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Report Title: Investigation of *Nosema fumiferanae* (Microsporidia: Nosematidae) infections in adult male populations of the spruce budworm (*Choristoneura fumiferana* (Clem.)) as an indicator of population health

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Investigation of *Nosema fumiferanae* (Microsporidia: Nosematidae) infections in adult male populations of the spruce budworm (*Choristoneura fumiferana* (Clem.)) as an indicator of population health.

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Abstract

Diagnostic evaluations were done for the presence of the protozoan pathogen, *Nosema fumiferanae* (Thompson) on male moths of spruce budworm (*Choristoneura fumiferana* (Clem.)) that were available from surveys conducted within New Brunswick from 1994 to 2001 (1999 data were missing). Evaluations for 1996 and 1997 have not yet been completed. In 1994 and 1995, the incidence of infection across the Province was about 20%, declining to about 5% in 1998, and remaining at this low level in 2000 and 2001. The intensity of infection in infected moths also declined over this period. In general, similar trends were seen in each county and in each of J.D. Irving, Limited's sites. The results are discussed in relation to current trends in spruce budworm populations in the Province. It is recommended that moths captured in future years should also be examined for *Nosema* infections to quantify the response of *Nosema* to rising host populations as a possible indicator of changes in population health.

Introduction

Local populations of the spruce budworm, *Choristoneura fumiferana* (Clem.), periodically erupt to high densities at which time large-scale protection programs may become necessary to ensure the attainment of long-term fibre and non-fibre sustainable objectives that are expected from the Province's forests. When and where protection is required is determined through the use of various survey methods designed to detect and monitor changes in populations. When populations are high, aerial defoliation surveys, and egg mass or L2 surveys, are usually employed. However, when populations are low, such as currently exist in New Brunswick and other eastern jurisdictions, populations are monitored by a system of pheromone traps that catch adult male moths. Although these survey methods provide information on numerical changes in spruce budworm populations, they do not provide any information on the health of the populations. Detecting annual changes in local population health may prove useful as an additional tool in annual monitoring surveys.

Recognizing this need, we undertook a study to examine the incidence of *Nosema fumiferanae* (Thompson) infection in male moths caught in pheromone traps placed at numerous locations over several years within New Brunswick. This protozoan pathogen, hereafter referred to as *Nosema*, is one of the most common natural enemies of the spruce budworm, with incidences of over 80% being reported (Thompson 1960). It is efficiently transmitted both vertically, from one generation to the next through eggs, but not through sperm, and horizontally through the larval instars within a generation (Wilson 1982). Consequently, the incidence of infection has been reported to increase across generations (Wilson 1977). High intensity infections can cause death, whereas low (sub-lethal) intensity infections tend to prolong larval development (Bauer and Nordin 1988), reduce pupal (and hence adult) weight and shorten adult longevity (Wilson 1981), and lower fecundity (Bauer and Nordin 1989). Such a debilitating pathogen could also make the spruce budworm more susceptible to insecticides and thus incidences of infection should be considered when contemplating protection measures, especially those involving biologicals (e.g. *Bacillus thuringiensis*).

The only other study conducted on the incidence of *Nosema* infections in field populations of male and female spruce budworm moths revealed that the incidence of infection in females, but not males, was positively correlated with annual changes in moth density (Eveleigh *et al.* in prep). However, that study covered only a small range of years from outbreak to the decline of a local population; no long-term study on *Nosema* infections, covering the endemic (low density) and increasing phases of a population cycle, has been conducted. Thus, our overall objectives were to (1) determine annual changes in the incidence and intensity of infection in low-density male moth populations, and (2) determine if changes in the incidence of infection can be used as an index of population health. Results from this investigation form a baseline set of data against which future changes can be compared as populations rise in the next outbreak.

Materials and Methods

Annual moth density estimates. Male spruce budworm moths were caught in pheromone traps deployed throughout the Province of New Brunswick from 1994 – 2001, (1999 moths inadvertently discarded) by the Provincial Department of Natural Resources & Energy (DNRE) as part of their annual spruce budworm surveys, and by J. D. Irving, Limited on their freehold limits and at their tree seed orchards (Carter 2001). Each year, traps were placed in the field

prior to moth eclosion in the local areas and retrieved in late August – October, well after moth flight period had ended. Moths caught in each trap were counted, placed in appropriately labeled envelopes, and frozen for subsequent examination. Geo-referenced coordinates were recorded for each location and this facilitated assigning locations to counties within the Province (Fig. 1). Changes in overall Provincial moth densities in DNRE annual surveys are summarized Table 1.

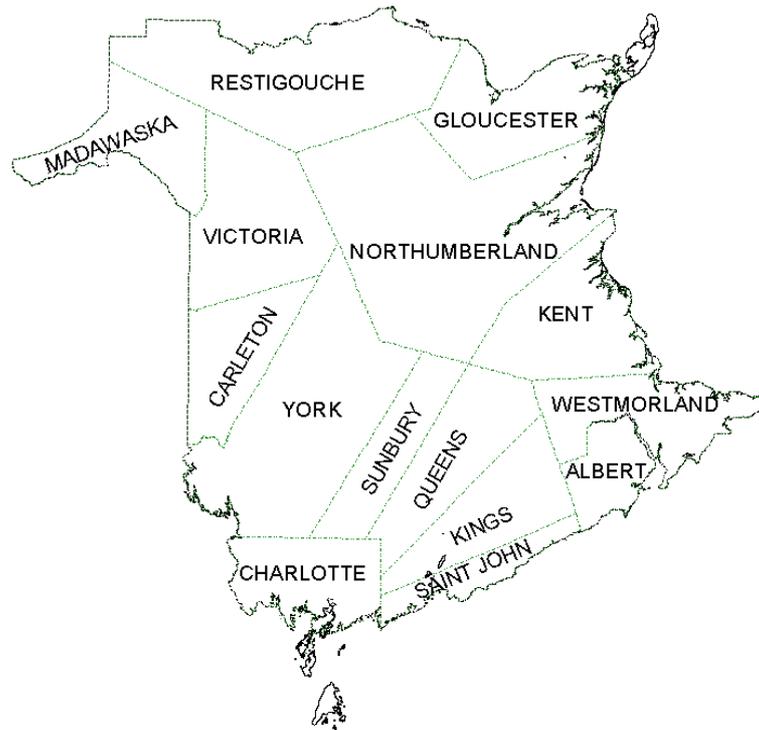


Figure 1. New Brunswick counties.

Table 1. Number of spruce budworm male moths caught in DNRE's operational pheromone trap survey in New Brunswick from 1996 to 2001.

Year	Number of traps	Number of moths/trap			Maximum trap catch	Mean trap catch
		Nil	1-10	>10		
1995	296	42%	50%	8%	47	3.27
1996	99	53%	41%	6%	54	3.24
1997	148	73%	27%	0%	6	0.49
1998	148	67%	33%	0%	10	0.95
1999	155	59%	41%	<1%	12	1.05
2000	154	55%	42%	3%	25	1.67
2001	197	42%	50%	8%	32	2.90

***Nosema* diagnosis.** Specimens from each trap were removed from storage and either all, or a random sub-sample, were selected for examination. A portion of the abdomen of each specimen was macerated in distilled water on a glass slide and the homogenate examined at 400X magnification for the presence of *Nosema* spores. Infections were scored as either light or moderate-heavy relative to the number of spores observed in each sample.

***Nosema* incidence.** Because individual trap catches were often less than 10 individuals, the data for all traps in a county were combined to give the overall incidence of *Nosema* in each county. Similarly, the data for all trap catches at each of J.D. Irving, Limited ‘sites’ were combined to give the overall incidence for each site.

Results

Data for 1996 and 1997 are not yet available (and 1999 data are missing). The incidence of *Nosema* infections in male spruce budworm moths caught in pheromone traps in each county and at each J. D. Irving, Limited site is shown in Table 2. On a Province-wide basis, the incidence of *Nosema* declined from ~20% in 1994 and 1995 to <10% from 1998 to 2001. This trend was also seen in the incidence of *Nosema* in moths caught in counties for which there were sufficient annual data (e.g., Madawaska, Northumberland, and Restigouche). Although the annual incidence of *Nosema* on J.D. Irving, Limited sites was lower than that for all counties combined, the overall trend was similar. The Province-wide percentage of infected moths with moderate-heavy infections decreased from ~ 45 % in 1994 to ~ 12% from 1998 to 2001 with a concomitant increase in light infections from ~ 55% to ~ 88%.

Discussion

In the latest spruce budworm outbreak in New Brunswick, populations reached outbreak levels in the early to mid-1980s, and declined to endemic levels in the late 1980s to the early 1990s (DNRE annual survey data). Although we did not monitor the incidence of *Nosema* infection during the increasing phase of this outbreak, the incidence of *Nosema* in local spruce budworm populations has been reported to increase as the age of the infestation increases (Wilson 1977). At the peak of the outbreak, the annual incidence of *Nosema* in pupal populations near Fredericton ranged from 20-30% (1983-1987) and from 31-48% (1988-1992) in populations near St-Quentin, NB (Fig. 2; E. Eveleigh, unpublished data). Data in the present study indicate that after host densities declined to current endemic levels, the incidence of *Nosema* in the population slowly declined, reaching very low incidences at low host population levels. Thus, it appears that the incidence of *Nosema*, because of its strong dependence on the host, increases and declines with the rise and fall of spruce budworm populations during an outbreak cycle.

The last detectable defoliation in NB occurred in 1995 and the last operational control was applied that year on J.D. Irving, Limited freehold lands in the northwestern part of the Province (Carter 2001). Interestingly, in 1994, the incidence of *Nosema* in these freehold lands, and in northern counties in general (e.g., Madawaska, Restigouche and Northumberland), was higher than in other areas of the Province, reflecting the higher spruce budworm populations in these areas. However, by 1998 the incidence in these counties dropped to levels similar to that of other counties.

After the decline in spruce budworm populations, the intensity of infection in infected moths also declined, with fewer moths having moderate-heavy infections when host populations are very low. Thus, at endemic host population levels both the incidence and intensity of infection become very low.

The increase in moth catches in recent years (Table 1) has not resulted in a detectable increase in either the incidence or intensity of *Nosema* infections. This suggests that *Nosema*

infections may take a few years to increase after the spruce budworm populations start to increase as seen in typical predator (pathogen)-prey relationships. Nevertheless, it is important to examine moths captured throughout the Province in the next few years to determine where within the Province, and at what host population levels, *Nosema* infections start to increase.

Summary

1. Male spruce budworm moths, caught in pheromone traps distributed throughout NB by DNRE and J.D. Irving, Limited from 1994 - 2001, were assessed for infection by the protozoan pathogen, *Nosema fumiferanae*. Results from 1996 and 1997 are not yet processed.
2. Data available so far indicate that the overall Provincial average annual incidence of infection declined from a high of 20.7 % in 1995 to a low of 3.8 % in 2001.
3. The overall Provincial average annual percentage of infected moths with moderate-heavy infections decreased from 46.3 % in 1994 to 10.3 % in 2000 with a concomitant increase in light infections from 53.7 % in 1994 to 89.7 % in 2000.
4. The annual incidence of infection on J.D. Irving, Limited sites was lower than all counties combined, but followed the same overall trend.
5. The increase in Provincial moth catches in recent years did not result in a detectable increase in the incidence of infection using the methods employed in this study.

Conclusion

The incidence and intensity of *Nosema fumiferanae* infections in male spruce budworm moths declined to very low levels after spruce budworm populations declined to endemic levels. Thus, in terms of *Nosema* infections, endemic spruce budworm populations in NB are relatively healthy and are likely to remain so until populations increase again in the next outbreak. We recommend that moths captured in future years should be examined for *Nosema* infections to quantify the response of *Nosema* to rising host populations as a possible indicator of changes in population health.

Literature cited

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Deliverables

1. Microscopic diagnosis of *Nosema* infection in male spruce budworm moths provided by DNRE and J.D. Irving, Limited.
2. Results of incidence of *Nosema* infection summarized and assessed along with other pertinent spruce budworm population data.

Summary of Expenditures

Salary for student assistant (diagnostics)	\$4400.00
Glass slides	\$ 338.79
Glass cover slips	\$ 99.82
Microcentrifuge tubes	\$ <u>181.53</u>
Total expenditures	\$5020.14
Amount received from Fundy Model Forest	\$4500.00
Hold-back payment outstanding	\$ 500.00

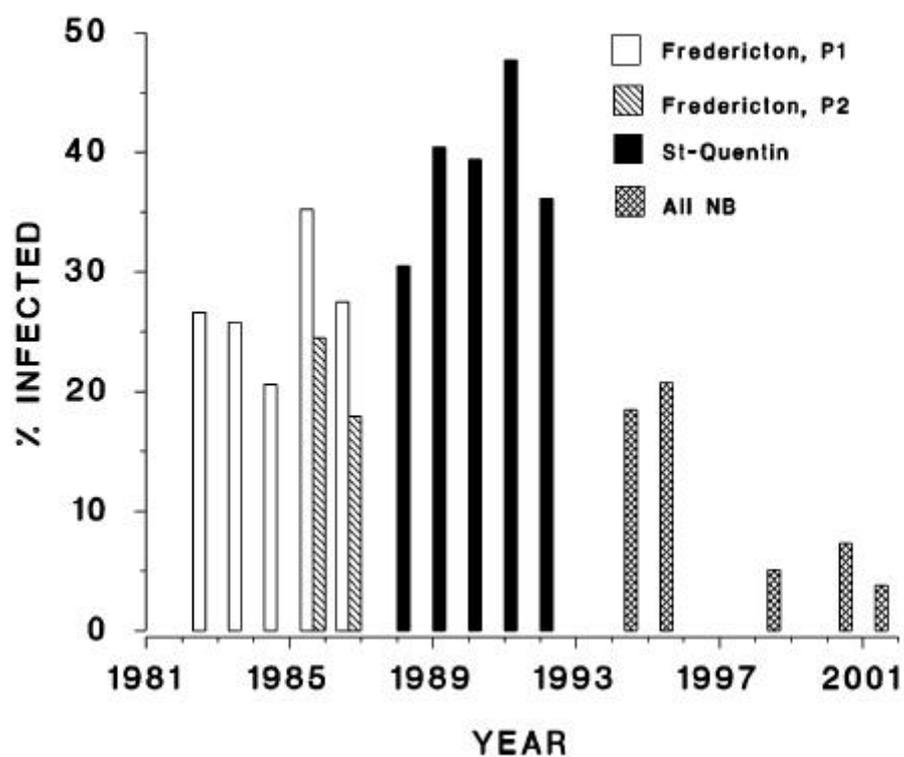


Figure 2. Incidence of *Nosema fumiferanae* infections in spruce budworm pupae collected in two plots (P1 & P2) near Fredericton (1983 –1987) and in one plot near St-Quentin (1988-1992) (E. Eveleigh, unpublished data), and incidence of infection in male spruce budworm moths collected across New Brunswick (1994 -2001). Note that data for pupae were collected when spruce budworm populations were at outbreak levels

Table 2. Incidence and intensity of *Nosema fumiferanae* infections in male spruce budworm moths caught in pheromone traps distributed across New Brunswick and on J.D. Irving, Limited sites from 1994 - 2001.

County / JDI sites	Year														
	1994			1995			1998			2000			2001		
	n	% infected	% mod-heavy Infection ¹	n	% infected	% mod-heavy infection	n	% infected	% mod-heavy infection	n	% infected	% mod-heavy infection	n	% infected	% mod-heavy infection
County															
Albert	- ²	-	-	24	12.5	0	-	-	-	-	-	-	2	0	0
Carleton	13	15.4	0	23	56.5	69.2	1	100	0	15	6.7	0	18	0	0
Charlotte	-	-	-	1	0	0	5	0	0	10	10	0	3	33.3	0
Gloucester	41	7.3	33.3	115	23.5	29.6	2	0	0	12	16.7	0	6	0	0
Kent	-	-	-	2	0	0	-	-	-	3	33.3	0	3	0	0
Kings	2	0	0	103	17.5	11.1	1	0	0	-	-	-	-	-	-
Madawaska	18	22.2	25	252	20.6	46.2	32	6.3	0	54	5.6	33.3	53	3.8	0
Northumberland	50	34	82.4	199	27.1	35.2	9	11.1	0	26	7.7	0	22	9.1	0
Queens	3	66.7	0	26	7.7	0	2	50	0	-	-	-	2	0	0
Restigouche	80	26.2	23.8	518	21.6	37.5	33	6.1	0	37	5.4	0	55	3.6	0
Saint John	-	-	-	-	-	-	-	-	-	-	-	-	3	0	0
Sunbury	-	-	-	4	0	0	-	-	-	5	0	0	7	0	0
Victoria	-	-	-	140	15	57.1	23	4.3	0	17	0	0	29	3.4	0
Westmorland	-	-	-	34	23.5	12.5	-	-	-	-	-	-	5	0	0
York	-	-	-	9	33.3	0	11	0	0	22	13.6	0	32	6.3	50
All counties	207	23.7	42.9	1450	21.6	37.4	119	6.7	0	201	7.5	13.3	240	4.2	20
JDI sites															
Deersdale	-	-	-	-	-	-	11	9.1	100	34	14.7	20	33	9.1	0
Juniper	7	0	0	3	0	0	-	-	-	-	-	-	-	-	-
Irving limits	223	20.2	48.9	5	40	0	-	-	-	-	-	-	50	0	0
Parkindale Orchard	200	13.5	48.1	100	10	40	43	0	0	134	4.5	0	43	0	0
Sussex tree nursery	18	0	0	14	0	0	-	-	-	20	15	0	10	0	0
Sussex District	-	-	-	-	-	-	2	0	0	8	0	0	21	9.5	0
All JDI sites	448	16.1	48.6	122	9.8	50	56	1.8	100	196	7.1	7.1	157	3.2	0
Total Province	655	18.5	46.3	1572	20.7	37.8	175	5.1	11.1	397	7.3	10.3	397	3.8	13.3

1 % of infected moths that had moderate-heavy infections

2 - indicates that no moths were available for examination; in most cases, no moths were caught