Interactions of the Parasite *Pediobius foveolatus* (Hymenoptera: Eulophidae) with Two *Nosema* spp. (Microsporida: Nosematidae) of the Mexican Bean Beetle (Coleoptera: Coccinellidae)

OWN S. OWN[1] AND WAYNE M. BROOKS

Department of Entomology, North Carolina State University, Raleigh, North Carolina 27695-7613

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ABSTRACT The hymenopterous parasite *Pediobius foveolatus* (Crawford) was highly susceptible to *Nosema epilachnae* Brooks, Hazard and Becnel and *N. varivestis* Brooks, Hazard and Becnel, two naturally occurring microsporidia of the Mexican bean beetle, *Epilachna varivestis* Mulsant. After female parasites oviposited in late-instar larvae exposed previously to spores of each microsporidium as either early instar larvae or as late-instar larvae 24 h before parasite oviposition, progeny of the parasites were infected directly by the microsporidia and infection appeared to be systemic in nature. In heavily infected hosts, incidence of infection by both microsporidia in the parasites' progeny approached 100% and percentage mortality was also high. Mortality occurred primarily in the pupal stage and incidences of infection and mortality were directly related to degree of host infection or microsporidian virulence. Most infected adults were normal in appearance but some had malformed wings, greatly swollen abdomens, or both. Infection did not adversely affect the development period of emerging adults but adult longevity was significantly reduced. Adults were also susceptible to both microsporidia per os. Relatively few female parasites of the P generation that were infected with *N. epilachnae* were capable of ovipositing in host larvae, and only one female transmitted the pathogen transovarially to some of her progeny. Female parasites, infected with *N. varivestis*, successfully transmitted the microsporidian transovarially to F_6 individuals at rates varying from 5.8 to 70.0%. Both microsporidia were also transmitted mechanically from diseased to healthy hosts during parasite oviposition.

THE MEXICAN BEAN beetle (MBB), *Epilachna varivestis* Mulsant, is the most serious insect enemy of beans in infested areas of the United States (Aldred et al. 1980, Barrows and Hooker 1981, Michels and Burkhardt 1981). It is also an important pest of soybeans in many parts of the United States (Waddill and Shepard 1974). In recent years its importance as a soybean pest has increased, especially in the Southeast and along the East Coast (Aldred et al. 1980).

As a result of its economic importance, many papers dealing with the control and biology of *E. varivestis* have been published (see bibliography by Nichols and Kogan 1972). Although most crops can be adequately protected against the MBB by a number of chemical insecticides, considerable efforts have also been made to utilize various natural enemies of the MBB as biological control agents (Schroder 1981). Inundative release programs with the eulophid parasite *Pediobius foveolatus* (Crawford) have been particularly successful (Stevens et al. 1975a, Coulson 1976, Barrows and Hooker 1981), and the parasite is the primary factor around which several pest management programs for the MBB have been structured recently (Coulson 1976, Schroder 1981). However, such programs are dependent on the successful rearing of the parasite under laboratory conditions.

During laboratory rearing of MBB for the production of this parasite, researchers at Clemson University and subsequently at North Carolina State University discovered a microsporidium that appeared to be a limiting factor in maintaining not only the MBB colony itself but also cultures of *P. foveolatus* (Brooks et al. 1980). While studying the prevalence of infection in laboratory and field populations of *E. varivestis*, they also found a second species in the MBB. These two species have been recently described, respectively, as *Nosema epilachnae* and *N. varivestis* (Brooks et al. 1985).

Since MBB larvae are used as hosts for mass rearing *P. foveolatus*, the purpose of this study was to determine the specific effects of these two species of microsporidia on *P. foveolatus* and the general nature of the host/parasite/pathogen interrelationships involved.

Materials and Methods

Production of the Pathogens. Isolates of the two microsporidia used in this study were derived from strains previously reported by Brooks et al. (1980)

[1] Current address: Faculty of Science, El Fateh Univ., Tripoli, Libya.

that had been maintained by frequent passage through larvae of *E. varivestis*. Suitable quantities of spores of each strain were produced periodically by feeding neonate MBB larvae with disks cut from lima bean leaves inoculated with ca. 0.1 ml of stock spore suspensions of each species. Spores were harvested from heavily infected cadavers by maceration in distilled water, filtration through cheese cloth, and differential centrifugation. Clean spore suspensions of each species in distilled water were stored at 4°C for up to 3 months before utilization.

Production of *E. varivestis*. Egg masses of the MBB were obtained as needed from a microsporidian-free stock culture maintained at North Carolina State University. Upon hatching, the larvae from several egg masses were placed on a flat of 3- to 5-week-old lima beans and housed in a saran-screened, wooden-frame cage under greenhouse conditions. The plants were watered daily until late third or early fourth instars were obtained, generally between 9 and 14 days. Samples of these larvae were checked routinely to assure their microsporidian-free status before use as hosts for parasite production.

Production of *P. foveolatus*. The culture of *P. foveolatus* used in this study was obtained from the North Carolina Department of Agriculture, Raleigh, and was maintained using procedures similar to those described by Stevens et al. (1975b).

Host/Parasite/Pathogen Interactions. Female parasites were allowed to oviposit in third- or fourth-instar MBB previously exposed to spores of the microsporidia according to the following protocol. The spore concentration in stock suspensions of each microsporidian species was determined with a bacteria counter (Petroff-Hauser), and adjustments were made to obtain concentrations of 1.0×10^5 spores per ml of *N. epilachnae* and 1.0×10^6 spores per ml of *N. varivestis*. Preliminary infectivity tests established that late-instar, infected larvae could be obtained by feeding neonates on leaf disks (21 mm in diam) inoculated with 0.1 ml of each spore suspension. Control disks were inoculated with distilled water only. Each leaf disk was placed in a 30-ml plastic jelly cup containing moistened filter paper. Larvae were exposed in groups of five per disk for 48 h at 26.5°C. Thus, each larva was exposed per os to ca. 2×10^3 and 2×10^4 spores of *N. epilachnae* and *N. varivestis*, respectively, assuming each larva ate about ⅕ of the treated disk. Tests were also conducted with both microsporidia utilizing late-instar larvae exposed individually to the microsporidium 24 h before parasite exposure. In these exposure tests, host larvae were either inoculated with 5×10^3 spores of *N. epilachnae* or 5×10^4 spores of *N. varivestis*. However, one test series was also conducted with *N. epilachnae*, in which larvae were exposed individually to between 5×10^4 and 2×10^5 spores.

In tests involving neonates, larvae were transferred to petri dishes containing fresh lima bean leaves after the 48-h exposure period and leaves were changed daily until larvae reached the third or fourth instar. Larvae were then placed individually in a 35-ml plastic vial with a small circular hole in the bottom covered by a piece of fine-mesh organdy cloth. A terminal lima bean leaflet was added to each exposure chamber, along with a 1- to 12-day-old female parasite from the stock culture. Each larva was exposed to the parasite for 24 h. After removing the parasite, lima bean foliage was added to each chamber daily until the larva mummified or pupated. A drop of honey was placed on the organdy mesh in each vial to serve as food for the emerging parasites.

Records were maintained on the developmental period of emerging adults, the sex and condition of the adults, date of adult death, and the incidence of infection in both emerged and non-emerged parasites as well as the host cadaver. Evidence of infection was determined by the examination of wet-mount preparations of tissue smears with phase microscopy. For histological observations, prepupal and pupal stages of *P. foveolatus* that had developed in uninfected and microsporidian-infected hosts were prepared as described by Brooks et al. (1985).

The susceptibility of adults to each microsporidium was also determined per os by adding spores of each species to honey placed as food in vials containing newly emerged adults. Adults were exposed to concentrations of 7.5×10^5 spores of *N. epilachnae* and 5.5×10^6 spores of *N. varivestis*. Each adult was examined for infection at death.

Efforts were also made to determine if the parasite could transmit each species transovarially or mechanically. Female parasites that had emerged from infected hosts were allowed to oviposit immediately in healthy third or early fourth instars. Female progeny of subsequent generations were allowed to oviposit in healthy hosts before examining them for infection. Records were maintained on the incidence of infection in the parasite's progeny as well as the host cadaver. In the mechanical transmission study, 1- to 12-day-old female parasites were first confined individually in sterile plastic 35-ml vials, each containing one heavily infected host larva. Each female was allowed to remain therein for 1–2 h, a time sufficient for the parasite to sting the host larva repeatedly, thereby contaminating their ovipositor. Each female was transferred to a new vial containing a single late third- or early fourth-instar healthy host larva and allowed to remain therein until she had apparently oviposited in the host or for about 1 h. In the first test seven females were each exposed successively to eight host larvae; however, only five hosts were exposed sequentially to each of 10 females in a second trial. Each exposed larva was maintained separately. Dead hosts were dissected to ascertain parasitization, and wet-mount preparations of host larvae and parasite progeny were prepared to check for infection.

Table 1. Development of *P. foveolatus* in larvae of *E. varivestis* infected with *N. epilachnae*

Host category	No. hosts parasitized	No. progeny per host ($\bar{x}$ ± SD)	% progeny per host				
			Mortality			Adult emergence ($\bar{x}$ ± SD)	Infection ($\bar{x}$ ± SD)
			Larva ($\bar{x}$ ± SD)	Pupa ($\bar{x}$ ± SD)	Adult ($\bar{x}$ ± SD)		
Infected							
(Group A)[a]	41	15.0 ± 6.5a	23.5 ± 40.9a	70.3 ± 39.5a	1.6 ± 5.0ab	4.6 ± 10.7a	96.0 ± 8.7a
(Group B)[b]	17	19.2 ± 8.4b	0b	28.6 ± 24.8b	2.8 ± 3.3ab	68.6 ± 25.1b	32.3 ± 26.6b
(Group C)[c]	19	19.9 ± 8.7b	0b	63.0 ± 40.6a	4.3 ± 6.8a	32.7 ± 40.5c	72.7 ± 40.5c
Control	147	20.2 ± 7.1b	0.1 ± 0.3b	0.8 ± 2.8c	0.5 ± 3.6b	98.6 ± 4.6d	0d

Means in a column followed by the same letter are not significantly different ($P = 0.01$; Duncan's [1955] multiple range test).

[a] Exposed as groups of five neonate larvae per leaf disk to 1×10^4 spores per disk and allowed to reach the third or early fourth instar before parasite exposure.

[b] Exposed individually as late instars to 5×10^3 spores per leaf disk for 1 day before parasite exposure.

[c] Exposed individually as late instars to 5×10^4 to 2×10^5 spores per leaf disk for 1 day before parasite exposure.

Statistical Analysis. The data were analyzed using a one-way analysis of variance and Duncan's (1955) multiple range test in conjunction with Kramer's (1956) procedure for comparing means of unequally replicated treatments.

Results

Interactions with *N. epilachnae*

Parasite Susceptibility and Pathological Effects of Infection. As shown in Table 1 (Group A), parasites that developed in hosts exposed as first instars were highly susceptible to *N. epilachnae*. Significantly fewer parasite progeny were produced from infected hosts on the average than healthy hosts. An average of 96% of the progeny became infected, and most were unable to complete their development and emerge as adults from host cadavers. However, a majority of the few parasites that emerged as adults escaped infection, as the incidence of infection in the dead (nonemerging) progeny (98.7%) was much higher than in those that emerged successfully as adults (40.6%). The high virulence of *N. epilachnae* for *P. foveolatus* is also reflected by the average mortality rate of 95.4% for parasites developing in infected hosts compared to only 1.4% mortality for the control group.

The significant relationship between parasite susceptibility and degree of host infection was demonstrated in trials where the parasites were allowed to develop in late-instar larvae exposed to the microsporidium only 24 h before oviposition (Table 1, Groups B and C). In larvae exposed to the lowest dosage of spores (Group B), only 32.3% of the progeny were infected on the average; in contrast, an average infection rate of 72.7% was obtained in progeny that developed in host larvae exposed to the higher spore dosages (Group C). The less severe degree of host infection in these two groups was also correlated significantly with higher average numbers of progeny per host as well as higher average percentages of the progeny that were able to complete their development when compared to those in Group A.

Parasite mortality due to microsporidiosis occurred primarily in the pupal stage (Table 1). In parasites that developed in hosts exposed to the microsporidium as early instar larvae (Group A), pupal mortality averaged 70.3% compared with 23.5% in the larval stage and only 1.6% in the adult stage. Parasite mortality levels in control hosts were very low. The less severe nature of the infection accompanying the development of parasites in late-instar larvae exposed to the microsporidium 24 h before parasitization is also reflected by similar data in this table (Groups B and C). None of these progeny died as larvae, and most of those that emerged successfully as adults were individuals that escaped infection.

Despite the high-mortality characteristics of infection by *N. epilachnae* in *P. foveolatus*, a small percentage of the individuals developing in infected hosts either escaped infection or were able to emerge as infected adults. Many of the infected adults were normal in appearance macroscopically but 34% (16 of 47) were obviously malformed. These adults were marked most conspicuously by malformed wings, greatly swollen abdomens, or both. A few adults were so severely affected that they died during pupal eclosion or while exiting from their hosts.

Observations from wet-mount preparations of tissue smears and histological preparations revealed that *P. foveolatus* was infected systemically upon development in larvae infected with *N. epilachnae*. Spores were observed in all body regions of adult parasites and were definitely present in the epidermis, fat body, and muscles of sectioned pupae.

The lengths of the developmental periods of both the uninfected and infected adults that successfully emerged from infected hosts were not significantly different, averaging about 14 days. However, the average longevity of the infected adults was significantly reduced (Table 2), with none living longer than 4 days.

When newly emerged adults ($n = 164$) were exposed per os to spores, the longevity of those that became infected was also reduced significantly.

Table 2. Longevity of *P. foveolatus* reared from larvae of *E. varivestis* uninfected and infected with *N. epilachnae*

Host category	♂♂			♀♀		
	n	$\bar{x}$ (days)	SD	*n*	$\bar{x}$ (days)	SD
Exposed as first instars	2	<1a		11	2.5a	1.3
Exposed as third or fourth instars[a]	5	2.8a	1.3	17	3.5a	1.6
Controls[b]	88	17.9b	9.9	257	36.4b	26.6

Means in a column followed by the same letter are not significantly different ($P = 0.01$; Duncan's [1955] multiple range test).

[a] Data represent a composite of all parasitization trials involving late instars exposed to spores 1 day before parasite exposure (Groups B and C, Table 1).

[b] Based on parasites that emerged from 25 healthy hosts.

Infected male ($n = 35$) and female ($n = 98$) parasites lived for 5.8 and 6.1 days, respectively, compared to 15.8 days for control males ($n = 52$) and 36.3 days for control females ($n = 140$); 82.1% of the exposed adults became infected.

The relatively few infected adults that emerged from infected hosts and their short-lived nature precluded detailed observations on adult mating success, fecundity, and transovarian transmission. However, limited tests with some of the infected adults revealed that only 2 of 15 infected female parasites successfully parasitized healthy host larvae to which they were exposed. However, these two host larvae died prematurely and none of the immature parasites within were found to be infected. Despite this lack of evidence for transovarian transmission, at least one infected female parasite in preliminary tests was found to have transmitted the microsporidium transovarially to some of its progeny. Vegetative stages and spores were found in smear preparations stained with Giemsa's solution in a few of this parasite's progeny.

Transmission During Parasite Oviposition. *N. epilachnae* was transmitted mechanically during oviposition by 6 of 7 parasites in the first test and by 9 of 10 females in the second test. The most efficient vector transmitted the pathogen to 75% (three of four) of its parasitized hosts. However, the average rate of transmission to the parasitized hosts was only 38.7%. Most of the parasites' progeny that developed in these infected hosts escaped infection, but an average of 12.4% (0–54.5%) of the progeny from such hosts did become infected. No logical sequence in the mechanical transmission of the pathogen was apparent.

Table 4. Longevity of *P. foveolatus* reared from larvae of *E. varivestis* uninfected and infected with *N. varivestis*

Host category	♂♂			♀♀		
	n	$\bar{x}$ (days)	SD	*n*	$\bar{x}$ (days)	SD
Exposed as first instars	73	4.8a	3.1	243	7.9a	5.7
Exposed as third or fourth instars	10	8.4a	6.8	34	17.1ab	14.0
Controls[a]	192	17.1b	8.8	502	37.4b	25.2

Means in a column followed by the same letter are not significantly different ($P = 0.01$; Duncan's [1955] multiple range test).

[a] Based on parasites that emerged from 46 healthy hosts.

Interactions with *N. varivestis*

Parasite Susceptibility and Pathological Effects of Infection. *P. foveolatus* also proved to be highly susceptible to *N. varivestis*, especially when allowed to parasitize late-instar hosts exposed to the microsporidium as first instars (Table 3, Group A). All of the progeny produced in such infected hosts were infected and only 48.9% of them were able to emerge successfully as adults. The average number of progeny per infected host was also significantly lower than the control group. Compared with an average mortality rate of only 1.3% in the control, 51.1% of the progeny produced in the infected hosts died, primarily in the pupal stage of development.

Although *P. foveolatus* is highly susceptible to *N. varivestis*, the relatively low virulence of this microsporidium was more readily apparent when the parasite was allowed to develop in late-instar hosts exposed only 24 h before parasite oviposition

Table 3. Development of *P. foveolatus* in larvae of *E. varivestis* infected with *N. varivestis*

Host category	No. hosts parasitized	No. progeny per host ($\bar{x} \pm$ SD)	% progeny per host				
			Mortality			Adult emergence ($\bar{x} \pm$ SD)	Infection ($\bar{x} \pm$ SD)
			Larva ($\bar{x} \pm$ SD)	Pupa ($\bar{x} \pm$ SD)	Adult ($\bar{x} \pm$ SD)		
Infected							
(Group A)[a]	43	17.3 ± 6.5a	1.5 ± 6.8a	45.0 ± 35.4a	4.6 ± 8.2a	48.9 ± 37.0a	100a
(Group B)[b]	16	19.8 ± 4.9ab	0a	2.1 ± 3.7b	0.4 < 0.1b	97.5 ± 4.6b	29.7 ± 25.4b
Control	154	20.4 ± 7.4b	0.1 < 0.1a	0.9 ± 3.6b	0.3 ± 1.7b	98.7 ± 3.9b	0c

Means in a column followed by the same letter are not significantly different ($P = 0.01$; Duncan's [1955] multiple range test).

[a] Exposed as groups of five neonate larvae per leaf disk to 1×10^5 spores per disk and allowed to reach the third or early fourth instar before parasite exposure.

[b] Exposed individually as late instars to 5×10^4 spores per leaf disk for 1 day before parasite exposure.

Table 5. Fecundity and parasitization capacity of *P. foveolatus* as influenced by infection with *N. varivestis*

Parasite category	Parasite to host ratio	No. larvae exposed	% larvae parasitized[a] ($\bar{x}$ ± SD)	No. progeny produced per	
				Parasitized larva ($\bar{x}$ ± SD)	Parasite used ($\bar{x}$ ± SD)
Infected (P)	1:1	132	28.0 ± 45.1	12.2 ± 6.7	3.4 ± 6.5
Infected (F_1)	1:1	27	55.6 ± 50.2	14.9 ± 4.5	8.3 ± 8.2
Control	1:1	129	88.4 ± 32.2	20.0 ± 8.4	17.7 ± 10.2
Infected	1:3	84	26.2 ± 32.8	16.9 ± 8.2	13.3 ± 17.7
Control	1:3	84	69.0 ± 29.6	12.4 ± 3.5	25.8 ± 10.7

[a] Based only on larvae from which parasites emerged.

(Table 3, Group B). In these hosts, only 29.7% of the progeny produced were infected and 97.5% were able to emerge successfully as adults. The infection rate in emerging adults was only 31.3% and the average percentage mortality rates were not significantly different from those of the control (2.5 versus 1.3%). There were also no significant differences in the average number of progeny per host or in the number of progeny per host able to reach the adult stage. Even in the relatively few progeny that failed to emerge as adults, only 12.5% were infected. Thus, most of the progeny either escaped infection or were able to emerge as adults despite being infected.

Most of the infected adults were macroscopically normal in appearance. However, 12.2% (49 of 402) of the parasites that emerged from host larvae exposed to the microsporidium as early instars and 3.6% (3 of 83) emerging from hosts exposed as late instars were malformed. Deformities were again manifested primarily as malformed wings and greatly swollen abdomens. Limited histological observations indicated that infection was systemic, with spores detected in adipose tissue, muscle, midgut epithelial cells, and in the ventral nerve cord.

The lengths of the developmental periods of both the uninfected and infected parasites that emerged successfully as adults were not significantly different, averaging ca. 14 days. However, adult longevity (Table 4) was significantly reduced; infected males lived an average of <5 days and females <8 days upon emerging from hosts exposed as first instars to the microsporidium. Although the average longevity of adults that emerged from hosts exposed as late instars was about twice as long, the differences were not significant. And, only the longevity of the males was significantly less than that of the control adults.

The adults (65.8% of 144) also proved to be susceptible per os to infection by *N. varivestis* when allowed to feed on honey water containing spores. However, neither male nor female longevity was reduced significantly over that of the controls.

Effects on Parasite Fecundity and Mating Success. Although their longevity was significantly reduced, most adult parasites that emerged from hosts exposed to *N. varivestis* as early instars lived sufficiently long to mate and oviposit, thus allowing observations on fecundity and parasitization capacity (Table 5). At a parasite to host ratio of 1:1, only 28% of the host larvae were parasitized by infected female parasites when placed with their host for 24 h, in contrast to an 88.4% parasitization rate for healthy females. The infected parasites also produced an average of only 12.2 progeny per host compared to 20.0 progeny per host for the controls. This reduction in parasitization capacity is more readily visualized by comparing the average number of progeny produced per parasite used (3.4 versus 17.7). When the parasite to host ratio was modified to 1:3, similar reductions in fecundity and parasitization capacity were obtained, particularly when the average number of progeny produced per parasite used (13.3 versus 25.8) was compared. The average number of progeny produced per parasitized host was higher for the infected females, but they successfully parasitized only 26.2% of their hosts compared with a 69.0% parasitization rate for the control females. Although the fecundity and parasitization capacity records for infected F_1 females are lower than for the control parasites (Table 5), the parasitization rate and average number of progeny produced per parasite used were about twice those of the parental generation. Upon dissection postoviposition, these F_1 females were found to be generally less heavily infected than those of the parental generation.

Observations on mating success were obtained by using infected females (those emerging from hosts exposed as first instars) that were allowed to remain within rearing vials with males at a known average sex ratio for 4–12 days. Although the average sex ratios of males to females for both the infected and control parasites were fairly similar (1:3.8 versus 1:4.8, respectively), the infected females produced more males than did control females (a male to female ratio of 1:1.7 versus 1:5.4, respectively). The production of a higher number of males by the infected females indicates that fewer fertilized eggs were laid by the infected females, possibly as a result of poor mating success among the infected parasites.

Transovarian and Mechanical Transmission. As shown in Table 6, 23 of 83 infected females in the parent (P) generation transmitted the pathogen transovarially to an average of 29.4% of their progeny. Transmission rates of F_1–F_4 females were similar, averaging between 22.9 and 31.4% of the

Table 6. Incidence of transovarian transmission of *N. varivestis* in six generations of *P. foveolatus*

Parasite generation no.	No. infected ♀♀		% parasite offspring infected[b]		
	Ovipositing in host larvae	Transmitted pathogen transovarially	$\bar{x}$	SD	Range
P[a]	83	23	29.4	18.5	8.3–70.0
F_1	15	4	22.9	11.2	11.1–38.1
F_2	6	1	31.4	—	—
F_3	8	5	24.7	16.8	6.7–41.7
F_4	10	5	26.3	18.3	7.7–44.8
F_5	8	2	5.8	0.7	5.3–6.3

Third or fourth instars of *E. varivestis* used as host insects.

[a] Parent females had developed in infected hosts that had been exposed to the pathogen as first instars.

[b] Percentage based on only the females that actually transmitted the microsporidium to at least one of their progeny.

progeny produced by those females that transmitted the microsporidium. However, the infection rate fell greatly with the F_5 females and the culture was discontinued with the production of F_6 parasites when only one male and one female were infected. Except for the P-generation parasites (i.e., those emerging from infected hosts exposed as early instars to *N. varivestis*), the maximum rate of transovarian transmission by any one female was always to <50% of her progeny. Efforts were not made to obtain complete records on all parameters that would accurately reflect the effects of infection on the transovarially infected parasites. However, the only noticeable effect was found in the 15 F_1 females that produced an average of only 14.9 progeny per host. The average number of progeny produced per host for the F_2–F_5 females ranged from 17.2 to 28.4, which more closely corresponds to the number of progeny produced by healthy females. In addition, most of the transovarially infected adults appeared normal, not obviously short-lived, and the average percentage mortality among the progeny of infected females was low, ranging from 1 to 12.6%.

Of seven female parasites that were placed with an infected host for 1 h to allow for oviposition, only two females successfully transmitted the pathogen during the act of oviposition. The most efficient of these two vectors transmitted the microsporidium to two of three parasitized hosts. In both of these instances of mechanical transmission, the first host parasitized by each female represented two of the three hosts to develop an infection. In addition, none of the progeny produced in these mechanically infected hosts became infected or exhibited any obvious adverse effects from having developed in the infected hosts.

Discussion

As suggested by Brooks et al. (1980), *P. foveolatus* proved to be highly susceptible not only to *N. epilachnae* but also to the other microsporidian species, *N. varivestis*, of the MBB. The host/parasite/pathogen interactions are similar in many respects to other systems where the parasite has been shown to be directly invaded by a microsporidium or some other protozoan of the host insect itself (Allen and Brunson 1945, Allen 1954, Tanada 1955, McLaughlin and Adams 1966, Hostounsky 1970, Smirnoff 1971a,b, Brooks and Cranford 1972, Brooks 1973, Bell and McGovern 1975, Andreadis 1980). Such infected parasites usually die as larvae or pupae or the adults may be malformed and short-lived (Brooks 1973).

The specific aspects of the host/parasite/pathogen interrelationships involved in this study were most obviously related to the degree of host infection and to the differential virulence of the two *Nosema* species. Thus, the pathological manifestations exhibited by *P. foveolatus* were greater in hosts exposed as neonate larvae to each microsporidian species before parasitization as late instars and in hosts infected with *N. epilachnae*, the more virulent of the two species. For example, in heavily infected hosts (i.e., those exposed as neonates), females of *P. foveolatus* produced significantly fewer progeny per host and infection rates among the progeny approached 100% with both species of microsporidia (Tables 1 and 3, Group A, respectively). However, there was no significant difference in the number of progeny produced in hosts exposed to either microsporidium as late instars only 1 day before parasitization, and the infection rates among the progeny of these hosts were significantly lower (Table 1, Groups B and C; Table 3, Group B). Similarly, mortality rates for *P. foveolatus* were significantly higher in the progeny of hosts exposed as neonate larvae to both species of microsporidia, and the overall average mortality rates were higher for progeny developing in hosts exposed to *N. epilachnae* even though lower spore dosages were generally used to infect these hosts than for those exposed to *N. varivestis*.

Although most of the infected progeny died as pupae, some infected as well as uninfected adults were able to emerge from host cadavers, especially those infected with *N. varivestis*. A few adults infected with either species were malformed but most were normal in appearance, as also observed in other microsporidian-infected parasites by Allen (1954) and Brooks and Cranford (1972). And, as noted in several other studies (Allen and Brunson 1945, McCoy 1947, Allen 1954, Brooks and Cranford 1972, Brooks 1973, Andreadis 1980), the longevities of infected male and female adults were significantly lessened over that of control adults, with the specific reductions again related to the degree of host infection and the differing virulence of the two *Nosema* species. Interestingly, adults also were found to be susceptible per os to both microsporidia, but adult longevity was only reduced significantly by *N. epilachnae*. The only other adult parasite reported to be susceptible per

os to its host protozoan is *Bracon mellitor* Say (McLaughlin and Adams 1966).

Lowered fecundity of infected parasites is also a common consequence of protozoan infection (Allen and Brunson 1945, McCoy 1947, Issi and Maslennikova 1966, Brooks 1973). In *P. foveolatus*, infected females that emerged from hosts exposed to either species of *Nosema* as first instars parasitized fewer hosts and produced fewer progeny per host at a parasite to host ratio of 1:1. Similar results were obtained with female parasites infected with *N. varivestis* at the parasite to host ratio of 1:3 (Table 5). Using this same parasite-host ratio (1:3), Stevens et al. (1975b) found that healthy female parasites produced an average of 7.5 progeny per host used or 25.2 individuals per female parasite used—data comparable with that of the control in this study of 8.6 parasites per host exposed or 25.8 progeny per female used.

Fecundity was also reduced in F_1 females infected with *N. varivestis* (Table 5). However, the fecundity of subsequent generations appeared to be normal, apparently due to the dilution of the degree of infection in parasites of the subsequent generations.

Few observations on the mating success of adult parasites as affected by protozoan infection have been reported. In the progeny of females infected with *N. varivestis*, the sex ratio averaged 1 male: 1.7 females compared with a ratio of 1 male: 5.4 females for controls. Stevens et al. (1975b) reported an overall sex ratio of 1 male: 6.75 females for laboratory reared parasites. Moreover, Stevens et al. (1977) found that, as the number of parasites emerging per host exceeded 20 adults, the sex ratio was almost halved instantly and continued to decline as numbers increased. The sex ratio of progeny of *N. varivestis*-infected adults, however, increased as the number of emerging parasites per host increased. This inconsistency is probably due to the fact that females that produced an average of >20 progeny per host were apparently less severely infected and, as a result, more may have been able to mate successfully than the severely infected female parasites.

Observations reported herein on the transmission of both *Nosema* species mechanically during oviposition and on the transovarial transmission of *N. varivestis* by *P. foveolatus* are similar to those reviewed by Brooks (1973). There was no logical sequence of mechanical transmission and the rapid dilution of the degree of infection appeared to negate any detrimental effects in transovarially infected adults under field conditions. However, the high susceptibility of *P. foveolatus* to both *N. epilachnae* and *N. varivestis*, and the detrimental effects of infection by both species, indicate that this parasite should be mass-produced for inundative release programs using only healthy MBB larvae. Thus, colonies of the MBB should be checked periodically to maintain their microsporidian-free status, and new individuals added to a culture should be obtained from eggs of either field-collected or laboratory reared adults that are also free of infection.

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