage II.

Stage II Review and Funding

The Executive Director may obtain at least two reviews of each Proposal – one from a scientist and one from a user. The final proposals and the reviewers' comments are then provided to the Steering Committee to determine which projects will receive support for funding in the upcoming year

Conflict of Interest Guidelines

Recognizing that members of the SERG-I Steering Committee may also be participants on SERG-I-supported projects, the following rules will apply:

1. Said members will declare themselves in conflict when evaluating SERG-I proposals (Stage II – Terms of Reference) of which they are collaborators or which have the same scientific goals as SERG-I proposals/ projects of which they are a collaborator.
2. Project-team members will be excluded from the Stage II evaluation of their SERG-I project.

Rules for SERG-I Stage II Evaluation

1. Prior to the Stage II evaluation meeting, principal investigators will be notified of the date – time for the SERG-I review.
2. Principal investigators will avail themselves, either in person or by telephone, for questions that may arise during the Stage II review.
3. Attendance to the full review meeting will be restricted to the SERG-I Steering Committee, their alternates and invitees.
4. The Stage II review meeting may also have an open format during which specific questions relating to project proposals will be discussed.

SERG-I is not a funding agency with an allotment of money to be awarded through a competitive-process. Rather, SERG-I provides a means for the members to work cooperatively on research projects through the sharing of expertise, financial resources, in-kind resources, staff, and the results to achieve common goals consistent with specified SERG-I General Areas of Research.

At Stage II, the Steering Committee will review the final proposals and the comments of the reviewers in considering the proposals for funding by the members. Each member will decide which specific proposals to support, and what type (e.g., financial, in-kind, coordination, advisory) and amount of support will be provided. Opportunities for cooperation and efficiencies will be explored and encouraged by the Steering Committee.

Proposal Endorsement and Conditions

The Executive Director will inform each proponent in writing of the status of the Proposal (e.g. approved, not approved, approved with conditions, or revise and re-submit). Proposals approved at Stage II will be assigned a Project Number.

SERG-I research projects will be completed according to SERG-I Field Guidelines available on the SERG-I website (www.sergreport.net).

For most projects, SERG-I will arrange for a holdback of at least 10% of funds. This holdback will be released upon receipt and acceptance by the funding agencies of the final or interim report and a presentation preferably by the Principal Investigator at the SERG-I Workshop. Proponents are expected to manage their finances by taking this 10% holdback into consideration. Each year, a project report must be received and accepted by the funding agencies before funding will be granted for subsequent years.

APPENDIX C

Reporting Policy for SERG-I

1. Introduction

SERG International (SERG-I) has an excellent reputation for supporting applied research and technology for use in forest pest management and other related fields. As a service to the technical community, SERG-I maintains on-line access to reports produced through the SERG-I process and distributes these as PDF files upon request. Typically, SERG-I facilitates some 30 research and development projects annually.

2. SERG International Reporting Requirements

As part of the agreement with a Principal Investigator (PI) who participates in the SERG-I process, a final report (and yearly interim reports in the case of multi-year funding) is required by mid-January of year following acceptance of proposal and an oral presentation at the annual Workshop. Final acceptance of report submissions is the responsibility of SERG-I funding members.

Final Reports

- **SERG-I Report**
  A detailed project report is acceptable as a final report. This report should follow a classic scientific format with abstract, introduction, objectives etc. through to results and conclusions. It should be written in plain, understandable language and terminology. The report must include a bilingual executive summary; translation is the responsibility of the Principal Investigator. It will be the PI's responsibility to identify any disclaimers on the front page of the report (e.g. do not cite, do not copy, refer all questions to author, etc.). The report should be signed-off by the PI's manager. The funding members of the project will arrange for a review of the final report.

  Only FINAL SERG-I reports will be bound in SERG-I covers and be submitted to the Canada Institute for Scientific and Technical Information (CISTI).

- **Peer review article**
  Principal Investigators receiving funding are encouraged to produce peer reviewed articles. SERG International should be acknowledged.

  Peer reviewed journal articles will be accepted as final reports if the funding members agree the subject matter is relevant. A suggested format to fulfil SERG-I reporting requirements is a bilingual executive summary of the project and a brief (one page) introduction explaining how the peer review article resulted from the original project. These cover pages should be bound with a reprint of the article between SERG-I covers and submitted to the SERG-I Executive. (For electronic distribution, a link or web page address can be used to link the interested party to the publishing society to obtain the article.)

Peer review requirements are often external to SERG-I (e.g., academic employers or federal research managers, etc.) Anonymous peer review is the best way to insure that results are correct and of high quality. However, it is recognized that the time frame surrounding peer review publications can legitimately take two years from submission to publication. This time frame will be considered in the proposal evaluation process. At the time a submission is made for journal publication, the draft manuscript should be forwarded to the Executive Director. If this is unacceptable, this needs to be explored up front between funding members and the Principal Investigator involved. One option in some circumstances is for funding members to hold and use data internally while treating it as proprietary to the Principal Investigator.

Failed or abandoned peer review. Peer review publications may be turned down for many reasons. Failed or abandoned peer review projects should be reformatted into Project reports and evaluated for suitability as Final reports.

Copies of final reports will be distributed by the PI in the following manner:

1. All Executive Committee members and funding agencies will receive one hard copy each (names and addresses of members can be obtained from the Executive Director) bound between SERG-I covers.
2. The SERG-I Executive Director will receive 2 bound copies and a PDF version. A copy of the report will be filed with CISTI and distributed (PDF version) upon request.

Interim Reports

All formats discussed above (with a bilingual abstract) are appropriate for interim reporting. It is suggested further that reporting requirements be discussed in the proposal and upon funding. As a minimum reporting requirement, an interim report will be required every year. Interim reports include

- **Data Report**
  Unless otherwise stated, data generated in a specific project are the property of the funding members. The final report may be a data report if the funding members agree. This report must include a restatement of objectives, an executive summary and a methods section. Raw data should be included as an Appendix.

- **Conference/Workshop Proceedings**
  Proceedings reports can fulfil interim reporting requirements. Proceeding papers that undergo anonymous peer review and meet the requirements for such may be simply formatted into a Final Report as described above if this is acceptable to the funding members.

- **Other agency reports**
  Other agency reports may be accepted as interim reports. Reformatting may be required to meet SERG-I reporting requirements to be considered as final reports. Since most reports follow some semblance of a classic scientific outline, this should not be an egregious requirement.

Proprietary Research

Proprietary industrial projects (as opposed to proprietary academic rights in order to protect publication potential) will be handled as a separate entity. In this case, an explicit contract will be entered into between funding members and Principal Investigator and the deliverables will be stated. This is the only case where a final report may not be required based on the specific contract.

3. Acceptance of SERG-I Reports

For most Projects, SERG-I will hold back a minimum 10% of allocated funds. These will be released upon acceptance of reporting requirements. Ultimately, acceptance of all SERG-I reports rests with the funding members. If a majority of the funding members accept a Final Report then the project is considered complete. If there is a dispute or in the unusual case of a deadlock, the SERG-I Executive Director will cast the deciding vote. The balance of project funds will be withheld and released only on report completion unless negotiated beforehand (i.e. in the case of the prospect of a long peer review process, the letter of submission to a journal would release the final balance of funds).

4. Delinquency

It will be the responsibility of the Funding members working with the Executive Director to obtain project reports. Reporting delinquency is a problem and can, in some cases, result in the inability of a funding member to generate additional funds for contribution to SERG International. Principal Investigators who don’t fulfil yearly interim or final reporting requirements will not receive further funding from SERG-I until reporting requirements have been completed.

In the event a delinquent report is not ‘in the works’ (i.e., actively being worked on by data analyses and writing, and has a scheduled reporting date), or is not likely to be prepared as acknowledged by the original PI, then that PI will be obliged to provide the raw data and associated results that were collected, the objectives of the project, and as much detail as possible about the material and methods that were used. This information may be made available to one or more of the original funding members of the project and subsequently made available by them to another researcher duly recognized by them and/or the SERG-I Executive who has interest in further analyses and reporting, though not necessarily to the exclusion of the original PI.

APPENDIX D

SERG-I Executive Director Responsibilities

Overall Responsibility

Under the authority of the Executive Members of SERG-I, the Executive Director has the responsibility to plan, organize, manage and control all the activities defined in SERG-I's Terms of Reference.

Specific Responsibilities

1. Chairs the SERG-I Steering Committee: liaises with other research organisations/ networks; ensures that meetings of the Steering Committee are held as required, workshops are held annually, research proposals are submitted for review either by request or letter call, and research reports are submitted and distributed.

2. Maintains milestones for all new research projects to ensure compliance to report production schedules.

3. Calls periodic meetings of the Steering Committee and sub-committees as required to identify problems and opportunities, prepare research proposals, plan research activities, and recommends priorities accordingly.

4. Ensures distribution of research reports and facilitates communication and collaboration among scientists, applicators, and forest managers.

5. In consultation with the Steering Committee, assists the Executive in updating annually a Long-Term plan which includes each activity area addressed by SERG-I, clearly identifying individual studies and defining general and annual objectives.

6. Maintains and circulates to the Steering Committee a status report on publications and reports prepared or anticipated from studies supported by SERG-I.

7. Represents SERG-I at meetings and submits reports to the Steering Committee.

8. Ensures that minutes for Steering Committee Meetings are taken and distributed.

9. Reviews progress on current research studies supported by SERG-I.

10. Presents new research proposals recommended by the Steering Committee and distributes them to the membership.

11. Identifies potential sources of funding for research proposals and, where appropriate, assists the Steering Committee in obtaining commitments from these sources

12. Relays the decisions and recommendations of the Steering Committee to the SERG-I mailing list and proponents.

13. Stimulates communication among the members to share resources.

14. Produces an annual workshop proceedings summarizing SERG-I activities and distributes it to SERG-I members and other stakeholders through the SERG-I web site.

15. Maintains the SERG-I website.

16. Prepares an annual Executive Director's operating budget for consideration by the Steering Committee prior to the commencement of the next fiscal year.

APPENDIX E

SERG International
Graduate Student Awards Fund (GSAF)

Context

SERG International (SERG-I) would like to honour the hard work and dedication of many practitioners of forest pest management by establishing an annual Graduate Student Awards Fund (GSAF) to support graduate students who are doing research directly related to SERG-I's areas of interest [www.serg-i.org]. The main purpose of the Fund is to support students early in their careers by providing an opportunity to attend the annual SERG-I Workshop where they can present their own research and interact with many of the key researchers and managers responsible for forest pest management in Canada and the United States as well as some of the leading manufacturers of pest control products and related services. The Annual Workshop is hosted by different member agencies; hence, its location varies annually across Canada and northern United States.

Terms of Reference

1. Annual selection of GSAF Honourees

a. On or about October 1st of each year, the Executive Director of SERG-I will canvass by email the Steering Committee Members and universities/colleges for nominations for the GSAF. .

b. All GSAF applications received by the Executive Director by the November 15th deadline (as noted in 4e of the Application Process) will be forwarded within a week to Executive Committee members for review. The Executive will evaluate all applications and make their recommendation(s) known at the SERG-I Annual General Meeting, normally held in the first week of December in conjunction with the annual Forest Pest Management Forum.

c. The Executive Director will subsequently communicate with the successful student(s) regarding their awards and the requirement to attend and participate at the SERG-I Annual Workshop normally held in February of the ensuing year.

3. Award Value

a. Annual awards of up to $2,000 each may be made available for up to 5 students (i.e., maximum of $10,000 annually subject to Executive approval). The Executive may approve, subject to funds available and/or other relevant circumstances, additional awards in years when there are more than 5 students who qualify based on recommendations by the Selection Committee.

b. SERG-I reserves the right to modify the conditions of the GSAF or to discontinue the initiative entirely.

4. Application Process

a. The availability of the SERG-I GSAF will be made known to potential applicants through the Steering Committee members and university contacts and will be posted on the SERG-I website.

b. Awards are open to post-graduate students who are engaged in research in one or more of the four themes of research specified in the Terms of Reference for SERG-I. Students may apply more than once during their degree program.

c. The major supervisor of the student applying for an award <u>must</u> provide a Letter of Support outlining the merits of the nominee and this Letter <u>must</u> be included in the application which is due by November 15th.

d. Each applicant <u>must</u> enclose, along with the Letter of Support, a Student Summary Statement in which they discuss, in no more than two pages, their research interests, enthusiasm and interest for attending and presenting at the SERG-I Workshop, and how their project adheres to one or more of the SERG-I research themes.

e. Complete applications, including the Letter of Support and the Student Summary Statement must be submitted by <u>email</u> to the Executive Director of SERG-I by November 15th in each award year. The Executive Director will forward applications to the Executive Committee in advance of the SERG-I Annual General Meeting held in conjunction with the annual Forest Pest Management Forum (usually the first week of December). Successful GSAF winners will be notified by the SERG-I Executive Director shortly thereafter as noted in 2c above.

f. Incomplete applications will not be considered.

5. Award Conditions

a. Award to consist of $500 cash, waiver of SERG-I Workshop Registration Fee and up to $1,500 towards reimbursement of expenses incurred for attending the Workshop including: economy return air fare; hotel; taxi; and per diems (at federal rates). (<u>Note:</u> all original receipts are required except for per diem items).

b. Each successful Award winner is **<u>required</u>** to attend the annual SERG-I Workshop and make an oral presentation of their research work. Associated abstracts and summary reports are to be provided for inclusion in the SERG-I Workshop Proceedings and may be posted on the SERG-I website.

c. Awards will be presented at the SERG-I Annual Workshop.

5. Award Restrictions

a. A candidate can only receive a Graduate Student Award once during each graduate degree.
b. A candidate receiving funding as PI for a SERG-I project can not apply for a Graduate Student Award.

6. Generalized Timeline

January	February	March	April	May	June	July	August	September	October	November	December
		-------------- Student study & research period ------------- Letter of Support & Student Summary Statement to be prepared with Application to be submitted by deadline of November 15th.								Applications received by SC by Nov. 15th; Reviews done.	GSAF Awards announced at AGM.F

2017/18 SERG International Work Plan

Advances and Implementation of New Technologies

2016/03-2017-107

Effect of dosage and treatment frequency on efficacy of TreeAzin® Systemic Insecticide for protecting foliage from the beech leaf-mining weevil and preventing mortality of American beech in high value urban environments.

***Investigators*:** Jon Sweeney, Cory Hughes, Tarryn Goble, Joe Meating, Ed Czerwinski, Joel Goodwin, Simon Pawlowski, N. Kirk Hillier, Rob Johns

Objective: 1. Determine the effect of dosage and frequency of treatment on the efficacy of TreeAzin for protecting foliage from the beech leaf-mining weevil and preventing tree mortality.

Funding:

Financial	NS DNR $2K, OMNRF $2K
Total	$ 4K

2017/02-2017-122

Predation rates in endemic and outbreak forest tent caterpillar populations

***Investigators*:** Emma Despland, Chris JK MacQuarrie

Objective:

1. Quantify predation rates on early instar caterpillars in endemic and outbreaking populations.
2. Compare forest tent caterpillar mortality between regenerating and mature stands.
3. Assess relative importance of epigeic (ground-based, e.g. ants, spiders) and flying (e.g. beetles, stinkbugs, parasitoids, birds) natural enemies

Funding:

Financial	AB AAF $2K, SOPFIM $2K, SK MOE $2K, NSERC $6K
Total	$ 12K

Emerald Ash Borer

2017/05-2017-125

Validation of emerald ash borer branch sampling and estimation of 2 year life cycle in northern populations

Investigators: Chris JK MacQuarrie, Krista Ryall.

Objectives:
1. Assess and validate the EAB branch sampling method in a new infestation.
2. Quantify the frequency of the two year life cycle in emerald ash borer.

Funding:

Financial	OMNRF $2.5K, MB CWS $4K, SK MOE $1K,
Total	$ 7.5K

2008/06-2017-707

Investigations into the Chemical Ecology of the Emerald Ash Borer (EAB) *Agrilus planipennis*: development of tools for sampling and management of EAB.

Investigators: Krista L. Ryall, Peter Silk

Objectives: To develop and improve tools for early detection and management of the emerald ash borer, by chemically identifying, synthesizing, and field testing semiochemicals that elicit sex-based mate location. First, we intend to further optimize the synthetic lactone analogs for use as a lure in early detection surveys; our research has shown that traps baited with saturated analog capture similar numbers of EAB as compared to traps baited with the lactone pheromone. Newly discovered fluorinated analogs also show high antennal activity and potential to attract adults in trapping experiments. These analogs would represent a less-expensive and easier to synthesize option for surveys, and greatly increase our understanding of the pheromone ecology of this invasive pest. Second, we propose to determine the feasibility of slowing the spread of EAB through the application of semiochemical-based strategies, such as a ‘push-pull’ strategy. Non-host volatiles are being tested for their efficacy in disrupting orientation of EAB to traps and ash trees. Ultimately, we want to integrate these tools with others (e.g., stem-injected insecticides) in the context of an IPM strategy.

Funding:

Financial	MB-CWS $4K, SK-MOE $2K, USDA-FS $5.2K
Total	$ 11.2K

2017/06-2017-126

Modelling the development of emerald ash borer in its expanding range

Investigators: C J K MacQuarrie, D Inward, N Straw, S Smith, K Dearborn

Objectives: 1. Determine developmental rates for EAB and BBB eggs, larvae and pupae under controlled rearing condition
2. Develop predictive models for EAB population development for regions outside the invaded range, and for BBB in potentially invaded areas in the UK

Funding:

Financial	OMNRF $2K, MB CWS $6K, SK MOE $1K, USDA FS $1.3K, DEFRA $38K, UofT $15K
Total	$ 63.3K

2017/09-2017-129

Individual-based approach to monitor potentially invasive Europena *Agrilus*

Investigators: Marc Rhainds, Troy Kimoto

Objectives: Develop pro-active monitoring protocols in the event of European *Agrilus* introduction and the associated potential invasion risk they might pose..

Funding:

Financial	OMNRF $4K
Total	$ 4K

2015/05-2017-903

Release of exotic parasitoids for biological control of the emerald ash borer in Canada

Investigators: Krista Ryall, Chris J.K. MacQuarrie, Amanda Roe

Objectives:

- Parasitoid releases in 2017 will be completed at release sites already established.
- New release sites will be established to increase the geographic distribution of the parasitoids within the infested region in Canada.
- Release sites will be selected to evaluate the effect of landscape type on successful establishment of the parasitoids, including forested, agricultural, and urban landscapes.

- Release data will be uploaded into the mapBioControl.org database.
- Post-release assessment of establishment for the original 2013-2016 release sites will continue in the fall of 2017, as required. Assessments will involve tree removals, rearing and dissections.
- Releases of *S. galinae* will be conducted upon approval by CFIA
- The development of rearing technology and techniques for *T. planipennisi* will continue, in collaboration with the Insect Production and Quarantine Laboratory at the Great Lakes Forestry Centre, Sault Ste. Marie, Ontario to provide a source for parasitoids for releases in Canada.

Funding:

Financial	OMNRF $11K, MB CWS $1K, SK MOE $1K, USDA FS $2K, SPA ISC $1.8K
Total	$ 16.8K

Early Intervention Strategies To Suppress A Spruce Budworm Outbreak

2014/05-2017-100

Early Intervention to Suppress a Spruce Budworm Outbreak

***Principal Agency*:** Forest Protection Limited

Objective: Implementing an EIS to cope with the impending SBW outbreak in NB. The ultimate goal is to prevent or limit the spread of SBW in NB and mitigate the negative socio-economic effects from a reduced forest sector within the Province.

Components

2.2.1: Dynamics and management of rising spruce budworm outbreaks

***Investigators*:** Régnière J, Delisle J, Martel V, Seehausen ML, Boulanger Y, Béchard A, Labrecque A, Trudeau S, St-Amant R.

Objective: Addresses questions regarding what SBW density to initiate an EIS, what products may be most effective, and what are the consequences of pesticide treatments (Btk, tebufenozide and pheromones) on natural enemy populations attacking SBW in subsequent years.

Funding:

Financial	AIF/FPL $161.9.1K, C/O $6.2K
Total	$168.1K

2.2.2: Landscape level impacts of EIS on SBW, other herbivores, & associated natural enemies

***Investigators*:** V. Martel & R. Johns

Objective: Test the efficacy of pesticide use and evaluate possible unintended impacts on very low density SBW and its parasitoid complex. It will include detailed assessment of proposed EIS and other pesticide including pheromone trials of increasing sizes from 2014 to 2017..

Funding:

Financial	AIF/FPL $355.6K, C/O $13.5K
Total	$369.1K

2.2.3: Innovative DNA-based diagnostic for SBW, its natural enemies and other conifer-feeding species

***Investigators*:** M. Alex Smith, Eldon Eveleigh

Objective: Develop novel genomics tools to quantify and identify parasitism of SBW larvae and pupae. This will permit identification of parasitoids in larvae killed by pesticide treatments and explicitly evaluate the impact pesticide treatment has on the natural enemy community of SBW.

Funding:

Financial	AIF/FPL $189K, C/O $7.2K
Total	$196.2K

2.2.4: Aerial application of pesticides including pheromones

***Investigators*:** Peter Amirault and Luke Amos-Binks

Objective: Begin an EIS SBW trial on 5,000 ha in northern NB in 2014 and conduct Bt and pheromone control trials on low populations in QC. Continued trials using increasing sized blocks of pesticide as outbreak progresses.

Funding:

Financial	AIF/FPL $3845.7K, C/O $146.5K
Total	$3992.2K

2.2.5: Epicenter formation and migratory behavior of adult SBW: Implications for the rise and spread of outbreaks and EIS

***Investigators*:** Deepa Pureswaran, Rob Johns

Objective: Study migratory behavior from ‘epicenters’ (QC) & associated formation of epicenters (NB)

Determine methods to differentiate resident & migrant SBW moths, & contribution of migrant moths to outbreaks

Funding:

Financial	AIF/FPL $361.5K, C/O 13.8K
Total	$375.3K

2.2.6: Spruce Budworm Mating Disruption Trials with DISRUPT BIO-FLAKE®

***Investigators*:** Peter Silk, Wayne MacKinnon, Eldon Eveleigh

Objective: Develop and register a more potent 4-component sex pheromone blend for use in mating disruption of SBW and evaluate whether pheromones promote dispersal of female moths.

Funding:

Financial	AIF/FPL $253.2K, C/O $9.7K
Total	$262.9K

2.2.7: Evaluating the inoculation of seedlings with endophytic fungi to improve tolerance of conifers to spruce budworm

***Investigators*:** Dan Quiring, Greg Adams, Sara Fraser, David Miller, Andrew McCartney

Objective: Inoculate spruce seedlings with insect toxin-producing endophytic fungi
First application in forest trees

Funding:

Financial	AIF/FPL $172.1K, C/O $6.5K
Total	$178.6K

2.2.8: Early intervention strategies against spruce budworm: modeling & DSS analyses

***Principal Investigator*:** David A. MacLean, Jeremy Gullison, Chris Hennigar, Bo Zhang

Objective: Develop SBW population-derived defoliation scenarios for alternative EIS strategies, integrate insecticide efficacy models, develop and test models of SBW ‘hot-spot’ protection decisions and optimum operational blocking, and evaluate effects of alternative SBW control strategies in NB timber supply, cost-benefits, and economy-wide impact. Monitor with LiDAR, hyperspectral scanner, satellite imagery

Funding:

Financial	AIF/FPL $242.9K, C/O $9.3K	
Total	$252.2K	
Total	$5794.6K	(AIF, FPL , NRCaN, C/O)

2015/11-2017-910

Testing new approaches for detecting and locating early increasing populations of the Spruce Budworm for implementing an Early Intervention Strategy

***Investigators*:** Christian Hébert, Jean-Michel Béland and Sébastien Bélanger

Objectives:

1) test new versions of the Luminoc® trap for sampling the SBW and detect early population increases and moth immigration;
2) identify areas with highest probabilities to show SBW population increases to optimally deploy the new trap.

Funding:

Financial	NL DNR $3K, NS DNR $6K, SOPFIM $7K , iFor $7K
Total	$ 23K

2015/13-2017-912

Numerical descriptors of spruce budworm immigration on the west coast of Newfoundland

***Investigators*:** Marc Rhainds, Dan Lavigne, Troy Rideout

Objectives: Improve the monitoring of budworm migrations to facilitate early detection

Funding:

Financial	NL DNR $12K, SOPFIM $2K
Total	$ 14K

2015/08-2017-907

Measuring the active space of a spruce budworm pheromone trap: Bridging the gap between chemical ecology and estimates of absolute population density.

***Investigators*:** C J K MacQuarrie, J D Allison, M C Bouwer, R Johns, D Pureswaran

Objectives:

1) Develop and refine methods for trapping array study and walking bioassay
2) Determine the baseline relationship between absolute density of SBW and pheromone-baited trap catches
3) Examine variation in (2) among different stand types
4) Use relationships developed in (2 & 3) to provide quantitative estimates of SBW throughout eastern Canada from trap-catch data collected by provincial agencies and studies in ACOA 2.2.5

Funding:

Financial	FPL $3K, AB AAF $1K, NS DNR $2K, OMNRF $1K, SK MOE $1K, USDA FS $2.6K
Total	$ 10.6K

2015/09-2017-908

Using landscape level forest management and biotic interaction to reduce the intensity of spruce budworm outbreaks

***Investigators*:** Dan Kneeshaw, Louis De Grandpré, Deepa Pureswara, Dave MacLean, Brian Sturtevant, Laura Kenefic, Patrick James, Steven Kembel

Objectives:

1) Evaluate interactions between topography, climate and forest management on SBW outbreaks.
2) Identify landscape scale risk factors to use in designing pest management strategies.
3) Identify factors influencing plant- and budworm-associated microbial communities.
4) Identify microbial taxa associated with budworm vigour

Funding:

Financial	AB AAF $2K, NS DNR $6K, OMNRF $10K, SOPFIM $5K
Total	$ 23K

2017/03-2017-123

Understanding overwintering survival as a component of pest management strategies for spruce budworm

***Investigators*:** Joe Bowden, Eric Moise , Michael Stastny

Objectives:

1) To identify landscape- and stand-level variables that influence overwintering survival in eastern spruce budworm.
2) To use these variables to refine density-defoliation relationships.
3) To integrate our findings into risk management models via consultation with pest management groups.

Funding:

Financial	NL DNR $18K. SK MOE $2K
Total	$ 20K

2009/08-2017-759A

Optimization of SBW pheromone application for mating disruption

***Investigators*:** C. M. Riley

Objectives: To evaluate one or more aerial application practices and use strategies for reducing operational costs and optimizing the effectiveness of sprayable pheromone formulations for the management of spruce budworm populations..

Funding:

Financial	NL DNR $10K
Total	$ 10K

2012/09-2017-806

Comparisons of Btk aerial spraying strategies against the eastern spruce budworm based on protection timing and intensity during a complete outbreak episode

***Investigators*:** Alain Dupont, Éric Bauce, Alvaro Fuentealba Morales, Christian Hébert and Richard Berthiaume

Objectives: To assess / compare forest protection scenarios (strategies) regarding wood losses (growth, mortality, and forest yield), wood quality, carbon balance and aerial spray investments. Using a benefit / cost analysis, the project will lead us to determine the more efficient strategy or complementary strategies relevant to different field situations and / or protection objectives (wood production areas, wildlife habitats, seed orchards, thinned stands, plantations, forest aesthetic values, etc).

Compare with early intervention strategy developed in New Brunswick regarding results, costs and operational feasibility.

Funding:

Financial	FPL $10K, NLDNR $5K, C/O $0.5K, USDA-FS $2K, Canada Economic Development $200K
Total	$ 217.5K

2016/06-2017-114

Validation of a spruce budworm phenology model across environmental and genetic gradients: applications for budworm control and climate change predictions

Investigators: Jean-Noël Candau

Objectives: Quantify the variability of spruce budworm development rates across its distribution.
Relate the variability in development rates to environmental and/or genetic gradients.
Validate Régnière phenology model across budworm distribution

Funding:

Financial	AB AAF $5K, NS DNR $5K, OMNRF $5K, SOPFIM $5K, SK MOE $2K
Total	$ 22K

2017/04-2017-124

Testing the efficacy of Trichogramma minutum in the context of an 'Early-intervention Strategy' against the spruce budworm using different release methods

Investigators: Véronique Martel, Simon Trudeau, Rob Johns, Emily Owens, Sandy Smith, Guy Boivin

Objectives:

1. Determine whether the use of *T. minutum* significantly increases egg mortality in low to moderate density spruce budworm populations.
2. Determine the impact of *T. minutum* releases on the populations' growth (L2-L2).
3. Compare the efficacy of both release methods (cards vs. drones).

Funding:

Financial	OMNRF $2K
Total	$ 2K

2017/07-2017-127

Optimization of trapping parameters for spruce budworm sampling

Investigators: Chris JK MacQuarrie, Jeremy Allison, Drew Carleton, Rob Johns

Objectives:

1. Quantify trap interference for SBW lures in bucket-style traps

2. Determine release rates for SBW lures
3. Quantify the effect of lure age on trap catch
4. Determine a sampling design for year-over-year trap calibration

Funding:

Financial	AB AAF $1K, NL DNR $2K, NS DNR $1K, OMNRF $1K
Total	$ 5K

Silviculture Practices

2016/05-2017-112

The impact of silviculture practices on the development of a recalcitrant understory layer: Are we creating a problem for future rotations?

***Investigators*:** Kierann Santala, Isabelle Aubin, Michael Hoepting

Objectives:

1. Do some silviculture practices negatively impact tree growth in the long-term by favoring the development of a recalcitrant understory layer?
2. Are some practices more susceptible to the development of a recalcitrant layer?
3. What are the mechanisms for the formation of such a layer and can we prevent it while still achieving enhanced crop tree production?
4. Are there some sites that are more susceptible to layer formation (e.g. productive sites)?

Funding:

Financial	OMNRF $8K
Total	$ 8K

2016/01-2017-101

Using the functional traits of soil fungi to improve post-disturbance pine regeneration.

***Investigators*:** Nadir Erbilgin and Jonathan Cale

Objectives:

1. To test if soil fungal communities can be remediated to improve post-disturbance forest recovery
2. To characterize how disturbances affect the functional roles soil fungi play in forest regeneration
3. To identify the components of fungal communities which promote lodgepole pine regeneration following disturbances
4. To determine how soil fungal communities are affected by an array of individual and cumulative forest disturbances

.

Funding:

Financial	AB AAF $4K, OMNRF $2K, SK MOE $2K, NERC-Strategic Partnership Grant $198.4K
Total	$ 206.4K

Beetle Population Detection and Suppression

2014/02-2017-830

Improving monitoring tools to detect mountain pine beetle at low and high densities in novel habitats

Investigators: Nadir Erbilgin, Jennifer Klutsch

Objectives: Develop two types of lures to monitor mountain pine beetle activities at low and high density beetle populations in novel habitats.

Funding:

Financial	AB AAF $4K, OMNRF $5K, MB CWS $4K, SK MOE $1K, fRI Research $17K
Total	$ 31K

2010/07-2017-782

Mountain Pine Beetle Cold Tolerance and Seasonality: Implications for Population Dynamics and Range Expansion in Canada

Investigators: K. Bleiker, G. Smith

Objectives:

1) Determine lethal cold temperature thresholds for different life stages of MPB;
2) Examine the potential for host tree species (pure jack versus lodgepole pine) to affect the cold tolerance of MPB larvae *(added in 2015)*;
3 a) Define conditions (i.e., cooling rate, diurnal temperature variation) required for cold acclimation in the fall and if de-acclimation in the spring is reversible;
3 b) Determine if a "chilling period" is required for the acquisition of maximum cold hardiness and the length of time that maximum cold hardiness can be maintained *(added in 2015)*;
4) Determine mortality or sub-lethal fitness costs associated with cold events that vary in severity, duration and number of exposures;
5) Quantify MPB winter survival in the field and associated under-bark temperature regimes and tree moisture;
6) Identify critical factor(s) regulating MPB development and phenology that will determine the beetle's ability to maintain an adaptive seasonality in new habitats and climates

Funding:

Financial	AB AAF $2K, OMNRF $8K, MB CWS $3K, SK MOE $2K, Foothills Research Institute $ $20K
Total	$ 35K

2016/07-2017-117

Simulating MPB invasive spread control in Saskatchewan using SpaDES

***Investigators*:** Alex M. Chubaty, Eliot J. B. McIntire, Barry J. Cooke

Objectives:
1) How do stand dynamics influence outbreak potential?
2) Are there critical thresholds of pine volume connectivity that can be defined to guide operational management of MPB infestations in novel habitats?
3) To what extent should pest managers target well-established hindflank sources of long-range migrants versus frontal populations that are in the process of early establishment?.

Funding:

Financial	AB AAF $8K, OMNRF $7K, MB CWS $2K, SK MOE $8K
Total	$ 25K

2017/01-2017-121

Formulations of emamectin benzoate (TREE-äge® and TREE-äge® G4) and propiconazole (Propizol®) for protecting Engelmann spruce from mortality attributed to spruce beetle and associated fungi

***Investigators*:** Christopher J. Fettig, Darren C. Blackford, Donald M. Grosman, A. Steven Munson

Objectives: To determine the efficacy of TREE-äge® and TREE-äge® G4 each alone or combined with Propizol® for protecting Engelmann spruce from mortality attributed to spruce beetle and associated fungi

Funding:

Financial	BC MFLNR $9.5K, Arborjet Inc $4K, USDA FS $10K
Total	$ 23.5K

2017/08-2017-128

Temperature Regulation of Spruce Beetle’s Life Cycle and its Potential Role in Outbreaks

***Investigators*:** K. Bleiker, V. Rezendes, D. Huber, J. Robert

Objectives:

1. Elucidate temperature conditions influencing one- and two-year life cycle in spruce beetles (i.e., non-diapausing versus diapausing larvae);

2. Identify the larval instar(s) sensitive to the thermal conditions that induce diapause; and
3. Analyze the association between temperature and occurrence of spruce beetle outbreaks in western Canada

Funding:

Financial	AB AAF $8K
Total	$ 8K

2016/02-2017-102

Effect of trap height, trap color and trap lure on efficacy of detecting species of bark- and wood boring beetles (Cerambycidae Buprestidae Scolytinae).

***Investigators*:** Jon Sweeney, Peter Silk, Peter Mayo, Gaetan Leclair, Cory Hughes, Kate Van Rooyen, Jerzy M. Gutowski, Tomasz Mokrzycki, Dan Miller, Meng Qingfan, Li Yan, Joe Francese

Objectives:

1. Determine the efficacy of green, purple, and black traps, placed both in the tree canopy and the understory, for detecting species of bark and wood boring beetles in the Cerambycidae, Buprestidae, and Scolytinae.
2. Determine the combination of trap colors, trap heights, and lures that results in the greatest number of target species detected per sampling effort.
3. Improve early detection and surveillance of exotic and potentially invasive bark and wood boring insects in North America.

Funding:

Financial	OMNRF $7K, USDA FS $2.6K, C/O $2.5
Total	$ 12.1K

2017/18 Total Funding $6609.5K