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Comparative Effects of *Nosema epilachnae* and *Nosema varivestis* on the Mexican Bean Beetle, *Epilachna varivestis*

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In comparative tests with two species of microsporidia of the Mexican bean beetle, *Epilachna varivestis*, *Nosema epilachnae* was decidedly more virulent than *N. varivestis*, with the lowest dosage rate of *N. epilachnae* (1×10^3 spores per larva) resulting in an adult emergence rate of only 50% in contrast to a 96.3% survival rate of larvae exposed to the highest dosage rate of *N. varivestis* (1×10^6 spores per larva)—a 1000-fold difference in spore dosage rates. The adults infected with *N. varivestis* also survived an average of two to three times longer than those infected with *N. epilachnae*. Although both species produced significant reductions in adult longevity and fecundity, the test individuals were exposed to *N. epilachnae* as either late-stage larvae or as newly emerged adults while those exposed to spores of *N. varivestis* were inoculated as neonate larvae. Reductions in fecundity were manifested as lower average numbers of egg masses per adult and egg masses per adult per day; however, neither species reduced the average number of eggs per mass on a consistent basis. Reductions in adult longevity and fecundity by *N. epilachnae* were related to adult age at exposure. Both species were also transmitted transovarially, but the average rate of transmission of *N. varivestis* was low and did not increase in time. In contrast, the incidence of transovarian transmission of *N. epilachnae* increased with time to levels approaching 100% and was accompanied by a dramatic decline in egg hatching rates. The highly virulent nature of *N. epilachnae* for both larvae and adults of the MBB indicates that further effort to evaluate its potential as a microbial control agent is warranted.

KEY WORDS: Mexican bean beetle; *Epilachna varivestis; Nosema epilachnae; Nosema varivestis;* Microsporidia, comparative pathogenicity.

INTRODUCTION

The Mexican bean beetle (MBB), *Epilachna varivestis*, is a serious pest of bush beans and soybeans in the United States (Aldred et al., 1980; Barrows and Hooker, 1981; Michels and Burkhardt, 1981) and has become the focus of increasing attention by workers interested in biological and microbial control. The eulophid parasite *Pediobius foveolatus* is the primary factor around which several pest management programs for the MBB have been recently structured (Stevens et al., 1975; Coulson, 1976; Schroder, 1981), while microbial control efforts have involved preparations of *Bacillus thuringiensis* that contain a heat-stable exotoxin (Cantwell and Cantelo, 1982; Cantwell et al., 1985). However, such formulations of *B. thuringiensis* are not yet registered for use in the US.

The MBB has been considered to be virtually free of naturally occurring pathogens in the US (Aldred et al., 1980), but early reports of a few bacteria and fungi of uncertain taxonomic status and more recent reports of viruses and a fungus associated with the MBB are briefly reviewed by Brooks et al. (1985). In addition, two microsporidia initially reported as pathogens of the MBB in a note (Brooks et al., 1980) have been recently described as new species of the genus *Nosema* (Brooks et al., 1985). Their interrelationships with *P. foveolatus* have also been recently described (Own and Brooks, 1986).

The purpose of the present paper is to describe the host–pathogen relationships

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of these two microsporidia, *Nosema epilachnae* and *N. varivestis*, with the MBB and to enhance awareness of the potential of at least one of the species as a microbial control agent for the MBB.

MATERIALS AND METHODS

Isolates of the two microsporidia were derived from strains previously reported by Brooks et al. (1980) that had been maintained by frequent passage through larvae of *E. varivestis*. Suitable quantities of spores of each species were produced periodically by feeding neonate MBB larvae with disks cut from lima bean leaves inoculated with ca. 0.05 ml of stock spore suspensions of each species. Spores were harvested from heavily infected cadavers by masceration 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 1 month before utilization in infectivity studies.

Mexican bean beetles were obtained as needed from a microsporidian-free stock culture maintained at North Carolina State University on flats of 3- to 5-week-old lima beans (Fordhook 242). Samples of larvae and adults were checked routinely to assure their microsporidian-free status before use in various infectivity studies.

The primary pathogenic effects of each microsporidian species on the MBB were determined by exposing neonate larvae to varying spore dosages of each *Nosema* species. One to three replicates of 25 larvae each were exposed in each treatment; replicates were combined for analysis. However, because of the high virulence of *N. epilachnae* for the MBB, tests carried out to determine its influence on adult longevity and fecundity also involved the exposure of late-stage larvae and newly emerged adults to spores of this species.

In the tests involving neonates, leaf disks (21 mm in diameter) were inoculated with either 0.05 or 0.025 ml of each spore suspension, placed in a 30-ml plastic jelly cup containing a thin layer of 2% agar, and allowed to dry for ca. 1 hr prior to the addition of the neonate larvae. Larvae were exposed in groups of five per disk for 48 hr at 26.5°C. Since each larva ate about ⅕ of the treated disk, spore dosage was calculated on the basis of a further ⅕ dilution of the original spore count per milliliter of stock suspension. The spore concentration in stock suspensions of each species was determined with a bacterial counter (Petroff-Hauser), and adjustments were made to obtain the desired concentration per milliliter. In tests involving late-stage larvae or adults, only one individual was placed on a single leaf disk treated and handled in a similar manner for a 24- to 48-hr exposure period. Only individuals or groups of larvae that consumed more than 90% of the leaf disks within 48 hr were used in the various treatments in each test. In tests with newly emerged adults, each individual was allowed to feed for 24 hr prior to exposure to the microsporidium and was mated immediately after removal from the exposure chamber. After the exposure period, each larva or pair of mated adults was maintained in a rearing room at 26.7°C, 60% RH, and 16L:8D photoperiod within separate plastic Petri dishes (15 × 100 mm) with fresh leaves added daily for the duration of each test. Of several options compared in preliminary tests, these conditions proved to be the most satisfactory for rearing individual larvae to adults that were long lived and reproductively active.

In tests designed to determine effects on adult longevity and fecundity, MBB were exposed either as late-stage larvae or as adults to spores of *N. epilachnae*. The first test with this species involved the following treatments: infected females (I♀) (exposed as late-stage larvae) × healthy males (H♂); I♀ (exposed as newly emerged adults) × H♂ ; H♀ × infected males (I♂) (exposed as late-stage larvae; and H♀ × H♂. Since the infected adults died fairly rapidly in this test (most within 3 weeks), the second test involved only females that were exposed as

newly emerged adults after intervals of 1 (T_1), 4 (T_4), or 7 (T_7) days post adult emergence. A recently emerged and healthy male was placed with each female immediately after the exposure period. An appropriate control was included in the test. Two tests with *N. varivestis* were also conducted utilizing beetles inoculated only as neonate larvae. The tests differed in the spore dosage to which the neonate larvae were exposed and in the number of days post adult emergence during which data were obtained on ovipositional activities.

Because of the extensive effort required and often poor results obtained, only limited efforts were directed at obtaining data on egg hatch and transovarian transmission of each microsporidian species. Individual egg masses attached to the leaf surface on which they had been oviposited were transferred to a Petri dish which contained a fresh lima bean leaf (periodically replaced) to provide adequate atmospheric moisture within the dish. These conditions proved to be the best of several techniques tried to obtain data on egg hatch. Attempts to surface-sterilize the eggs or to provide moisture on filter paper or via cotton wicks resulted in extremely poor egg hatching rates. Observations on transovarian transmission were obtained by dissecting approximately 10 neonate larvae per egg mass or by examining the entire egg mass as a squash preparation when the eggs failed to hatch.

Records were maintained on the length of the larval and pupal period; date of larval, pupal or adult death; number of egg masses per female; number of eggs per mass; and egg hatching rate. However, no effort was made to relate the developmental period or adult longevity to the sex of the adult, except in those tests designed specifically to measure adult fecundity and longevity. All individuals were examined at death or at the end of each test for microsporidian infection in wet-mount preparations of tissue smears by phase microscopy.

The data were analyzed using a one-way analysis of variance and Tukey's procedure for comparing means of unequally replicated treatments. In one test, means were compared directly using Student's t test.

RESULTS

As summarized in Table 1, *N. epilachnae* was much more virulent for the MBB than *N. varivestis*. Of the neonate larvae exposed to the highest dosage of spores of *N. varivestis*, 96.3% of the infected individuals emerged as adults while only 50% of those infected at the lowest dosage of *N. epilachnae* successfully emerged as adults. Infection rates for both species were directly related to spore dosage although there was little difference between the percentage infection with *N. epilachnae* at the two highest dosage rates. Larval mortality was also directly related to spore dosage for *N. epilachnae*, but pupal mortality was the same for the two lowest spore dosage rates. There were no differences in the pupation and adult emergence rates of larvae infected with *N. varivestis*. The average larval and total developmental periods of individuals infected with *N. epilachnae* in all three treatments were similar and significantly longer than those infected with *N. varivestis* or control beetles; however, only the average pupal period of those infected with the lowest dosage rate of *N. epilachnae* was lengthened significantly. The average larval, pupal, and total developmental periods of infected beetles in both treatments of *N. varivestis* were similar and not significantly different from those of control beetles. The adults in all of the treatments were held until death, with some of the noninfected or control beetles surviving for as long as 95 days. Adults infected with *N. epilachnae*, however, were short-lived (averaging only 5 to 10 days in the two treatments in which some adults successfully emerged), while those infected with *N. varivestis* lived significantly longer (averaging 27 to 35 days). Only those infected adults exposed to the highest spore

TABLE 1

SUMMARY OF THE PATHOGENIC EFFECTS OF *Nosema epilachnae* AND *Nosema varivestis* ON THE MEXICAN BEAN BEETLE, *Epilachna varivestis*[a]

Treatment[b] (spores/ larva)	No. larvae per treatment	No. (%) infected	No. (%) pupation	Larval period (days) $\bar{x} \pm$ SD	No. (%) adult emergence	Pupal period (days) $\bar{x} \pm$ SD	Developmental period (days) $\bar{x} \pm$ SD	Adult longevity (days) $\bar{x} \pm$ SD
Nosema epilachnae								
1×10^3	43	24 (55.81)	23 (95.83)	18.35 ± 3.71a	12 (50.00)	6.00 ± 0.85a	22.00 ± 2.70a	4.92 ± 5.30a
1×10^4	69	60 (86.95)	36 (60.00)	17.58 ± 2.74a	18 (50.00)	5.33 ± 0.77b	21.11 ± 1.99a	10.11 ± 12.33a
1×10^5	23	22 (95.65)	3 (13.64)	18.00 ± 1.00a	1 (4.55)	—	—	<1.0
Nosema varivestis								
1×10^4	67	36 (53.73)	35 (97.22)	13.86 ± 0.85b	35 (95.24)	5.14 ± 0.36b	19.00 ± 0.84b	35.12 ± 13.71b,c
1×10^6	57	54 (94.74)	53 (98.15)	14.33 ± 0.89b	52 (96.30)	5.06 ± 0.57b	19.40 ± 0.96b	26.95 ± 12.59b
Control	73	0	72 (98.63)	13.90 ± 0.63b	69 (94.52)	5.14 ± 0.52b	19.00 ± 0.75b	42.54 ± 22.68c

[a] Means followed by the same letter are not significantly different at the 5% level, as determined by Tukey's procedure.

[b] Exposed as neonate larvae.

concentration of *N. varivestis* lived for a significantly shorter period than did the control adults. (Although not included in Table 1, the data on the average larval, pupal, and total developmental periods as well as adult longevity of the exposed but noninfected beetles in each treatment were similar to each other and not significantly different from those of the control.)

The first experiment designed to determine the influence of *N. epilachnae* on adult longevity and fecundity (Table 2) was concluded 31 days post adult emergence due to the apparent mortality of most of the infected females in each treatment. At this time 20 female beetles in the control were still alive. In contrast, only 12 of the remaining 39 females in the three treatments exposed to *N. epilachnae* were found to be infected. Although male beetles in only one treatment (H♀ × I♂) were exposed directly to the microsporidium, many of the males in the other two treatments as well as some of the unexposed females in this treatment also become infected as a result of their having been confined with their infected mates. In these cases it was impossible to determine exactly when these individuals became infected. In addition, not all of the beetles exposed per os to the microsporidium became infected; thus the data included in Table 2 is based only on those individuals that were infected at death or upon dissection. Again, the data (not included in Table 2) on the exposed but noninfected individuals were statistically similar to those of the control.

In this test (Table 2) female longevity was significantly shorter only in the treatment where they had been exposed to *N. epilachnae* as late-stage larvae (I♀ × H♂). The average longevity of the infected males in the same treatment was also signficantly shorter than that of the control males as was that of the males exposed directly as late-stage larvae (H♀ × I♂). And although not compared statistically, males lived slightly longer than females, except where the males had been exposed directly to the microsporidium as late-stage larvae (H♀ × I♂). The ovipositional activities of the females infected by *N. epilachnae* in all three treatments were not significantly different, but in all treatments the average numbers of egg masses per female and egg masses per female per day were significantly less than those produced by control females. Infected females in two of the treatments also produced significantly smaller average egg masses than did the controls. In addition, many infected females produced no eggs in two of the treatments.

A more extensive effort was made in the second experiment (Table 3) to determine the effects of *N. epilachnae* on adult fecundity and to measure parameters indicative of transovarian transmission of the microsporidium. When terminated at 46 days post adult emergence, only 5.8% (4 of 69) of the infected females in all three of the treatments were still alive in contrast to 54.2% (13 of 24) of the control females. Many males confined with infected females also became infected and their average longevity was slightly longer than their female counterparts. In this test the exposure of females at 1, 4, and 7 days post adult emergence generally resulted in higher infection rates than those exposed to the same spore dosage as larvae or newly emerged adults in the first test. In addition, most of the infected females were reproductively active. The data (not included in Table 3) from the only treatment (T_7) in which there were many noninfected but exposed adults were found to be statistically similar to those of the control.

The average longevities of the infected males and females were statistically similar but they were both significantly shorter than the control, except those in treatment T_7 where the females were exposed 7 days post adult emergence. Females in treatment T_1 produced significantly fewer average numbers of egg masses per female and egg masses per female per day than did those in treatment T_7 or the control. The females in treatment T_4, however, were in-

TABLE 2

EFFECT OF *Nosema epilachnae* ON THE LONGEVITY AND FECUNDITY OF *Epilachna varivestis* (EXPERIMENT I)[a]

Treatment	Longevity[d] Males No.	Males $\bar{x}$ ± SD	Females No.	Females $\bar{x}$ ± SD	No. females ovipositing	No. egg masses per female ($\bar{x}$ ± SD)	No. egg masses per female per day ($\bar{x}$ ± SD)	No. eggs per mass ($\bar{x}$ ± SD)
I♀[b] × H♂	16	21.56 ± 8.02a	19	15.79 ± 8.61a	7	5.00 ± 3.92a	0.186 ± 0.12a	43.43 ± 7.96a,b
I♀[c] × H♂	18	27.72 ± 2.35b	26	24.15 ± 6.20b	22	2.73 ± 1.98a	0.104 ± 0.07a	33.80 ± 11.17a
H♀ × I♂[b]	13	17.69 ± 8.43a	13	23.08 ± 7.92b	7	4.00 ± 4.16a	0.160 ± 0.15a	33.13 ± 11.49a
H♀ × H♂	26	27.65 ± 6.82b	26	27.12 ± 7.02b	24	9.42 ± 3.30b	0.318 ± 0.10b	46.09 ± 7.59b

[a] Means followed by the same letter are not significantly different at the 5% level, as determined by Tukey's procedure.
[b] Exposed as late-stage larvae to 1×10^4 spores/larva.
[c] Exposed as neonate adults to 1×10^4 spores/adult.
[d] Test concluded after 31 days.

TABLE 3

EFFECT OF *Nosema epilachnae* ON THE LONGEVITY AND FECUNDITY OF *Epilachna varivestis* (EXPERIMENT II)[a]

Treatment[b]	Longevity[c] Males No.	Males $\bar{x}$ ± SD	Females No.	Females $\bar{x}$ ± SD	No. females ovipositing	No. egg masses per female ($\bar{x}$ ± SD)	No. egg masses/♀/day ($\bar{x}$ ± SD)
T_1	17	32.24 ± 4.48a	23	27.96 ± 7.62a	21	5.19 ± 2.04a	0.178 ± 0.06a
T_4	17	35.76 ± 5.78a	22	32.77 ± 7.22a	21	8.38 ± 3.23a,b	0.249 ± 0.06a,b
T_7	5	35.40 ± 0.89a,b	9	36.33 ± 3.87a,b	9	11.78 ± 3.56b	0.306 ± 0.06b,c
Control	25	41.12 ± 6.44b	24	39.25 ± 10.60b	22	16.14 ± 5.76c	0.371 ± 0.11c

[a] Means followed by the same letter are not significantly different at the 5% level, as determined by Tukey's procedure.
[b] Female beetles were exposed 1, 4, and 7 days post adult emergence to 1×10^4 spores/adult and then mated with healthy male beetles.
[c] Test concluded after 46 days.

termediate in their ovipositional activities and thus the average egg mass per female and egg mass per female per day were statistically similar to T_1 and T_7 but were significantly different from the control. These ovipositional indices of treatment T_7 were the same as those in T_4 and the control. Unlike the females in the first test (Table 2), there were no significant differences in the average number of eggs per mass of the females in any of the treatments. There was, however, a significant difference in the average preovipositional period between T_1 and the control but the other exposure timings produced no significant differences from the control; T_7 was also the same as T_1.

Mean egg hatching rates for the infected females were lower than those of the control but the differences were only significant for females in treatments T_1 and T_4. While not evident in the data means included in Table 3, data for individual females of each treatment indicated trends in the prevalence of transovarian transmission and in the egg hatching rates associated with microsporidian infection. Once an infected female began producing infected egg masses, only rarely did she produce a subsequent egg mass that was completely free of the microsporidium. Also, in contrast to the controls in which egg hatching rates were consistently high throughout the 6- to 7-week ovipositional period, the egg hatching rates among the infected females exhibited a dramatic decline once an infected egg mass was produced. These trends were particularly evident in the females from treatments T_1 and T_4 where high egg hatching rates were obtained for the first several egg masses prior to the production of the first infected egg mass. Thereafter, the infection rate in newly hatching larvae usually increased rapidly and often reached 100% in egg masses from which some of the larvae were able to hatch. Simultaneously, the egg hatching rate decreased, and generally none of the eggs within the last two or three egg masses produced by an infected female hatched. These masses were consistently found to be infected when the whole mass was examined in a wet-mount, squash preparation. More specific data on the incidence of transovarian transmission are provided, for example, by the females in treatment T_1. Based on the sampling procedure used where 10 neonate larvae per mass were examined for infection, the prevalence of infection ranged from 10 to 100% within egg masses where some larve hatched and at least 1 larva was infected. The average rate of transmission was 56.5% in similar egg masses produced by all of the females in this treatment. However, the average rate of infection in the first egg mass producing at least one infected larvae was 45.6% while the average rate in the last egg mass from which any larva was able to hatch was 66.7%.

The average number of days to the production of the first infected egg mass per female and the average number of egg masses produced per female to the production of the first infected egg mass were correlated with the timing of adult exposure. The differences between treatments T_4 and T_7 were nonsignificant, but these parameters were significantly lower for the females in treatment T_1.

Because of the low virulence of *N. varivestis* for the MBB, only limited data were obtained on its influence on adult fecundity (Table 4). Infection rates among the adult females were low even though they had been exposed to the microsporidium as neonate larvae, and the generally low hatching rates of eggs produced by the control females limited the observations largely to the ovipositional activities of the females. In addition, egg production rates by females in the second test were generally lower than those in the first, although this difference is likely related to the higher dosage of spores to which these females had been exposed as neonate larvae.

In the first test, concluded 22 days post adult emergence, there was no significant

TABLE 3a
EFFECT OF *Nosema epilachnae* ON THE LONGEVITY AND FECUNDITY OF *Epilachna varivestis* (EXPERIMENT II)[a]

No. eggs per mass $\bar{x}$ ± SD	% Egg hatch/♀ $\bar{x}$ ± SD	Preovipositional period (days) $\bar{x}$ ± SD	No. days to first infected egg mass $\bar{x}$ ± SD	No. egg masses to first infected egg mass $\bar{x}$ ± SD
42.20 ± 6.46a	47.57 ± 16.86a	11.86 ± 2.71a	16.74 ± 3.66a	1.79 ± 1.13a
42.60 ± 7.08a	52.14 ± 16.10a	10.00 ± 1.15b	22.63 ± 6.47b	5.11 ± 2.35b
43.40 ± 4.25a	69.83 ± 10.38b	11.00 ± 1.32a,b	23.71 ± 5.02b	6.71 ± 1.80b
47.99 ± 8.77a	72.55 ± 12.80b	9.64 ± 0.85b	—	—

[a] Means followed by the same letter are not significantly different at the 5% level, as determined by Tukey's procedure.

difference in the average longevities of the infected and control females. However, the infected females produced significantly fewer average numbers of egg masses per female and egg masses per female per day than did the controls. These differences were also significant and even more pronounced in the second test that was concluded 46 days post adult emergence. The average longevity of the infected females, however, was significantly lower in this test than those of the control. In addition, the average number of eggs per mass of the infected and control females did not differ significantly in either test.

Observations on transovarian transmission of *N. varivestis* indicated that the incidence of transmission ranged from 10 to 80% in egg masses that produced at least one infected larva. However, the average rate of transmission, 24.6%, was low, and in only 3 of 32 egg masses did the rate of transmission exceed 40%. Infection rates did not increase in time and no relationship was apparent between microsporidian infection and low egg hatching rates.

DISCUSSION

Like most species of the entomophilic protozoa, microsporidia generally produce chronic, sublethal infections in their hosts characterized by such symptoms as irregular growth, retarded larval development, incomplete metamorphosis, and reduced adult vigor, fecundity, and longevity (Brooks, 1974; Canning, 1981). And, while most species are preceived as important

TABLE 4
EFFECT OF *Nosema varivestis* ON THE LONGEVITY AND FECUNDITY OF *Epilachna varivestis*[a]

Treatment	No. females ovipositing	Female longevity $\bar{x}$ ± SD	No. egg masses/♀ $\bar{x}$ ± SD	No. egg masses/♀/day $\bar{x}$ ± SD	No. eggs per mass $\bar{x}$ ± SD
			(Test 1[b])		
Infected	13	17.92 ± 3.64a	4.46 ± 2.30a	0.238 ± 0.10a	40.11 ± 9.54a
Control	21	19.33 ± 4.15a	7.67 ± 3.15b	0.380 ± 0.13b	42.75 ± 8.12a
			(Test 2[c])		
Infected	16	20.19 ± 8.40a	3.44 ± 2.39a	0.158 ± 0.06a	33.55 ± 12.24a
Control	14	32.28 ± 12.28b	11.00 ± 3.84b	0.341 ± 0.08b	36.04 ± 13.10a

[a] Means within the columns for each test followed by the same letter are not significantly different, as determined by Student's *t* test.

[b] Each adult female had been exposed to 1.9×10^5 spores as a neonate larva; test concluded 22 days post adult emergence.

[c] Each adult female had been exposed to 1.29×10^6 spores as a neonate larva; test concluded 46 days post adult emergence.

natural mortality factors but of only limited value as candidates for short-term, microbial insecticides, several species have been evaluated for use in applied pest control programs (see reviews by McLaughlin, 1971; Brooks, 1980; Henry, 1981; Canning, 1982). Of these, *Nosema locustae* has already been developed as a control agent for rangeland grasshoppers (Henry, 1981) and *Vairimorpha necatrix* possesses high potential as a short-term control agent for use against lepidopterous pests (Maddox et al., 1981).

Of the two species involved in this study, *N. epilachae* is decidedly more virulent for the MBB than is *N. varivestis* and appears to possess the greater potential as a microbial control agent (Table 1). At relatively low spore doses, larval mortality ranged from 50 to 95% and most of the successfully emerging adults die within an average of 10 days or less. The average developmental period of these adults was also significantly lengthened over those infected with *N. varivestis* or the controls. In contrast more than 95% of the neonate larvae exposed to the highest dosage of *N. varivestis* emerged as adults, and the adults survived at least two to three times longer on the average than did those infected with *N. epilachnae*. In fact, the only significant difference between the controls and those beetles infected with *N. varivestis* was in the average adult longevity of those exposed to highest dose of spores.

Because the MBB is long-lived and feeds extensively in both the larval and adult stage, any potential microbial control agent should also be effective against the adults as well as the larvae. In this respect *N. varivestis* is, again, decidedly less attractive than *N. epilachnae*. Adult longevity was only reduced significantly by *N. varivestis* when MBB were exposed as neonates to the highest spore dose (Table 4). And, while egg production was also significantly lower, it is highly unlikely that similar results would have been obtained had the beetles been exposed as late-stage larvae or as adults.

On the other hand, female beetles exposed to *N. epilachnae* as either late-stage larvae (Table 2, treatment I♀ × H♂) or as newly emerged adults (Table 3, treatments T_1 and T_4), exhibited significant reductions in both their longevity and fecundity as compared to the controls despite the lower spore dosage rates used. The only treatment in which female longevity and fecundity were not significantly lowered was in T_7 (Table 3) where the females were exposed 7 days post adult emergence. The effect of *N. epilachnae* on adult longevity is further indicated by the fact that the average longevity of the infected males was generally less than that of the control males even though they were not exposed directly to spores of the microsporidium. Although the males in each treatment generally survived slightly longer than did their respective female counterparts, this difference may have been due to delayed innoculation from their indirect exposure to the microsporidium via spores liberated in the feces of the infected females.

Despite the significant reductions in adult longevity and fecundity by both species of microsporidia, one parameter indicative of fecundity (number of eggs per mass) was not consistently affected. There was no difference in the number of eggs per mass in the tests with *N. varivestis* (Table 4) or in the more extensive test with *N. epilachnae* (Table 3). Differences noted in the other test with *N. epilachnae* (Table 2) may have resulted from a small sample size in two of the treatments or abnormal variation in the data. Regardless, the general lack of significant differences in the number of eggs per mass is consistent with the results of Kitayama et al. (1979) who found that this parameter for the MBB was not affected by host plant stage. They concluded that a certain level of nutrients was required to produce an egg mass and that oviposition would not occur until that level was reached.

While *N. epilachnae* is obviously pathogenic to adult MBB, the responses obtained in this study also indicate that they

were related to adult age at exposure (Table 3). Thus, the reductions in adult longevity and fecundity were greatest in females exposed 1 day post adult emergence (treatment T_1) and the reductions noted in the other two treatments (T_4 and T_7) were obviously correlated, respectively, to the delays of 3 and 6 days before their exposure to the microsporidium as adults. This apparent increase in the resistance of females with age might, however, be overcome with higher spore dosage rates. Higher spore rates might also considerably lower adult fecundity and longevity.

The eggs produced by adults infected with *N. epilachnae* also exhibited reductions in their average hatching rates which were correlated with adult age at exposure. Again, as the reductions were only significant for the females exposed 1 and 4 days post adult emergence, reductions in the egg hatching rates for older females could probably only be achieved through the use of higher spore dosage rates.

Another attribute of *N. epilachnae* as a potential microbial control agent is its capacity to be transmitted transovarially at relatively high rates and its ultimate effect on egg hatching rates of the MBB. The length of time and the number of egg masses to the production of the first infected egg mass were correlated, again, with adult age at exposure; however, once an infected egg mass was produced, the incidence of infection generally increased in subsequent masses accompanied by dramatic decline in egg hatching rates. Thus, at least some infected progeny would be produced and serve as foci of inocula for further horizontal as well as transovarian transmission in field populations of the beetle. Although *N. varivestis* is also transmitted transovarially, the average rate of transmission was low and did not appear to increase in time. Observations were too limited to determine any potential influence on the egg hatching rates with this species.

The highly virulent nature of *N. epilachnae* for both larvae and adults indicates that further efforts to evaluate its potential as a microbial control agent of the MBB are warranted. This species is also infectious for the squash beetle, *Epilachna borealis* (Brooks et al., 1980), the Colorado potato beetle, *Leptinotarsa decemlineata,* and several species of Lepidoptera (Brooks et al., 1985). Preliminary results indicate that this microsporidium can be mass produced in larvae of the corn earworm, *Heliothis zea,* and may be useful as a control agent of the Colorado potato beetle (unpublished observations). On the other hand, *N. varivestis* is less attractive as a control agent because of its low virulence for the MBB and the difficulties involved in its propagation. However, both species are present at low levels in natural populations of the MBB and, at least under laboratory conditions, may pose serious problems in maintaining laboratory colonies of the MBB (Brooks et al., 1980) or the eulophid parasite, *P. foveolatus* (Own and Brooks, 1986).

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REFERENCES

ALDRED, J. M., SHEPARD, M., AND HOLMES, P. T. 1980. A stochastic model of parasitism of the Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), in soybeans by *Pediobius foveolatus* (Crawford) (Hymenoptera: Eulophidae). *Res. Popul. Ecol. (Tokyo),* **21,** 286–299.

BARROWS, E. M., HOOKER, M. E. 1981. Parasitization of the Mexican bean beetle by *Pediobius foveolatus* in urban vegetable gardens. *Environ. Entomol.,* **10,** 782–786.

BROOKS, W. M. 1974. Protozoan infections. *In* "Insect Diseases" (G. E. Cantwell, ed.), vol. 1, pp. 237–300. Marcel Dekker, New York.

BROOKS, W. M. 1980. Production and efficacy of Protozoa. *Biotech. Bioeng.,* **22,** 1415–1440.

BROOKS, W. M., MONTROSS, D. B., SPRENKEL, R. K., AND CARNER, G. 1980. Microsporidioses of coleopterous pests of soybeans. *J. Invertebr. Pathol.,* **35,** 93–95.

BROOKS, W. M., HAZARD, E. I., AND BECNEL, J 1985. Two new species of *Nosema* (Microsporidia: Nosematidae) from the Mexican bean beetle *Epi-*

lachna varivestis (Coleoptera: Coccinellidae). *J. Protozool.*, **32**, 525–535.

CANNING, E. U. 1981. Insect control with Protozoa. *In* "Biological Control in Crop Production," Beltsville Symposia in Agricultural Research (G. C. Papavizas, ed.), Vol. 5. pp. 201–216. Allanheld, Osmun, New Jersey.

CANNING, E. U. 1982. An evaluation of protozoal characteristics in relation to biological control of pests. *Parasitology*, **84**, 119–149.

CANTWELL, G. E., AND CANTELO, W. W. 1982. Potential of *Bacillus thuringiensis* as a microbial agent against the Mexican bean beetle. *J. Econ. Entomol.*, **75**, 348–350.

CANTWELL, G. E., CANTELO, W. W., AND SCHRODER, R. F. W. 1985. The integration of a bacterium and parasites to control the Colorado potato beetle and the Mexican bean beetle. *J. Entomol. Sci.*, **20**, 98–103.

COULSON, J. R. 1976. Programs in the United States. Federal programs. Importation of natural enemies. *In* "Organized Programs to Utilize Natural Enemies of Pests in Canada, Mexico, United States" pp. 6–10. USDA, APHIS, 81–28, Wash., D.C.

HENRY, J. E. 1981. Natural and applied control of insects by Protozoa. *Ann. Rev. Entomol.*, **26**, 49–73.

KITAYAMA, K., STINNER, R. E., AND RABB, R. L. 1979. Effects of temperature, humidity, and soybean maturity on longevity and fecundity of the adult Mexican bean beetle, *Epilachna varivestis*. *Environ. Entomol.*, **8**, 458–464.

MADDOX, J. V., BROOKS, W. M., AND FUXA, J. R. 1981. *Vairimorpha necatrix*, a pathogen of agricultural pests: Potential for pest control. *In* "Microbial Control of Pests and Plant Diseases 1970–1980" (H. D. Burges, ed.), pp. 587–594. Academic Press, New York.

MCLAUGHLIN, R. E. 1971. Use of protozoans for the microbial control of insects. *In* "Microbial Control of Insects and Mites" (H. D. Burges and N. W. Hussey, eds.), pp. 151–172. Academic Press, New York.

MICHELS, G. J., AND BURKHARDT, C. C. 1981. Economic threshold levels of the Mexican bean beetle on pinto beans in Wyoming. *J. Econ. Entomol.*, **74**, 5–6.

OWN, S. O., AND BROOKS, W. M. 1986. Interactions of the parasite *Pediobius foveolatus* (Hymenoptera: Eulophidae) with two *Nosema* spp. (Microsporidia: Nosematidae) of the Mexican bean beetle (Coleoptera: Coccinellidae). *Environ. Entomol.*, **15**, 32–39.

SCHRODER, R. F. W. 1981. Biological control of the Mexican bean beetle, *Epilachna varivestis* Mulsant, in the United States. *In* "Biological Control in Crop Production," Beltsville Symposia in Agricultural Research (G. C. Papavizas, ed.), Vol. 5. pp. 351–360. Allanheld, Osmun, New Jersey.

STEVENS, L. M., STEINHAUER, A. L., AND COULSON, J. R. 1975. Suppression of the Mexican bean beetle on soybeans with annual releases of *Pediobius foveolatus*. *Environ. Entomol.*, **4**, 947–952.