JOURNAL OF INVERTEBRATE PATHOLOGY 52, 335–342 (1988)

Mortality of Grasshoppers (Orthoptera: Acrididae) Inoculated with a Canadian Isolate of the Fungus *Verticillium lecanii*

D. L. JOHNSON, H. C. HUANG, AND A. M. HARPER

Forage and Field Crop Science Section, Agriculture Canada Research Station, Lethbridge, Alberta T1J 4B1, Canada

Received August 17, 1987; accepted February 21, 1988

The ability of *Verticillium lecanii* to infect the grasshoppers *Melanoplus bivittatus, M. packardii,* and *M. sanguinipes* was determined in laboratory and field cage experiments. Four types of inoculation were investigated: grasshoppers sprayed with a spore suspension (3.2×10^7 spores/ml), grasshoppers fed on wheat leaves sprayed with a spore suspension, grasshoppers fed wheat bran colonized by *V. lecanii,* and grasshoppers confined with cadavers of conspecifics infected and killed by *V. lecanii.* The first three treatments resulted in measurable rates of infection and a statistically significant increase in mortality over mortality of untreated grasshoppers. No transmission of the disease from infected cadavers occurred. In field cage experiments, spores sprayed onto grasshoppers confined on pasture grass resulted in ca. 40% mortality. Application of 2.5 g of mycelium-infested wheat bran/m^2 resulted in 48% mortality of grasshoppers. Although moisture was a significant factor in determining pathogenic efficacy in the laboratory experiments, this factor did not strongly affect the rate of infection in the field cages.

KEY WORDS: *Verticillium lecanii;* biological control; grasshoppers; mycosis; inoculation; entomopathogen.

INTRODUCTION

The fungus *Verticillium lecanii* is a widespread entomopathogen. It may provide control of homopteran pests, notably the whitefly *Trialeurodes vaporariorum* (see Hall, 1982), aphids such as *Myzus persicae* (see Hall and Burges, 1979), and the scale-insect *Coccus hesperidum* (see Samšiňáková and Kálalová, 1975). It also is known to infect certain beetles (Barson, 1976; Samšiňáková, 1977). In 1984, an isolate of *V. lecanii* from a soil sample from Mount Allan in the Rocky Mountains of Alberta, Canada, was identified and cultured by J. Bissett at the Biosystematics Research Institute, Ottawa, Ontario. This isolate was tested for activity against nine insect pest species by Harper and Huang (1986) and showed potential for infecting the migratory grasshopper *Melanoplus sanguinipes.* All grasshoppers dipped in a spore suspension of 8.4×10^7 spores/ml for 30 sec died within 10 days, whereas 8, 21, and 37% of the grasshoppers kept in three cages with plants sprayed with the spore suspension died.

Grasshoppers are the most prominent insect pests of grasses and other crops on the Canadian Prairies. In 1986, of 156,600 km^2 of agricultural land surveyed in Alberta, 19,350 km^2 were rated as severely infested with grasshoppers (Johnson and Andrews, 1987). An additional 40,900 km^2 were moderately infested. Although grasshoppers tend to be primarily a dryland pest, they also attack irrigated crops in Alberta, especially alfalfa and soft white spring wheat, in which fungi could be valuable for integrated control.

We performed three experiments designed to expand on the initial findings concerning the effects of *V. lecanii* on grasshoppers. Our objectives were to assess various means of inoculation of grasshoppers with *V. lecanii,* and to determine whether field applications of *V. lecanii* were practical.

MATERIALS AND METHODS

Preparation of Inoculum

V. lecanii (DAOM 179104) was main-

tained on potato dextrose agar (PDA) in slant cultures. For experiment 1, three substrates (wheat bran, rolled oatmeal, and corn meal) were used to increase *V. lecanii* for the feeding test. The autoclaved, moistened substrates were kept in 40-ml Erlenmeyer flasks, and each flask was inoculated with 3 ml of a suspension containing 1.9×10^7 spores/ml of *V. lecanii* collected from 1-week-old cultures on PDA plates. The flasks were kept at 20°–22°C for 13 days until the fungus colonized the entire substrate. The inoculated substrate was removed from the flasks, dried for 5 hr under a transfer hood, and crumbled into small particles ca. 1–3 mm in diameter. Noninoculated substrates prepared by the same methods were used as controls.

For experiments 2 and 3, 13-day-old cultures of *V. lecanii* on PDA plates were used to prepare a spore suspension (3.2×10^7 spores/ml) for inoculating grasshoppers or infesting wheat seedlings. Five flats (each 26×52 cm) of wheat seedlings at the three- to four-leaf stage (ca. 4 seedlings/cm^2) were sprayed with 60 ml of the suspension per flat, using an atomizer. For inoculation of grasshoppers, 260 insects in a 4-liter glass jar were sprayed with 20 ml of the suspension.

Grasshopper cadavers infected with *V. lecanii* were produced by dipping living grasshoppers in a spore suspension as described by Harper and Huang (1986).

Experiment 1. Consumption of Mycelium in Food

The grasshoppers used in this experiment were reared in the laboratory from eggs laid in rearing cages by adults collected in the field 6 months before the experiment. Before hatching, the eggs were stored for 80 days at 0°–1°C to terminate diapause.

In this experiment, each of 90 adult *M. sanguinipes* was confined in a translucent 250-ml polyethylene cage, and received one of six diets: wheat bran, corn meal, or rolled oats, with or without the colonization of *V. lecanii* in the food. The 90 cages were randomly arranged on a single shelf in a dimly lit controlled environment room kept at a constant temperature of 25°C. After 72 hr, all grasshoppers were taken off the grain diets and provided with clean lettuce leaves, replaced every second day. Records of mortality and behavior were made daily for 16 days. Cadavers of the grasshoppers that died during the experiment were surface-sterilized by immersion in 95% ethanol for 90 sec, incubated at room temperature for 5 days, and examined for the presence of *V. lecanii* conidiophores. The numbers of the grasshoppers successfully infected by feeding on the three substrates were compared using χ^2 contingency tests.

Experiment 2. Laboratory Assessment of Methods of Inoculation

This experiment was designed to assess four methods of inoculation of grasshopper nymphs with *V. lecanii*. A total of 900 second-instar *M. packardii* were treated. The grasshoppers were reared in the laboratory (in $37 \times 37 \times 26$-cm cages) from eggs collected at a field site near Lethbridge, Alberta. An experimental unit consisted of a single grasshopper in a 300-ml sealed transparent plastic cage with a 2-cm sand layer on the cage floor. Each cage was randomly assigned to one of five treatments: (1) primary contact: before confinement in the cage, the grasshoppers were sprayed to runoff with a spore suspension of 3.2×10^7 spores/ml with a hand-pump mist sprayer; (2) spores on food: wheat leaves were sprayed with the spore suspension and fed to unsprayed grasshoppers; (3) mycelium in food: *V. lecanii* grown on wheat bran was included in the cages as food; (4) horizontal transmission: infected cadavers, obtained by dipping grasshoppers in the spore suspension the week before commencement of the experiment, were added to cages containing uninoculated grasshoppers; (5) control: uninoculated grasshoppers were provided clean wheat leaves and sterile wheat bran.

Primary contact refers to a condition in which the conidia, in aqueous spray, initially contact the integument. Secondary contact occurs when conidia initially contact noninsect surfaces such as plants or soil, and are secondarily transferred to the insect's integument, or to the insect mouthparts and gut during feeding.

The five treatments were crossed with a moisture treatment (moist vs dry), resulting in a total of 10 treatments. To provide two different moisture regimens, the oven-dried sand on the floors of the cages received either 0 or 3 ml of distilled water on the first day of the experiment. The resulting initial relative humidity was 10–18% and 95–97%, respectively. By the end of the experiment, the addition of wheat leaves as food increased the moisture in the dry cages, resulting in a relative humidity of 40–50%. A total of 90 caged second-instar grasshoppers were randomly assigned to each of the 10 treatments. The 900 cages were arranged in three layers (i.e., randomized complete blocks) in a phytotron (16:8 L:D, 25°:15°C day:night). The cabinet provided lighting of 310 $\mu E\ m^{-2}\ sec^{-1}$ photon flux density, from cool white fluorescent and incandescent light bulbs (2:1 power ratio).

The grasshoppers were examined every 4 days, and mortality was recorded. The experiment was terminated 16 days after application of the treatments.

The effectiveness of the treatments, and their interactions with environmental moisture, were assessed with analysis of variance appropriate to a factorial design in randomized complete blocks (GLM, SAS Institute Inc., 1982), and with stepwise logistic regression of mortality events (1,0), as a function of block, method of treatment, and moisture regime (BMDPLR, Dixon and Brown, 1979). Specific hypotheses were tested with orthogonal comparisons. Pairwise comparisons of mean mortality rates were made with Tukey's HSD test (Steel and Torrie, 1980). A descriptive statistic that estimates the mortality in the treated group after adjusting for mortality in the control group was calculated from the modified Abbott's formula:

$$100\left[1 - \frac{T_2C_1}{T_1C_2}\right],$$

where T_1 and T_2 are the number of subjects alive in the treated group at the start and finish of the period of interest, and C_1 and C_2 are the corresponding numbers in the untreated group (Abbott, 1925; Connin and Kuitert, 1952). The initial values, T_1 and C_1, were unequal only in cases in which grasshoppers escaped, resulting in some treatments having slightly fewer than the original 90 grasshoppers included in the assays.

Experiment 3. Cages on Pasture Grass

Fifty screened, open-bottomed cages, each 1 meter in height and covering 1 m^2 of grass, were arranged in five randomized complete blocks on 1 hectare of crested wheat grass (*Agropyron cristatum*) pasture, at the Lethbridge Research Station. The wood frame bottoms of the cages were pushed into the sod and banked with 2–5 cm of soil to preclude the possibility of escape. Thirty field-collected third-instar grasshoppers of each of two species, *M. bivittatus* and *M. packardii*, were added to each cage before application of the treatments. The grasshoppers were collected from an untreated grassland site with a sweepnet, and held at 16°C for 2 days before introduction to the cages. The five inoculation treatments were: (1) 1.0×10^7 spores suspended in 20 ml of distilled water/m^2 sprayed onto the grass in the cage; (2) 1.0×10^8 spores suspended in 20 ml of distilled water/m^2 sprayed onto the grass in the cage; (3) infested bran scattered over the grass in the cages at a rate of 0.25 g/m^2; (4) infested bran at 2.5 g/m^2; and (5) an uninoculated control group. The treatments were applied once, between 5:00 and 7:00 PM on June 13, 1985.

We also included ambient moisture (dry vs low moisture) as a treatment in the field cage experiment. In each block of 10 cages, there were two cages for each of the inoc-

ulation treatments. One of these received 1 liter of distilled water every second morning for 6 days, applied with a watering can, and the other cage remained dry. This amount simulated trace rainfall in 25 of the cages and dry prairie conditions in the remaining 25 cages.

Five days after the date of treatment application, the surviving grasshoppers were counted, but not removed. Fifteen days after initiation of the experiment, the surviving grasshoppers were removed from the cages with vacuum cleaners and counted. Since all of the 50 cages started with an equal number of grasshoppers (60/cage), analysis of variance of the $\log_e$-transformed number surviving per cage was used to assess the effects of the inoculation and moisture treatments. The percentage mortality in each treatment was calculated by applying Abbott's adjustment to the untransformed counts in the appropriate check and treatment groups.

RESULTS AND DISCUSSION

Experiment 1. Consumption of Mycelium in Food

Although the grasshoppers appeared to consume the wheat bran more readily than corn meal or rolled oats, there were no significant differences in the observed grasshopper mortality or in the number of infections among the three substrates colonized by *V. lecanii* ($\chi^2 = 1.68$, $P > 0.4$). The numbers found dead are shown in Figure 1. The cumulative mortality in the treated and untreated groups differed significantly on sampling dates after 10 days ($\chi^2 = 6.94$, $P < 0.01$). Cadavers removed from the treated cages 6 days after treatment were infected. By the 16th day, internal *V. lecanii* infection occurred in 34 of the 45 grasshoppers that were provided food containing mycelium. Seventeen of the 34 infected grasshoppers died during the experiment (Fig. 1). None of the living or dead grasshoppers in the control group were infected by *V. lecanii*.

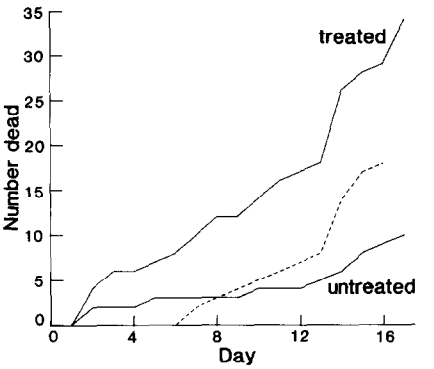

FIG. 1. The number of dead grasshoppers in the *Verticillium lecanii*-treated and untreated groups in experiment 1. Initially there were 45 grasshoppers in each group. The dotted line indicates the number of dead grasshoppers from the treated group that were found to be infected.

Most of the surviving grasshoppers were lethargic, and some exhibited degeneration of the tarsi and tibiae. Biting of the tibiotarsal region, probably indicating irritation, was common behavior among heavily infected grasshoppers.

None of the 45 grasshoppers in the control group were infected with *V. lecanii*, although virtually all dead grasshoppers contained *Rhizopus* sp., *Penicillium* sp., or *Aspergillus* sp. These fungi are common nonlethal contaminants in grasshopper gut contents and feces.

Experiment 2. Laboratory Assessment of Methods of Inoculation

Of the four inoculation methods tested in laboratory cages, only confinement with infected cadavers did not result in grasshopper mortality greater than that of the untreated group (Table 1). Confinement with cadavers was included as a treatment to provide an estimate of the rate of transmission of infection from one grasshopper to another by contact, cannibalism of infected tissue, or other types of spore dispersal. Observations made on sampling dates indicated that, although cannibalism among grasshoppers is common in nature (Henry, 1972), the confined grasshoppers were not feeding on the infected cadavers. The lack

TABLE 1
THE PERCENTAGE (UNADJUSTED) OF GRASSHOPPERS KILLED BY *Verticillium lecanii* UNDER LABORATORY CONDITIONS (EXPERIMENT 2)

Treatment	*n*	Days after treatment application[1] 4	8	12
Spores, contact spray	179	90	99	99
Spores on wheat leaves	180	20	69	87
Mycelium in bran	178	31	72	85
Confined with cadavers	180	11	33	51
Untreated group	179	19	44	61

[1] The proportion is the average of *n* quantal observations. The results of significance tests are given in the text. The adjustments for the mortality in the untreated population are shown in Figure 2.

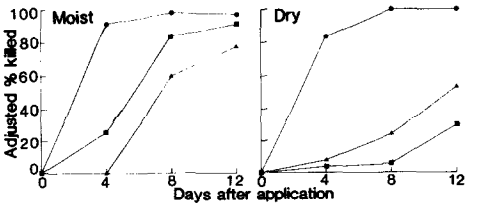

FIG. 2. The adjusted percentage mortality attributable to the *Verticillium lecanii* treatments applied in experiment 2: spore suspension spray (●), spores on wheat leaves (▲), and mycelium in bran (■). The percentages on the 16th day were not calculated, because of high mortality in the untreated group.

of transmission of the pathogen during confinement with infected cadavers, even in the moist treatments, could be due to little or no direct contact between healthy grasshoppers and the cadavers. Some of the cadavers had *V. lecanii* conidiophores clearly visible on the integument.

Moisture significantly altered the relative effectiveness of the methods of inoculation 8 days after treatment (contact vs noncontact methods, orthogonal comparisons of the treatment by moisture interactions, $P < 0.01$). Stepwise logistic regression indicated that the probability of death was not affected by moisture during the first 4 days after treatment ($P = 0.25$), but that the moisture treatment significantly increased the infection rate after this date ($P < 0.05$). The effects of environmental moisture on mortality were most apparent in the bran treatment and least apparent in the treatment in which insects were sprayed with spore suspension. Only direct contact of the spores, in the aqueous spray treatment, resulted in rapid infection and mortality under both moisture regimes (Fig. 2). It is possible that the water provided by the spray was sufficient to allow infection to occur. This explanation is consistent with recent findings of Drummond et al. (1986) that high humidity is more important for the establishment of *V. lecanii* infection of the whitefly than for the subsequent growth and sporulation of the fungus.

The spores sprayed onto wheat leaves did not result in mortality significantly different from the untreated group until 8 days after treatment ($P < 0.05$), with or without added water. The bran treatment appeared to respond to moisture more rapidly, but the overall effectiveness of the bran and wheat leaf treatments did not differ significantly after 8 days (orthogonal comparison, $P > 0.1$). Wheat bran was chosen as the mycelium carrier since it is readily accepted by grasshoppers.

The proportion of grasshoppers that died in all fungus-treated groups was significantly greater than the proportion that died in the untreated group, on all sampling dates. However, statistical analysis of the data after 8 days must be interpreted with caution due to the mortality in the untreated group (Table 1). Although the treated and untreated groups can be expected to experience the same background mortality, it reduces the power of the experiment to discriminate treatment effects. During adverse weather conditions, mortality of grasshopper nymphs can be high. However, under more usual conditions, natural mortality in the field is not as high as in confinement.

Shelf layer (bottom, middle, or top block) did not have a significant effect on mortality ($P > 0.05$).

Experiment 3. Cages on Pasture Grass

During the field experiment, the maxi-

mum and minimum air temperatures were 31.5° and 1.5°C, with typical daytime temperatures in the range 18–26°C. A total of 177.3 hr of sunshine were recorded during the 15 days. Only 1.4 mm of rain fell (recorded on the seventh day after treatment application). The grass and leaf litter were relatively dry, because the total rainfall in May and June was 74 mm, only 57% of the May and June average for the preceding 50 years. The crop height at the research pasture averaged 16 cm, with a measured standing crop of 24.4 g/m^2 dry weight (n = 40 clipped 1-m^2 samples, SEM = 1.14 g/m^2).

The number of grasshoppers that died during the 15 days did not differ between the two species ($P > 0.1$). Consequently, we analyzed the total number of survivors without respect to species. Mortality attributable to the effects of the treatments in the field was only about half as great as in the laboratory experiments (Table 2).

Unlike results in the laboratory, the moisture treatment (simulated trace rainfall) was not a significant factor in determining the success rate of the treatments applied in the field cages, nor did moisture interact with the relative efficacy of the methods of inoculation ($P > 0.5$). If the addition of water improves the activity of *V. lecanii* on these species, it does so at higher levels of moisture than were applied to the cages in this experiment (1 liter/m^2 every 48 hr). Six days after treatment, the spores sprayed in water resulted in detectable mortality, and performed significantly better than the bran treatment ($P = 0.01$). Since the grasshoppers were in the cages when the treatments were applied, the spore suspension spray treatments allow for both primary and secondary contact with spores.

After 15 days, the efficacy of the spore and bran treatments did not differ significantly ($P > 0.6$). On neither date did the higher rate of spores (1×10^8 spores/m^2) perform better than the lower rate (1×10^7 spores/m^2) ($P > 0.7$). Apparently the rate of infection of the insects by *V. lecanii,* and subsequent growth and mycosis, was limited by factors other than the quantity of spores present.

After 15 days, the higher rate of mycelium-infested bran (2.5 g/m^2) resulted in significantly more mortality than the lower rate ($P = 0.01$). The effects of the lower rate did not differ from the results in

TABLE 2
THE EFFECT OF *Verticillium lecanii* ON SURVIVAL OF GRASSHOPPERS UNDER FIELD CONDITIONS[1]

Treatment	After 6 days		After 15 days	
	Number alive	Percentage mortality[2]	Number alive	Percentage mortality[2]
Mycelium in bran:				
0.25 g/m^2	23.8 ab (1.7)	11%	20.0 ab (1.8)	10%
2.5 g/m^2	18.9 ab (1.8)	29%	11.4 c (2.3)	48%
Spores sprayed:				
1.0×10^7/m^2	17.3 b (2.4)	35%	13.8 abc (2.7)	38%
1.0×10^8/m^2	17.8 b (1.6)	33%	13.0 bc (1.5)	41%
Untreated	26.7 a (1.8)		22.1 a (1.4)	

[1] The mean numbers of grasshoppers (and SEM) surviving in the field cages after treatment with *V. lecanii.* The effects of water were not statistically significant, so this factor is not shown in the table. Each of the means shown above is based on the results of n = 10 cages. Means within a column followed by the same letter do not differ significantly (Tukey's HSD, $\alpha = 0.05$).

[2] Adjusted for mortality in the untreated groups by Abbott's formula. Since the grasshoppers were confined in 1-m^2 caged areas, reductions in abundance were considered to represent mortality.

the untreated cages (Tukey's HSD, $P > 0.05$). The lower rate, equivalent to 2.5 kg/hectare, is a realistic field application rate used for insecticides and *Nosema locustae* on bran carrier (Johnson and Henry, 1987). However, it was not enough for infection of grasshoppers by *V. lecanii* in our field experiment. At the end of the 15-day experimental period, only the higher rate of spore application and the higher rate of bran application resulted in significantly reduced survival of grasshoppers ($P < 0.05$; Table 2). Because of the nature of the pathogen, the bran treatment is not strictly a per os treatment, and may also include the effects of contact with spores produced on the bran or at the soil surface.

The increment in mortality from the 6th to the 15th day did not differ among treatments. All of the effects of the fungus treatments on the grasshoppers occurred within 6 days of treatment application. This indicates that there was effectively no transmission from infected grasshoppers to the survivors in the field cages within the 15 days available, a period that is probably not long enough for the infected insects to produce spores that would spread the disease. Our assessment was designed to measure the short-term effects of the pathogen, and not the long-term epizootiology.

We expect that *V. lecanii* would be more widely transmitted among grasshoppers after wet weather, or during periods of overcast and high humidity. However, this does not preclude its use as a microbial insecticide. Applications made during the evening or early morning may achieve sufficient initial infection to have a detectable impact on the populations. Application during the early season (during May and early June, on the Canadian prairies) could prove more effective, since rainfall is higher, daily temperature maxima are lower, and grasshoppers are younger and more susceptible than in late June and July. In some experimental alfalfa insect control programs (e.g., control of spotted alfalfa aphid by Hall and Dunn, 1958), distribution of entomopathogenic fungi has been restricted to fields under irrigation. *V. lecanii* may provide reductions in insect abundance under these conditions. The efficacy is low, however, and the treatment would be useful only in situations in which population density is not extremely high and rapid and highly effective control is not required.

ACKNOWLEDGMENTS

We thank R. C. Andrews, M. J. Kokko, and P. Story of the Agriculture Canada Research Station, Lethbridge, Alberta, for technical assistance throughout the experiments. The *Verticillium lecanii* isolate (DAOM 179104) was supplied by J. Bissett, Biosystematics Research Institute, Ottawa, Ontario.

REFERENCES

ABBOT, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18**, 265–267.

BARSON, G. 1976. Laboratory studies on the fungus *Verticillium lecanii*, a larval pathogen of the large elm bark bettle (*Scolytus scolytus*). *Ann. Appl. Biol.*, **83**, 207–214.

CONNIN, R. V., AND KUITERT, L. C. 1952. Control of the American grasshopper with organic insecticides in Florida. *J. Econ. Entomol.*, **45**, 684–687.

DIXON, W. J., AND BROWN, M. B. 1979. "Biomedical Computer Programs, P-Series." Univ. California Press, Berkeley, California.

DRUMMOND, J., HUSSEY, N. W., AND HEALE, J. B. 1986. The effect of humidity on the pathogenicity of *Verticillium lecanii* towards the glasshouse whitefly *Trialeurodes vaporariorum*. *In* "Fundamental and Applied Aspects of Invertebrate Pathology" (R. A. Samson, J. M. Vlak, and D. Peters, Eds.), p. 248. Fourth International Colloquium of Invertebrate Pathology, Wageningen.

HALL, I. M., AND DUNN, P. H. 1958. Artificial dissemination of Entomophthorous fungi pathogenic to the spotted alfalfa aphid in California. *J. Econ. Entomol.*, **51**, 341–344.

HALL, R. A. 1982. Control of whitefly, *Trialeurodes vaporariorum* and cotton aphid, *Aphis gossypii* in glasshouses by two isolates of the fungus, *Verticillium lecanii*. *Ann. Appl. Biol.*, **101**, 1–12.

HALL, R. A., AND BURGES, H. D. 1979. Control of aphids in glasshouses with the fungus, *Verticillium lecanii*. *Ann. Appl. Biol.*, **93**, 235–246.

HARPER, A. M., AND HUANG, H. C. 1986. Evaluation of the entomophagous fungus *Verticillium lecanii* (Moniliales: Moniliaceae) as a control agent for insects. *Environ. Entomol.*, **15**, 281–284.

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

JOHNSON, D. L., AND ANDREWS, R. C. 1987. "The 1987 Alberta Grasshopper Forecast." Canadex. Agriculture Canada, Ottawa, Ontario, in press.

Johnson, D. L., and Henry, J. E. 1987. Low rates of insecticides and *Nosema locustae* (Microsporidia: Nosematidae) on baits applied to roadsides for grasshopper (Orthoptera: Acrididae) control. *J. Econ. Entomol.*, **80**, 685–689.

SAMŠIŇÁKOVÁ, A. 1977. The effect of fungi preparations on larvae of the Colorado beetle, *Leptinotarsa decemlineata*. *Acta. Entomol. Bohemoslov.*, **74**, 76–80.

SAMŠIŇÁKOVÁ, A., AND KÁLALOVÁ, S. 1975. Artificial infection of scale-insect with entomophagous fungi *Verticillium lecanii* and *Aspergillus candidus*. *Entomophaga*, **20**, 361–364.

SAS INSTITUTE INC. 1982. "SAS User's Guide: Statistics." Cary, North Carolina.

STEEL, R. G. D., AND TORRIE, J. H. 1980. "Principles and Procedures of Statistics: A Biometrical Approach," 2nd ed. McGraw–Hill, New York.