PATHOGENICITY OF *NOSEMA FUMIFERANAE* (THOMSON) (MICROSPORIDA) IN SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEMENS), AND IMPLICATIONS OF DIAPAUSE CONDITIONS[1]

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Abstract

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A standardized bioassay procedure was used to determine median lethal doses (LD_{50}) of the microsporidium, *Nosema fumiferanae* (Thom.), on newly molted fourth- and fifth-instar eastern spruce budworm larvae (*Choristoneura fumiferana* (Clem.)). The LD_{50} for fifth-instar larva was $1.23 \times 10^6 \pm 2.82 \times 10^5$ spores. The fourth-instar LD_{50} was $2.23 \times 10^4 \pm 4.30 \times 10^3$ spores per larva for populations experiencing prolonged post-diapause cold storage or an elevated temperature during diapause and $2.00 \times 10^5 \pm 6.66 \times 10^4$ spores per larva for populations not experiencing stressful conditions during and after diapause. Median lethal times (LT_{50}) ranged from 6 to 19 days, depending on instar and dose level. Sublethal responses of fourth- and fifth-instar larvae inoculated with serial dilutions of spores were estimated by significant linear models. These regressions were negative for pupal weight and adult longevity and positive for development time (duration of instar VI). Inoculations of newly molted sixth-instar larvae produced similar models, although development time was not significantly affected. Insects reared following stress during and after diapause had consistently longer developmental times. The importance of prolonged developmental time on disease expression and insect susceptibility is discussed.

Résumé

On a utilisé une méthode standard pour déterminer les doses létales médianes (LD_{50}) de la microsporidie *Nosema fumiferanae* (Thom.) pour des larves nouvellement muées des stades quatre et cinq de la tordeuse des bourgeons de l'épinette, *Choristoneura fumiferana* (Clem.). La dose létale médiane (LD_{50}) pour les larves de cinquième stade était $1{,}23 \times 10^6 \pm 2{,}82 \times 10^5$ spores. La LD_{50} pour les larves de quatrième stade était $2{,}23 \times 10^4 \pm 4{,}30 \times 10^3$ spores par larve chez des populations ayant subi un refroidissement post-diapause prolongé ou une élévation de température durant la diapause, et $2{,}00 \times 10^5 \pm 6{,}66 \times 10^4$ spores par larve n'ayant pas subi de conditions stressantes durant, ou après la diapause. Les délais de mortalité (LT_{50}) ont varié de 6 à 19 jours selon le stade et la dose. On a caractérisé les réactions sub-létales des larves des stades quatre et cinq inoculées avec des dilutions en série des spores par des modèles de régression linéaire significatifs. Ces régressions étaient négatives dans le cas du poids des pupes ou de la longévité adulte, et positives pour la durée de développement (au stade VI). L'inoculation de larves de stade six nouvellement muées a révélé des modèles similaires, bien que la durée du développement n'ait pas été affectée. Les insectes ayant subi un stress durant ou après la diapause montraient invariablement un prolongement du développement. On discute de l'importance du prolongement du développement pour l'expression de la maladie et la susceptibilité de l'insecte.

Introduction

Nosema fumiferanae (Thom.) (Microsporida: Nosematidae) is the causal agent of a chronic and ubiquitous disease of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.). This lepidopteran is considered the most destructive native defoliator of North American boreal forests (Bean and Mott 1972). Although Neilson (1963) reported a wide

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range of pathogens in this insect host, none of these approaches the incidence rate of *N. fumiferanae*. Numerous studies have shown that incidence of this pathogen increases over time in spruce budworm populations (Wilson 1977). Its virulence is low (Wilson 1985) and transmission is efficient both vertically and horizontally (Thomson 1958*a*; Wilson 1982). The role of this pathogen as a natural regulator of spruce budworm populations, however, has not been quantified (Royama 1984) or incorporated into spruce budworm management strategies (Wilson 1981). In fact, its presence is generally overlooked in studies of the variables that affect spruce budworm performance.

Many debilitating effects of *N. fumiferanae* on spruce budworm are well documented. Most studies, however, have evaluated the impact using naturally infected insects (Thomson 1958*b*; Wilson 1980) or insects inoculated using spore concentrations applied to foliage or diet surface (Wilson 1974). The quantity of inoculum consumed was presumed to be proportional to the concentration applied. Such an assay, however, cannot be replicated because the actual number of spores ingested can vary, depending on uniformity of coverage on diet or foliage surface and the surface area consumed by each larva.

For studies seeking to quantify pathogen impact and to understand variables affecting susceptibility, it is essential to have a repeatable bioassay procedure that provides the insect host with a known dose in a short time period. An increase in the rate of dose consumption improves precision by reducing the variability in host response (Burges and Thomson 1971). Also, uniformity in the physiological state of insect hosts at the time of treatment is important because it helps to reduce variability in pathogen impact. Failure to control host variables makes it difficult to compare assays, even in the same pathogen–host system. However, the significance of conditions during diapause, which generally occurs well before inoculation, has not been considered. In the boreal environment, the extended larval diapause of spruce budworm (ca. August–May) may be a stress factor in disease expression.

The purpose of this study was to quantify the dose–mortality response and sublethal impact of *N. fumiferanae* on spruce budworm development time, fresh pupal weight, and adult longevity. The implications of post-diapause storage and temperature during diapause on mortality response are also discussed. A bioassay procedure is described that provides a known spore dosage to fourth- and fifth-instar larvae in 24 h or less on artificial diet.

Materials and Methods

Insects. We obtained microsporidia-free, second-instar eastern spruce budworm larvae in hibernacula that had satisfied diapause conditions, and kept them in refrigerated storage (6 ± 2°C) until needed. For identification purposes, each shipment was labelled as a separate population (SBWI, SBWII, SBWIII, SBWIV). Results of assays from a total of four populations utilized during a 22-month period (1984–1986) are reported. Before experiments were begun, samples from each population were examined microscopically to verify the absence of microsporidia, nuclear polyhedrosis virus, and cytoplasmic polyhedrosis virus.

Post-diapause larvae were reared at a density of eight larvae per 30-mL plastic creamer on standard spruce budworm diet (McMorran 1965) without the antibiotic Aureomycin. Assays used larvae within 24 h of ecdysis and, following dose consumption, they were reared individually at 20 ± 1.5°C with a photoperiod of 16L:8D. Pupae and adults were maintained at 22 ± 2°C.

Pathogen. Fresh spores of *N. fumiferanae* were produced in spruce budworm larvae according to techniques described by Wilson (1976). The triangulation method (Cole 1970) was used to obtain a pure spore suspension. Spore concentration in stock suspensions was determined using a Petroff-Hausser counting chamber. Spores were refrigerated (6 ± 2°C) in sterile distilled water for up to 6 weeks.

Bioassay. The bioassay procedure for fourth- and fifth-instar larvae was modified from that described by Nordin (1976). To accommodate these smaller and more active larvae, we used a smaller diameter glass assay tube (3 mm I.D.), fitted with a rubber serum bottle stopper at one end. Standard spruce budworm diet, minus antimicrobial agents, was poured in a thin layer (ca. 5 mm deep) into plastic trays. By pressing an assay tube into the surface, an area of diet was delineated. Spore doses were applied to this area in a 2-μL droplet of suspension and allowed to air dry under a sterile hood. Sterile distilled water was used for controls. Pre-cut diet discs (1 mm thick by 4 mm diameter) were used to inoculate sixth-instar larvae. A dosed disc and larva were placed together in 17-mm (I.D.) tissue culture plate wells. During the inoculation period, trays were inverted and maintained at $22 \pm 2°C$ with a photoperiod of 24L:0D. Only those larvae that consumed the entire dose in a 24-h period, or less, were included in the assays.

Dose–Mortality Response, Sublethal Effects. The median lethal dose (LD_{50}) was determined for fourth- and fifth-instar larvae with four levels of inoculum. The number of insects per dose level ranged from 30 to 60 larvae. Median lethal times (LT_{50}) were calculated separately for dosages that were sufficiently lethal.

Sublethal effects were quantified on two populations, each inoculated as fourth, fifth, and sixth instars at four dose levels. Insect response variables were monitored daily and included duration of each instar, pupal period, fresh pupal weight, and adult longevity. Pupae were weighed the day following pupation. Results from the two populations were not significantly different (Student's t-test, $P \leq 0.05$) and were pooled at each dose level.

Diapause Conditions. Diapause duration. We could not determine the exact length of time larvae had been in cold storage before they arrived, but it was at least 18 and no more than 30 weeks (D. Grisdale, personal communication). After they arrived we monitored the duration of refrigerated storage for each population. One population (SBWI) was used for fourth-instar assays at 3 and 10 weeks post-arrival, and comparisons were made between them for median lethal doses and times. Fresh spore batches were used for each assay. In addition, we evaluated the effect of post-diapause refrigerated storage on healthy individuals by comparing data generated from cohorts that received different durations of post-diapause storage.

An experiment was designed to test the effect of prolonged post-diapause storage using separate populations maintained in refrigerated storage ($6 \pm 2°C$) for 2, 7, and 15 weeks following arrival. The populations were reared simultaneously, and an even-aged set of larvae ($n = 50$) was selected from each population and inoculated with the same spore batch during a 2-day period. To determine if dose effect was related to larval weight, we obtained fresh weights of fifth-instar larvae for each population; both lethal and sublethal responses were compared.

Diapause temperature. Due to incubator failure, one population of spruce budworm (SBWII) received an elevated temperature during diapause of ca. 6°C (D. Grisdale, personal communication). Because this population had been used for bioassays to quantify the dose–mortality response of fourth- and fifth-instar larvae to *N. fumiferanae*, it was then possible to compare the median lethal doses and times with those generated from populations that had satisfied diapause requirements at a temperature of 2°C (SBWIII).

Statistics. Maximum likelihood estimates of median lethal doses and times were calculated with probit analysis (Finney 1971). Regression analyses were used to develop models estimating the effect of *N. fumiferanae* (log dose) on response variables. The sublethal effect of post-diapause treatments on response variables was analyzed using ANOVA procedures. The significance of paired multiple comparisons among treatments where F-values were significant was based on Duncan's multiple-range test. Student's t-test was used to determine the significance of post-diapause conditions on pupal weights and larval

Table 1. Summary of regression analysis estimates of the sublethal responses of spruce budworm. Response variables (y) include development time (duration of instar VI), pupal fresh weight (mg fw), and adult longevity for larvae surviving inoculations with serial dilutions of spores of *Nosema fumiferanae* (x) at fourth, fifth, and sixth instars

		Females			Males		
Stadium	Response variable	Slope estimate	y-intercept estimate	R^2	Slope estimate	y-intercept estimate	R^2
IV*	Development time (days)	3.6	−4.3	0.82	1.7	2.3	0.98
	Pupal weight (mg fw)	−24.5	218.3	0.80	−17.2	146.3	0.98
	Adult longevity (days)	−1.5	14.5	0.88	−0.9	8.3	0.67
V†	Development time (days)	2.3	1.7	0.89	2.3	−0.8	0.98
	Pupal weight (mg fw)	−31.9	267.8	0.92	−12.6	129.2	0.98
	Adult longevity (days)	−1.9	17.6	0.96	−0.5	6.8	0.47
VI‡	Pupal weight (mg fw)	−15.5	199.5	0.70	−6.7	115.0	0.99
	Adult longevity (days)	−0.5	9.5	0.54	−1.2	12.5	0.76

*Log_{10} doses used in regression analysis: 4.0, 4.7, 5.0, 5.7; female $n = 80$, male $n = 109$.
†Log_{10} doses used in regression analysis: 5.0, 5.7, 6.0, 6.7; female $n = 103$, male $n = 112$.
‡Log_{10} doses used in regression analysis: 5.7, 6.0, 6.7, 7.0; female $n = 121$, male $n = 138$.

periods. The Chi-square procedure was used to evaluate the effects of post-diapause storage times on percentage mortality. All statistics were computed using the Statistical Analytical System (SAS Institute 1982).

Results

Sublethal Impact. An infection rate of 100% was determined for all dose levels used in this study. Spruce budworm female and male pupal weights, duration of instar VI, and adult longevity were significantly correlated with dose when inoculated as newly molted fourth- and fifth-instar larvae (Table 1). This relation was negative for pupal weight and adult longevity and positive for development time. Inoculation of newly molted sixth instars produced similar models for pupal weights and adult longevity (Table 1), although development time was not significantly affected. All regressions were significant ($P \leq 0.001$) and no significant lack-of-fit was determined for these models ($P \leq 0.05$).

At each instar of inoculation, female pupal weight varied more (lower "R^2") and was more sensitive (larger coefficient of determination) than male pupal weight (Table 1). Larvae became increasingly resistant to sublethal effects with increasing age. Duration of the pupal stadium was not significantly affected.

Dose–Mortality Response. Assays performed on newly molted fourth-instar larvae were replicated four times, and significant differences in mortality response were found between different populations. A possible cause may be found in the diapause and post-diapause storage conditions of each population (Table 2). The Chi-square test for goodness of fit showed no evidence of heterogeneity within the replicates. Populations SBWI and SBWIII, following a standard diapause temperature of 2°C and assayed soon after receipt, had a similar dose–mortality response. A pooled LD_{50} estimate for these populations is 2.00×10^5 (Table 2). Population SBWI, assayed again following 10 weeks of refrigerated post-diapause storage, responded similarly to SBWII, a population stored at a temperature of 6°C to satisfy diapause requirements. The pooled LD_{50} for these latter two populations was 2.23×10^4 (Table 2). This LD_{50} is 10-fold lower than populations experiencing less stress during diapause. The higher slope associated with the lower LD_{50} indicates a more homogeneous larval susceptibility and not higher pathogen virulence, which would have been expressed as a parallel downward shift of the regression line.

Table 2. Maximum likelihood estimates of the median lethal doses (LD_{50}), 95% fiducial limits, and slopes (± SE) of bioassays of *Nosema fumiferanae* fed to newly molted fourth- and fifth-instar eastern spruce budworm*

Stadium	Population (I.D.)	Diapause temp. (°C)	Refrig. storage (weeks)	LD_{50}†	95% fiducial limits	Estimated slope ± SE
IV	SBWIII	2	1	2.05×10^5	1.65–2.48	1.93 ± 0.195
	SBWI	2	3	1.94×10^5	1.34–2.56	1.71 ± 0.198
	Pooled	"Nonstressed"		2.00×10^5	1.59–2.47	1.77 ± 0.178
IV	SBWI	2	10	2.06×10^4	1.36–2.74	2.92 ± 0.625
	SBWII	6	0	2.41×10^4	1.56–3.37	2.12 ± 0.386
	Pooled	"Stressed"		2.23×10^4	1.81–2.67	2.32 ± 0.306
V‡	SBWIV	2	1	2.29×10^6	1.25–6.71	1.06 ± 0.249
	SBWI	2	10	1.49×10^6	1.02–2.28	2.00 ± 0.409
	SBWII	6	0	1.13×10^6	0.50–5.26	0.80 ± 0.198
	Pooled	All		1.23×10^6	1.00–1.57	1.34 ± 0.167

*Data are reported by population with respect to temperatures during diapause at the Insect Rearing Section, Forest Pest Management Institute, Sault Ste. Marie, and the duration of refrigerated post-diapause storage (6 ± 2°C) following arrival.
†Spores per larva.
‡No significant difference was detected between diapause treatments for fifth-instar inoculations.

Insects designated SBWI and SBWIII appeared fully hydrated when they were received and after they emerged. They took approximately 4 days to emerge from hibernacula at room temperature. SBWI (after 10 weeks of post-diapause refrigerated storage) and SBWII (upon arrival) were small, dehydrated, and emerged from hibernacula immediately on warming. They were designated as populations "stressed during diapause". Following establishment on diet, insects from all populations appeared similar in quality and size. However, mortality and development times prior to selection of a homogeneous set of larvae for assay purposes were not quantified.

Between-population differences in fifth-instar LD_{50}s were not significant, although we observed a 2-fold increase in susceptibility for those receiving greater stress during or after diapause (Table 2). The pooled LD_{50} was 1.23×10^6 spores per larva. In contrast with fourth-instar inoculations, mortality occurred in the pupal stage. It accounted for a maximum of 44% of the total mortality at the highest dosage. However, the incidence of pupal mortality was variable between replicate assays.

Median lethal times, at comparable dosages, were significantly shorter for larvae subjected to stress during or after diapause when compared with non-stressed populations (Table 3). No significant differences were found in lethal times due to storage conditions during diapause for fifth-instar inoculations, and the three replicates were pooled (Table 3). At similar dosages, the LT_{50} for larvae inoculated at fourth instar was significantly shorter than for larvae inoculated at fifth instar. The LT_{50} for each stadium was significantly and inversely correlated to dose level.

Diapause Conditions. Because populations stressed during diapause were consistently more susceptible to *N. fumiferanae* in separate assays than populations not stressed during diapause, a single-dose experiment (1×10^6 spores per fifth-instar larva) was conducted. Percentage mortality tended to increase with the duration of post-diapause storage (Table 4). Comparisons of sublethal disease effects between insects surviving the dosage at each duration of storage showed female larval development time was significantly longer for the population stored 15 weeks. Males showed a similar trend. No significant differences were found in fresh weights of larvae prior to inoculation or of pupae surviving the dosages at each storage treatment. Unfortunately, we did not have enough insects to establish a control group. Therefore, we measured response variables for cohorts from other populations that were disease-free and reared following stress during or after diapause. Larval

Table 3. Maximum likelihood estimates of median lethal times (LT_{50}), 95% fiducial limits, and slopes (± SE) for spruce budworm larvae inoculated with *Nosema fumiferanae* as newly molted fourth- or fifth-instar larvae. Linear models estimate LT_{50} (y) regressed on $\log_{10}$ dose (x) within each group of insects at the $P<0.05$ level of significance

Instar	Dose (spores per larva)	n	LT_{50} (days)	95% fiducial limits (days)	Estimated slope ± SE
IV*	5×10^6	58	7.1	6.7– 7.4	5.67 ± 0.33
	1×10^6	56	11.8	10.8–12.8	6.52 ± 0.82
	5×10^5	54	13.2	12.8–13.7	6.84 ± 0.37
	1×10^5	19	14.4	13.7–15.0	9.40 ± 1.02
IV†	1×10^6	35	6.1	5.7– 6.5	7.12 ± 0.81
	5×10^5	68	9.1	8.8– 9.4	6.49 ± 0.34
	1×10^5	58	11.1	10.7–11.4	8.22 ± 0.57
	5×10^4	19	14.0	13.3–14.8	7.98 ± 0.81
	2×10^4	40	19.3	18.4–20.3	8.40 ± 0.81
V‡	5×10^6	32	8.5	7.9– 9.1	5.60 ± 0.61
	1×10^6	53	14.1	13.6–14.6	6.70 ± 0.37
	5×10^5	10	15.9	14.0–17.7	6.70 ± 1.35

*Nonstressed populations pooled. Regression equation: $y = -4.3x + 36.8$, $R^2 = 0.90$.
†Populations stressed during diapause were pooled. Regression equation: $y = -6.9x + 47.3$, $R^2 = 0.92$.
‡Populations stressed during diapause and nonstressed populations were pooled because diapause treatments were not significantly different at fifth-instar inoculations. Regression equation: $y = -7.5x + 58.9$, $R^2 = 0.98$.

development time was significantly longer for both females and males of cohorts experiencing prolonged post-diapause storage or higher temperatures during diapause (Table 5). Pupal weight was not significantly affected by stress during or after diapause.

Discussion

The expression of *N. fumiferanae* in spruce budworm is not the result of the actual dose provided, but of the numerical development and proliferation of the disease-causing organisms in insect tissues following infection. Under laboratory conditions, significant numbers of larvae died only when they were inoculated at an early instar and with a high dose. Insects that survived sustained a chronic and debilitating infection that prolonged larval development and reduced pupal weight and adult longevity. Within the range of doses provided, linear models accurately describe the sublethal impact of *N. fumiferanae* on these response variables. The response of female weight was greater and more variable

Table 4. Larval period estimates (duration of instars V–VI) (mean ± SE) and percentage mortality for three populations of spruce budworm inoculated with *Nosema fumiferanae* (1×10^6 spores per larva) following different durations of refrigerated post-diapause storage (6 ± 2°C)

Response	Refrigerated post-diapause storage			
	2 weeks	7 weeks	15 weeks	F*
Sexes pooled†				
% mortality	35.71	42.86	60.71	—
Females				
Larval period (days)	20.2 ± 0.7a (n = 20)	20.2 ± 0.3a (n = 20)	22.1 ± 0.6b (n = 17)	5.22
Males				
Larval period (days)	17.5 ± 0.5 (n = 21)	17.5 ± 0.3 (n = 15)	18.2 ± 0.7 (n = 14)	n.s.

*Within each sex and response variable, means followed by the same letters are not significantly different at the $P\leq0.05$ level Duncan's multiple-range test.
†Initial n = 50 fifth-instar larvae per group, $\chi^2 = 3.73$, not significant.

Table 5. Larval period estimates (duration of instars IV–VI) (means ± SE) of microsporidian-free spruce budworm reared following various diapause conditions*

	Refrigerated post-diapause storage			Temperature during diapause		
Response variable	3 weeks	10 weeks	P†	2°C	6°C	P†
Females						
Larval period (days)	16.9 ± 0.3 (n = 26)	18.5 ± 0.3 (n = 21)	<0.0001	17.4 ± 0.2 (n = 60)	19.7 ± 0.3 (n = 69)	<0.0001
Males						
Larval period (days)	14.7 ± 0.4 (n = 25)	16.7 ± 0.4 (n = 21)	<0.001	15.9 ± 0.3 (n = 58)	16.9 ± 0.2 (n = 59)	<0.0029

*Comparisons were made between cohorts of SBWI receiving 3 and 10 weeks of post-diapause storage at 6 ± 2°C and between two different populations (SBWII and SBWIII) following temperatures during diapause of 2°C and 6°C, respectively.
†Probability values from Student's t-test on post-diapause storage time or temperature during diapause at the P<0.05 level of significance.

than males, possibly because of their greater genetic potential to accumulate weight. The kinds of sublethal responses observed in this study are similar to those described by Wilson (1983) in spruce budworm and for other insect–microsporidium interactions (Henry 1981).

Increased resistance to microsporidiosis with each larval instar is well established in the literature (Wilson 1974; Weiser 1976). Thomson (1955) found that all host tissues could be infected, but that the earlier larvae were infected, the more extensive the invasion of their tissues. He believed that time available for microsporidian proliferation within the host was the determining factor in disease expression.

Wilson (1985) reported a fourth-instar LD_{50} at 23°C (2.81×10^6 spores per larva) that was at least 10 times the value we found. Several factors may have contributed to this discrepancy. Wilson used insect populations reared in the same laboratory, avoiding potential stress due to shipment or due to prolonged post-diapause storage. Higher rearing temperatures slow *Nosema* growth rates (Wilson 1979) and speed larval development, necessitating a larger dose for the same effect. In addition, the longer period allowed for dose ingestion (72 h) reduced the impact of the dosage due to increasing resistance (Bauer 1987).

The difference in response between replicate assays required a careful evaluation of the history of each population used. Differences in diapause and post-diapause conditions meant that emerging second-instar larvae entered the feeding period with various levels of energy reserves and water. Results from our study suggest that physiological changes during diapause may increase the susceptibility of these insects to *N. fumiferanae*. This knowledge is particularly important for laboratory studies that use cohorts of spruce budworm over a period of weeks or even months of refrigerated storage following completion of diapause requirements.

Rapid emergence following diapause and prolonged larval development, but not differential pupal weights, were the overt responses expressed by microsporidia-free insects to either elevated temperature during diapause or prolonged post-diapause storage time. Data presented by Renault (1972) show that the first insects to emerge following diapause took longer to develop than insects emerging just 2 days later. Harvey (1985) also reported longer developmental times for insects reared from genetically determined small-egg lines. Presumably these insects emerged following diapause with less energy reserves and took longer to establish on diet or required a longer feeding period.

The increased susceptibility to *N. fumiferanae* of insects stressed during or after diapause may be caused, in part, by these prolonged developmental periods. Weiser (1969) attributed rapid growth rate as the means of resistance to microsporidiosis in races of *Antheraea pernyi* Guerin and *Platysamia cercropiae* (L.). Rapid growth rates may enable insect growth to outpace *Nosema* proliferation in host tissues. Disease-induced spruce budworm mortality in the field during a 5-year period was lowest during warm, sunny

spring weather (Neilson 1963). The use of elevated temperatures to control microsporidiosis in laboratory cultures of insects (Hamm *et al.* 1971) also supports this hypothesis. Our study suggests that slowed insect growth rate, induced by unknown factors associated with stressful conditions during and after diapause, may be important in the host response. Additional research is necessary to establish whether conditions of diapause are artifacts of laboratory rearing conditions and to determine the underlying causes of these observed differences in spruce budworm susceptibility to *N. fumiferanae*.

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