Effect of *Nosema fumiferanae* (Microsporida) on Fecundity, Fertility, and Progeny Performance of *Choristoneura fumiferana* (Lepidoptera: Tortricidae)

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ABSTRACT Female eastern spruce budworm, *Choristoneura fumiferana* (Clemens), inoculated sublethally as fourth or fifth instars with *Nosema fumiferanae* (Thomson), exhibited significant reductions in size, fecundity, and total egg complement. Mating success and egg fertility were similar for treated and control insects. The presence of disease improved the positive correlation between fecundity or total egg complement and female pupal weight without significantly reducing the slope. Total egg complement was negatively correlated with disease load. A subsample of progeny reared from each fertile mating indicates 100% transovarial transmission efficiency at the spore dosages provided. Diseased progeny experienced twice the larval mortality, and surviving individuals were approximately 25% smaller and took 17% longer to complete development than healthy progeny. Maternal disease load was a significant, positive factor in percentage progeny mortality and male pupal weight.

KEY WORDS Insecta, *Choristoneura fumiferana*, *Nosema fumiferanae*, reproduction

Nosema fumiferanae (Thomson) is an intracellular, spore-producing, protozoan pathogen that maintains itself within eastern spruce budworm populations because it is typically nonvirulent and efficiently transmitted (Thomson 1958a, Wilson 1982). The incidence of this pathogen has been shown to increase during an outbreak in a density-dependent manner (Thomson 1958a; Neilson 1963; Wilson 1973, 1977; Burke 1980). Seasonal increase in disease incidence is caused by general environmental contamination with spores (Thomson 1958a, Wilson 1982). The only means demonstrated for maintaining *N. fumiferanae* across host generations is via transovarial transmission (Thomson 1958a, Wilson 1983).

Survival of larvae infected with *Nosema* depends on a complex interaction of the initial dosage (Thomson 1958b), larval age at infection (Wilson 1974), nutrition (Bauer & Nordin 1988a), climate (Neilson 1963, Wilson 1979), and other factors that influence insect developmental rate (Bauer & Nordin 1988b). Larvae sustaining sublethal infections may be characterized by prolonged developmental periods, suppressed pupal weight, reduced fecundity, and shortened adult longevity (Thomson 1958b, Wilson 1983, Bauer & Nordin 1988b). However, if the initial dose is low or received late in larval life, the insects may have no symptoms. These individuals, although overtly similar to healthy insects, may suffer some loss of reproductive function.

The objectives of this study were to quantify the effects of horizontally transmitted dosages of *N. fumiferanae* on parental fecundity, mating success, egg fertility, transmission efficiency to progeny, and the effects on F_1 progeny. These effects include stage-specific mortality, sublethal effects on developmental time and pupal weight, and correlation of progeny performance to parental response variables.

Materials and Methods

Experimental Insect. Diapause-free spruce budworm were used. Colony maintenance was similar to that described for diapausing populations (Grisdale 1970) except that following egg hatch, first instars were transferred to meridic diet (McMorran 1965) without the antibiotic aureomycin. Larvae were reared at 20 ± 1.5°C with a 16:8 (L:D) photoperiod. These insects were determined to be free of microsporidia, nuclear polyhedrosis virus, and cytoplasmic polyhedrosis virus by standard diagnostic procedures.

Bioassay Procedure for Parents. Spores of *N. fumiferanae*, reared in spruce budworm, were purified by the triangulation method (Cole 1970) and quantified with a Petroff-Hausser counting chamber (Improved Neubauer, C. A. Hausser & Sons, Philadelphia). The bioassay procedure, described in Bauer & Nordin (1988b), provided fourth and fifth instars with a known spore dosage on artificial diet in 24 h or less. Newly molted female fourth or fifth instars were selected from the colony and inoculated with 1×10^4 or 1×10^5 spores per larva, respectively. Control larvae were inoculated with sterile distilled water. Female spruce budworm larvae were identified by the absence of darkly pigmented testes which are visible in males. The selection was confirmed at pupation. Stage-

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specific mortality and insect development were monitored, and pupae were weighed the day after pupation.

Disease-free males were reared at a reduced temperature (approximately 18°C) to achieve synchronous emergence with females. Within 24 h of adult eclosion, one female and two males were placed in autoclaved pint Fonda cartons fitted with Petri dish covers at 22 ± 2°C. Fluted wax paper was used as a substrate for oviposition. Females were permitted to lay eggs until they died. The dead females from fertile matings were frozen to permit later determination of the numbers of mature (unlaid) eggs. Counts were done under a dissecting microscope, and mature eggs were detected by the abrupt change in egg size and bright green coloration within the pedicel region of the ovarioles (Outram 1971a). In addition, spore concentrations of *N. fumiferanae* were determined for each fertile female insect by using a Petroff-Hausser counting chamber; they were reported on the basis of pupal fresh weight.

Egg masses were placed in 30-ml polystyrene cups where they hatched. Unhatched eggs and larvae were counted under a dissecting microscope. Fecundity was defined as the total number of eggs laid by successfully inseminated females. Total egg complement was defined as the number of eggs laid plus eggs unlaid. Percentage egg fertility was calculated as the ratio of eggs hatched to fecundity. Matings were considered fertile if larval development was visible within the egg under a dissecting microscope. The ratio of fertile matings to initial matings represents the percentage fertile matings, with the understanding that some fertile matings may have been counted as sterile if embryos died before the visible stage.

Progeny. Within 3 d of egg hatch, F_1 progeny were placed on diet at a density of three larvae per cup up to a maximum of 50 larvae per cohort. These populations were subsampled for determinations of larval development time and fresh pupal weight. Upon daily observations, all larvae found dead were staged and evaluated microscopically for the presence of spores of *N. fumiferanae*. Transovarial transmission efficiency was determined by examining microscopically the midguts and salivary glands of 10 adult progeny per family for the presence of spores.

Pupal weight and developmental time measured for progeny reared from both maternal disease treatments did not differ significantly ($P \leq 0.05$); the results were pooled and are termed "diseased." Control progeny are termed "healthy."

Statistics. Student's t test was used to determine the significance of differences measured between sterile and fertile females or between each treatment and the control ($\alpha = 0.05$). The χ^2 procedure was used to compare differences in progeny mortality, parental mortality, fertile matings, and egg fertility. Regression analysis and the Spearman rank-order correlation were used to evaluate relationships between parental or progeny response variables for healthy and diseased populations (both disease treatments were pooled). The Statistical Analysis System (SAS Institute 1982) was used for all statistical analyses.

Results

Parents. The dosages provided to fourth and fifth instars caused about twice the mortality measured in the control groups (Table 1). The proportion of fertile matings was not significantly lowered by the disease treatments. The mean number of *N. fumiferanae* spores per adult, a measure of disease intensity, was similar for both *Nosema* treatments.

Diseased fertile females from both treatments were significantly smaller and less fecund than the controls (Table 1). An average of both *N. fumiferanae* treatments shows 10% reduction in pupal weight, 22% reduction in total egg complement, and 35% reduction in fecundity. Egg fertility was unaffected by the treatments.

Regression analysis indicated that parental responses differed relative to the health of the population (Table 2). The regression of total egg complement versus pupal weight was similar for healthy and diseased females ($P \leq 0.05$), although it was more highly correlated for the diseased population. Fecundity versus pupal weight regressions were significant for diseased insects but not for the healthy population. The number of unlaid eggs was positively correlated with weight for both groups. Diseased females (slope, 0.35 ± 0.03), when compared to healthy (slope, 0.63 ± 0.07), however, contained almost half the number of unlaid eggs, as a function of pupal weight ($P \leq 0.05$). Total egg complement was inversely correlated with final spore load in the diseased population.

Progeny. Based on microscopic evaluations of sampled progeny ($n = 10$) from each family, the efficiency of transovarial transmission from maternal parent to F_1 progeny was 100% for both maternal pathogen dose levels. Mortality of diseased progeny was twice as high as that of healthy progeny (Table 3). Surviving diseased progeny had a 27 and 22% reduction in pupal weight for females and males, respectively, compared with the healthy progeny. Developmental time was prolonged by 15% for females and 13% for males.

Significant correlations between progeny and parental response variables were present in the diseased population (Table 4). These comparisons were not significant for healthy progeny. Pupal weight of male progeny was positively correlated with maternal pupal weight. Maternal spore load was a significant and negative factor in male progeny pupal weight. Regressions of female progeny responses showed similar trends but were not significant. Progeny survival was correlated with the mean pupal weight of surviving progeny within each family. This correlation was significant for siblings of both sexes.

Table 1. Larval mortality, fresh pupal weight, total egg complement, fecundity, egg and mating fertility, and spore load (mean ± SE) of female spruce budworm following inoculation with *N. fumiferanae* as newly molted fourth or fifth instars

Maternal response	Treatments		Pooled controls (*n*)
	Fourth instar (*n*)	Fifth instar (*n*)	
Mortality (%)	17.7 (86)[a]	18.6 (94)[b]	7.8 (78)
Pupal wt (mg)	127.5 ± 5.2*	127.7 ± 3.8*	141.4 ± 3.4
TEC[c]	117.1 ± 9.9*	127.7 ± 6.9*	157.7 ± 7.8
Fecundity	87.9 ± 7.2*	92.6 ± 7.2*	139.0 ± 9.0
Egg fertility (%)	29.4 (21)	26.4 (29)	28.2 (31)
Fertile matings (%)	37.5 (56)	36.7 (79)	44.3 (70)
Spores ($\times 10^6$)	1.8 ± 0.1 (14)	1.4 ± 0.1 (19)	—

*, each treatment significantly different from the control at the $P \leq 0.01$ level of significance using Student's t test.
[a] $\chi^2 = 3.23$, $P < 0.07$ (fourth-instar treatment versus control).
[b] $\chi^2 = 4.18$, $P < 0.04$ (fifth-instar treatment versus control).
[c] TEC, total egg complement.

Discussion

Sublethal dosages of *N. fumiferanae* induced significant suppression of spruce budworm fecundity and total egg complement after inoculation at fourth or fifth instar. Reductions in fecundity caused by microsporidiosis in spruce budworm also have been reported by Thomson (1958a), Neilson (1963), and Wilson (1982).

No reduction in percentage fertile matings occurred at the dosages reported in this study. Adults produced from bioassays with higher dosages (LD_{50}) (unpublished data) were half the weight of controls and experienced a 90% reduction in fertile mating. The consistent trend across treatments and controls for sterile females to be smaller, however, suggests that mating success may be limited by female size.

Problems associated with the use of pupal weight as a predictor of insect fecundity between populations with different infestation histories have been discussed by Prebble (1941), Miller (1957), and Lorimer & Bauer (1983). Sublethal disease levels did not significantly alter the slope of this regression, but they did improve correlation. Apparently environmental conditions, such as lack of moisture for imbibing (Miller 1987), can limit adult longevity and the time available for egg maturation and oviposition. At death, healthy insects contained twice the number of unlaid eggs per milligram of pupal weight than diseased insects. Those insects with more eggs to lay, therefore, were more limited by conditions of the oviposition period. Infection by *N. fumiferanae* improved the fecundity versus pupal weight correlation by setting a stringent upper limit on egg production, presumably caused by energy store depletion (Nolan & Clovis 1985).

Suppression of potential fecundity by *N. fumiferanae* in field budworm populations may account for part of the 49% loss of expected eggs ($n = 200$) reported by the Green River life table studies (Morris 1963). This loss was attributed previously to population density, food quality and quantity, weather, mating failure, predation, dispersal, and genetic weakness (Blais 1952, 1953; Miller 1957, 1963a,b; Greenbank 1963). The significant negative correlation between spore load and total egg complement indicates that *N. fumiferanae* is an additional factor. Consideration of the quantitative response of budworm to the proliferation of spores of *N. fumiferanae* may improve the predictive value of fecundity versus pupal weight regressions.

A determination of the impact of *N. fumiferanae* on egg fertility is confounded by the process of continuous oögenesis in spruce budworm (Outram 1971a). In nature, females mate repeatedly to provide adequate sperm for the entire oviposition period (Outram 1968, 1971b). However, females continue to lay eggs until death, even if adequate sperm for insemination is lacking and the eggs are infertile. This may have been the principal contribution of percentage egg infertility in the healthy group.

The impact of *N. fumiferanae* transmitted transovarially to F_1 progeny was expressed as increased larval mortality, longer developmental time, and reduced pupal weight. Thomson (1958b),

Table 2. Regression analysis of total egg complement, fecundity, or unlaid eggs versus maternal fresh pupal weight or spores per milligram of tissue

Comparison	Diseased				Healthy			
	Slope (±SE)	*y* int.	*r*	*P*	Slope (±SE)	*y* int.	*r*	*P*
TEC[a] vs pupal wt	1.20 (0.15)	−29.85	0.75	0.0001	1.40 (0.37)	−35.57	0.56	0.0005
Fecundity vs pupal wt	0.84 (0.19)	−6.23	0.37	0.0001	0.78 (0.50)	23.05	0.26	NS[b]
Unlaid eggs vs pupal wt	0.35 (0.03)	−24.04	0.38	0.01	0.63 (0.07)	−58.61	0.38	0.02
TEC[a] vs spores/mg	−0.21 (0.08)	159.06	0.37	0.01	—	—	—	—

[a] TEC, total egg complement.
[b] Not significant.

Table 3. Larval mortality, fresh pupal weight, and development time (mean ± SE) of F_1 progeny transovarially infected with *N. fumiferanae*

Response variables	F_1 progeny	
	Diseased (*n*)	Healthy (*n*)
Sexes pooled		
Second–fourth instar mortality (%)	66.7 (533)	33.5 (427)[a]
Female[b]		
Pupal wt (mg)	91.3 ± 2.7 (71)*	125.2 ± 3.1 (143)
Larval period (d)	33.7 ± 0.5*	28.5 ± 0.5
Male[b]		
Pupal wt (mg)	63.4 ± 1.6 (87)*	81.5 ± 1.7 (141)
Larval period (d)	30.0 ± 0.3*	26.2 ± 0.4

* Diseased insects significantly different from healthy ones at the $P \leq 0.0001$ level of significance using Student's *t* test.
[a] $\chi^2 = 308.8$, $P \leq 0.001$.
[b] Pupal weights and larval periods from each treatment were compared with pooled controls.

studying naturally infected spruce budworm, reported similar findings. Additionally, these transovarially transmitted infections induced significant correlations between progeny and parental response variables that did not occur between progeny and parents within the healthy populations. Because progeny mortality is positively correlated with final maternal disease load (spores per milligram), the determination of adult disease load may prove to be a useful predictor of disease-induced mortality.

Regulating insect populations through shifts in the intrinsic quality of individuals has been suggested for various insect species (Wellington 1960, Leonard 1970). Spruce budworm populations evaluated over several generations show a steady decline in fecundity and pupal weight and an increase in the correlation coefficients for this regression (Campbell 1962). These changes were attributed to genetic selection (Campbell 1962) or genetic weakness (Greenbank 1963) developing within the population. In this study, transovarially transmitted infections of *N. fumiferanae* produced similar trends between two generations, which could be interpreted as genetic change or "weakness." Because of its subtle and asymptomatic nature, microsporidiosis in insect populations is overlooked frequently and may be the underlying cause of deteriorating population quality.

Table 4. Regression analysis and Spearman rank-order correlation of diseased F_1 progeny versus maternal response variables and progeny mortality versus progeny pupal weight, paired by family

Comparison	Diseased				
	n	Slope	*y* int.	*r*	*P*
Progeny vs maternal parent					
First instar (n) vs pupal wt[a]	52	0.50	−23.76	0.38	0.005
Wt (♂) vs pupal wt[a]	46	0.18	40.40	0.30	0.03
Wt (♂) vs spores/mg[a]	46	−0.08	74.59	0.33	0.03
Survival (%) vs spores/mg[b]	40	—	—	0.41	0.05
Progeny vs progeny					
Survival (%) vs female wt[b]	43	—	—	0.37	0.05
Survival (%) vs male wt[b]	46	—	—	0.45	0.05

[a] Linear regression.
[b] Spearman rank-order correlation.

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