JOURNAL OF INVERTEBRATE PATHOLOGY 57, 23–36 (1991)

Pathogenicity and Transmission Potential of *Nosema locustae* and *Vairimorpha* n. sp. (Protozoa: Microsporida) in Mormon Crickets (*Anabrus simplex;* Orthoptera: Tettigoniidae): A Laboratory Evaluation

CHARLES M. MACVEAN* AND JOHN L. CAPINERA†

Universidad del Valle de Guatemala, Apartado Postal No. 82, 01901, Guatemala, Central America, and †Department of Entomology and Nematology, 3103 McCarty Hall, University of Florida, Gainesville, Florida 32611

Received December 8, 1989; accepted March 26, 1990

Nosema locustae did not produce detectable infections in Mormon crickets in any stage of development. While nymphal development was retarded among insects inoculated as first to third instars with high spore concentrations (10^6/mg bran), no reductions in survival or oviposition were obtained. A new species of microsporidian (*Vairimorpha* n. sp.) was isolated from Mormon crickets near Dinosaur National Monument. In laboratory cage tests, this pathogen produced significant mortality among first to third instar Mormon crickets treated with a bran bait containing 10^6 spores/mg. Lower concentrations (10^4/mg and 10^2/mg) did not significantly affect survival, but retarded nymphal development and yielded heavy infections. Crickets inoculated as seventh instars to adults did not exhibit significant changes in survival, infection, or oviposition relative to controls. Before *N. locustae* can contribute to Mormon cricket management, a "cricket strain" of this pathogen must be isolated and tested. However, *Vairimorpha* n. sp. appears to be a promising biocontrol agent for both short-term host density reductions and longer-term reductions in host development coupled with enhanced transmission potential.

KEY WORDS: Mormon cricket, *Anabrus simplex;* microsporidans, *Nosema locustae, Vairimorpha* n. sp., pathogenicity of; infection; transmission potential; biological control.

INTRODUCTION

The host range of the orthopteran pathogen *Nosema locustae* includes many genera of short-horned grasshoppers (Acrididae), certain crickets (Gryllidae), pygmy grasshoppers (Tetrigidae), and the Mormon cricket, *Anabrus simplex* (Tettigoniidae) (Henry, 1969; Henry and Oma, 1981). A number of authors have suggested that *N. locustae* holds potential as a biocontrol agent for long-term suppression of pest acridids, by virtue of population reductions within the season of inundative release (application of infective spores on bait) and suppression in the following season (Henry and Oma, 1974, 1981; Ewen and Mukerji, 1980; Henry and Onsager, 1982a; Erlandson et al., 1985, 1986), as well as sublethal effects such as reductions in feeding and growth as a result of infection (Oma and Hewitt, 1984; Johnson and Pavlikova, 1986).

In the initial isolation of *N. locustae* from Mormon crickets in Montana, Henry and Oma (1981) found spores in the fat body as well as in gut tissue. However, in a field trial where *N. locustae* produced in acridids was applied to Mormon crickets in Colorado, Henry and Onsager (1982b) found spores only in gut tissue, raising the question of strain differences in *N. locustae* and/or the cricket hosts. This study suggested that third instar Mormon crickets were susceptible to infection via application of treated bran bait; however, due to movement of cricket bands into and out of the treated area and the lack of good controls, the impact of *N. locustae* on the cricket population could not be discerned.

Given a history of chemical control measures for these insects, combined with a recent population outbreak of crickets in and around Dinosaur National Monument (MacVean, 1987), the National Park Ser-

0022-2011/91 $1.50

vice showed interest in testing biocontrol agents, particularly a registered pathogen, for cricket control in this area. These concerns and the preliminary results given by Henry and Onsager (1982b) prompted us to undertake a more thorough evaluation of *N. locustae*'s pathogenicity in this potential rangeland pest.

In 1985 we found an undescribed microsporidian in adult Mormon crickets near Dinosaur National Monument (Colorado–Utah). Very high numbers of spores were present in a large proportion of the cricket aggregation, associated with sluggish behavior and a moribund condition in many of the insects.[1] Because of the high mortality potentially due to this pathogen, we tested it in the same manner as *N. locustae*. This paper presents the results of a laboratory study on both pathogens' infectivity and effects on Mormon cricket survival, growth, and oviposition. Field-test data will be reported separately.

METHODS AND MATERIALS

The spores of *N. locustae* used in our studies were obtained from two sources: Dr. John Henry of the ARS Rangeland Insect Laboratory in Bozeman, Montana, and from Evans BioControl, a commercial producer of *N. locustae* in Broomfield, Colorado. Production of spores follows very similar procedures at both facilities, using the same host, *Melanoplus differentialis*. Live infected grasshoppers were received from Bozeman, then frozen as they died. Spore preparations were obtained by homogenizing the cadavers in distilled water, followed by filtration, rinsing, and centrifugation as given by Henry (1971) and Henry et al. (1973), and then stored in water at −10°C. Spores from Evans BioControl were received frozen in water and stored at −10°C. Spore suspensions from both sources were thawed for titering, then refrozen until needed. Spore concentrations were determined by counts on a Petroff–Hausser cell. Aside from the first batch of material from the Bozeman laboratory, newer preparations (shorter storage time) were available from Evans and were utilized for our assays.

To ascertain gross differences in infectivity between spores from the two sources, two comparative lettuce-disk assays (Henry and Oma, 1974) were conducted 2 weeks apart with third to fourth instar *M. differentialis* inoculated at 10^5 spores per individual. Infection was monitored during 8 weeks following inoculation by examining whole-insect homogenates and recording infection levels. Differences in infection levels due to sources were tested by log-linear analysis. Details of infection diagnosis and log-linear analysis are described below. All statistical analyses were conducted with SPSS PC+ version 2.0 (SPSS Inc., 1987); only the specific module used for a test is indicated in parentheses throughout the rest of this discussion, e.g. (HILOGLINEAR) for analysis of infection data.

Spores of *Vairimorpha* n. sp. were obtained from naturally infected Mormon crickets collected in the vicinity of Dinosaur National Monument. Purification and storage of spores were accomplished as described above for *N. locustae*.

For tests with Mormon crickets, spores were formulated on flaky wheat bran at several concentrations, then fed to groups of insects. The choice of bran inoculation to groups of test individuals, rather than individual inoculations on lettuce disks, for example, was based on the premise that the former more closely approximates the inoculation of a field population. Bran has historically been the bait material of choice in applications of chemical agents (Wakeland, 1959) and *N. locustae* (Henry and Oma, 1981) for cricket and grasshopper control

[1] Description of this species by Drs. John Henry and Doug Streett is in progress at the ARS Rangeland Insect Laboratory, Bozeman, Montana. It exhibits dimorphic development, with both diplokaryotic and monokaryotic spores present together in the same host; it will be placed in the genus *Vairimorpha*.

because it is readily consumed by many species, is inexpensive, and has good dispersal qualities for either ground or aerial application. Precise individual dosages on lettuce disks are well suited for laboratory comparisons of different spores or host strains and for LD_{50} studies, but are of limited value in predicting the fate of a field inoculation because one has little control over individual dose in the field. The parameters relevant to a field application of a pathogen bait are the concentration of infective stages in the bait (spores/mg bran, in this case) and the application rate (kg/ha, for example), both of which can be manipulated in lab experiments. Therefore, data based on group inoculations should be more general and more useful, although these values are not strictly comparable to data from individual inoculations.

Spores were applied to bran by tumbling the bran in a 4-liter plastic jar turned by an electric roller and simultaneously spraying a concentrated aqueous suspension of spores into the mouth of the jar through an artist's air brush. No stickers or adjuvants were added to the spores. The concentrations prepared were 10^6, 10^4, and 10^2 spores/mg bran. All spore suspensions were sprayed in equal volumes of water for each trial and were prepared within 12 hr of feeding to the test insects. The proportion of spore suspension (or pure water for controls) to bran varied from 5 to 10% (v:w) among trials, with an application time of about 10 min, which resulted in thorough coverage and tumbling but did not result in wet or soggy bran. Prepared baits were either fed immediately to test crickets or refrigerated for a few hours until needed.

Since *N. locustae,* like most insect pathogens, appears to be most pathogenic to acridid hosts in the early instars but reproduces more actively in later instars (Canning, 1962; Henry et al., 1973; Henry and Oma, 1981), we tested its effects in three age categories of Mormon crickets: instars one to three, four to five, and seven to young adult (Mormon crickets develop through seven stadia before the final molt to adults). *Vairimorpha* was tested in the first to third instar and seventh to adult instar age groups, but not in the fourth to fifth instar group. Insects were field collected in or near Dinosaur National Monument, in the vicinity of Dinosaur, Colorado, and Vernal, Utah, and transported to a field station in Dinosaur. Since nymphal development is fairly synchronous, the age span in a population is only two to three instars. Mormon crickets are difficult to rear for more than one generation and a laboratory colony is not available at this time. Therefore, it was not feasible to test all three age groups simultaneously, and assays were conducted sequentially over the course of the 1986 and 1987 field seasons.

Groups of 30 insects (25 in the case of seventh instars and adults) of the appropriate age category were placed in 29 × 29 × 29-cm aluminum-screened cages (BioQuip) for inoculation. Three replicate groups were used for each concentration of spores, including controls. Each cage received the amount of bran that could be consumed in 24 hr by 30 insects of the particular age category, calculated from consumption rates given by Cowan and Shipman (1947) for the various instars and weighted by the proportion of each instar in the group. These were 5, 12, and 85 mg bran/cricket for the first to third, fourth to fifth, and seventh to adult instar groups, respectively. All pathogen concentrations were tested in the youngest age group, while only the two highest concentrations were fed to fourth to fifth instars and seventh to adult instars. A 24-hr feeding period was chosen to roughly approximate a field application of bait, where visual observations suggest that bran particles are available to crickets for no more than about 24 hr, after which the bait is depleted via consumption by target and nontarget organisms, blown away by wind, or pummeled by rain. Each cage was provided with water (250-ml bottle with sponge wick) but with no food besides the treated bran during the inoculation period. Most of

the bait was consumed during the first 12 hr and it was totally consumed after ca. 36 hr. At this time, a normal feeding regime consisting of fresh winter rye sprouts, dry wheat bran, clippings of native food plants (e.g., flowering heads of *Balsamorrhiza sagitatta* (Pursh) or *Senecio integerrimus* Nutt.), and water was begun.

Tests were conducted at a field station (Dinosaur, Colorado) using a complete randomized block design in a temperature-controlled mobile home; shelves at different heights in the room constituted the blocks, thus accounting for any vertical temperature gradients. Crickets were exposed to a day–night regime of 20–30°C, 15–40% relative humidity, and approximately 14:10 L:D. Survival in each cage was monitored by censusing on alternate days for 4 weeks. The area under the survival curve for each replicate (cricket-days) was then calculated and treatment effects were examined through analysis of variance (MANOVA) with cricket-days as the dependent variable. Curve areas provide a convenient way to utilize all census data in an aggregate measure of survival (Southwood, 1978).

To test for slowed development as a result of infection, the age structure of each cage population (i.e., number of insects in each developmental stadium) was recorded at 4–5 weeks postinoculation in the first to third instar and the fourth to fifth instar treatment groups. The resulting frequency tabulations were then analyzed with log-linear models for multiway contingency tables (Fienberg, 1980) in which the effects of pathogen species and the dose of inoculation on age distribution were determined (HILOGLINEAR). In this type of analysis, null hypotheses of independence between explanatory variables (e.g., pathogen species, dose) and a dependent variable (e.g., age distribution) can be tested. Significant effects (nonindependence) arise from interaction terms in the model involving the explanatory variables and the dependent variable; that is, when the observed number of individuals in any category of the dependent variable for a particular combination of the classification variables is significantly lower or higher than expected from the null model, the hypothesis of independence is rejected. Since the number of insects falling into categories defined by pathogen species and dose were not random response variables but rather fixed by experimental design (e.g., "fixed marginals," Fienberg, 1980) and affected by differential treatment mortality, the significance of the pathogen-by-dose interaction was not tested and this term was simply maintained as a constant in the model (Fienberg, 1980).

Cadavers were removed daily, frozen, and later examined individually for the presence of spores through phase-contrast microscopy. In the *Nosema* treatments and controls, midgut homogenates were prepared in 0.2–0.5 ml water and examined separately from the whole-insect homogenates, prepared in 1 ml (first to third instars), 2 ml (fourth to fifth instars), 4 ml (sixth to seventh instars) or 5 ml (adults). Separate midgut examinations follow from Henry and Onsager's (1982b) observation that *N. locustae* infection in Mormon crickets is restricted to midguts only. In the *Vairimorpha* treatments, only a whole-body homogenate was prepared since this pathogen infects fat body tissue and is easily detected in a whole-body preparation. Since *Vairimorpha* occurs naturally and could affect susceptibility to *Nosema*, levels of *Vairimorpha* were recorded for the *Nosema*-treated insects and for controls. Conversely, although no naturally occurring *N. locustae* has been reported in crickets in Colorado, we inspected all whole-insect homogenates for the presence of *N. locustae* spores. Given the developmental time of 13 days from spore to spore for *N. locustae* (Henry and Onsager, 1982b) and approximately 7 days for *Vairimorpha* n. sp. (Henry and Street, pers. commun.), only insects dying after the required time for spore production were examined in the

treatment groups, e.g., 14 days and 8 days postinoculation. However, all control insects, which could harbor natural infections contracted at unknown times, were examined.

In all cases, a 25-μl droplet of the homogenate was smeared under a coverslip and 20 fields were examined at 400×. Taxonomic diagnosis was based on spore morphology (size and color), aided by frequent measurements with an ocular micrometer and reference samples identified as *N. locustae* or *Vairimorpha* n. sp. by Dr. John Henry, of the Rangeland Insect Laboratory (Bozeman, Montana). *N. locustae* spores average 5.2 μm in length (Henry and Oma, 1981) and possess a yellowish, opalescent appearance, while *Vairimorpha* spores seldom exceed 2.6 μm in length and are pale grayish-white in color. *N. locustae* spores are also somewhat wider than those of *Vairimorpha* n. sp. (2.8 μm vs 2 μm). *Vairimorpha* produces octospores, or "packages" of eight spores within a vesicle (Larsson, 1986; Malone and Canning, 1982), in addition to the free spores. Octospores were comparatively rare and were not used in diagnosis. *N. locustae* produces only single, free spores.

Infection levels were rated from 0 to 5 using the procedure given by Henry (1971), except that we did not use hanging-drop slides. However, based on comparisons using serial dilutions of known concentrations, the wet-mount procedure produces the same ratings as the hanging-drop method and allows phase-contrast resolution (MacVean, unpubl.). Moreover, the rating assigned to a given sample is largely insensitive to variations of 1 or 2 ml in homogenate volume; this results from the broad intervals of spore numbers that define each rating level. Individual homogenates were further classified into one of three frequency categories: uninfected, lightly infected (levels 1–2 of Henry (1971)), or heavily infected (levels 3–5 of Henry). Grouping was necessary as a result of low observed values in many cells when using all five rating categories. To test for changes in infection patterns over time, infection was scored separately for insects dying during the first 4 weeks after inoculation (period 1) and those surviving longer than 27 days postinoculation (period 2). As with age distributions, the resulting frequency tabulations were analyzed with log-linear models (HILOGLINEAR) for multiway contingency tables, with species of inoculation, dose, and period as explanatory (classification) variables and infection (with three levels) as the dependent variable. The constant term in this model was the species-by-period-by-dose interaction, corresponding to effects fixed by experimental design. Separate analyses were performed for each inoculation age group to avoid uninterpretable fourth- and fifth-order interactions which could result from including the age group as a fifth variable in the contingency table. Moreover, separate analyses were called for because the different age groups were tested at different times, using different cohorts of insects.

Fecundity was measured by providing a 500-ml oviposition cup filled with a 50:50 mix of sand and vermiculite in each cage. Mormon crickets lay eggs singly which are easy to count by sifting the substrate. Cups were placed in the cages when mating was first observed and removed at the end of the experiment. Fecundity was measured for groups of crickets instead of individual females because these mate repeatedly during the oviposition period (Cowan, 1929) and because pairing for copulation does not occur randomly but is determined by male choice (Gwynne, 1981, 1984), such that some females are rejected. We elected to let insects in groups make these choices rather than select arbitrary pairs. Frequent mating was in fact observed in the cages. Allowing for the possibility that plastic cups might not represent the ideal oviposition substrate from a cricket's point of view, we dissected all females at the end of the experiment and counted the number of mature eggs remaining in the ovaries; these

were added to the count for that replicate. Since mortality rates and the number of females in each cage varied, the total number of eggs obtained was adjusted to a per female-day basis by dividing by the total adult female-days during the oviposition period for each replicate. These data were then transformed by $\log_{10}(x + 1)$ to correct for nonnormality among very small numbers (Zar, 1984) prior to analysis of variance.

RESULTS AND DISCUSSION

No significant differences in infectivity between the two sources of *N. locustae* spores used in these experiments were detected, although the Evans spores produced somewhat higher rates of overall infection as well as heavier infections relative to spores from the Rangeland Insect Laboratory (Table 1). Data from the two separate assays were pooled for analysis, as heterogeneity χ^2 testing did not indicate significant differences between the two ($X^2 = 1.509$, $df = 2$, $p > 0.50$).

The effects of *N. locustae* and *Vairimorpha* n. sp. on survival of first to third instar Mormon crickets are described in Figure 1 and analysis of variance is shown in Table 2. Treatment with the highest concentration of *N. locustae* (10^6 spores/mg bran) generally reduced survival (cricket-days), but this reduction was not statistically significant (Table 2, control vs *N. locustae* 10^6/mg). The highest concentration of *Vairimorpha* (10^6 spores/mg bran), however, produced high, rapid mortality (Fig. 1) which was statistically significant (Table 2, control vs *Vairimorpha* 10^6/mg). Accordingly, the difference between the two species' effects on cricket-days were highly significant (*N. locustae* vs *Vairimorpha*, Table 2). Polynomial contrasts showed that increasing the pathogen concentration (of both species) on a log scale reduced cricket-days in a manner consisting of both linear and quadratic components. Moreover, the significant linear-by-species interaction confirmed that the linear decline in cricket-days due to increasing *Vairimorpha* concentration was significantly sharper than that for *Nosema*.

Nosema produced no significant effects on survival in the fourth to fifth instar group (Table 3), and neither pathogen signifi-

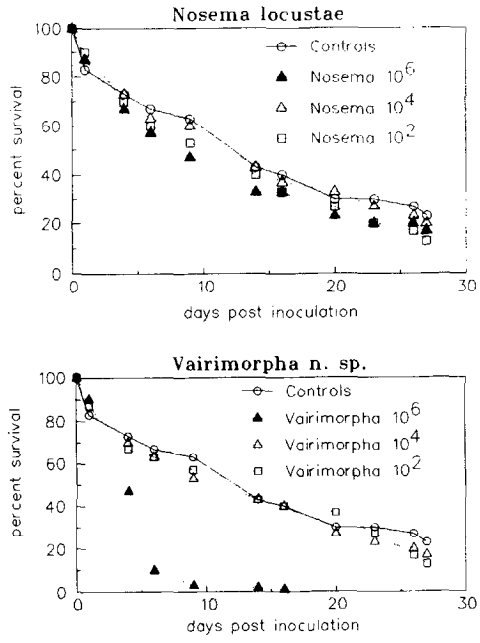

FIG. 1. Survival of first to third instar Mormon crickets following inoculation with *Nosema locustae* or *Vairimorpha* n. sp. on wheat bran. Each curve represents the mean survival of three replicates of 30 crickets each. Dose shown in spores/mg bait.

TABLE 1

COMPARATIVE INFECTIVITY OF *Nosema locustae* SPORES FROM TWO SOURCES, THE ARS RANGELAND INSECT LAB (RIL) AND EVANS BIOCONTROL (EBC), IN *Melanoplus differentialis* INOCULATED INDIVIDUALLY WITH 10^5 SPORES AS THIRD INSTARS

	Infection level (% of insects)			
Source	Uninfected	Light	Heavy	*N*
Controls	97	3	0	34
RIL	51	17	31	35
EBC	36	22	42	36

Note. Data include all insects dying during the experiment (>14 days postinoculation) and those surviving to the end of assay (57 days postinoculation) pooled. χ^2 for test of independence between source and infection: 1.694, $df = 2$, $P = 0.429$.

TABLE 2
ANALYSIS OF VARIANCE FOR EFFECTS OF *Nosema* AND *Vairimorpha* ON SURVIVAL (CRICKET-DAYS) OF CRICKETS INOCULATED AS FIRST TO THIRD INSTARS

Effect	MS	*df*	*F*	*P*
Overall ANOVA				
Residual	3853.28	12		
Blocks	1882.33	2	0.49	0.625
Treatments	29819.78	6	7.74	0.001
Contrasts for treatment effects				
Control vs *N. locustae* 10^6/mg	6208.17	1	1.61	0.228
Control vs *Vairimorpha* 10^6/mg	120700.17	1	31.32	<0.001
Nosema vs *Vairimorpha*	21770.89	1	5.65	0.035
Linear[a] effects of dose	60492.00	1	15.70	0.002
Quadratic[a] effects of dose	30508.44	1	7.92	0.016
Linear by species interaction	46128.00	1	11.97	0.005

[a] On a $\log_{10}$ scale.

cantly reduced survival in the seventh to adult age group (Fig. 2 and Table 3).

Both pathogen species significantly retarded nymphal development in the first to third instar inoculation group, as indicated by increasing proportions of insects remaining as third to fourth instars, and a smaller proportion of the sample reaching sixth instar, with increasing pathogen concentrations (Fig. 3). The dose-by-age group interaction was highly significant (X^2 = 30.597, df = 6, $P < 0.001$). There were no highly significant differences between *Nosema* and *Vairimorpha* in their effects on development, as shown by the species-by-age group interaction in log-linear analysis (X^2 = 4.962, df = 2, P = 0.084). *Nosema* did not significantly affect development in the fourth to fifth instar inoculation group, as indicated by a nonsignificant dose-by-age group interaction (X^2 = 2.526, df = 2, P = 0.283). *Vairimorpha* was not tested in this age group. These results sug-

TABLE 3
ANALYSIS OF VARIANCE FOR EFFECTS OF *Nosema* AND *Vairimorpha* ON SURVIVAL (CRICKET-DAYS) OF CRICKETS INOCULATED AS FOURTH TO FIFTH OR SEVENTH TO ADULT INSTARS

Effect	MS	*df*	*F*	*P*
Crickets inoculated as fourth to fifth instars				
Overall ANOVA				
Residual	5207.29	4		
Blocks	9202.33	2	1.77	0.282
Treatments	1094.33	2	0.21	0.819
Crickets inoculated as seventh instars to adults				
Overall ANOVA				
Residual	5694.78	8		
Blocks	5086.87	2	0.89	0.447
Treatments	5543.93	4	0.97	0.473

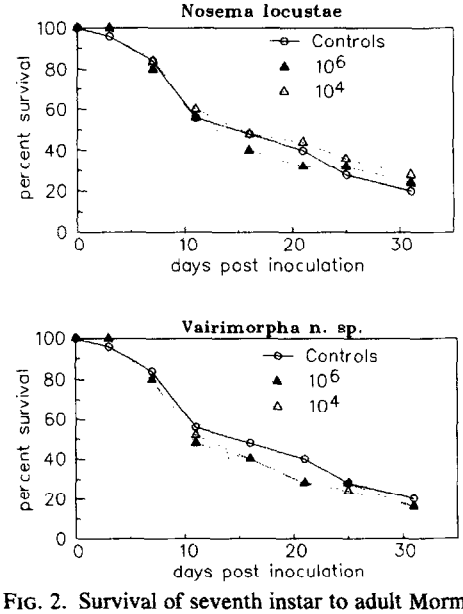

FIG. 2. Survival of seventh instar to adult Mormon crickets following inoculation with *Nosema locustae* or *Vairimorpha* n. sp. on wheat bran. Each curve represents the mean survival of three replicates of 30 crickets each. Dose shown in spores/mg bait.

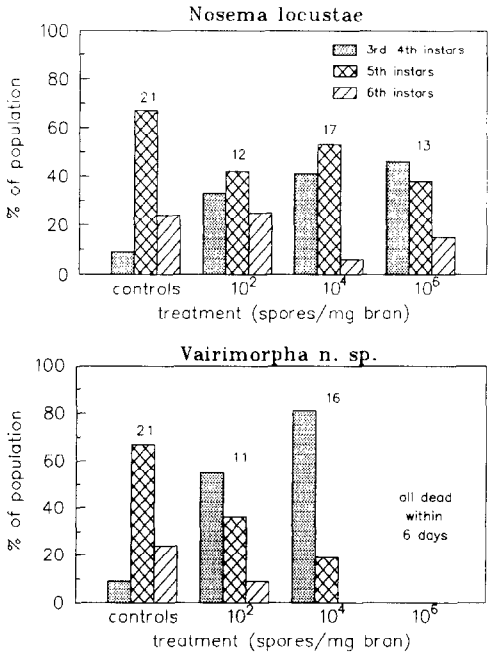

FIG. 3. Instar distribution 30 days postinoculation among Mormon crickets inoculated with *N. locustae* or *Vairimorpha* as first to third instars. Sample sizes for each treatment group are shown above bar clusters.

TABLE 4

EGG PRODUCTION (EGGS/FEMALE/DAY) BY FEMALES INOCULATED AS FOURTH TO FIFTH INSTARS WITH *Nosema* OR INOCULATED AS SEVENTH INSTARS TO ADULTS WITH *Nosema* OR *Vairimorpha*

Treatment	Mean	SE	*n*
Crickets inoculated as fourth to fifth instars			
Controls	0.44	0.15	3
Nosema			
10^4/mg	0.92	0.35	3
10^6/mg	0.48	0.23	3
Two-way ANOVA (on $\log_{10}(x + 1)$): $F_{trtmnts} = 1.21, p = 0.388$			
Crickets inoculated as seventh instars to adults			
Controls	3.74	2.06	3
Nosema			
10^4/mg	5.50	4.80	3
10^6/mg	2.54	1.12	3
Vairimorpha			
10^4/mg	4.51	2.34	3
10^6/mg	3.05	1.88	3
Two-way ANOVA (on $\log_{10}(x + 1)$): $F_{trtmnts} = 0.08, p = 0.98$			

gest that while *Nosema* does not reduce survival of Mormon crickets, it and *Vairimorpha* could significantly reduce the proportion of insects reaching the adult, reproductive stage and thus reduce fitness of a cohort treated with the pathogens in the early instars. This would constitute a valuable sublethal impact on a population of Mormon crickets on rangeland, where a low forage value implies that immediate density reductions are not as critical as long-term dampening of population outbreaks.

Neither pathogen exerted significant effects on oviposition (Table 4) in either fourth to fifth instar or seventh instar-adult treatment groups. Relative to the average fecundity of 2.3 eggs/female/day which can be calculated from Cowan's (1929) data, the oviposition was low in the younger inoculation group, though not in the older group. Reasons for low fecundity are unclear but reflect the frequently cited difficulties in rearing Mormon crickets (Cowan, 1929; Wakeland, 1959; Henry and Onsager, 1982).

In view of the effects on development, it was puzzling to find a total lack of infection by *Nosema* (e.g., spores) in any of the inoculation groups, except for two individuals in the seventh to adult group which had trace infections. Since diagnosis is based on presence of progeny spores, requiring a full life cycle to be completed in the host, conservative estimates of infection may be obtained if the pathogen undergoes only a partial reproductive cycle. It is conceivable that the effects on development described above arise from such a process or simply from the trauma of massive filament extrusion by infective spores in the midgut following inoculation. We did not examine gut or other tissues for nonspore stages and do not know the extent of parasite development, if any. In any event, Mormon cricket appears to be an extremely poor host for *N. locustae,* at least for the "acridid strain" of this pathogen. The barrier to infection is

unknown. While Henry and Onsager (1982) found spores of *N. locustae* in Mormon crickets following aerial treatment of a large geographic area with spores produced in acridids, infections were limited to the midgut and occurred at very low levels. In contrast, Henry and Oma's (1981) initial report of natural occurrence of *N. locustae* in Mormon crickets was based on high levels of infection in the fat body of crickets in Montana. As suggested by Henry and Onsager (1982) and the results of the present study, these inconsistencies strongly suggest strain differences or perhaps even species differences among the pathogens labeled "*N. locustae*" based on external spore morphology. Unfortunately, no material from the original population of the pathogen infecting the fat body of Mormon crickets is available and no new field isolations have been made with which to perform comparative studies.

Infection data for *Vairimorpha* are much more interesting. In the following discussion, "infection" will be used to define the proportions of insects showing light or heavy spore levels in contrast to uninfected individuals. In interpreting the influence of various factors on infection, it is important to bear in mind that in contingency-table analysis significance (nonindependence) arises from *changes* in proportions of insects in the three infection categories across levels of the explanatory variable, not from unequal proportions. That is, no significance results as long as proportions of insects in the three infection categories remain similar across levels of the classification variable, no matter how unequal the proportions may be at any one level.

As mentioned previously, we measured infection by *Vairimorpha* in all insects treated with *Nosema,* anticipating that natural infections could affect susceptibility to *Nosema,* possibly in the antagonistic way reported between other pathogens and baculoviruses (Harper, 1986). Since no *Nosema* infection was obtained, regardless of whether the insects were infected with *Vairimorpha* or not, we cannot evaluate this interaction; however, the opposite situation emerged quite unexpectedly. That is, inoculation with *Nosema* was associated with significant *Vairimorpha* infections in both first to third and seven to adult treatment groups, particularly in the second infection period. Figure 4 illustrates this relationship for first to third instars. Less surprising were the increases in *Vairimorpha* infection over natural (control) levels resulting from inoculation with this species (Figs. 5, 6). Since we did not test *Vairimorpha* in the fourth to fifth instars, we cannot compare infection associated with *Nosema* inoculation vs *Vairimorpha*. However, increases in *Vairimorpha* infection did not occur as a result of *Nosema* inoculation in this age group.

Tests of significance for the various factors influencing infection by *Vairimorpha* are presented in Table 5. In the first to third instar group, an increasing dose of inoculation (i.e., concentration of spores in the

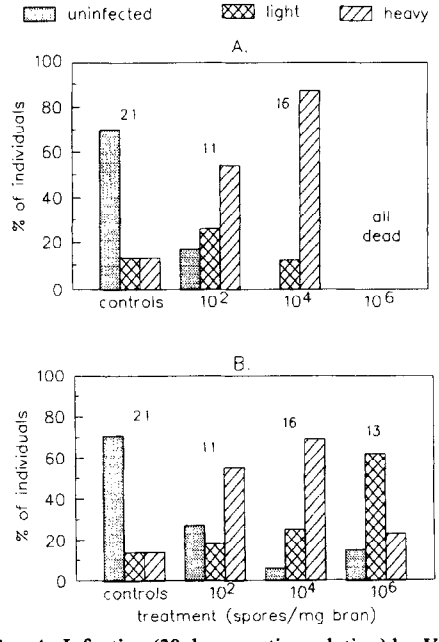

FIG. 4. Infection (30 days postinoculation) by *Vairimorpha* in Mormon crickets inoculated as first to third instars with *Vairimorpha* (A) or *Nosema* (B). Sample sizes for each treatment group are shown above bar clusters.

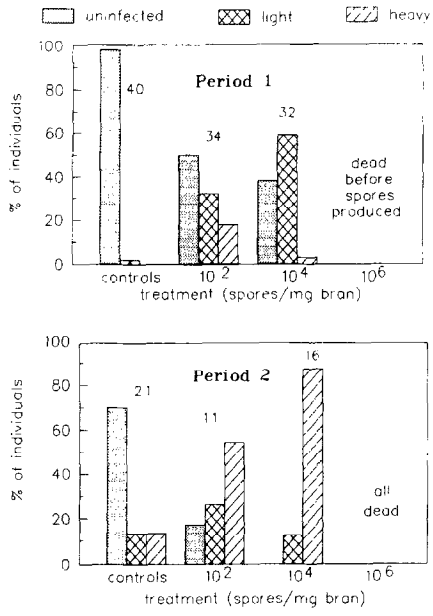

FIG. 5. Infection by *Vairimorpha* during the first 27 days postinoculation (period 1) and after 27 days postinoculation (period 2) in Mormon crickets inoculated as first to third instars. Sample sizes for each treatment group are shown above bar clusters.

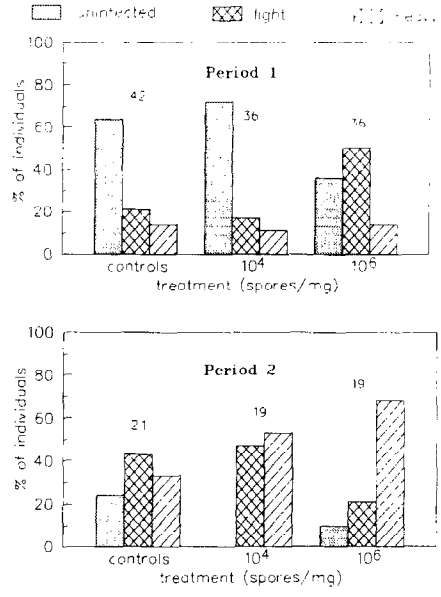

FIG. 6. Infection by *Vairimorpha* during the first 27 days postinoculation (period 1) and after 27 days postinoculation (period 2) in Mormon crickets inoculated as seventh instars to adults. Sample sizes for each treatment group are shown above bar clusters.

bran) significantly increased infection, as shown by the significant infection-by-dose term in the log-linear model. Similar results were reported by Henry and Oma (1974). The time period was also highly significant, with higher infection levels developing in the second period. The significant three-way interaction of infection-by-period-by-dose shows that the effects of increasing dose on the increase in proportions of light and heavy infections were different in the two time periods, i.e., more pronounced in the second period (Fig. 5). The infection-by-species term is marginally significant, suggesting that differences in *Vairimorpha* infection arising from inoculation with one pathogen vs the other are not great. In fact, species differences disappeared altogether in the seventh instar to adult treatment group, such that *Vairimorpha* infection levels after 27 days postinoculation (second period) were very similar whether insects are challenged with *Nosema* or *Vairimorpha*. As with the first to third instars, the period of time after inoculation highly influenced infection levels. Despite an increase in heavy infections with increasing dose (Fig. 6), this effect (infection-by-dose) was not statistically significant in seventh in-

TABLE 5

LOG-LINEAR ANALYSIS OF *Vairimorpha* INFECTION LEVELS BY SPECIES USED FOR INOCULATION (*Nosema* OR *Vairimorpha*), DOSE OF INOCULATION, AND PERIOD OF TIME POSTINOCULATION FOR MORMON CRICKETS INOCULATED AS FIRST TO THIRD INSTARS OR AS SEVENTH INSTARS TO ADULTS

Effect	*df*	χ^2	*P*
Crickets inoculated as first to third instars			
Infection × species × period	2	3.467	0.177
Infection × species × dose	6	3.335	0.766
Infection × period × dose	6	15.977	0.014
Infection × species	2	5.772	0.056
Infection × period	2	120.661	<0.001
Infection × dose	6	115.569	<0.001
Crickets inoculated as seventh instars to adults			
Infection × species × period	2	0.041	0.980
Infection × species × dose	6	4.177	0.653
Infection × period × dose	6	15.437	0.017
Infection × species	2	0.853	0.653
Infection × period	2	97.451	<0.001
Infection × dose	6	6.675	0.352

stars and adults. This was probably due to high natural (control) infection levels in seventh instars and adults—40% prevalence in the first period—precluding much response to additional inoculum. In the first to third instar group, however, background (control) *Vairimorpha* prevalence was low—only 2% in the first period—allowing the bran inoculum to produce new infections. It is difficult to ascribe much biological meaning to the significant three-way interaction of infection-by-period-by-dose, except that time effects were dependent on dose, e.g., most pronounced in the 10^4 spores/mg concentration (Fig. 5).

The interaction between *Nosema* and *Vairimorpha* infection suggests that, although *Nosema* cannot successfully reproduce in Mormon crickets, it can sufficiently stress or weaken them to facilitate reproduction by *Vairimorpha* already residing in the host or, alternatively, stimulate *Vairimorpha* in some fashion. Harper (1986) and Benz (1987) describe interactions between baculoviruses and other entomopathogens and abiotic factors, some of which suggest activation of one pathogen by another. Our example is certainly not a case of "latency" as described by Tanada and Fuxa (1987) since *Vairimorpha* infection and reproduction occur independently of *Nosema*, but might be considered akin to environmental stresses which can "activate" disease agents (Benz, 1987). Given that the levels of *Vairimorpha* "induced" by *Nosema* are similar to those produced by *Vairimorpha* itself, it is probable that the apparent effect of *Nosema* on nymphal development was actually due to facilitated *Vairimorpha* infection rather than to *Nosema* parasitism (infection rates for insects shown in Fig. 3, top panel, are given in Fig. 4B).

Arguments for *N. locustae*'s effectiveness in Acrididae rely on horizontal and vertical transmission, via cannibalism (Henry, 1972; Henry and Oma, 1981) and transovarial transmission (Henry, 1972; Ewen and Mukerji, 1980), respectively. There is also evidence for reservoirs of infective spores in feces and soil (Henry, 1972; Germida et al., 1987) which might serve to infect newly hatched nymphs. While limited LD_{50} (e.g., Bucher, 1958) and no ID_{50} data are available, high doses of *N. locustae*, on the order of 10^4 spores per individual, are generally required to successfully infect (per os) and weaken (reduce longevity, feeding, and growth) an acridid host (Henry and Oma, 1974; Johnson and Pavlikova, 1986). It is also known that microsporidan spores are subject to rapid degradation by abiotic and biotic agents (Brooks, 1980; Maddox, 1973; Germida et al., 1987; Henry and Oma, 1974). Therefore, it is logical to assume that large numbers of spores must be produced in infected insects in order to supply and maintain transmission mechanisms to the extent that pathogenic effects are expressed in the host population. In fact, spore production on the order of 10^9 spores per individual is not unusual in *Melanoplus* grasshoppers 3–4 weeks postinoculation in either lab or field treatments (Henry, 1985, 1972). This level of spore production is certainly high enough to allow for significant horizontal transmission, likely reflected in the increasing prevalence of *N. locustae* during the 4–6 weeks following initial inoculation of field populations (Henry and Oma, 1981). However, given the unsuitability of Mormon crickets as hosts for *N. locustae* in our studies, it seems very unlikely that this pathogen could become established via the transmission mechanisms mentioned above and significantly impact the host population.

Although Henry and Onsager (1982b) found spores of *N. locustae* in crickets 1 year following application, it is almost impossible to attribute this, and apparent density reductions, to the application of the pathogen because, as Henry and Onsager (1982b) acknowledge, dispersing bands of Mormon crickets move long distances within just a few days or weeks (MacVean, 1987); therefore, the crickets sampled 1

year after application could easily represent an entirely different population than the one treated. Moreover, *N. locustae,* or a *locustae*-like microsporidian, occurred naturally in two individuals of a control replicate in the seventh instar to adult inoculation group of our study.

Before *N. locustae* can contribute to Mormon cricket management, particularly density reductions, an appropriate "cricket strain" must be isolated and propagated. Unfortunately, no significant infections (e.g., in fat body) have been found or selected in Mormon crickets since the initial field isolation in Montana (Henry and Oma, 1981; Henry and Onsager, 1982b), leaving acridid-reared *N. locustae* as the only current option for mass inoculation.

The new species of *Vairimorpha* appears somewhat more promising as a management tool in that it offers potential for both short-term host density reduction and longer-term sublethal effects and transmission. Typically, the pathogenicity and transmission potential of grasshopper microsporidians have been presented as mutually exclusive, species-specific traits; a highly pathogenic parasite is not likely to produce high levels of spores due to early death of the host, while a species that builds up high levels of spores and offers greater potential for transmission and establishment is necessarily low in pathogenicity and yields only slow, debilitating effects (Henry, 1977, Erlandson et al., 1985). Because of this dichotomy, the literature suggests that a choice must be made between short-term pathogenicity and longer-term transmission likelihood when designing parasite augmentation campaigns, and a corresponding choice of parasite species must follow (Henry and Oma, 1974). However, as is well known for many parasites, the expression of pathogenic effects and propagule production are highly dependent on the dose of inoculation and the development stage of the host at infection (Bucher, 1958; Steinhaus, 1949; Tanada, 1976; Henry et al., 1973; Tanada and Fuxa, 1987). Thus, while pathogenicity (e.g., LD_{50}) measured under standardized conditions for strain or species comparisons is probably a fairly constant parameter, the magnitude of pathogenic effects (such as percentage mortality or reduction in growth) or transmission potential (e.g., spore production) are not fixed and can be manipulated through changes in dose of inoculation and the targeted host stage. *Vairimorpha* n. sp. offers an example of a parasite which, although not very pathogenic in a strict sense, can exert high mortality if administered at high rates to early instars of the Mormon cricket, but can also provide high spore production if applied at lower rates to young insects or variable rates to older insects.

Consequently, the dichotomy between pathogenicity and transmission potential does not force a choice between different species of pathogens, but rather implies a choice of inoculum levels and timing of applications of a single species, depending on management objectives. Some pathologists may frown on considering within the scope of microbial control the use of high doses of spores (such as 10^6/mg bran) to achieve host mortality since this probably represents massive septicemia and not true parasitism by the microsporidian (Vavra and Maddox, 1976). Nevertheless, the end goal of host suppression through biological means is achieved. The more economically inclined will argue that it is financially impossible to produce and sell such high concentrations of a pathogen; however, this is not an inherent shortcoming of the biocontrol agent but rather a challenge in production efficiency, successful marketing, and careful definition of the target pest population to reduce costs.

ACKNOWLEDGMENTS

This research was supported by a grant from the National Park Service. We also thank Bill Barnes, Tom Weissling, and Dave Horton for their technical assistance and their many contributions to this project.

REFERENCES

BENZ, G. 1987. Environment. *In* "Epizootiology of Insect Diseases" (J. R. Fuxa and Y. Tanada, Eds.), pp. 177–214. Wiley, New York.

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

BUCHER, G. E. 1958. General summary and review of utilization of disease to control insects. *Proc. 10th Int. Congr. Entomol. Montreal,* **4**, 695–701.

CANNING, E. U. 1962. The pathogenicity of *Nosema locustae* Canning. *J. Insect Pathol.*, **4**, 248–256.

COWAN, F. T. 1929. Life history, habits, and control of the Mormon cricket. *USDA Tech. Bull.*, 161.

COWAN, F. T., AND SHIPMAN, H. J. 1947. Quantity of food consumed by Mormon crickets. *J. Econ. Entomol.*, **40**, 825–828.

ERLANDSON, M.A., MUKERJI, M. K., EWEN, A. B., AND GUILLOT, C. 1985. Comparative pathogenicity of *Nosema acridophagus* Henry and *Nosema cuneatum* Henry (Microsporida: Nosematidae) for *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae). *Canad. Entomol.*, **117**, 1167–1175.

ERLANDSON, M. A., EWEN, A. B., MUKERJI, M. K., AND GUILLOT, C. 1986. Susceptibility of immature stages of *Melanoplus sanguinipes* (Fab) (Orthoptera: Acrididae) to *Nosema cuneatum* Henry (Microsporida: Nosematidae) and its effects on host fecundity. *Canad. Entomol.*, **118**, 29–35.

EWEN, A. B., AND MUKERJI, M. K. 1980. Evaluation of *Nosema locustae* (Microsporida) as a control agent of grasshopper populations in Saskatchewan. *J. Invertebr. Pathol.*, **35**, 295–303.

FIENBERG, S. E. 1980. "The Analysis of Cross-Classified Categorical Data," 2nd ed. MIT Press.

GERMIDA, J. J., EWEN, A. B., AND ONOFRIECHUK, E. E. 1987. *Nosema locustae* Canning (Microsporida) spore populations in treated field soils and resident grasshopper populations. *Canad. Entomol.*, **119**, 355–360.

GWYNNE, D. T. 1981. Sexual difference theory: Mormon crickets show role reversal in mate choice. *Science*, **213**, 779–780.

GWYNNE, D. T. 1984. Sexual selection and sexual differences in Mormon crickets (Orthoptera: Tettigoniidae, *Anabrus simplex*). *Evolution*, **38**, 1011–1022.

HARPER, J. D. 1986. Interactions between baculoviruses and other entomopathogens, chemical pesticides and parasitoids. *In* "The Biology of Baculoviruses" (R. R. Granados and B. A. Federici, Eds.), Vol. II, pp. 133–149. CRC Press, Boca Raton, FL.

HENRY, J. E. 1969. Extension of the host range of *Nosema locustae* in Orthoptera. *Ann. Entomol. Soc. Amer.*, **62**, 452–453.

HENRY, J. E. 1971. Experimental application of *Nosema locustae* for control of grasshoppers. *J. Invertebr. Pathol.*, **18**, 389–394.

HENRY, J. E. 1972. Epizootiology of infections by *Nosema locustae* Canning (Microsporida: Nosematidae) in grasshoppers. *Acrida*, **1**, 111–120.

HENRY, J. E. 1985. Effect of grasshopper species, cage density, light intensity, and method of inoculation on mass production of *Nosema locustae* (Microsporida: Nosematidae). *J. Econ. Entomol.*, **78**, 1245–1250.

HENRY, J. E., AND OMA, E. A. 1974. Effects of infections by *Nosema locustae* Canning, *Nosema acridophagus* Henry, and *Nosema cuneatum* Henry, (Microsporida: Nosematidae) in *Melanoplus bivittatus* (Say) (Orthoptera: Acrididae). *Acrida*, **3**, 223–231.

HENRY, J. E., AND OMA, E. A. 1981. Pest control by *Nosema locustae*, a pathogen of grasshoppers and crickets. *In* "Microbial Control of Pests and Plant Diseases 1970–1980" (H. D. Burges, ed.), pp. 573–586. Academic Press, New York.

HENRY, J. E., TIAHRT, K., AND OMA, E. A. 1973. Importance of timing, spore concentration, and levels of spore carrier in applications of *Nosema locustae* (Microsporida: Nosematidae) for control of grasshoppers. *J. Invertebr. Pathol.*, **21**, 263–272.

HENRY, J. E., AND ONSAGER, J. A. 1982a. Large-scale test of control of grasshoppers on rangeland with *Nosema locustae*. *J. Econ. Entomol.*, **75**, 31–35.

HENRY, J. E., AND ONSAGER, J. A. 1982b. Experimental control of the Mormon cricket *Anabrus simplex* by *Nosema locustae* (Microspora: Microsporida), a protozoan parasite of grasshoppers (Ort.: Acrididae). *Entomophaga*, **27**, 197–201.

JOHNSON, D. L., AND PAVLIKOVA, E., 1986. Reduction of consumption by grasshoppers (Orthoptera: Acrididae) infected with *Nosema locustae* Canning (Microsporida: Nosematidae). *J. Invertebr. Pathol.*, **48**, 232–238.

LARSSON, R. 1986. Ultrastructure, function, and classification of Microsporidia. *Prog. Protistol.*, **1**, 325–390.

MACVEAN, C. M. 1987. Ecology and management of Mormon cricket, *Anabrus simplex* Haldeman. *In* "Integrated Pest Management on Rangeland: A Shortgrass Prairie Perspective" (J. L. Capinera, Ed.), pp. 116–136. Westview Press.

MADDOX, J. V. 1973. The persistence of the Microsporida in the environment. *Misc. Publ. Entomol. Soc. Amer.*, **9**, 99–104.

MALONE, L. A., AND CANNING, E. U. 1982. Fine structure of *Vairimorpha plodiae* (Microspora, Burenellidae), a pathogen of *Plodia interpunctella* (Lepidoptera, Phycitidae), and infectivity of the dimorphic spores. *Protistologica*, **18**, 503–516.

OMA, E. A., AND HEWITT, G. B. 1984. Effect of *Nosema locustae* (Microsporida: Nosematidae) on food consumption in the differential grasshopper

(Orthoptera: Acrididae). *J. Econ. Entomol.*, 77, 500–501.

SOUTHWOOD, T. R. E. 1978. "Ecological Methods, with Particular Reference to the Study of Insect Populations." Wiley, New York.

SPSS Inc. 1987. "SPSS/PC+ V2.0 for the IBM PC/XT/AT and PS/2." Chicago, IL.

STEINHAUS, E. A. 1949. "Principles of insect pathology." McGraw–Hill, New York.

TANADA, Y. 1976. Epizootiology and microbial control. *In* "Comparative Pathobiology" (L. A. Bulla and T. C. Cheng, Eds.), Vol I, pp. 247–280. Plenum, New York.

TANADA, Y., AND FUXA, J. R. 1987. The pathogen population. *In* "Epizootiology of Insect Diseases" (J. R. Fuxa and Y. Tanada, Eds.), pp. 113–158. Wiley, New York.

VAVRA, J., AND MADDOX, J. V. 1976. Methods in microsporidiology. *In* "Comparative Pathobiology" (L. A. Bulla and T. C. Cheng, Eds.), Vol I. Plenum, New York.

WAKELAND, C. 1959. Mormon crickets in North America. *USDA Tech. Bull.*, 1202.

WEISER, J. 1958. Protozoan diseases in insect control. *Proc. 10th Int. Congr. Entomol. Montreal*, **4**, 681–685.

ZAR, J. H. 1984. "Biostatistical Analysis." 2nd ed. Prentice–Hall, Englewood Cliffs, NJ.