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Abstract

The onset of a new jack pine budworm outbreak in NW and NE Ontario in 2004 provided an opportunity to investigate the presence and prevalence of the microsporidium *Nosema fumiferanae*. This parasite is the most ubiquitous pathogen that has been implicated as a regulatory factor in spruce budworm populations but requires years of high-density populations to reach high prevalence levels. Because outbreak densities of jack pine budworm larvae typically persist for only 2-4 years, *Nosema* may not be able to build up in those populations unless transmission is much more efficient than in the spruce budworm-fir-spruce system. We examined presence of *Nosema* in ~15,000 second-instar larvae collected as part of annual operational surveys of overwintering larvae from 2004-2009.

In the NE region, the infestation stayed relatively small (maximum of 72,000 ha with severe defoliation); mean larval densities in the various outbreak areas did not exceed 10 larvae per branch; and *Nosema* was present at relatively high levels across the region (with the exception of the Timmins district), with average prevalence increasing from 1.6% in 2004 to around 25 % in 2005-2007. In the NW region, the infestation erupted to ~720,000 ha of severe defoliation during its peak (2006); mean larval densities in the various outbreak areas reached highs of 60 larvae per branch; but regional averages of *Nosema* prevalence remained below 15%. The exception was the Red Lake district where prevalence reached a high of 31% despite mean larval densities of less than 10 larvae per branch. *Nosema* prevalence reached highs of 83% and 96% in individual samples sites in the NE and NW, respectively.

Rapid build up of *Nosema* in jack pine budworm outbreak populations was evident from:i) changes in regional- and district- wide prevalence as the outbreak progressed,

ii) an increase in the proportion of monitored sites that was infected,

iii) an average 10- to 13-fold increase in % infected larvae from one year to the next in sample plots that were monitored for two consecutive years, and

iv) the relationship between density of overwintering larvae and their infected proportion, showing low prevalence in early outbreak years and highest levels in late outbreak years.

We postulate that transmission of *Nosema* in jack pine budworm is more efficient relative to spruce budworm because the life cycle of this species presents two opportunities for horizontal transmission: during the pollen cone-feeding stage of early instars and during the shoot-feeding stage of late instars, while larva-to-larva transmission in spruce budworm populations is limited to the shoot-feeding stage of late instars. Our data suggest that the role of *Nosema* in jack pine budworm outbreak dynamics should be considered and investigated in more detail.

Résumé

Le début d’une nouvelle infestation de tordeuse de pin gris dans le nord-ouest et le nord-est de l’Ontario en 2004 a constitué une occasion d’explorer la présence et la prévalence de la microsporidie *Nosema fumiferanae*. Ce parasite est le pathogène le plus omniprésent comme facteur de régulation des populations de tordeuse des bourgeons de l’épinette, mais il faut une forte densité des populations durant des années avant d’atteindre des niveaux de prévalence élevés. Comme l’intensité des infestations de larves de tordeuse de pin gris persiste généralement de 2 à 4 ans seulement, il se pourrait que le *Nosema* ne s’accumule pas dans ces populations à moins d’une transmission beaucoup plus efficace que ce qu’on observe dans le système tordeuse des bourgeons de l’épinette-sapin-épinette. Nous avons examiné la présence du *Nosema* dans ~15 000 larves au deuxième instar recueillies dans le cadre des relevés opérationnels annuels de larves hivernantes de 2004 à 2009.

Dans le nord-est, l’infestation est demeurée relativement petite (maximum de 72 000 ha touchés par une défoliation intense), les densités larvaires moyennes dans les différents secteurs touchés par une infestation n’ont pas excédé 10 larves par branche, et les niveaux de *Nosema* étaient relativement élevés dans la région (à l’exception du district de Timmins), la prévalence moyenne augmentant pour passer de 1,6 % en 2004 à environ 25 % en 2005-2007. Dans le nord-ouest, l’infestation a provoqué une défoliation intense sur ~720 000 ha à son point culminant (2006) et les densités larvaires moyennes dans les divers secteurs infestés ont atteint des sommets de 60 larves par branche, alors que les moyennes régionales de prévalence du *Nosema* sont demeurées sous 15 %. L’exception était le district de Red Lake, où la prévalence a atteint un sommet de 31 % malgré des densités larvaires moyennes de moins de 10 larves par branche. La prévalence du *Nosema* a atteint des maximums de 83 % et de 96 % dans les sites d’échantillon individuels dans le nord-est et le nord-ouest respectivement.

Plusieurs preuves d’une accumulation rapide de *Nosema* dans les infestations de tordeuse de pin gris ont été observées:

i) changements dans la prévalence à l’échelle de la région et du district à mesure que l’infestation progressait,
ii) une augmentation de la proportion de sites surveillés qui étaient infectés,
iii) une augmentation moyenne de 10 à 13 fois du pourcentage de larves infectées d’une année à l’autre dans les parcelles d’échantillon surveillés durant deux années consécutives;
iv) le rapport entre la densité des larves hivernantes et leur proportion infectée, qui montrait une faible prévalence dans les premières années d’infestation et les niveaux les plus élevés dans les dernières années d’infestation.

Nous posons comme postulat que la transmission du *Nosema* dans la tordeuse de pin gris est plus efficace que pour la tordeuse des bourgeons de l’épinette parce que le cycle de vie de cette espèce présente deux possibilités de transmission horizontale : durant les premiers stades larvaires où elle se nourrit de cônes à pollen et durant les stades larvaires plus tardifs où elle se nourrit de pousses, tandis que la transmission de larve à larve dans les populations de tordeuse des bourgeons de l’épinette se limite aux stades larvaires plus tardifs, lorsque la larve se nourrit de pousses. Nos données semblent indiquer que le rôle du *Nosema* dans la dynamique des infestations de tordeuse de pin gris vaut la peine d’être exploré plus en détail.

Introduction

The jack pine budworm and eastern spruce budworm share a nearly identical complex of natural enemy regulators (Nealis 1991; 1995). *Nosema fumiferanae* is the most ubiquitous pathogen in spruce budworm populations, and has been implicated as a regulatory factor (Royama 1984; Régnière 1984). A similar *Nosema* was described from jack pine budworm (Thomson 1959), which could be the same species (Kyei-Poku et al., 2008). It was found in jack pine budworm populations during the 1984-87 outbreak in Ontario, but its incidence was always low (<5%) (Nealis 1995). Microsporidian infections are assumed to play a diminished role in jack pine budworm outbreak dynamics as high larval densities typically persist for only 2-4 years, as compared to often more than 10 years for spruce budworm (Nealis1995).

Lack of specific surveys to date precludes a definitive conclusion that *Nosema* does not reach high prevalence in jack pine budworm populations. It is possible that *Nosema* transmission is much more efficient in the jack pine budworm system than in the spruce-fir-spruce budworm system, allowing the pathogen to reach high prevalence in fewer years of high-density populations. The onset of a new jack pine budworm outbreak in NW and NE Ontario provided an opportunity to conduct a more thorough assessment of how prevalent *Nosema* really is. We used larvae from operational annual surveys of overwintering second-instars (L2) to quantitatively assess prevalence of *Nosema* during the course of the outbreak.

Why do we want to know if *Nosema* is a prevalent pathogen? Because if it is, disease prevalence may have to be considered as a factor in the development of jack pine budworm Decision Support Systems (*e.g.*, inclusion of prevalence in overwintering L2s might improve forecasts of infestation severity), and possibly in the planning of foliage protection or population suppression activities (*e.g.*, remove heavily diseased populations from spray plans).

Methods

Overwintering second-instar larvae were obtained from washed branch samples as part of the 2004, 2005, 2006, 2007, 2008 and 2009 operational L2 surveys conducted in NW and NE Ontario. In each year, OMNR and BioForest Technologies personnel collected 10 branches from each sample site. Larvae were removed by washing in dilute sodium hydroxide, counted and preserved in 70% ethanol. From each location a subsample of up to 30 larvae was collected, using only those locations with populations that exceeded 5 larvae per branch. A sample of 30 larvae from each location was smeared individually on a 30-well microscope slide stained with Naphthalene Black dye and microscopically examined (using bright-field optics) for presence of spores. Infection intensity was recorded by scoring relative abundance of spores in 20 fields of view (~20% of total smear).

Results

An annual survey of overwintering larvae was instigated in response to a small pocket of severe defoliation observed in 2004 in the Northeast (NE) region of Ontario. Severe defoliation in the Northwest region (NW) was first recorded in 2005. The areas of severe defoliation mapped through aerial surveys reflect drastically different outbreak patterns in the two regions (Table 1). In the NE, the infestation stayed relatively small with total severe defoliation never exceeding 72,000 ha, while in the NW the outbreak exploded, reaching a maximum of more than 700,000 ha in 2006 (Fig. 1). In both regions, the outbreak varied between districts from year to year (Table 1). In the NW, the outbreak moved from the Fort Frances district in 2005-2006 to the Kenora and Dryden districts in 2006-2007 and the Sioux Lookout and Red Lake districts in 2007-2009. In the NE, the main outbreak shifted from the Sudbury district in 2006-2007 to the Timmins and Parry Sound districts in 2007-2010.

A total of at least 1500 sites were sampled between 2004 and 2009, and about 15,000 larvae were smeared and rated for presence of *Nosema* spores. Summary statistics (Table 2) show that larval densities were generally lower in the NE than in the NW, while *Nosema* prevalence reached higher levels in the NE. Densities averaged across all sites ranged from 0.4-6 L2/branch in the NE as compared to 2-43 in the NW. Maximum larval densities observed in any given sample site were 124 L2/branch or less in the NE as compared to 232 in the NW. In the NE, infection of those larvae increased from 1.6% in 2004 to around 25% in 2005-2007, while *Nosema* prevalence remained at much lower levels (below 13%) throughout most of the NW (with the exception of the 2008 and 2009 infestation in the Red Lake and Sioux Lookout districts). *Nosema* prevalence reached highs of 83.3% and 96.6% in individual samples sites in the NE and NW, respectively. The same patterns are reflected in the 75% quartiles, ranges and maximum observed values presented in the box plots of Fig. 2.

Densities varied among the districts within each region (Fig. 3 A and B), roughly reflecting spatial variation in the infestation from year to year. In the NE region, relatively small infestations persisted in the Sault Ste. Marie (SSM) district throughout the observation period with average densities peaking at 9.5 L2/branch in 2006. In the Sudbury (SUD) district, the infested area increased 50-fold from 2004 to 2008 (Table 1) but average larval densities remained below 7.4 larvae/branch in any given year. Larval densities reached a similar high in the Timmins district (TIM, Gogama) in 2007 and 2008. In the Chapleau district, average populations remained below 1.0 larva per branch (data not shown). Samples collected in the North Bay, Parry Sound and Pembroke districts were too few in number to allow meaningful comparisons. In the NW region, average larval densities reached the highest level in the Fort Frances (FF) district in 2005 (61.2 larvae/branch). Populations peaked later in the Dryden (DR) and Kenora (KE) districts, reaching highs of 23.6 and 28.6 larvae/branch in 2006, respectively. Populations remained at much lower levels in the Sioux Lookout (SL, data not shown) and Red Lake (RL) districts, with maximum average densities of 4.8 (2006) and 7.1 (2008) larvae/branch, respectively.

Nosema was present at relatively high levels across the NE region, with the exception of the TIM district (Fig. 3C). Most of the sites monitored for *Nosema* in the SSM and SUD districts were infected, with average prevalence reaching highs of 23.8% (2006) in SSM and 31.1% (2007) in SUD. The interquartile range (Q1-Q3) of % infection in those districts ranged between 10 and 60%. In the TIM district, average prevalence did not exceed 3.5% (2008). In the NW region, *Nosema* was present in 26-33% of the sample sites at the start of the outbreak (2004-2005), and became much more widespread as the outbreak progressed with 96-98% of the monitored sites being infected in 2008-2009 (Table 2). Prevalence increased rapidly in each of the NW districts, but overall infection levels remained much lower than in the NE (Fig. 3D). Average prevalence reached a high of 10.9% in FF (2006), 12.2 % in DR (2007), and 11.3 % in KE (2007), with the interquartile range generally remaining below 20%. Exceptions were the SL and RL districts: average prevalence reached levels that were comparable to those in the NE districts, reaching highs of 15.8 and 31.4 %, respectively.

Rapid build up of *Nosema* in outbreak populations is also suggested by the relationship between density of overwintering larvae and their proportion that was infected (Fig. 4). Generally, *Nosema* prevalence was low in early outbreak years even in sites where overwintering densities were high (100-250 larvae/branch), and reached its highest levels in late outbreak years when larval densities were generally low (<50 larvae/branch). Such patterns were evident when data were pooled for the entire NW region (Fig. 4A), as well as for the DR (not shown), FF and KE districts (Fig. 4B,C). Although it is hazardous to make inferences about temporal patterns from spatially diverse data, the patterns suggest a build up of *Nosema* prevalence in summer populations resulting from the initial high-density L2 populations (survey t = 0), which becomes apparent in overwintering larvae of the next generation (survey t = 1). As the outbreak progresses, observed larval densities decrease as a result of host feedback (reduced production of pollen cones in the year following heavy defoliation), while the disease prevalence increases as a result of build up in the high larval populations in the preceding summer. The most recent incursion of the NW infestation into the RL district (Fig. 4D) does not fit this pattern at all: *Nosema* prevalence reached high levels early in the outbreak (2007, 2008), despite the fact that larval populations never exceeded 50 larvae/branch. In the NE region (Fig. 4E,F) *Nosema* prevalence was high in early outbreak years but still exhibited a tendency to increase in later outbreak years. Further analysis is underway to examine relationships between disease prevalence, larval density, defoliation and flowering conditions in the sampled stands.

Discussion

Our data indicate that *Nosema* builds up rapidly in outbreak populations of the jack pine budworm. Rapid build up is inferred from changes in regional- and district- wide prevalence as the outbreak progressed (Table 2, Fig. 2,3,4), as well as from the increase in the proportion of monitored sites that was infected (Table 2). A third line of evidence comes from a small subset of sites that were sampled for at least two consecutive years (Table 3). *Nosema* prevalence increased in 83% of those sites with a 10- or 13-fold increase on average (in NE and NW regions respectively) from one year to the next.

Such rapid build up differs from the generally gradual increase in infection levels reported for spruce budworm outbreaks (Wilson 1977, Thomson 1960) and was unexpected, considering the short duration of jack pine budworm outbreaks. We postulate that transmission of *Nosema* in jack pine budworm is more efficient because the life cycle of this species presents two opportunities for horizontal transmission: during the pollen cone-feeding stage of early instars and during the shoot-feeding stage of late instars. Jack pine budworm larvae feed gregariously on developing pollen cones during early larval stages (~instar 2 - 4), as opposed to the individual needle-mining and bud-boring feeding behaviours of early spruce budworm instars. The pollen cones, often harbouring tens of larvae per cone, provide a closed environment for efficient horizontal transmission early in larval development, so that *Nosema* can go through many replication cycles and build up high spore burdens in infected larvae. This is further aided by the longer larval development time (7 instars). Higher spore burdens in late instars means a higher load of spores in the environment, which should result in more efficient horizontal transmission during late larval stages, as well as higher infection intensities in adult moths. In spruce budworm populations, larva-to-larva transmission is limited to the shoot-feeding stage of late instars (as shown in Fig. 5, which was included from an earlier study for comparative purpose).

We undertook an intensive field study in 2007 to test these hypotheses by tracking *Nosema* prevalence and spore production from the onset of larval emergence to pupation in six selected populations in the Kenora area. Though each population had a low level of *Nosema* infection in overwintering larvae, prevalence did not increase in any of the study sites, for unknown reasons. In 2008, we used a different approach by collecting pollen cones soon after larval emergence from clusters of infested trees in each of three locations. The cones were placed on water in rearing boxes and maintained at 21 ^{o}C and a 16:8 L:D photo period. Initial prevalence was estimated by dissecting a subsample of cones right after collection when most larvae were in second instar. Final prevalence was estimated by sacrificing larvae (mostly fourth and fifth instars) right after they left the pollen cones in search of elongated shoots. *Nosema* prevalence increased 15-fold in the site with highest larval densities (Gogama), and doubled in one of the lower density sites (Sudbury), but not in the other (Nairn Centre) despite a much higher density of infected larvae in that site. We are planning laboratory experiments to investigate this in more detail in the near future.

Rapid build up of *Nosema* suggests that the pathogen could play a role in jack pine budworm population cycles, for example by exacerbating negative effects of reduced pollen cone density on early-instar survival (Nealis et al. 2003, van Frankenhuyzen et al. 2007) or on fecundity (Batzer and Jennings 1980). Furthermore, higher infection intensities in adult moths are likely to increase negative effects on net reproductive rates because the severity of sublethal debilitating effects is directly linked to spore loads (Bauer and Nordin 1989, van Frankenhuyzen et al. 2007). This means that the pollen-cone feeding behaviour of jack pine budworm sets the stage for *Nosema* infection to possibly have greater population impacts than is the case for spruce budworm.

Our results suggest that it might be useful to monitor *Nosema* prevalence in overwintering larval populations during an outbreak as an indicator of the extent to which the outbreak will develop. It is noteworthy that prevalence was low in districts where the infestation erupted into a large-scale outbreak (FF, KE, DR), and was relatively high in districts where the outbreak remained confined (SSM, SUD). However, the relationship was not conclusive because some infestations did not fit this pattern: the outbreak in TIM stayed small despite a low prevalence of *Nosema* while the outbreak in RL reached a considerable size even though *Nosema* prevalence was relatively high. Obviously the relationship between *Nosema* prevalence and changes in larval density is complex and a more detailed analysis of the survey data may shed further light on factors (flowering condition, previous defoliation, site characteristics) that affect that relationship.

Lack of site fidelity is also a key issue, as relationships between year-to-year changes in variables have to be inferred from averages calculated for broad geographic areas, like the districts used in operational forest health surveys. Even though the district designations happen to correspond roughly to coarse outbreak patterns (*e.g.* FF includes portion of outbreak that erupted first; SL and RL include portions of outbreak that erupted last; TIM includes discrete Gogama outbreak that flared in 2008), a more detailed delineation of sample sites by discrete outbreak patterns is likely to improve the analysis.

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Table 1

Hectares of moderate-to-severe defoliation caused by jack pine budworm in northeastern (NE) and northwestern (NW) Ontario between 2004 and 2010 by District

Region	District	2004	2005	2006	2007	2008	2009	2010
NW	Fort Frances	0	85,328	355,134	41,020	0	0	0
	Kenora	0	1,134	248,843	227,210	12,292	0	0
	Dryden	0	1,983	116,195	178,881	0	0	40
	Sioux Lookout	0	0	0	9,065	7,926	7,350	0
	Red Lake	0	0	0	6,783	115,041	147,204	204
	Thunder Bay	0	0	0	892	0	0	0
	Total	0	88,445	720,172	463,851	135,259	154,821	244
NE	Sudbury	851	2,599	14,038	42,775	4,092	2,664	14,657
	Sault Ste Marie	0	953	951	2,502	2,356	0	0
	Timmins	0	0	0	4,228	20,240	6,682	180
	North Bay	0	0	0	306	0	195	0
	Parry Sound	0	0	4,548	21,674	4,760	39,701	23,771
	Pembroke	0	222	407	530	262	80	137
	Total	851	3,774	19,944	72,015	31,710	49,322	38,745
Combined		851	92,219	740,116	535,866	166,969	204,143	38,989

Table 2

Summary data for plots that were sampled as part of the annual L2 survey and subset of plots used for monitoring prevalence of *Nosema* in northeastern (NE) and northwestern (NW) Ontario

Region		2004	2005	2006	2007	2008	2009
NE	No. plots sampled	52	135	94	99	104	80
	Mean L2/branch	3.8	4.1	6.2	3.8	3.1	0.36
	Max L2/branch	52	118	124	84	35	3
	No. plots monitored	8	13	17	20	27	0
	% Infected [a]	50.0	100.0	94.1	85.0	44.4	--
	Mean % infected [b]	1.6	25.4	27.1	23.8	7.2	--
	Mean % heavy [c]	nd	85.2	72.8	56.8	66.9	--
NW	No. plots sampled	24	184	271	219	122	118
	Mean L2/branch	7.8	42.8	16.7	2.2	6.0	2.6
	Max L2/branch	81	232	138	24	35	36
	No. plots monitored	6	114	167	69	49	28
	% Infected [a]	33.3	26.3	84.4	73.9	97.9	96.4
	Mean % infected [b]	2.4	1.2	9.9	12.6	31.2	28.2
	Mean % heavy [c]	nd	92.4	60.2	66.3	71.9	42.2

[a] Percentage of monitored plots that had infected larvae
[b] Region-wide mean (all plots) of % of smeared larvae that were infected
[c] Region-wide mean (all plots) of % of infected larvae that carried a heavy infection

Table 3
Year-to-year changes in *Nosema* prevalence in sites that were sampled for at least two consecutive years

Region	No. of sites	No. sites with Nf prevalence			Magnitude of increase[1]
		Increasing	No change	Decreasing	
NE	10	10	0	0	10.7 ± 3.5
NW	49	39	7	3	13.6 ± 2.9

[1] Mean –fold increase ± SE

Table 4

Increase in prevalence of *Nosema fumiferanae* during pollen cone feeding stage of jack pine budworm larvae. Cones were collected from a cluster of infested trees in each of three locations and maintained at 21 ^{o}C. Initial prevalence (P_i) was estimated by dissecting a subsample of cones right after collection when most larvae were in second instar. Final prevalence (P_f) was estimated by sacrificing larvae (mostly fourth and fifth instars) right after leaving the pollen cones in search of elongated shoots for feeding.

Location	**No. of flowers per cone**	**No. of L2 per cone**	**P_i (no. of larvae)**	**P_f (no. of larvae)**
Sudbury	11.1	5.7	26.0 (100)	51.8 (162)
Gogama	12.1	18.1	1.0 (100)	14.9 (348)
Nairn Centre	14.8	7.4	40.0 (87)	57.0 (128)

Figure 1

Areas of moderate-to-severe defoliation during peak of outbreak in 2006 and 2007

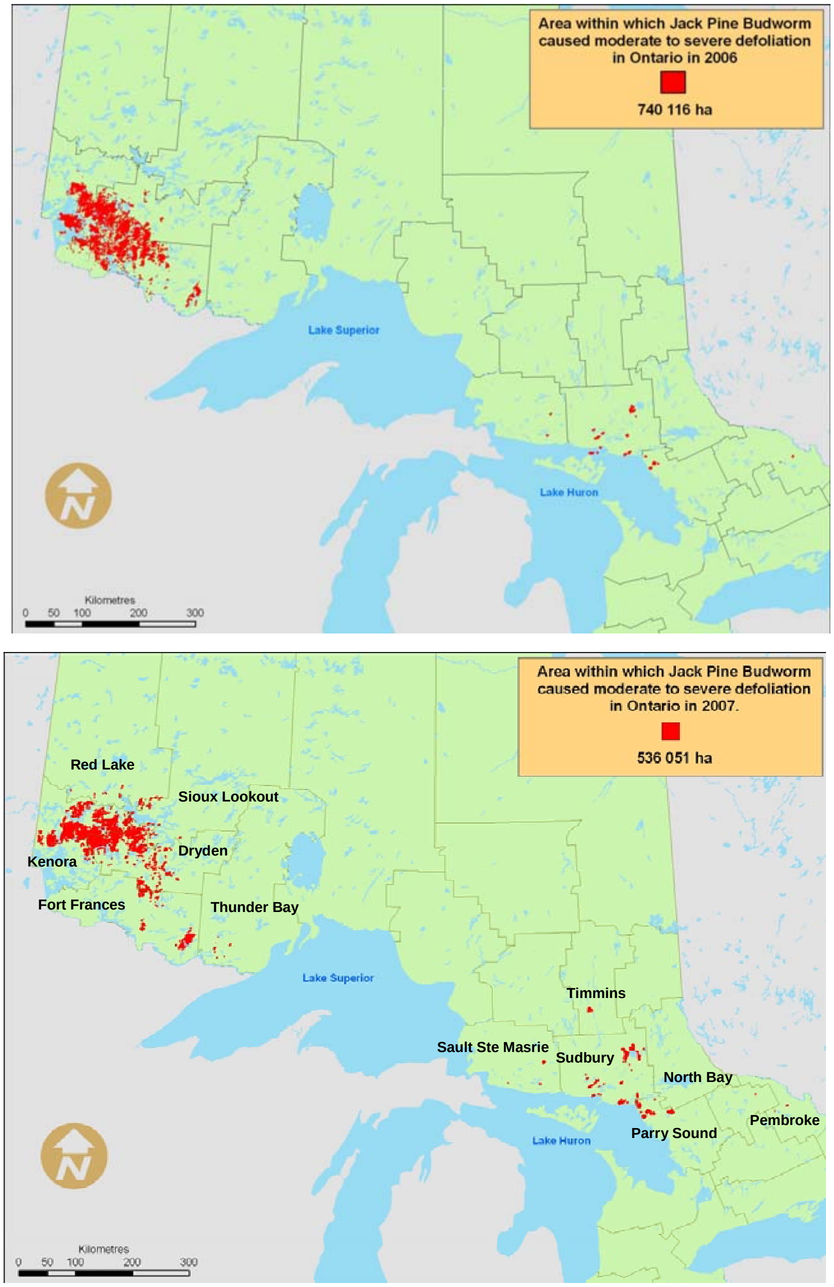

Figure 2
Box plots of L2 density (panel A and B) and of *Nosema fumiferanae* prevalence (% of L2 infected) (panel C and D) for each year across the NE (not including Chapleau, North Bay and Parry Sound districts) and NW regions (not including Thunder Bay and Sioux Lookout districts).

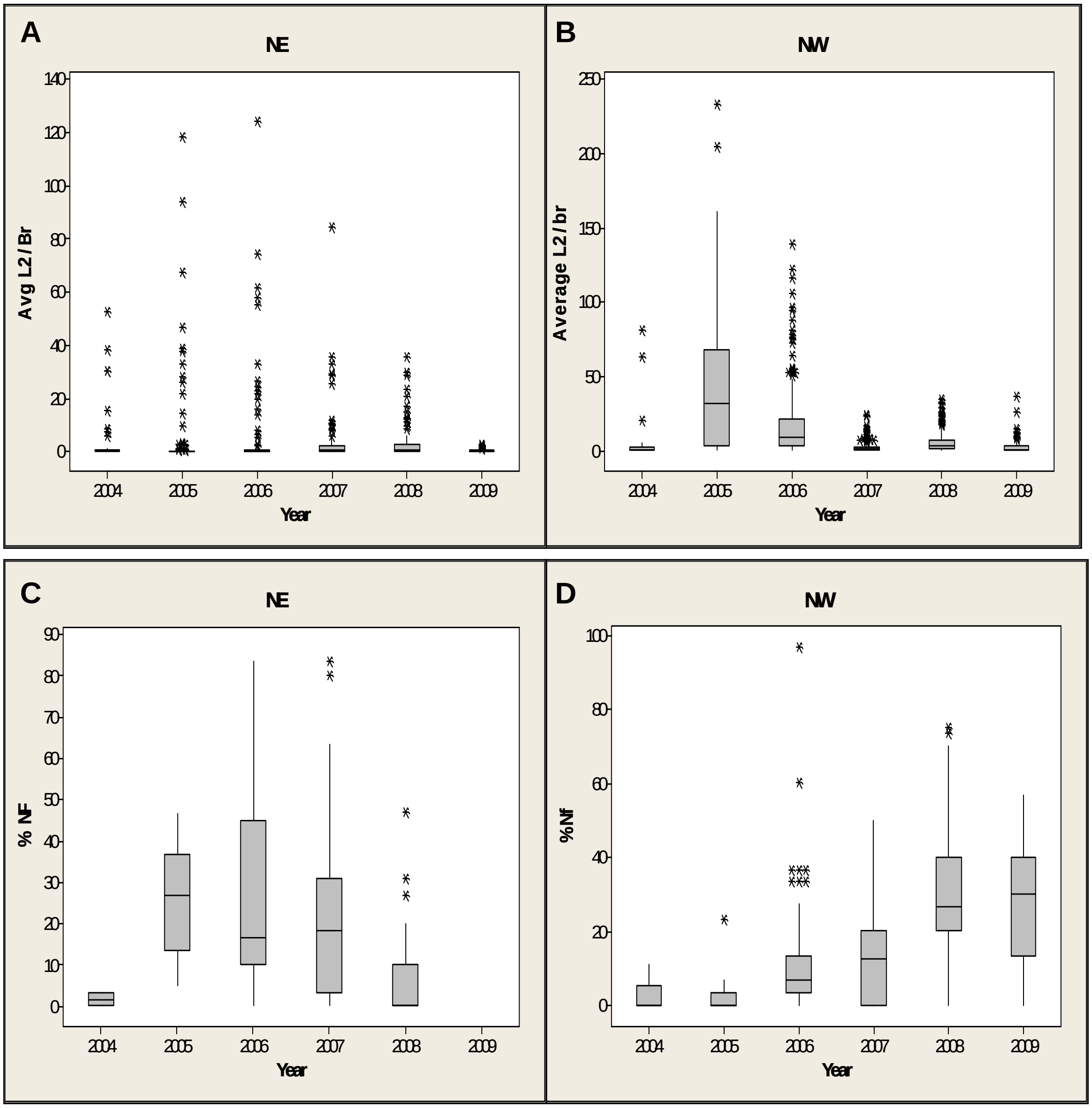

Figure 3
Box plots of L2 density (panel A and B) and of *Nosema fumiferanae* prevalence (% of L2 infected) (panel C and D) by year and district in the NE and NW regions. SSM = Sault Ste. Marie, SUD = Sudbury, TIM = Timmins, DR = Dryden, FF = Fr Frances, KE = Kenora, RL = Red Lake

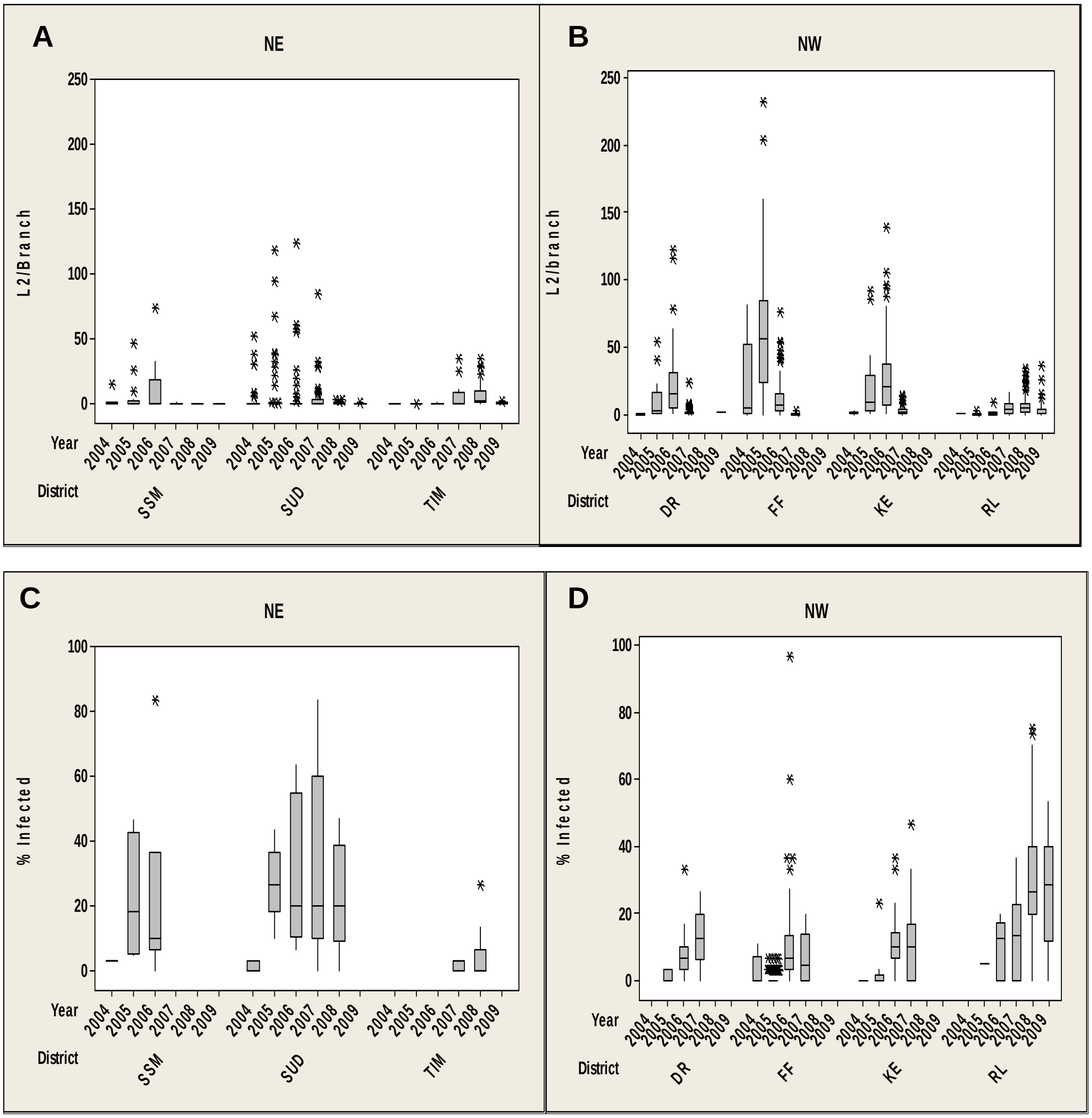

Figure 4
Relationship between L2 density and prevalence of *Nosema fumiferana* (% NF) in sample sites by year for the entire NW and NE region (districts pooled) and for selected individual districts.

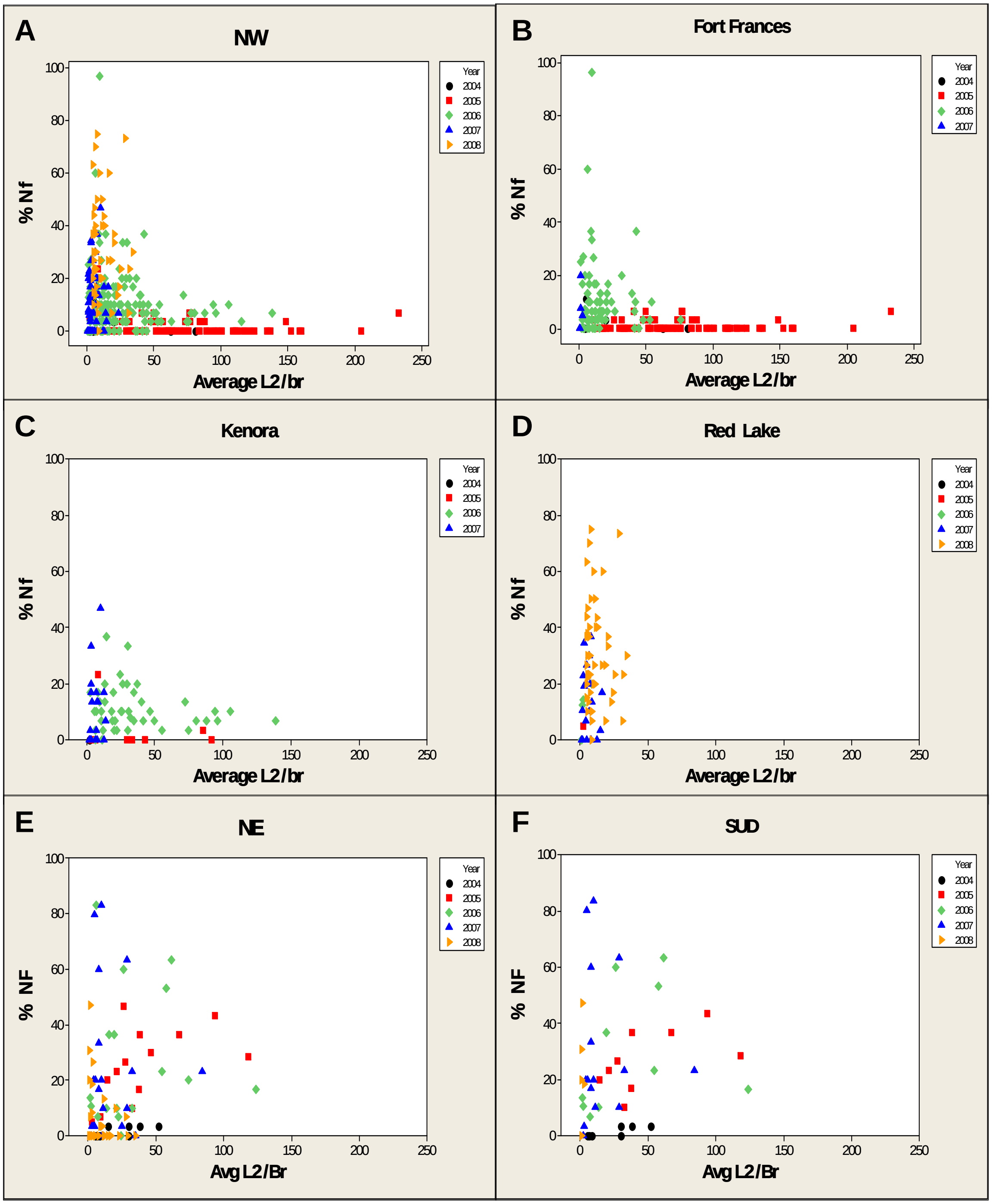

Figure 5

Transmission of *Nosema fumiferanae* in an outbreak population of spruce budworm near Sault Ste. Marie (2006) in relation to larval phenology (% instar).