

# I. Biological Control

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Many wildlife species, like this lark bunting, choose grasshoppers as food for their young. Favoring bird populations can help limit grasshoppers in a complementary effort with other control methods. (Photograph by chapter author Lowell C. McEwen, of Colorado State University; used by permission.)



## I.1 Biological Control: An Introduction

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DeBach (1964) defined biological control as “the action of parasites, predators, or pathogens (disease-causing organisms) in maintaining another organism’s population density at a lower average than would occur in their absence.” A more recent definition proposed by the National Academy of Sciences (1987) for biological control is “the use of natural or modified organisms, genes, or gene products to reduce the effects of undesirable organisms (pests), and to favor desirable organisms such as crops, trees, animals, and beneficial insects and microorganisms.”

While many people may share the wider view of biological control that encompasses the methods broadly defined by the National Academy of Sciences, Garcia et al. (1988) make some valid arguments for using DeBach’s definition because it emphasizes the concepts of self-sustaining and density-dependent regulation of one species by another. For land managers’ purposes, the more traditional definition of biological control proposed by DeBach will be used in this introduction.

Constraints on the use of chemical pesticides may benefit the development of biological control options and their implementation in an integrated pest management (IPM) program. The U.S. Department of Agriculture’s (USDA) Animal and Plant Health Inspection Service (APHIS) (1994 unpubl.) defines IPM as “the selection, integration, and implementation of pest management tactics in a systems approach on the basis of anticipated biological, economic, ecological, and sociological indicators.” For a more thorough discussion of IPM, refer to the excellent review article by Cate and Hinkle (1993) describing the history and progression of IPM.

Biological control is usually achieved through one or a combination of the following approaches: conservation, augmentation, and classical biological control.

- Conservation is an approach whereby management systems are manipulated to enhance or conserve naturally occurring biological control agents.
- The augmentation approach includes both inoculative and inundative releases of biological control agents. An inoculative release depends upon the biological control agent reproducing, persisting, and spreading on its own accord in the pest population. Inundative releases are more of a short-term control measure with biological

control agents causing a more immediate reduction in the pest population but lacking the ability to persist or spread in the environment.

- In the classical approach, exotic (not native) pest species are controlled by the introduction and establishment of exotic biological control agents. Classical biological control has been extremely successful at controlling pests, and current Federal regulations are adequate to monitor and safeguard the importation of biological control agents (Soper 1992).

The approach to classical biological control proposed by Hokkanen and Pimentel (1984, 1989) involves the selection of promising biological control agents from exotic sources for the control of native pest species. Major premises for this approach are a greater likelihood for success using this new association and the ability to control native pests, which represent 60–80 percent of all pest species (Hokkanen and Pimentel 1989).

In the early 1990’s, a parasitic wasp and a fungus from Australia were imported into the United States for evaluation as biological control agents against rangeland grasshoppers in the Western United States. Some scientists raised concerns regarding whether the importation of exotic agents would result in some risk to the environment. While concerns about the release of exotic biological control agents are sensible, no major problems are reported from the use of these agents in the United States (Carruthers and Onsager 1993). For a more detailed discussion of this issue, see Lockwood (1993a, b) or Howarth (1991) and Carruthers and Onsager (1993) and/or chapters VII.4 and VII.6 in the Future Directions section of this handbook.

Here in section I, some review chapters on the current status of biological control of grasshoppers discuss the potential of parasites, predators, and pathogens. Various authors in this section describe some research projects funded during the USDA, APHIS, Grasshopper Integrated Pest Management (GHIPM) Project. Topics include identification of fungal pathogens, laboratory assays to assess the effectiveness of *Nosema locustae*, and construction of bird nest boxes. These chapters provide a solid foundation of knowledge on the biological control of grasshoppers. Basic and applied research will continue to be essential in the development and implementation of biological control strategies.

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## I.2 *Nosema locustae*

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### Introduction

Grasshoppers are the most economically important insect pests on rangeland in the Western United States (Hewitt and Onsager 1982). A conservative estimate for the average value of rangeland forage loss to grasshoppers in the West each year is about \$393 million (Hewitt and Onsager 1983). Since the late 1960's, controlling major infestations of grasshoppers on rangeland has involved the use of chemical insecticides, primarily malathion and carbaryl. However, increasing awareness of the environmental risk associated with the exclusive use of chemical insecticides led to the establishment of the Grasshopper Integrated Pest Management (GHIPM) Project.

Disease-causing micro-organisms have been investigated as potential biological control agents of grasshoppers for many years. Probably the most well-known case has been the parasite *Nosema locustae*, a pathogen that was selected in the early 1960's for development as a microbial control agent for use in long-term suppression of grasshoppers (Henry 1978, Onsager 1988). *Nosema locustae* is the only registered microbial agent that is commercially available for control of rangeland grasshoppers.

*Nosema* has been studied more than any other microbial control agent for the suppression of grasshopper populations. Applications of *Nosema* formulated on a wheat bran bait have resulted in numerous successful introductions of the pathogen into field populations. However, while this parasite has proven a potentially effective tool in grasshopper management, several questions have been raised regarding the effectiveness of *Nosema* in the field.

### Unpredictability of *Nosema*

Vaughn et al. (I.4) attributed the apparent failures of *Nosema* to low-quality material, equipment failure, poor formulation, inappropriate target species, and unreasonable expectations by users. Onsager (1988) also discussed some of the reasons for this lack of confidence in *Nosema* for controlling grasshopper populations. He noted that the traditional sampling approach used to estimate grasshopper reductions in field trials with chemical insecticides may not be appropriate to assess the effectiveness of *Nosema*. Typically *Nosema* requires much

longer to kill a grasshopper than chemicals. Grasshoppers are then able to disperse and conceal differences between treated and control plots.

Reuter et al. (1990) suggested that the standard application rate of *Nosema* ( $1 \times 10^9$  spores/acre) was too low to induce immediate grasshopper population suppression. In a field evaluation, an untreated control plot was compared to plots receiving either the standard rate ( $1 \times 10^9$  spores/acre) or a higher (100 $\times$ ) rate ( $1 \times 10^{11}$  spores/acre) of *Nosema*. Density estimates were taken weekly, and bottomless field cages and small rearing cages were used to estimate mortality. The lack of treatment replication, the small plot size, and the close proximity of plots made it impossible to draw firm conclusions about the grasshopper densities or relative rates of suppression after treatment. However, significant mortality was observed at the higher application rate for *Melanoplus sanguinipes* in the small rearing cages 7 weeks after application (Reuter et al. 1990). These preliminary mortality results lend support to Henry's (1981) contention that applying higher dosages of *Nosema* will not necessarily produce a commensurate gain in density reduction.

A more immediate density reduction has been demonstrated in field studies using wheat bran bait formulations of *Nosema* and carbaryl in which significant short-term response to carbaryl was followed by a later response to *N. locustae* (Onsager et al. 1981). Further studies on the response of grasshoppers to higher application rates of *Nosema* may be warranted.

A review of the literature on the effectiveness of *Nosema* in the field identifies dispersal as a common problem. Movement between plots was cited as affecting results in six of eight studies that evaluated the effects of *Nosema* in the field (Henry 1971; Henry and Oma 1974, 1981; Henry and Onsager 1982; Henry et al. 1973, 1978). Only Johnson and Henry (1987) suggested that there was little movement of infected individuals into control plots within 31 days of application.

### Detection of *Nosema locustae*

In the past, visual examinations with phase contrast microscopy for spores have been required to detect *Nosema* infection in grasshoppers. Generally, *Nosema*

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spores are detectable about 21 days after application (Henry and Oma 1974). Most protocols recommend microscopic examinations at 28 days following application (Henry 1978). Thus, it has not been possible to assess some of the earlier events in a *Nosema* treatment program.

Dispersal and death that occur prior to the detection of *Nosema* reduce estimates of its presence in the field. Early detection of *Nosema* infections is therefore necessary to obtain unbiased estimates of initial prevalence. Scientists have developed a sensitive nucleic acid probe for the detection of *Nosema* in grasshoppers. Data indicate that the probe can reliably detect *Nosema* in grasshoppers within 7–10 days after infection. Use of a probe to estimate infection rates should eliminate much of the inherent bias associated with visual examination.

## ***Nosema* Transmission**

A recent laboratory study by Raina et al. (1995) has reported transovarial transmission of *N. locustae* in *Locusta migratoria migratorioides* with the incidence of infection ranging from 72 percent to 92 percent among progeny up to the F7 generation. *N. locustae* spores also were found in all nymphal instars for the F1 and F2 generations.

The mechanisms and rates of *Nosema* transmission in the field have not been addressed adequately. Spores have been observed in feces (Henry 1969 unpubl.), but the scavenging of *Nosema*-infected cadavers by healthy grasshoppers may represent the greatest potential for transmission to uninfected grasshoppers of the same generation. Scavenging of cadavers is common in many species of grasshoppers (Lavigne and Pfadt 1964, Lockwood 1988). Henry (1969 unpubl.) observed feeding on *Nosema*-infected cadavers in the field. Scavenging may offer a very efficient means for transmission of *Nosema* during the year of treatment and possibly into later generations (O'Neill et al. 1994).

Spores of *Nosema* have been observed in ovaries from and in eggs produced by infected females (Henry 1969 unpubl.). Although Ewen and Mukerji (1980) were unable to find spores in eggs collected from *Nosema*-treated plots, they did observe *Nosema* infection among

nymphs raised from field-collected eggs. Henry and Onsager (1982) also reported infection in grasshopper populations during the year after treatment. These observations indicate that transmission to subsequent generations is indeed likely, but the details of *Nosema* transmission in field populations of grasshoppers have never been fully explained.

## **Effect on Grasshopper Egg Production**

*Nosema*-infected females produce fewer eggs than healthy females (Henry and Oma 1981). Henry (1969, 1971) reported detecting little ovarian or egg debris in infected grasshoppers that were ground up, which suggests that infected females fail to develop reproductively. Ewen and Mukerji (1980) reported substantially lower rates of egg laying after applications of *Nosema* in the field. Henry and Oma (1981) suggested the need to measure the effects of *Nosema* on egg numbers and egg viability. Lockwood and Debrey (1990) also observed some evidence of lower egg production in higher populations (greater than 11.5 grasshoppers/yard<sup>2</sup> or 9.6 grasshoppers/m<sup>2</sup>) of grasshoppers treated with *Nosema*.

## **Conclusions**

Until the reasons for the inconsistent response of *Nosema* to grasshoppers are better understood, its effectiveness will probably continue to be disputed (See I.4.). The grasshopper species complex, the age of the grasshoppers, and population density can affect the response to a *Nosema* application. Therefore, a more comprehensive approach is needed to adequately assess *Nosema* against grasshoppers. This approach must include a better understanding of the major disease processes of *Nosema*. Vaughn's team (I.4) recommends that *Nosema* be used to suppress rangeland grasshoppers in environmentally sensitive areas where cost and acute insecticide control are not primary concerns and proposes the use of higher rates and/or multiple applications when environmental issues outweigh the economic issues.

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## I.3 Laboratory Bioassays of *Nosema locustae*

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### Introduction

The use of living insect pathogens as biocontrol agents for insects requires that the virulence (killing power) of these agents must be monitored occasionally, especially just prior to their distribution into the environment. Evaluation of an agent's virulence can be accomplished through the use of laboratory bioassays involving the target insects (raised in the laboratory) and the biocontrol agents that are to be tested.

The first biocontrol agent registered by the U.S. Environmental Protection Agency for grasshopper pests was the protozoan *Nosema locustae*. Grasshoppers acquire *N. locustae* infections by eating its spore stage.

*N. locustae* infects the fat bodies of grasshoppers and is only mildly pathogenic to its host. For several years, our lab at South Dakota State University (SDSU) has been bioassaying the viability and virulence of *N. locustae* spores supplied commercially to the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA, APHIS, PPQ) Grasshopper Integrated Pest Management (GHIPM) Project. Laboratory-reared third-instar *Melanoplus sanguinipes* grasshoppers were used as the target insect for these bioassays, and the grasshoppers were fed the *Nosema* spores on small disks cut from romaine lettuce. The grasshoppers were then kept in the lab for 20 days, and LD<sub>50</sub> (the calculated dose of pathogen or toxin that kills half of the bioassayed grasshoppers) values were calculated based upon the percentage of grasshoppers that had died by the end of the time period.

When *Nosema* is used to control grasshoppers, spores are typically applied on rangelands with a wheat-bran bait. Lettuce bioassays can be used only to measure the viability of spores prior to the spores' addition to wheat bran. The purpose of this chapter is to describe the protocol used in our laboratory to measure the virulence of *N. locustae* spores stored in water and applied to lettuce disks, and also to describe a bioassay protocol that we've used for measuring the virulence of these spores after their addition to wheat bran. Representative results from these bioassays are reported in this chapter.

### Lettuce Bioassay

**Methods.**—*Nosema locustae* spores used for these studies were provided by various commercial sources. All spores were stored in distilled water at  $-4^{\circ}\text{F}$  ( $-20^{\circ}\text{C}$ ) until use. *Melanoplus sanguinipes* grasshoppers used in the studies were a lab-reared Canadian strain that had been maintained at SDSU for several years. These grasshoppers were reared according to the recommendations provided by Henry (1985).

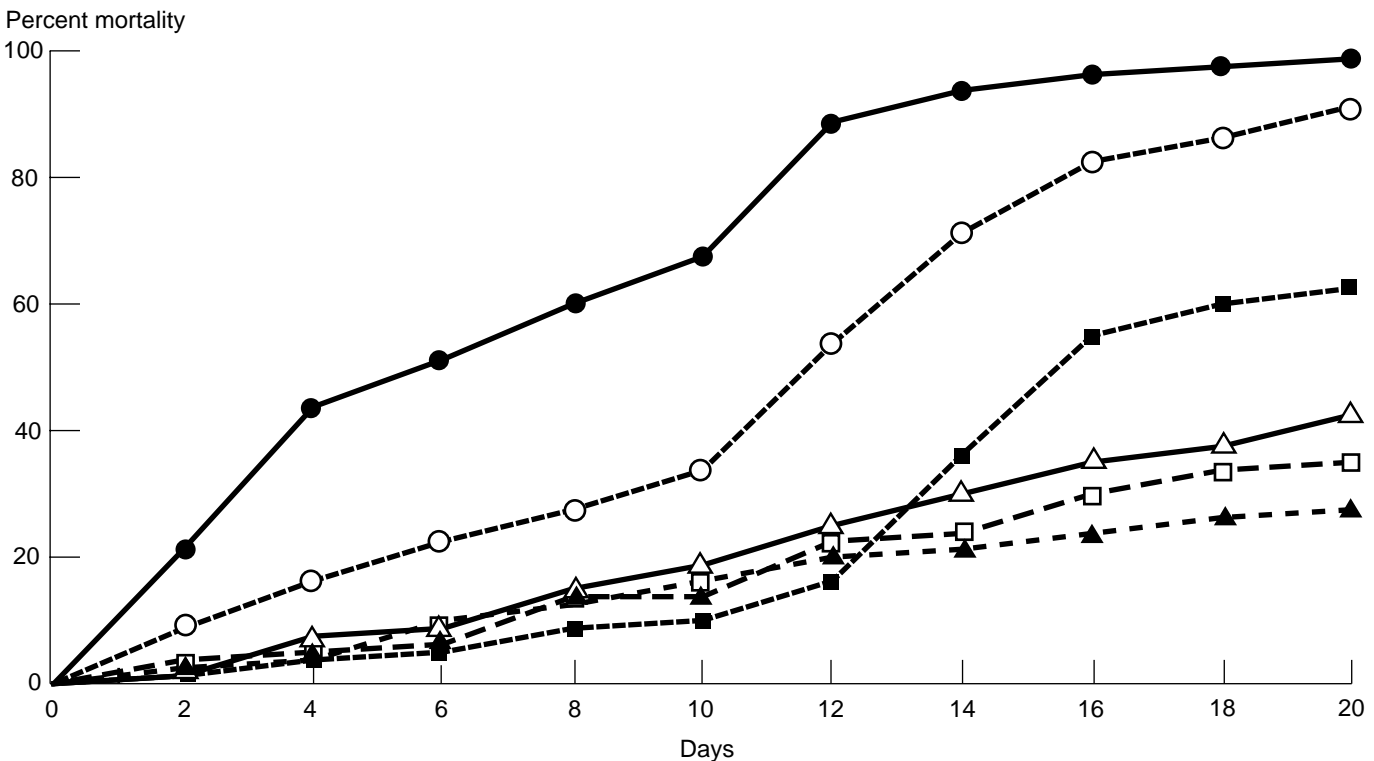
The lettuce bioassay was based upon recommendations supplied by John Henry (personal communication). Spores were counted in a hemocytometer (a special slide used in hospitals to count blood cells) and applied to freshly cut lettuce disks approximately one-third inch (7 mm) in diameter disks using a 10  $\mu\text{L}$  (microliter) pipettor. Six dosages of *N. locustae* spores in 10  $\mu\text{L}$  distilled water (e.g., 0,  $1 \times 10^4$ ,  $10^{4.5}$ ,  $10^5$ ,  $10^{5.5}$ ,  $10^6$  spores;  $1 \times 10^{4.5}$  is equal to  $3.162 \times 10^4$  or 31,620) were added to the disks (120 disks per dosage), and allowed to dry for 1 to 4 hours. Each disk was fed individually to a third-instar grasshopper that had been previously starved for 1 day in glass vials at approximately  $86^{\circ}\text{F}$  ( $30^{\circ}\text{C}$ ). To distribute the grasshoppers into vials, the insects needed to be cooled briefly from ambient  $86^{\circ}\text{F}$  ( $30^{\circ}\text{C}$ ) to approximately  $39^{\circ}\text{F}$  ( $4^{\circ}\text{C}$ ). Before adding the appropriate lettuce disks to the vials, the vials were randomly sorted and divided into the appropriate six dosage groups. Once 80 grasshoppers from each group had eaten an entire disk, they were placed in groups of 5 into 16 bioassay tubes (8 inches or 20 cm long, 2.75 inches or 7 cm in diameter) constructed of 0.08-inch (0.02-mm) sheet acetate with screened ends. Generally, grasshoppers ate an entire disk within 2 hours or did not eat it even after 12 hours. The 16 bioassay tubes were divided into 4 replicates of 4 tubes each. In the bioassay tubes, grasshoppers were fed laboratory-reared rye grass daily along with triple sulfa-coated rolled oats (Henry and Oma 1975) and maintained under continuous fluorescent illumination at approximately  $86^{\circ}\text{F}$  ( $30^{\circ}\text{C}$ ).

Each day, we counted the number of dead grasshopper carcasses in the bioassay tubes. Grasshoppers frequently cannibalized other grasshoppers in the tubes, and portions of carcasses often were found. Therefore, we verified the number of living grasshoppers remaining in each tube to

not overlook cannibalized individuals. We calculated LD<sub>50</sub> values by using the software package POLO-PC (LeOra Software, Inc., Cary, NC).

**Results.**—An example of the typical results obtained from the 22 bioassays conducted in our lab during the past 5 years is shown in figure I.3–1. A few of the uninfected control grasshoppers always died during the 20 days of each bioassay. For all of the 22 bioassays, generally less than 20 percent of the control grasshoppers died before the end of the bioassay. Inoculation of grasshoppers with increasing numbers of *N. locustae* spores consistently increased the mortality rate for grasshoppers infected with  $1 \times 10^6$ ,  $1 \times 10^{5.5}$ , and  $1 \times 10^5$  spores. Grasshoppers inoculated with  $1 \times 10^6$  *Nosema* spores typically started to die sooner than the control grasshoppers within the first 8 days postinoculation (p.i.); the

largest number of deaths normally occurred between days 10 and 14 p.i. By 20 days p.i., 70 to 100 percent of the grasshoppers infected with 1 million ( $1 \times 10^6$ ) spores had died among the various bioassays performed. It typically took grasshoppers inoculated with  $1 \times 10^{5.5}$  spores longer to die than it did for grasshoppers infected with  $1 \times 10^6$ , and fewer grasshoppers had died by the 20-day bioassay period (generally 40 to 90 percent). The mortality rate for grasshoppers dosed with  $1 \times 10^5$  spores tended to separate gradually from the control mortality, and usually became consistently apparent only after 16 days p.i. Mortality in grasshoppers infected with the two lower concentrations ( $1 \times 10^4$  and  $10^{4.5}$ ) did not consistently differ from those of the controls even at 20 days p.i. The calculated LD<sub>50</sub> for the bioassay shown in figure I.3–1 was  $1.19 \times 10^5$  at 20 days p.i.



**Figure I.3–1**—Percent mortality of a 1990 tube bioassay involving third-instar *Melanoplus sanguinipes* grasshoppers treated with varying dosages of *Nosema locustae* spores on lettuce disks (e.g., 0,  $1 \times 10^4$ ,  $10^{4.5}$ ,  $10^5$ ,  $10^{5.5}$ ,  $10^6$ ) and maintained for 20 days postinoculation at approximately 86 °F (30 °C). Solid triangle = 0 spores/grasshopper, open square =  $1 \times 10^4$  spores/grasshopper, open triangle =  $1 \times 10^{4.5}$  spores/grasshopper, solid square =  $1 \times 10^5$  spores/grasshopper, open circle =  $1 \times 10^{5.5}$  spores/grasshopper, and solid circle =  $1 \times 10^6$  spores/grasshopper.

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## Bran Bioassay

**Methods.**—To calculate the theoretical quantity of spores present on average-size flakes of commercially formulated bran, we filtered several grams of the bran through a series of wire sieves with diminishing pore sizes. Most of the flakes were collected on three sieves with pore sizes of 2.36 mm (mesh 8), 2.00 mm (mesh 10), and 1.70 mm (mesh 12). From each of these sieves, 100 flakes were weighed individually. The quantity of spores on each group of flakes was calculated based upon the assumption that each pound of bran contained  $1 \times 10^9$  spores.

As described in the results section, the theoretical concentration of spores on flakes of commercially formulated bran (an average 1-mg flake should contain  $2.2 \times 10^3$  spores) was roughly 100 times lower than the concentration of spores easily detected in laboratory bioassays ( $1 \times 10^{5.5}$  or  $3.16 \times 10^5$  spores could easily be detected based upon their effect on grasshopper deaths).

Therefore, in order to bioassay spores on a single bran flake, it was necessary to formulate new bran with spores at a concentration 100 times that of commercially formulated bran ( $10^{11}$  spores/lb instead of  $10^9$  spores/lb). The spores were sprayed onto wheat bran while continually mixing the bran with a small cement mixer. These spores had been recently recovered from grasshoppers and bioassayed on lettuce in our lab ( $LD_{50}$  value was  $3.29 \times 10^5$ ). In addition to the spores, the spray solution contained 0.2 percent weight to volume (w/v) hydroxymethyl cellulose in distilled water. Hydroxymethyl cellulose is thought to help the spores stick to the bran (Henry et al. 1973). An aerosol sprayer was used to spray the solution on the bran. The treated bran was then allowed to dry and was stored at 39 °F (4 °C).

Attempts were made to bioassay the 100×-treated bran using the same approach used for the lettuce bioassay. One week after formulation of the 100× bran, third-instar grasshoppers were cooled as described above and distributed individually into glass vials. The grasshoppers were starved for 24 hours, randomized, and divided into four groups. Treated bran flakes of different sizes (sieved through mesh 8, 10, or 12) were added to each appropriate vial. Untreated control flakes (sieved only through

mesh size 10) were added to the tubes containing control grasshoppers. Once 80 grasshoppers from each group had consumed all bran flakes, they were placed in groups of 5 into 16 bioassay tubes and maintained as described for the lettuce bioassay.

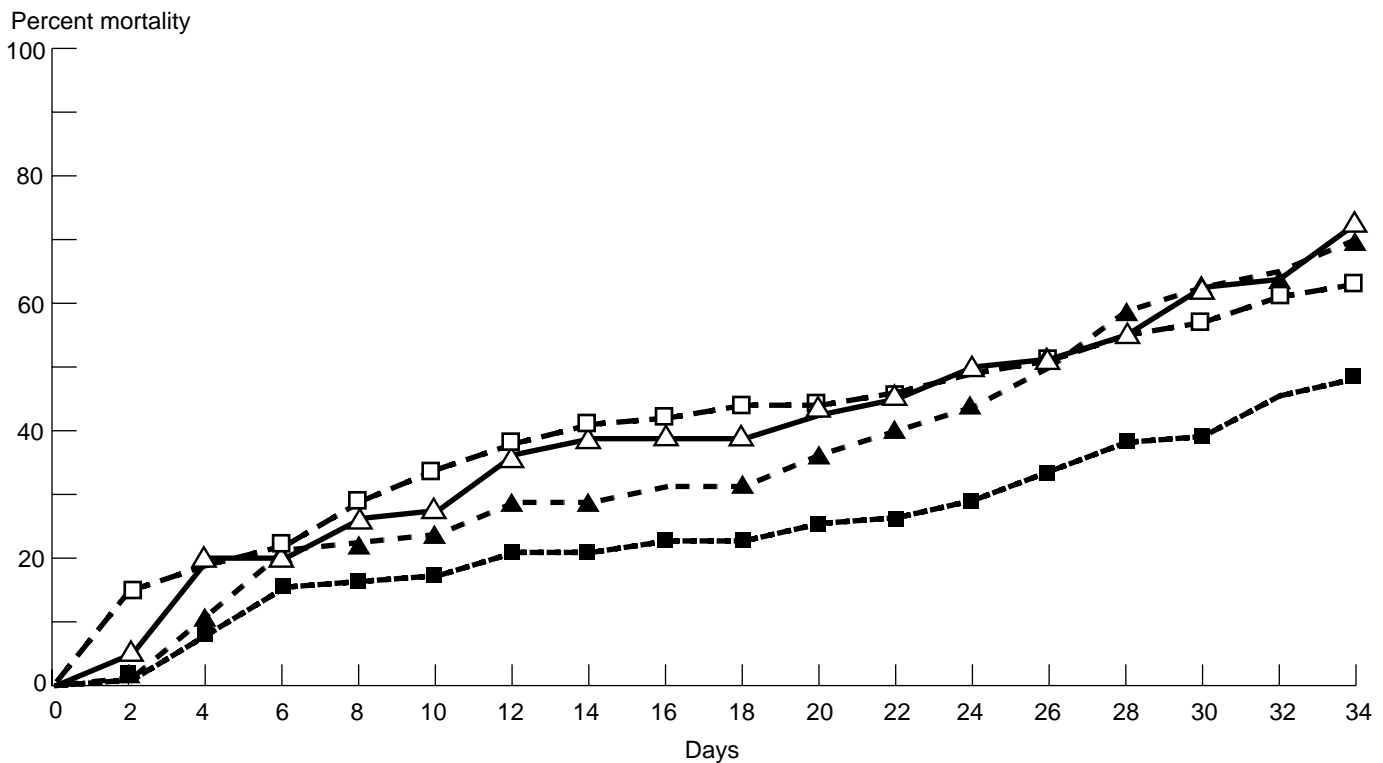
Results from the single-flake bran bioassay study suggested that each grasshopper needed to consume additional bran before any effect could be detected. Therefore, an attempt was made to enable each grasshopper to consume a maximum quantity of treated bran before inclusion in a second bioassay. For that bioassay, 100 grasshoppers were maintained in a large screened rearing cage (30 × 32 × 55 cm) for 48 hours. The only food source during this time was 2.0 g of control or treated bran contained in a standard petri dish. After 24 hours, the uneaten bran was replaced with fresh. Weights were determined from each container of bran and compared to the weights of similar bran maintained similarly just outside the cage. At the end of the bioassay period, the grasshoppers were maintained in bioassay tubes as described for the single-flake bioassay.

**Results.**—The average weight for each size of Nolo Bait® bran flakes and the estimated number of spores per flake are shown in table I.3–1. The average values ranged from 1.42 mg for larger flakes sieved through mesh 8 to 0.625 mg for flakes sieved through mesh 12. If  $1 \times 10^9$  spores are added to each pound of bran, then each milligram of flakes should contain  $2.20 \times 10^3$  spores; therefore, the largest flake weighed in this study (2.2 mg) should contain  $4.85 \times 10^3$  spores.

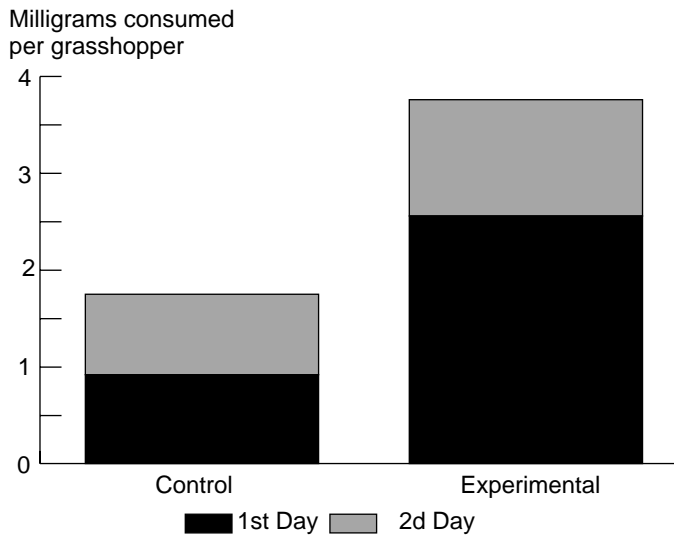
Figure I.3–2 illustrates the mortality rates of grasshoppers fed only one flake of 100×-treated bran from each of the various sieves. Because the average flake of bran weighed 1.05 mg, it should contain approximately  $2.32 \times 10^5$  spores. After 30 days, the mortality rates from the experimental groups of grasshoppers were not significantly greater than that of the controls. In fact, fewer of the grasshoppers receiving the small flakes of experimental bran died than did the control. Unfortunately, however, the mortality rate for the control grasshoppers in this experiment was twice that of previous experiments, and may have obscured any small effects caused by *Nosema*.

**Table I.3-1—Average weight in milligrams for each size of bran flakes and estimated spores per flake**

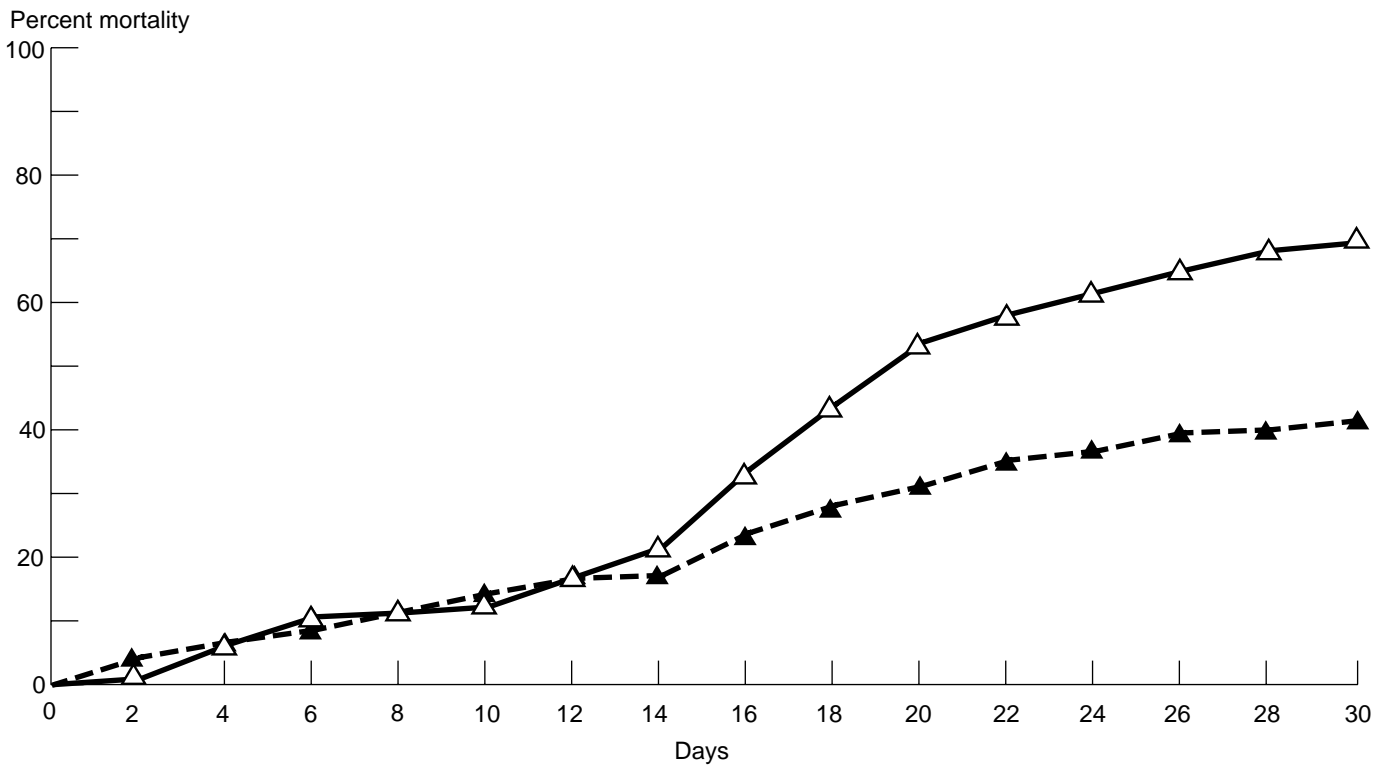
Mesh size	Average weight ± standard error of the means	Weight range	Estimated spores per flake
8	1.422 ± 0.0029 mg	0.9–2.2	3.132 × 10 <sup>3</sup>
10	1.107 ± 0.0031 mg	0.5–2.2	2.438 × 10 <sup>3</sup>
12	0.625 ± 0.0030 mg	0.1–1.1	1.377 × 10 <sup>3</sup>



**Figure I.3-2—Initial tube bioassay involving *N. locustae*-treated wheat bran flakes given individually to third-instar *M. sanguinipes* maintained for 34 days postinoculation. Solid triangle = grasshoppers given an untreated flake of bran; open triangle = grasshoppers given a treated bran flake that passed through a mesh 7 sieve but not the mesh 8 sieve; open square = flake passed through mesh 8 but not mesh 10; solid square = flake passed through mesh 10 but not mesh 12. Spores had been added to the bran at a concentration of  $1 \times 10^{11}$  spores per pound of bran.**



**Figure I.3-3**—Consumption of control and experimental (*Nosema*-treated) bran by 400 grasshoppers in each group during the first and second day of the inoculation period. Values are expressed in “grams consumed per grasshopper.”



**Figure I.3-4**—Tube bioassay involving *N. locustae*-treated wheat bran given ad lib (from a petri dish) to third-instar *M. sanguinipes* maintained for 30 days postinoculation. Solid triangle = grasshoppers given untreated flakes of bran; open triangle = grasshoppers given 100×-strength treated bran.

Grasshoppers given as much of the 100×-treated bran as they wanted for 2 days consumed an average of 2.56 mg on the first day and 1.20 mg on the second. Therefore, each experimental grasshopper consumed an average of 3.76 mg of treated bran (roughly 6 small flakes) or  $8.27 \times 10^5$  spores by the end of the second day. At the end of 2 days, control grasshoppers consumed less than half of the bran consumed by the experimental grasshoppers (fig. I.3–3). Mortality at 30 days p.i. was 75 percent higher for experimental grasshoppers than for those receiving control bran (fig. I.3–4). Mortality rates increased significantly in the experimental grasshoppers after 14 days p.i.

## Conclusions

The LD<sub>50</sub> values determined through the use of lettuce bioassays described in this chapter are generally similar to values reported in other studies. For example, Mussnug and Henry (1979) calculated the LD<sub>50</sub> for *N. locustae* in their study of *M. sanguinipes* to be  $1.5 \times 10^5$  spores based upon a bioassay conducted for 24 days. In lettuce bioassays conducted at SDSU, spore quantities below  $1 \times 10^5$  did not exhibit mortality rates that were consistently higher than those of the controls. Because the average bran flake from commercially prepared *Nosema*-treated bran theoretically contains only  $2.32 \times 10^3$  spores, each grasshopper would need to ingest 43 flakes of treated bran to become inoculated with  $1.0 \times 10^5$  spores. *Melanoplus sanguinipes* grasshoppers that were given only bran flakes during a 2-day period consumed an average of approximately six flakes of bran. In field studies, it is unlikely that many wild grasshoppers ingested more than 40 flakes of *Nosema*-treated bran; therefore, other factors must have influenced the reported effectiveness of *N. locustae* in the field (Henry 1971).

By formulating bran with *N. locustae* spores at a concentration 100 times that which is generally sold commercially ( $10^{11}$  spores/lb versus  $10^9$  spores/lb), it was possible to measure mortality rates caused by the resultant *Nosema* infections. The results generally are consistent with those reported by Reuter et al. 1990 (unpubl.) when the 100× rate—compared to the standard rate and untreated populations only—resulted in significant mortality to one of two field-treated species tested in cages.

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## Bioassays of *Nosema locustae*: An Outline of Procedures

**I. Purpose of the outline** is to describe two protocols to measure the virulence of *Nosema locustae* spores.

- A. First Protocol: used for spores stored in water
- B. Second Protocol: used for spores already adhered to bran

### II. Lettuce Bioassay

- A. Protocol
  1. Obtain 1,000 lab-reared, third-instar *Melanoplus sanguinipes* (Canadian strain) grasshoppers.
  2. Dilute spores to the following concentrations: 0,  $1 \times 10^4$ ,  $1 \times 10^{4.5}$ ,  $1 \times 10^5$ ,  $1 \times 10^{5.5}$ ,  $1 \times 10^6$  per 10 $\mu$ L distilled water.
  3. Apply 10 $\mu$ L of the appropriate concentration to 7-mm lettuce disks.
  4. Cool grasshoppers to 39 °F (4 °C), and distribute each grasshopper into a glass vial.
  5. Add disks to vials and wait until the entire disk is consumed.
  6. Distribute grasshoppers into appropriate bioassay tubes.
  7. Maintain grasshoppers for 20 days, daily feeding them lab-reared rye grass and sulfa-coated rolled oats.
  8. Record grasshopper deaths each day.
  9. Calculate the LD<sub>50</sub> value based upon the total mortality after 20 days p.i.
- B. Results
  1. Largest number of deaths in the grasshoppers infected with  $10^6$  spores occurred between 10 days and 14 days p.i.
  2. Calculated LD<sub>50</sub> for the bioassay reported in this study was  $1.19 \times 10^5$ .

### III. Bran Bioassay

- A. Protocol
  1. Formulate *Nosema locustae*-treated bran at a concentration of  $1 \times 10^{11}$  spores/lb (100 times higher than the concentration commercially available).
  2. Prepare two large rearing cages each containing 100 lab-reared, third-instar *Melanoplus sanguinipes* (Canadian strain) grasshoppers.
  3. Add 2 g of treated bran (in a petri dish) to one cage and 2 g of control bran to the other cage (add no other food source).
  4. After 24 hours, replace each petri dish with petri dishes containing another 2 g of appropriate bran.
  5. After another 24 hours, distribute grasshoppers into appropriate bioassay tubes, and maintain as described above for 30 days.
  6. Data can be reported only as net percent mortality.
- B. Results
  1. Consumption of control and treated bran can be measured by comparing the weight of the leftover bran inside each cage to the weight of similar bran stored outside the cage.
  2. In our first bran bioassay, on average 3.76 mg of treated bran and 1.90 mg of control bran was consumed by the grasshoppers during the 2-day infection period (theoretically  $8.27 \times 10^5$  spores consumed per grasshopper).
  3. Experimental grasshoppers exhibited a 75-percent increased level of mortality at 30 days p.i. compared with grasshoppers receiving control bran at rates near  $2.5 \times 10^9$  per ha on 2 kg (approx.  $1 \times 10^9$  spores/lb) wheat bran.





## I.4 Utility of *Nosema locustae* in the Suppression of Rangeland Grasshoppers

James L. Vaughn, Wayne M. Brooks, John L. Capinera, Terry L. Couch, and Joe V. Maddox

*Editorial note:* The authors served as an independent review team and prepared this report on *Nosema locustae* in 1991 at the request of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine's Grasshopper Integrated Pest Management Project. The internal report contains guidelines and recommendations for the use of *Nosema locustae* and is reproduced in the User Handbook because of the importance of the information the report contains. The present version has been edited to be consistent in style and tone with the User Handbook.

*Nosema locustae* is a microsporidium pathogenic (disease-causing) to a wide range of grasshoppers (more than 90 species are susceptible). It can be easily mass produced and formulated in baits for use as a biological control agent. Although many species of microsporidia are known to act as important naturally occurring biological control agents of insects, very few can be appropriately used as traditional microbial insecticides.

Laboratory studies, simulation models, and some field experiments suggest that *N. locustae* may be successfully utilized for longrange grasshopper control. But there is little or no evidence that *N. locustae* can be used effectively as a microbial insecticide for short-term control of grasshopper populations.

Inducing infections in insect populations is, at best, difficult. Many variables affect the onset and duration of an epizootic (disease outbreak). In the case of grasshoppers, the number and extent of variables are especially troublesome. The number of grasshopper species present, age of grasshoppers, and population density all influence the outcome of field applications. Therefore, the use of *N. locustae* as a grasshopper biological control agent should be considered as part of a long-term suppression effort but not as a microbial insecticide in direct competition with chemical pesticides.

Diseases that affect insects should have great potential for grasshopper control primarily because many grasshopper species readily eat bait into which pathogens can be incorporated. The extensive information generated by

*Nosema locustae* studies will be of great help in this area. Domestic and international efforts should be made to identify and isolate other grasshopper pathogens for use as biological control agents.

In preparation for the analysis that is the foundation for this chapter, we were provided with a number of documents, including representative scientific publications, annual reports, and technical reports (see attached list). In addition, we discussed selected questions with Jerome Onsager, Robert Staten, and Jan Meneley.

After consideration of this information, we made the following specific recommendations:

1. *Nosema locustae* should be used to suppress rangeland grasshoppers in environmentally sensitive areas where cost, rapid knockdown, and high levels of control are not primary concerns. In such areas where insecticidal applications are not possible, continued use of *N. locustae* may be warranted. In these areas it may aid in the long-term management of the pest, and its use may allow researchers to address some of the important ecological questions surrounding it. These subjects are discussed in the following section.
2. Higher rates and/or multiple applications should be used where environmental sensitivities outweigh the higher costs involved.

In most of the past field tests with *N. locustae*, the dosage rate of  $1 \times 10^9$  spores per acre appears to have been predicated more on the economics involved in a grasshopper control program rather than on the actual dose required for effective grasshopper suppression. As estimates of the number of spores per bran flake at this standard rate of application are considerably below  $LD_{50}$  (the dose where 50 percent of exposed individuals are killed) rates for *Melanoplus sanguinipes* and *M. bivittatus*, the effectiveness of higher dosage rates needs further evaluation. Laboratory bioassays support the enhanced effectiveness of *Nosema locustae* at higher dosages, although field studies have produced conflicting results.

In tests with up to five times the standard rate, greater reductions in grasshopper densities have not been obtained. However, in tests with 100 times the standard

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rate and where small field cages were also used to evaluate treatment effectiveness, grasshopper mortality was significantly higher, at least with *M. sanguinipes*. Despite the obvious costs of using higher dosage rates, the potential for enhancing the effectiveness of a readily available and registered biological control agent for use in environmentally sensitive areas may outweigh economic considerations.

In these sensitive areas where higher dosage rates and multiple applications of spores may be used, the methods of evaluation should be improved to include confinement of known numbers of the various grasshopper species in laboratory and field cages. Thus, along with monitoring population densities at appropriate time intervals in field plots, known numbers of treated and untreated grasshoppers should be confined in small field cages on untreated rangeland as well as under laboratory conditions. This evaluation plan will allow more accurate estimates of *N. locustae*'s primary effects on infection and mortality rates, as well the secondary effects on grasshopper food consumption, longevity, fecundity (reproductive capability), and vertical transmission.

3. Use of *Nosema locustae* at presently recommended dosages does not reliably provide an adequate level of suppression. *N. locustae* has been shown to induce measurable reductions in grasshopper longevity, fecundity, and consumption rates under controlled conditions in laboratory and field cages. Also, numerous examples from Canada and the United States indicate that it is possible to obtain significant reductions in grasshopper numbers and damage under field conditions using *Nosema*. However, results are not consistent. Reports of apparent failure also exist and many of the "testimonial-type" data are suspect. Reasons given for the apparent failure of *Nosema locustae* to suppress grasshoppers include

- a. Suboptimal applications of the product: low-quality spores, bad weather, equipment failure, etc.
- b. Poor targeting of the product: grasshopper species of low susceptibility or in the wrong development stage.

- c. Incorrect assessment of the product: inadequate sampling or poor experimental design.
- d. Unreasonable expectations of the product: applicators, evaluators, and land managers expect insecticidal activity from a product that inherently cannot provide rapid or high levels of control.

As long as there are available insecticides that do provide high levels of control (70–95 percent is normal), control by *N. locustae* (30–40 percent under the **best** of conditions) will appear inadequate to ranchers and others concerned with economical, reliable grasshopper suppression. Until the basis for the inconsistencies is better understood, *N. locustae* should be reserved for areas where high levels of control are not essential, or where chemical insecticide usage is not a viable option.

If *N. locustae* is used in ecologically sensitive areas, then research should be conducted to determine the stability characteristics of the formulated bran product. Although data in the literature support the conclusion that the *N. locustae* inoculum is active at the time of formulation, nothing in the literature describes the viability of the *N. locustae* formulations just prior to aerial application.

Pathogens that affect insects are markedly sensitive to elevated temperatures, and significant reduction of activity occurs at temperatures as low as 104 °F (40 °C). If no special handling of the *N. locustae* formulation is routinely done as part of the application program, it is conceivable that the bran formulation could be exposed to temperatures during transit and site storage which could cause a significant, serious biological degradation of the product. It is possible that, in several of the studies, site storage conditions could have had a severe negative effect on the formulation.

Therefore, the committee suggests that a thermal death time-study be developed for the *N. locustae* formulation and storage parameters be defined for the product. These steps will ensure that, if and when future applications are made, shipping specifications and site storage requirements of the formulations can be adjusted to preserve the material's efficacy. With handling protocols in place, the viability of the product can be assured up to the point of application.

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In addition, bioassays of samples of the *N. locustae* bran formulation from the aircraft hopper should accompany each application. Information from these assays will aid in determining if the formulation was shipped and stored under the proper conditions as specified by data obtained from the thermal death time-study.

Additional research on application techniques other than bait seem warranted given the dearth of information in the literature. In particular, conventional low-volume and ultralow-volume liquid applications, with various adjuvants (additives) to increase droplet deposition and decrease evaporation, should be investigated.

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*Availability note:* Several of the following citations come from annual reports prepared for the Grasshopper Integrated Pest Management Project but not distributed outside the Animal and Plant Health Inspection Service. Individual photocopies of these materials are available on request from USDA, APHIS, Plant Protection and Quarantine, 4700 River Road, Riverdale, MD 20737.

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## I.5 Identification of Fungal Pathogens of Grasshoppers

Michael J. Bidochka and Donald W. Roberts

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### Introduction

Grasshoppers are host to a wide range of micro-organisms that cause disease. Of these, the fungi provide spectacular appearance of disease symptoms. On a larger scale, fungi can devastate whole populations of grasshoppers. Some of these fungi cannot grow without a grasshopper host (obligate pathogens); other fungi are easily cultured in the laboratory and can infect a wide range of insects including grasshoppers (facultative pathogens). In this chapter, we will examine methods used to discriminate pathogenic fungal infections from bacterial or nonpathogenic fungal growth on a dead insect. We will also discuss the most probable fungal infections found in the field.

### Fungi Pathogenic to Grasshoppers

There are two main groups of fungi that have species pathogenic to grasshoppers: the zygomycetes and the deuteromycetes. Some zygomycete species are obligate pathogens of grasshoppers. The deuteromycetes that are pathogenic to grasshoppers are facultative pathogens.

**Zygomycetes (Entomophthorales).**—The pathogenic Entomophthorales are complex and poorly understood. The only confirmed pathogens that infect grasshoppers belong to the *Entomophaga grylli* complex. There are at least three pathotypes of the *E. grylli* complex. The term pathotype refers to the type of grasshopper that is infected. The three pathotypes also differ with respect to their life cycles, host grasshoppers, and growth requirements (Ramoska et al. 1988). Two of the pathotypes are native to North America, and a third pathotype has been isolated from a grasshopper species in Australia.

Pathotype 1 infects the bandedwinged grasshoppers (Oedipodinae). The grasshopper species most commonly infected are *Camnula pellucida* and *Dissosteira carolina*. Pathotype 2 infects melanopline grasshoppers (Melanoplinae) and the species most commonly infected are *Melanoplus* and *Hesperotettix* spp. Pathotype 3, the Australian isolate, infects bandedwinged and melanopline grasshoppers under laboratory conditions.

**Disease Characteristics.**—*Entomophaga* spp. are the most common and widespread pathogens of grasshoppers in North America. Disease symptoms in the advanced stage

are characteristic and easy to recognize. Shortly before death, infected grasshoppers crawl to the tops of plants, fenceposts, or any other elevated position. There they die with their legs wrapped around the plant stalk and heads pointed upward.

Examining the specimen found in the characteristic “summit disease” is simple. Open the abdomen or poke a hole in it with a sterile toothpick and a place sample of this on a microscope slide with a drop of water. The inside of the grasshopper may contain a variety of fungal bodies, but the most common are large (50  $\mu$ m in diameter), spherical, thick-walled resting spores. If the grasshopper is *Camnula*, the infection is probably pathotype 1; in a melanopline grasshopper, probably pathotype 2.

External sporulation is also used to discriminate between pathotype 1 and 2 infections. Grasshoppers suspect of *E. grylli* infection are placed in a humid environment, such as petri dish containing 1.5 percent agar. Within 24 hours some of the specimens will show sporulation (white rings) on the abdominal segments. Pathotype 1 will show external sporulation (conidia approximately 50  $\mu$ m in diameter) as well as the internal resting spores. Pathotype 2 will not show external sporulation.

**North Dakota Introductions.**—Recently, pathotype 3 (*E. praxibuli*) has been introduced into North Dakota from Australia. This fungus infects both oedipodine and melanopline grasshoppers. External growth on a melanopline grasshopper may be indicative of *E. praxibuli* infection. However, we caution against the use of morphology and growth characteristics as tools in differentiating the three *Entomophaga* pathotypes.

We have developed DNA (deoxyribonucleic acid) probes that could be used differentiate the three pathotypes (Bidochka et al., 1995). We have also devised a method by which the resting spores of these fungi can be fractured, and the DNA can be isolated and used as a template for the pathotype-specific probes.

**Deuteromycetes.**—Worldwide, the most common deuteromycete infections in grasshoppers are *Beauveria bassiana*, *Metarhizium anisopliae*, and *Aspergillus flavus*. In central Africa, *Metarhizium flavoviride* is found more commonly than *M. anisopliae*.

**Disease Characteristics.**—Grasshoppers that have an external white or green mycelial (filamentlike fungus) growth are also potential suspects of fungal infection. The most common non-*Entomophaga* infections found in the field are *B. bassiana*, *M. anisopliae* and *A. flavus*. *B. bassiana* infection is characterized by white mycelial growth on parts of the surface of the grasshopper; *M. anisopliae* and *A. flavus* infections are characterized by green surface growth. The conidia of these fungi are much smaller (approximately 5–10 µm in diameter) than the conidia of *Entomophaga grylli*. *M. anisopliae* conidia are rod shaped, but *M. flavoviride* conidia are more rounded or elliptical. *B. bassiana* conidia are globose (round or globelike), and *A. flavus* conidia are spherical. For more detailed descriptions and microphotographs of entomopathogenic fungi, refer to Samson et al. (1988) and Poinar and Thomas (1984).

**Isolating Pathogenic Deuteromycetes.**—Several selective media for the isolation of *B. bassiana* and *M. anisopliae* have been tested. The best medium for selective isolation of *B. bassiana* and *M. anisopliae* is 30 g of wheat germ in 1 L of water, autoclaved for 10 minutes and filtered through four layers of cheesecloth. To this are added 0.25 g chloramphenicol, 0.75 mg benlate (50 percent benomyl), 0.30 g dodine, 10 mg crystal violet, and 15 g agar (Chase et al. 1986).

The mycelia on the surface of the grasshopper can be picked with a sterile toothpick or sterile wire loop and streaked onto this agar-medium. The petri dishes should be wrapped in aluminum foil because exposure to light delays colony growth. Optimal growth occurs at 79 °F (27 °C) for these fungi. If the fungus grows, then it may be one of the pathogenic deuteromycetes. If the fungus does not grow, it may simply be a nonpathogenic fungus growing on the dead grasshopper.

*B. bassiana*, *M. anisopliae*, and *M. flavoviride* also can be differentiated based on patterns of DNA fragments generated by random amplification of polymorphic DNA (RAPD) and with molecular probes using the RAPD fragments (Bidochka et al. 1994).

Other fungi that may infect grasshoppers include *Verticillium lecanii*, *Nomuraea rileyi*, and *Paecilomyces* sp.

## Assessment of Fungal Disease

To prove that a certain fungal isolate is the causative agent in grasshopper death, lab personnel must follow these steps: (1) The fungus must be isolated from the grasshopper. (2) The fungus must be grown in media. (3) The fungus must cause disease either by injection of conidia into the body cavity or by exposing the insect to fungal conidia. Most entomopathogenic fungi normally infect by passing through the insect exoskeleton. It is preferable that the host insect from which the fungus was isolated be the test insect. This is particularly true for the Entomophthorales. For deuteromycetous fungi, a test insect such as wax moth larvae (*Galleria mellonella*) or silkworm larvae (*Bombyx mori*) may be used. (4) Finally, the fungus must be reisolated from the test insect.

The best diagnostic tools for differentiating *B. bassiana*, *M. anisopliae*, *M. flavoviride*, and the *Entomophaga* are molecular probes. The use of these probes is not difficult, and results are generally conclusive. In the near future, the use of such probes will be commonplace in fungal taxonomy.

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## I.6 Grasshopper Pathogens and Integrated Pest Management

Donald L. Hostetter and Douglas A. Streett

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### Introduction

Some 97 percent of all animals on Earth are invertebrates, and between 75 and 80 percent of these are insects. One of the most serious gaps in our knowledge of invertebrates in general, and insects specifically, is a thorough understanding of their diseases.

As would be expected, mankind's knowledge of insect parasites and predators preceded that of the disease-causing agents of insects. Although the early interests in insect pathology were primarily concerned with beneficial insects, such as the honeybee and the silkworm, many investigators recognized that harmful insects were subject to disease. Almost from the time of their discovery, insect diseases have been proposed as possible tools for controlling insect pests.

It was not until 1836 that Agostino Bassi, for whom the insect-infecting fungus *Beauveria bassiana* is named, suggested that microorganisms could be used to control destructive insects. Another 43 years would pass before Elie Metchnikoff published his account of a natural infection of the wheat cockchafer (*Anisoplia austriaca*) by the green-musccardine fungus (*Metarhizium anisopliae* [Metchnikoff]) and provided experimental methods for testing the possibility of controlling insects with fungi (Steinhaus 1956).

Micro-organisms with the ability to cause acute and chronic disease in grasshoppers and locusts currently are found among the bacteria, fungi, protozoa, rickettsia, and viruses (Bidochka and Khachatourians 1991).

### Bacteria

One of the first attempts to use bacteria as a control agent of insects was against grasshoppers in Mexico (d'Herelle 1911). The bacterium *Coccobacillus acridorum* d'Herelle was isolated from large numbers of dying grasshoppers that had migrated to Mexico from Guatemala. D'Herelle claimed to have begun epidemics among grasshopper populations in Mexico, Colombia, and Argentina, along with some success in Algeria and Tunisia. His results were not reproducible by others and soon viewed with doubt. This bacteria was later determined to be *Aerobacter aerogenes* (Kruse), a member of

the coliform group capable of invading warmblooded animals (Steinhaus 1949).

Another bacterium, *Serratia marcescens* Bozio, was isolated from desert locusts (*Schistocerca gregaria* [Forskäl]) raised in a laboratory. *S. marcescens* was cultured, formulated on a bran bait, and used in field tests against the desert locust in Kenya. The results were uncertain (Stevenson 1959). This gram-negative bacterium is found worldwide and is well known as a pathogen of laboratory insects.

The most promising bacteria currently being used for insect control belong to the spore-forming group *Bacillus thuringiensis* Berliner, often referred to as "Bt." A diamond-shaped crystalline toxin is produced within the bacteria as they mature and form spores. The toxin is the active ingredient that kills insect larvae. After it is consumed, the toxin is dissolved in the insects' alkaline gut juices and becomes activated. The gut is unable to process food, the larvae stop eating, and the gut ruptures, causing the larvae to die.

Grasshoppers have a built-in barrier against Bt because their gut juices are acidic, and the absence of an alkaline environment prevents the toxin from dissolving and becoming activated (Prior and Greathead 1989). Two isolates of Bt from the Dulmage Collection originally isolated from grasshoppers were inactive against *M. sanguinipes*, as were 26 other prospective isolates (Streett and Woods 1992 unpubl). Continued examination of the Bt group, along with advances in formulation chemistry and genetic manipulation, may produce future successes with these bacteria against grasshoppers.

### Fungi

More than 750 species of insect-infecting fungi have been documented (National Academy of Sciences 1979, Roberts and Humber 1984). Although fungi are among the best known and most often reported pathogens associated with grasshoppers and locusts, only a few different fungi have been recorded. The most common are *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metchnikoff) Sorokin, and *Entomophaga grylli* (Fresenius) Batko.

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Fungi are “contact” pathogens. They do not infect when they are eaten by the insect, as do other pathogens. Fungal infection may occur during the feeding process when conidia contact the mouthparts (Foster et al. 1991 unpubl.). The infection process begins after a spore comes in contact with a suitable host and germinates in the form of a “tube.” The tube penetrates the body wall, enters the body cavity, and releases a protoplast that begins asexual reproduction. Rapid growth of the fungus overwhelms the insect host and it dies. After death of the host, the fungus grows back through the body wall and forms vegetative stalks that produce primary spores (conidia) that are forcibly discharged into the atmosphere. These spores are capable of continuing the infection cycle. Toward the end of the season, or if environmental conditions are unfavorable for conidia production, “resting spores” are produced. Resting spores are the environmentally resistant or protective stage that overwinters in the soil litter or in dead grasshoppers.

*Beauveria bassiana* has been successfully developed and used as a microbial control agent of various insects in the Soviet Union and China (Goettel 1992). Interest in *B. bassiana* as a control agent for rangeland grasshoppers has been renewed with the recent isolation of a strain—virulent to some species of grasshoppers—from a grasshopper in Montana (Johnson et al. 1988 unpubl., Foster et al. 1992 unpubl.).

Extensive laboratory and field testing of this strain has indicated good potential for control of grasshoppers and resulted in the first aerially applied field tests of *B. bassiana* against grasshoppers on rangeland in the United States (Foster et al. 1991–93 unpubl.). Technology for mass production has been developed by Mycotech Corporation (Butte, MT), and a commercial product was registered for use against rangeland grasshoppers by the Environmental Protection Agency in 1995.

*B. bassiana* is expected to be competitive with current chemical insecticides and could be a very useful microbial control agent in future grasshopper integrated pest management (IPM) programs.

*Metarhizium anisopliae* is another fungus that has been isolated from grasshoppers and is known to have a worldwide distribution. It also can be mass produced and formulated as a microbial insecticide. One isolate has been used successfully as a control agent against the sugarcane spittlebug in Brazil (Roberts et al. 1991). It has not been tested in the field as a grasshopper control agent but should be considered as a potential tool that merits further tests.

*Entomophaga grylli*, formerly referred to as a complex of fungi composed of “pathotypes,” is now known to consist of at least four species: *E. calopteni* (Bessey) Humber, *E. macleodii*, *E. praxibuli*, and *E. asiatica*. *E. calopteni* is the only species that has been formally described to date (Humber 1989). *E. asiatica*, isolated from one grasshopper in Japan, was screened for activity and placed into the pathogenic insect fungus collection at the U.S. Department of Agriculture’s Agricultural Research Service laboratory in Ithaca, NY (Carruthers et al. 1989 unpubl.). All *Entomophaga* spp. isolated from grasshoppers and locusts are infective only for members of this group. This fungus also has a worldwide distribution. *Entomophaga* spp., unlike *B. bassiana* and *M. anisopliae*, cannot be produced in large quantities on or in artificial media at the present time. *Entomophaga* spp. cannot be used as microbial insecticides in large-scale spray applications now.

A classical introduction method uses individually infected grasshoppers, each injected with an amount of the infective stage (protoplasts) of *Entomophaga* sp. that will cause their death within 7 to 10 days. Before dying of the fungus disease, the infected grasshoppers are released into a susceptible population in the field. Distribution of the disease occurs and is dependent upon dispersal of spores from dead, infected grasshoppers to noninfected ones within the population. A series of biological and environmental factors must occur in sequence before such epidemics develop.

One of the native North American fungi, *Entomophaga macleodii* (pathotype I) infects grasshoppers from several genera and produces infective conidia as well as resting spores. The primary host of this fungus is the clear-winged grasshopper (*Camnula pellucida* [Scudder]), which belongs to the bandwinged group of grasshoppers.

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The other North American species is *E. calopteni* (pathotype II). It occurs only in a *Melanoplus* species (a member of the spurthroated group) and produces only resting spores upon death of the host.

The Australian fungus, *E. praxibuli*, was isolated from *Praxibulus* sp. grasshoppers in Australia in 1985 during a fungus epidemic. This fungus is similar to *E. macleodii* in producing both infective conidia and resting spores. Laboratory tests and field observations indicate that *E. praxibuli* has a greater host range than *E. macleodii* and is infective for at least 14 species of grasshoppers from the three major subfamilies: the spurthroated, slantfaced, and bandwinged grasshoppers.

Following a review of the known literature and a series of laboratory evaluations, the Australian isolate *E. praxibuli* was selected as a candidate for a classical biological control program for grasshopper populations in western North Dakota (Carruthers et al. 1989–91 unpubl.).

## Protozoa

The microsporidia comprise the most important group of the protozoan pathogens of insects with over 250 species currently documented (Maddox 1987). The most probable route of infection occurs when insects' food is contaminated with spores. Upon ingestion into the midgut of a host, the spores forcibly extrude a hollow filament that penetrates or is placed near the epithelial cells lining the gut. The infective sporoplasm travels through the tube and into the cell, where asexual reproduction of spores begins. Spores can be released prior to death of the infected host through regurgitation or in feces.

Microsporidia also can be passed on to the next generation of host insects on the surface of eggs, or within eggs laid by infected females. Some microsporidia may also be mechanically transmitted by the feeding or ovipositing activities of insect parasites of the infected host. Microsporidial infections can range from acute, leading to death in several days, to chronic, with little evidence of infection and prolonged life stages. Microsporidia can be serious pathogens in laboratory colonies of insects.

Within the family Microsporida, the genera *Nosema* and *Vairimorpha* have proven to contain the most promising

candidates for grasshopper and locust control. *Nosema locustae* (Canning) was first isolated from infected migratory locusts in a laboratory colony in Great Britain (Canning 1953). It has received the most attention as a biological control agent for grasshoppers. *Nosema* was thoroughly investigated in a series of laboratory and field evaluations, registered, and developed as the first commercial microbial product for grasshopper control (Henry 1978 and 1982, Henry and Oma 1981). Applications were difficult to evaluate and did not meet expectations. *N. locustae* was widely acclaimed but unfortunately is not extensively used in grasshopper control programs. For grasshopper control in environmentally sensitive areas, *N. locustae* is still worthy of consideration. In many cases, in sensitive areas, no action is chosen over *N. locustae* for economic reasons and because results with *Nosema* have been irregular (*See* I.4.).

*Nosema acridophagus* Henry and *N. cuneatum* Henry are two other grasshopper-isolated species of microsporidia that have potential as microbial control agents (Henry 1967, Henry and Oma 1974). Both have demonstrated variable virulence and have been adapted to production in surrogate hosts (certain species of caterpillars). These agents may have a place in future IPM programs (Streett 1987).

A *Vairimorpha* sp. was isolated from Mormon crickets (*Anabrus simplex* Haldeman) in Utah and Colorado during an epidemic in 1989. The crickets are very susceptible to this *Vairimorpha* and it may be considered as a control agent for Mormon crickets. Field observations indicate that infection causes increased mortality among crickets while decreasing development of nymphs and adult reproduction (Henry and Onsager 1989 unpubl.).

## Viruses

The only viruses isolated from grasshoppers and cricket species to date are members of the entomopoxvirus and crystalline array virus groups. The entomopoxviruses are the best known of the viruses reported from grasshoppers and crickets. The entomopoxviruses isolated from *M. sanguinipes* have received the closest examination and evaluation (Henry and Jutila 1966). Fewer than 10 entomopoxviruses have been isolated from grasshoppers (Streett et al. 1986). Two other poxviruses, one from

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*Arphia conspersa* Scudder and one from the African grasshopper *Oedaleus senegalensis* (Krauss), are potential microbial control agents (Streett 1987). These viruses were originally viewed with caution because of their resemblance to vertebrate orthopoxviruses (Bidochka and Khachatourians 1991). Examination of this group has revealed no biochemical similarity or infectivity of vertebrates, however (Arif 1984, Streett and McGuire 1990).

The crystalline array viruses do closely resemble the picornaviruses of vertebrates and are not currently considered to be exploitable as a microbial agent for grasshoppers (Greathead 1992).

Nuclear polyhedrosis viruses (NPV's), probably the most common of insect viruses, have not been isolated from grasshoppers or crickets. One report has documented transmission (by feeding) of an NPV from *Spodoptera littoralis* (a caterpillar) to both *Schistocerca gregaria* and *Locusta migratoria*, resulting in a phenomenon known as "dark cheeks" (Bensimon et al. 1987).

## Summary

Grasshoppers and locusts, like all other animals, are subject to pathogenic micro-organisms. Representatives from all of the major groups of known pathogens have been isolated from grasshoppers and crickets. The fungi *Entomophaga* spp. and *Beauveria* spp. are the most frequently reported and observed pathogens. Spectacular mortality due to *Entomophaga* sp. is often observed within grasshopper populations throughout the world. Fungi, at the current time and state of technology, probably have the greatest potential as microbial control agents.

Bacterial pathogens do not exhibit much promise as tools for grasshopper control now. Technological advances in molecular biology may lead to development of strains of *Bacillus thuringiensis* that will be active against grasshoppers. Efforts to isolate bacteria, particularly spore-formers, from grasshoppers and crickets on a worldwide scale should be supported.

Protozoans, particularly *Nosema* spp. and *Vairimorpha* spp., are also promising candidates for reducing grasshopper populations in environmentally sensitive areas. Although *Nosema locustae*, the first registered and commercially produced microbial control agent for grasshopper suppression, has not met expectations, it still remains a viable alternative to chemical control in long-term management programs.

Continued research with grasshopper and cricket viruses undoubtedly will result in new isolates that may be considered as management tools. Viruses have the potential to be "tailored" to fit specialized control requirements in localized areas and may become a tool of choice—with substantial research and development—for long-term population reduction in grasshoppers in the future. Insect pathogens will play a larger role in future grasshopper management strategies as requirements for control are redefined and evolve in the decades ahead.

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# I.7 Insect Predators and Parasites of Grasshopper Eggs

Richard J. Dysart

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## Introduction

The following remarks are intended to provide a brief overview of the life cycle and habits of the various insects that attack grasshopper eggs. Individually, these natural enemies may not seem significant, but collectively they determine how many grasshopper eggs will hatch and reach the nymphal stage. Also, it is important that land managers recognize the damage done to these beneficial insects when pesticides are sprayed during grasshopper control campaigns. Currently, when range-land grasshoppers are treated with pesticides, the chemical of choice is usually malathion because it is effective and inexpensive and relatively nontoxic to mammals and birds. However, malathion is not selective, killing virtually all of the exposed insects, including the beneficials.

## Grasshopper Egg Laying

Grasshopper eggs are normally deposited in clusters, called egg-pods, placed just below the surface of the soil. The egg-pod is covered by a fairly durable coating of soil particles mixed with a glutinous substance excreted by the female as she lays her eggs in the soil. The female thrusts her abdomen into the soil to a depth of an inch or two (5–10 cm) and starts laying her eggs. When the cavity formed by her abdomen is filled with eggs, she commonly blocks the hole above the eggs with a glandular secretion forming a “froth plug.”

The egg-pod may contain from 2 to more than 100 eggs, depending on the species of grasshopper. The eggs are quite tough and very resistant to cold. They are able to survive the most severe winters if the ground is not disturbed. Also, there is usually enough moisture in the surrounding soil to keep the eggs from drying out even in drought conditions. After the eggs have been deposited in a suitable spot, the female grasshopper provides no maternal or defensive care and merely abandons them.

## Natural Enemies of the Egg Stage

The eggs of some species hatch in a few weeks and thus escape destruction by many natural enemies. Most of the grasshoppers in the Western United States lay their eggs in summer and fall and they remain in the ground during

the winter in a state of suspended development called diapause, and they do not hatch until the following spring. These eggs are unprotected and exposed to their enemies for some 9 months of the year.

In spite of the fact that grasshopper eggs are available to natural enemies for such long periods, there are surprisingly few insect enemies of the egg stage. It must be noted that locating grasshopper egg-pods in the soil is usually a lengthy and difficult task. Because finding pods in soil and vegetation is so unpredictable, it is easy to miss egg-pods, and especially the larval stages of predators. Thus it is difficult to obtain accurate density counts per unit of area.

The insects that feed on grasshopper eggs can be divided into two groups, predators and parasites, based upon the insects' method of feeding.

## Egg Predators

Predators attack the egg-pod as a whole, feeding externally on the grasshopper eggs. Predators are capable of moving from one egg or egg-pod to another as they complete their development. Most insect predators of grasshopper eggs are generalists. They pose a threat to grasshopper egg populations, but in an undirected way. Some of these predators are no more than scavengers. They locate egg-pods somewhat at random, taking advantage of targets of opportunity. The following groups of grasshopper egg predators are discussed in their approximate order of importance in the Northern Plains.

**Coleoptera: Meloidae.**—In North America, the larvae of blister beetles (meloids) are an important group of predators of grasshopper eggs. However, in Australia, Africa, and other parts of the world, blister beetles are of little or no importance. The adult stages are called blister beetles because their body fluids can cause blistering of the human skin. Although the larvae of this group of beetles are predaceous, the adults feed exclusively on vegetation, and certain species can become numerous enough on crops such as alfalfa to require treatment with pesticides. In this family, the beneficial aspect of the larva frequently is offset by the destructive habit of the adult.

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Rees (1973) lists 26 species of meloids whose larvae are known to attack grasshopper eggs in North America. In early summer, the female blister beetle lays a group of 100–200 eggs in an earthen chamber. When the young larva hatches from the egg, it is quite mobile and begins to search through the soil for a grasshopper egg-pod. Once a pod is located, the meloid larva transforms into a fat white grub and usually eats all of the eggs within the egg-pod. In fact, if the larva still has not completed its development, it will seek out another egg-pod on which to feed. Some species require 2 years to complete their life cycle.

**Diptera: Bombyliidae.**—The larvae of certain bombyliid flies are also important predators of grasshopper eggs. As many as 13 genera have the habit of consuming acridid (grasshopper) eggs. The adults are called bee flies because certain species have furry bodies resembling a bumble bee. Also they hover in midair and dart swiftly from place to place, moving like bees. When the flies are at rest, the wings are held away from the body. Eggs are deposited in soil cracks and crevices in the vicinity of ovipositing grasshoppers.

After a brief incubation period, the eggs hatch and the larvae wander through the soil in a random search for food. Encounters with grasshopper egg-pods appear to be more or less accidental. A bee-fly larva can completely consume the contents of a pod, but at times only a few eggs are eaten in each of several pods. In this way, many egg-pods can be damaged, allowing the entry of other scavengers. The bee-fly larval stage can last for several years. The number of egg-pods destroyed per individual often exceeds three (Rees 1973). When the bee-fly larva is fully developed, it leaves the egg-pod and pupates near the surface of the soil.

**Coleoptera: Carabidae.**—Both the adult and larval stages of this family are predaceous on other insects, but members of the family are known as generalists in their choice of hosts. The adults are commonly called ground beetles. The larvae of carabid beetles are predaceous on grasshopper eggs, and in some local situations, they seem to be of importance (Greathead 1963).

**Miscellaneous Groups.**—On occasion, the larvae of certain members of the following families of beetles and flies have been noted as soil-inhabiting predators of acridid egg-pods, but none seem to be dependent on grasshopper eggs for their survival. These include three Coleoptera families (Cleridae, Tenebrionidae, and Trogidae) and three Diptera families (Asilidae, Calliphoridae, and Chloropidae) (Greathead 1992). Note: during the Grasshopper Integrated Pest Management Project study, larvae of two new chloropid flies were found to be predators of grasshopper eggs in the Northern Plains (Dysart 1991, Sabrosky 1991).

## Egg Parasites

Parasites feed internally and complete their development within a single egg. In general, parasites of the eggs of insects usually are tiny hymenopterous wasps that come from one of several different families. However, the eggs of grasshoppers are attacked by wasps of the family Scelionidae only.

**Hymenoptera: Scelionidae.**—Members of this group are the only true parasites of grasshopper eggs. The North American species of Scelionidae that develop as parasites in the eggs of grasshoppers belong to two genera: the genus *Scelio*, which contains about 19 species, and the genus *Synoditella*, represented by 2 species (Muesebeck 1972). *Scelio* species occur throughout the world wherever grasshoppers are found. Only a single wasp develops within a grasshopper egg. *Scelio* adults live only a very short time, usually no more than 3 weeks under the best conditions. The sex ratio varies among species, but there are usually more females than males by a considerable margin.

The factors involved in host selection are not entirely clear, but it seems certain that the adult female is attracted by some chemical in the egg-pod froth. After locating a suitable egg-pod, the female wasp chews a passageway through the froth until she encounters the grasshopper eggs. Then the wasp backs out, reenters the passageway tail first, and, using her long ovipositor, lays eggs in as many host eggs as she can reach. After the *Scelio* larva hatches, it feeds internally on the contents of the host egg.



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When mature, the larva pupates within the host egg shell, and the adult wasp emerges during the summer months. In the Northern Plains, *Scelio* species are thought to have only one generation per year. The most abundant and most widespread of the North American species is *Scelio opacus*. Host records from the literature and my own studies (Dysart 1995) show that it has been reared from eggs of nine different grasshopper species.

## Discussion

Many articles in the literature describe the habits and life history of grasshopper parasites and predators, but few good ecological studies describe the impact of these natural enemies on grasshopper populations.

In his general review of predators and parasites of North American grasshoppers, Rees (1973) speculated that grasshopper egg predators probably have more effect on grasshopper populations than do predators of nymphs and adults. Based on a 10-year study in North Dakota and Montana, Parker (1952) estimated that predators destroyed 20 percent of the eggs laid by grasshoppers. Parker and Wakeland (1957) cite results from a studies made at 16 sites in 7 States. Average annual destruction of egg-pods by predators was about 18 percent (9 percent by blister beetles, 6 percent by bee flies, and 3 percent by ground beetles).

Prior and Greathead (1989) estimated that, in Africa, scelionid egg parasites (*Scelio* spp.) were the predominant cause of egg mortality in solitary locust populations. However, scelionids were rather ineffective mortality factors in the egg beds of gregarious species, such as the desert locust. In Australia, parasitism by *Scelio* species at certain sites has been found in up to 90 percent of the egg-pods. In my study areas in Montana and North Dakota, *Scelio* parasitism never reached such high levels. I found that a complex of four species of *Scelio* parasitized about 11 percent of the egg-pods (Dysart 1995). Parasitism figures from the literature indicate that a range of 5 to 15 percent of pods are attacked by *Scelio* spp. in the Northern United States and the Prairie Provinces of Canada.

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# I.8 Natural Enemies Attacking Grasshopper Nymphs and Adults

D. L. Hostetter

## Introduction

There are 548 recognized species of North American grasshoppers, with about 400 of these occurring on the 650 million acres of rangeland in the 17 Western States (Pfadt 1988). Around two dozen of these are considered potential agricultural pests. Several species may be considered beneficial because of their preference for weeds, and the remainder are either harmless, cause only minor damage, or are beneficial as food sources for wildlife. Along with mammals, grasshoppers are the most significant grazers in the world's temperate grasslands, where people produce most of their food.

This large and diverse group of extremely successful insects occupies many habitats worldwide. Grasshoppers are a food source for equally large and diverse groups of parasites and predators—insects, spiders, and other animals collectively referred to as “natural enemies.” These complex animal groups maintain a continual pressure, although variable in degree, on grasshoppers throughout their range. Natural enemies significantly affect grasshopper populations and present the first line of defense before outbreak events. Natural enemies should be factored into regulatory strategies with efforts to conserve them when resorting to chemical control operations.

Decisionmakers need to consider the impact on nontarget and beneficial insects of chemical insecticides and application rates used to control rangeland grasshoppers. The effect of economically and politically expedient chemical control programs should be monitored constantly through the “window of natural enemies” in the process of regulation.

This chapter presents a brief review of life histories, occurrence, and distribution and briefly details facts relating to some of the more important arthropod natural enemies of grasshoppers in western North America.

## Grasshopper Parasites and Predators

### Order Diptera (flies).—

**Family Anthomyiidae.**—Members of the family Anthomyiidae are medium in size, about a quarter-inch (6 mm) long and closely resemble the common housefly.

Adults are often characterized by slender, dark-colored bodies and rarely possess any metallic coloration or noticeable “bristles” on their body (Cole 1969). Some species are of economic importance, but very few are true parasites. This family occurs worldwide with more than 550 species known in North America, and many are quite common (Borror and DeLong 1971). One species is reported to parasitize grasshoppers, and two species have been reported as egg predators of grasshoppers, although the validity of these reports has been challenged (Rees 1973).

*Acridomyia canadensis* Snyder is the only species in North America known to parasitize grasshoppers. It has been classified as an “important” parasite in Canada and mentioned as “occurring” in Montana and Idaho (Rees 1973). This fly is known to parasitize at least 16 species of grasshoppers within all 3 grasshopper subfamilies of the family Acrididae, the slantfaced, the spurthroated, and the bandwinged grasshoppers. *Melanoplus bivittatus* and *M. packardii* are reported as this parasite's preferred host species.

Details of the life history of this species are summarized by Rees (1973). Pupae overwinter in the soil, and adults emerge during June, July, and early August. *A. canadensis* typically has one generation per year; however, some adults do not emerge until the second year. Mating occurs upon emergence. After a short but unspecified gestation period, the female flies begin stalking hosts. Upon selection of a suitable host, the female uses its rasping mouthparts to penetrate the host's body and then feeds upon the body fluids. After feeding, the female inserts her ovipositor into the feeding wound and lays eggs in the body cavity. The ovipositor is barbed, which prevents the host from escaping once penetration has occurred. Up to 70 eggs are deposited in each host, and they hatch within 48 hours. Larvae (20–70 per host) develop simultaneously and complete three instars in about 16 to 20 days. Mature larvae then emerge from the host, enter the soil, and pupate. Death of the host usually precedes emergence of the larvae.

Two species of this family have been reported as predators of grasshopper eggs: *Hylemya angustifrons* (Meigen) and *H. platura* (Meigen) = *Delia platura* (Meigen). *H. angustifrons* was reported as a predator

only of *M. spretus* eggs. *D. platura*, the seedcorn maggot, may have been incorrectly associated with grasshopper eggs and confused with *Acridomyia canadensis*, which it closely resembles (Rees 1973). These assertions of predation recorded in the early literature should be viewed with caution in light of current systematic knowledge of this group.

**Family Calliphoridae.**—This is a common group occurring worldwide and well known as blow flies. Adults, about the size of the common housefly, are recognized by their abdomen. Adults of different species have abdomens of different colors—usually a variation of metallic blue or green. Most members of this group are scavengers that live in carrion and excrement. They are similar to flesh flies, family Sarcophagidae, which are important parasites of grasshoppers in North America.

Calliphorids and sarcophagids can be separated visually by structural differences of the antenna and thorax. It is uncertain whether differentiating *Calliphora vicina* Robineau-Desvoidy, the only species ever associated with grasshoppers in North America and collected by Riley (1877), from the now extinct Rocky Mountain locust, *Melanoplus spretus* (Walsh), was an error. Cole (1969) reported that Packard and Thomas, two other entomologists of that era, also recorded *C. vicina* as parasitic on *M. spretus* and indicated that reproduction was by paedogenesis (reproduction in the larval stage), suggesting to Cole that erroneous observations had been made.

**Family Asilidae.**—Members of the Asilidae are known as robber flies. These raptors of the insect world are strong fliers noted for their voracious appetites and predatory behavior toward flying insects (Rees and Onsager 1985). There are 856 species of Asilidae in North America; 26 are reported as predators of grasshoppers (fig. I.8–1). Six species exhibit a definite preference for grasshoppers (Rees 1973).

Asilids display a variety of identifying structural characters. Those that prey on grasshoppers are large with elongated, tapering bodies and long legs. Bright colors are rare in this group. Most species exhibit gray to silvery coloration, and nearly all are bearded and bristly (Cole 1969). Life cycles range from 1 to 3 years. The adults are very territorial and cannibalistic.



**Figure I.8–1**—Unidentified robber fly (Family Asilidae) feeding on a fifth-instar *Camnula pellucida*, Twin Buttes, Owyhee County, ID. (This photograph and all others in this chapter were taken by D. L. Hostetter of the U.S. Department of Agriculture’s Agricultural Research Service.)

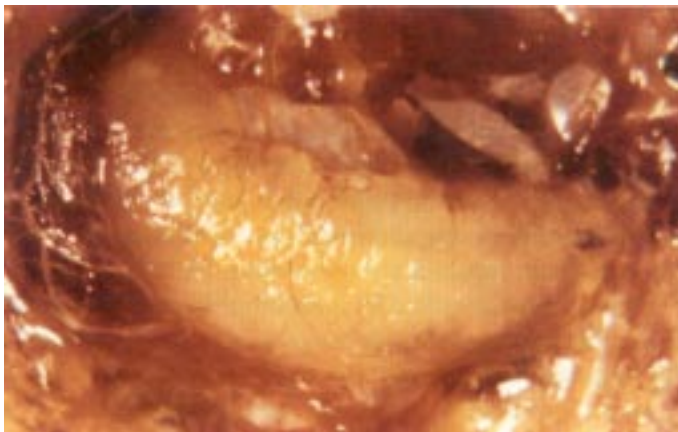
A 6-year study in Wyoming by Lavigne and Pfadt (1966) documented that three species, *Stenopogon coyote* Bromely, *S. neglectus* Bromley, and *S. picticornis* Loew, feed primarily on rangeland grasshoppers. These authors concluded that these species, along with 9 others associated with grasshoppers in Wyoming, can reduce grasshopper populations by 11 to 15 percent.

**Family Sarcophagidae.**—Most sarcophagids or flesh flies are scavengers as larvae, but some are parasites of insects. This family is distributed almost worldwide with more than 2,000 described species, most of which occur in tropical to warm temperate areas (Shewell 1987). There are about 21 to 23 species that are parasites of grasshoppers in North America. Sarcophagidae are without exception ovoviviparous, meaning that their eggs hatch within the uterus and the female deposits a live larva on the host (Shewell 1987).

The five most prominent North American species are *Acridophaga aculeata* (Aldrich), *Kellymyia kellyi* (Aldrich) = *Blaesoxipha kellyi* (Aldrich), *Opsophyto opifera* (Coquillett) = *Blaesoxipha opifera* (Coquillett), *Protodexia hunteri* (Hough) = *Blaesoxipha hunteri*, and *Protodexia reversa* (Aldrich) = *Blaesoxipha reversa* (Aldrich).

“Larviposition” by *A. aculeata* and *K. kellyi* occurs during flight of the fly with the selected grasshopper. This airborne interception often knocks the targeted grasshopper to the ground. The flies attack during natural flight or when the host has been flushed by livestock or otherwise disturbed. *Opsophyto opifera*, *P. hunteri*, and *P. reversa* all stalk grasshoppers on the ground. When within striking range, the female flips a larva from the tip of her abdomen onto the grasshopper. The larva quickly penetrates the host’s body through an intersegmental space and begins feeding on the body fluids and tissue (fig. I.8–2). One species, *Servaisia falciformis* (Aldrich) = *Protodexia* = *Sarcophaga falciformis* (Aldrich), possesses a sharp ovipositor that is used to insert a larva into the large muscle of the hind leg of the grasshopper. The larva begins to feed in the leg and eventually migrates into the body cavity, where it continues feeding until mature (Middlekauff 1959).

Sarcophagid larvae complete three instars (growth stages) in 6 to 9 days within the host before reaching maturity. The mature larva exits through a hole in the grasshopper body wall and pupates in the soil. One to three generations are possible, depending on the species, number of suitable hosts available, and environmental conditions. These flies target last-stage nymphs and adults and are generally considered the most effective group of grasshopper parasites (Rees 1973).



**Figure I.8–2**—Mature third-instar *Sarcophagidae* sp. larva prior to emergence from an adult *Melanoplus sanguinipes* (magnification 1.6×).

Reports documenting the incidence of parasitization in grasshoppers in the Northern United States and Western Canada vary from less than 1 percent to 50 percent (Middlekauff 1959, Lavigne and Pfadt 1966, Rees 1973). A detailed compilation of the North American species of Sarcophagidae associated with grasshoppers, preferred hosts, geographic distribution, and life histories and habits is presented in Rees (1973).

**Family Tachinidae.**—Tachinid flies also occur worldwide and represent the second largest family in the order Diptera with nearly 1,300 North American species (Borror and DeLong 1971). The larvae are primarily parasites of caterpillars in the order Lepidoptera. Most tachinids deposit their eggs directly on the surface of the host. Upon hatching, the larva burrows into the host and feeds internally on body fluids and tissue. The larva completes three instars feeding within the caterpillar. The host dies prior to emergence of the larva, which then pupates and overwinters in the soil. Six species have been reported from grasshoppers, but only the following three are considered important parasites in the United States and Canada (Smith 1958, Rees 1973).

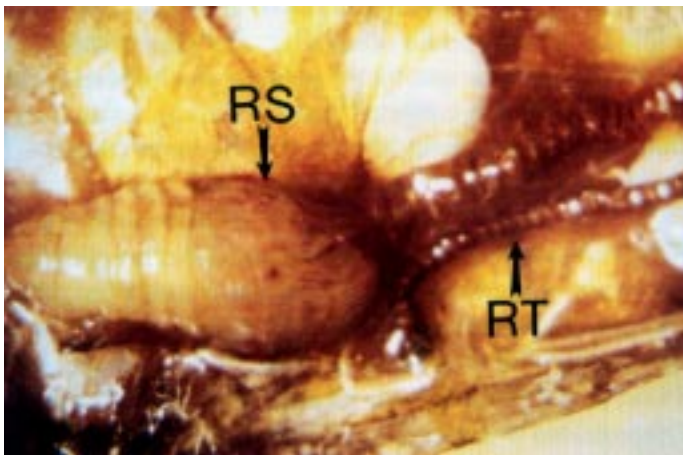
*Acemyia tibialis* is the principal tachinid parasite of grasshoppers and has been reported from *Melanoplus bivittatus* and *M. sanguinipes*. Canadian reports indicate parasitism ranges between 16 and 65 percent (Rees 1973). *Ceracia dentata* (Coquillett) and *Hemithrixion oestriforme* Brauer and Bergenstamm have been reported from grasshoppers collected in the United States and Canada with parasitism rates ranging between 1 and 5 percent (Rees 1973).

**Family Nemestrinidae.**—Members of this cosmopolitan family are commonly known as tangle-veined flies. They are medium-sized, stout-bodied, fast fliers that can hover persistently. There are only six North American species. Two, *Neorhynchocephalus sackenii* (Will.) and *Trichopsidea* (= *Parasymmictus*) *clausa* (Osten Sacken) (Smith 1958) are parasites of grasshoppers. *N. sackenii* is the smaller of the two species and is readily distinguished from *T. clausa* by having an elongated proboscis.

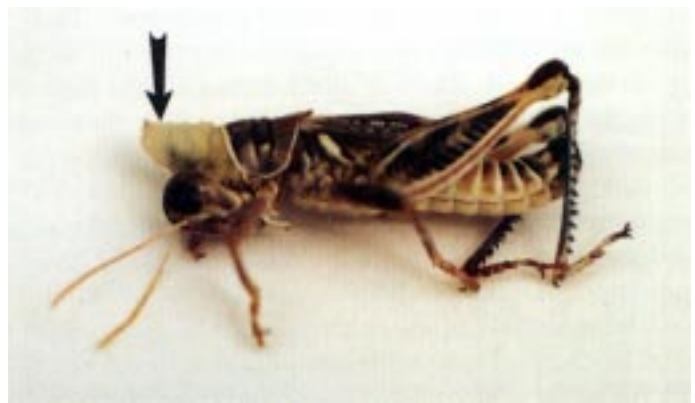
Nemestrinids have only one generation a year. They overwinter in the soil as mature larvae, pupate in the spring, and emerge as adults from late May through

mid-July (Smith 1958). Females deposit as many as 4,700 eggs in crevices or holes in dead weeds, fenceposts, and other similar structures at elevations ranging from 3 to 40 feet. Eggs hatch in 8 to 10 days, producing a small (0.5-mm) cream-colored larva. Larvae are thought to be distributed by the wind, a scenario enhanced by the fact that females prefer to lay their eggs on elevated sites (Prescott 1955). Contact with a host is thought to be a random event facilitated by the ability of larvae to survive up to 14 days in the free-living state.

When a suitable grasshopper host (fourth- and fifth-stage nymphs or adults) is contacted, penetration of the body wall occurs within 30 minutes. Once inside the host, the nemestrinid constructs an elongated, spiral respiratory tube attached internally to the body wall of the host (fig. I.8-3). The small end of the tube opens at the surface of the body wall and is the source of air for the larva. The larger end of the tube forms a respiratory sleeve, which fits snugly over the breathing spiracles on the rear of the larva. The larva feeds on the host's fat and reproductive tissue and completes four instars before emerging from the host (fig. I.8-4). The larva emerges just prior to death of the grasshopper and burrows into the soil, where it overwinters as a larva (Prescott 1955).



**Figure I.8-3**—Second-instar *Neorhynchocephalus sackenii* (Family Nemestrinidae) in adult *Oedaleonotus enigma*. Note the respiratory sleeve (RS) and respiratory tube (RT) attached to body wall (magnification 1.6×).



**Figure I.8-4**—Mature *Neorhynchocephalus sackenii* larva emerging between head and pronotum of mature brachypterous *Oedaleonotus enigma*.

Nemestrinids favor rangeland and “idle acres” habitats and those grasshoppers with similar habitat preferences. The flies are seldom found in cultivated areas or cropland (Prescott 1960). *Ageneotettix deorum* (Scudder), *Camnula pellucida* (Scudder), *Metator pardalinus* (Sausser), and *Aeropedellus clavatus* (Thomas) are preferred hosts of *N. sackenii* with parasitization rates between 30 and 95 percent (Prescott 1955). *Oedaleonotus enigma* (Scudder) is a preferred host of *N. sackenii* in south-central Idaho (Hostetter et al. 1991 unpubl.).

#### Order Hymenoptera.—

**Family Formicidae.**—This family consists of the ants, a large and very successful group found worldwide in almost every habitat. Ants can be formidable predators of hatchling grasshoppers if they are found in an ant colony's territory. Ants are localized, general predators and have little effect on grasshopper populations. Four species have been observed as predators of rangeland grasshoppers: *Formica rufa obscuripes* Forel, *F. obtusopilosa* Emery, *Myrmica sabuletti americana* Weber, and *Solenopsis molesta validiuscula* Emery (Lavigne and Pfadt 1966).

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**Family Sphecidae.**—This is a large family of solitary wasps consisting of eight subfamilies, most of which nest in wood, construct mud cells, or burrow in the soil.

Twenty-nine species are recorded as parasitizing grasshoppers in Canada and the United States (Rees 1973). Female “digger wasps” prefer grasshoppers as provisions for their nests and are sometimes mentioned as efficient grasshopper predators (Lavigne and Pfadt 1966). A typical species of this group, *Prionyx parkeri* Bohart and Menke, requires about 1 hour to capture, cache, and lay an egg on an adult grasshopper. Upon hatching, the wasp larva begins to consume the live grasshopper, which remains paralyzed. These wasps are generally rare in most grasshopper habitats, but there is a report in Idaho (Newton 1956) of three *Tachysphex* spp. reducing a population of *Oedaleonotus enigma* (Scudder) by 84 percent.

#### **Order Arachnida.**—

**Family Araneidae.**—The spiders are probably the least studied of the grasshopper predators. Nine species of spiders have been reported as predators of grasshoppers, but the list is known to be incomplete and undoubtedly is much longer (Lavigne and Pfadt 1966, Rees 1973). The wolf spider, *Schizocosa minnesotensis* Gertsch, and a jumping spider, *Pellenes* sp., are two species of nonweb-builders that are often quite abundant on rangeland and are reported (Lavigne and Pfadt 1966) as predatory on various rangeland grasshopper species. The black widow spider, *Latrodectus mactans* (F.), is also a common rangeland predator of grasshoppers in Wyoming and Idaho (Lavigne and Pfadt 1966, and my own personal observations).

The feeding habits and preferences of spiders in the rangeland ecosystem are largely unknown and difficult to measure. Most species are generalists and opportunistic feeders on grasshopper nymphs and adults.

**Family Trombidiidae.**—This is the most important of three known families of mites that have been reported as parasites of grasshoppers and locusts. Red mites have been universally observed attached to the wings of their host (Uvarov 1928). The most thorough biological studies of the commonly observed North American species

*Eutrombidium locustarum* (= *trigonum*) Walsh were by L. O. Howard (1918) and H. C. Severin (1944).

Adult mites appear early in the spring and begin searching for grasshopper egg-pods. Mites remain in the pods feeding on individual eggs until the mites become sexually mature. Mating takes place in the egg-pod, but eggs are laid in cells (300–700 per cell) in the soil. Larvae emerge after 28 to 30 days and actively seek a suitable host. Larvae usually attach at the base of the wings on adults. Feeding continues until the larva is engorged; it then drops off the host, burrows into the soil, and transforms into a “nymph,” an eight-legged immature mite that closely resembles the adult. Nymphs leave the grasshopper toward the end of the summer, when fresh egg pods become available for additional feeding. After more feeding on eggs, the nymph transforms into the adult and overwinters in the soil.

The value of these mites as regulators of the grasshopper is not significant, but they are minor factors in the grasshopper life cycle. Mites belonging to the genus *Gonothrombium* were collected from grasshoppers in Wyoming in 1963 (Lavigne and Pfadt 1966). The investigators reported that 21 of 35 species of grasshoppers (adults) collected during studies in Wyoming between 1959 and 1962 were infested with mites. Conversely, only 8 of 454 grasshopper nymphs collected during the same period were infested with mites. Numbers of mites per individual grasshopper ranged from 1 to 41 with 2 the most common number. No attempt was made to determine detrimental effects, but Lavigne and Pfadt concluded that the mites had little if any effect on the grasshopper hosts.

#### **Order Nematoda.**—

**Family Mermithidae.**—Three species of nematodes belonging to this family are parasitic on grasshoppers: *Agamermis decaudata* Cobb, Stienen, and Christie, *Agamospirura melanopli* Christie, and *Mermis subnigrescens* Cobb (Rees 1973). A *Hexamermis* sp. has also been recovered from the greenstriped grasshopper, *Chortophaga viridifasciata* (DeGeer), in Missouri (Blickenstaff and Sharifullah 1962, Puttler and Thewke 1971).

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Nematodes are long-lived animals with from 2 to 4 years required for each generation (Rees 1973). Spring rains and moist soil force the pregnant females from the soil to the vegetation. Eggs are deposited on foliage, where they remain alive for most of the summer. Grasshopper nymphs eat vegetation contaminated with these eggs. The infective nematode larva is released from the egg during the digestive process. The larva eventually penetrates through the host's gut wall into the body cavity, where it remains for 4 to 10 weeks. The mature larva exits the host (usually killing it) late in the summer and overwinters in the soil. The final molt, resulting in the adult, occurs in the spring.

Known North American distribution is limited to the upper Midwest, Northeast, and small, restricted areas in the Western United States. Moisture in the microhabitat, probably in the form of free water, is required for successful development of nematodes. When the required conditions occur, the incidence of infestation in localized areas can exceed 60 percent (Rees 1973).

**Family Gordiacea.**—Members of this class of roundworms (Nematomorpha) are known as horsehair worms or Gordian worms. They closely resemble nematodes in general body features (Hegner and Engemann 1968). Adults are free living and aquatic. Larvae are parasitic in crustaceans, grasshoppers, crickets, and beetles. Females lay thousands of eggs in long, gelatinous strings in water.

Upon hatching, larvae seek an immature form of aquatic insect as the primary host. Later the larvae become terrestrial and seek a secondary host (usually a cricket, grasshopper, or beetle), where they feed and continue to develop. The mature larva exits the host (causing death) and returns to an aquatic habitat (ponds, animal watering troughs, intermittent pools, streams, or similar area), where it reaches sexual maturity. Roundworms are opportunists that attack many hosts and are considered incidental parasites of grasshoppers and Mormon crickets (Rees 1973).

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## I.9 Mites and Nematode Parasites of Grasshoppers

G. E. Belovsky, D. Branson, J. Chase, J. Barker, and G. Hammond

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Very little is known about the nonfungal, nonbacterial, and nonprotozoan pathogens (macroparasites) of grasshoppers. Two major groups of macroparasites for grasshoppers are mites (Acarina) and roundworms (Nematoda). In some instances, the different species of these natural enemies of grasshoppers have not even been identified, let alone studied for their impacts upon grasshopper populations. Therefore, macroparasites are a largely unexploited set of biocontrol agents that might be used to manage grasshopper populations.

### Mites

Mites provide an excellent example of the potential opportunity for pest managers to exploit macroparasites in grasshopper control, as well as exemplifying the general lack of understanding about the ecology of parasites that prevents pest managers from using them.

At least two mite species are known to parasitize grasshoppers. The most common is the red mite (*Eutrombidium locustarum*) found on the wings of grasshoppers; another red mite is found on the legs and antennae of grasshoppers and has not yet been formally named. These mites have complex life cycles, going through at least three stages of development (larvae, nymph, and adult), and the complete life cycle requires from 2 months to a year (Rees 1973). Larvae of both mite species attach to the external surface (are ectoparasites) of grasshoppers and suck their blood (hemolymph). In addition, at least the wing mite as a nymph and adult also preys upon grasshopper eggs.

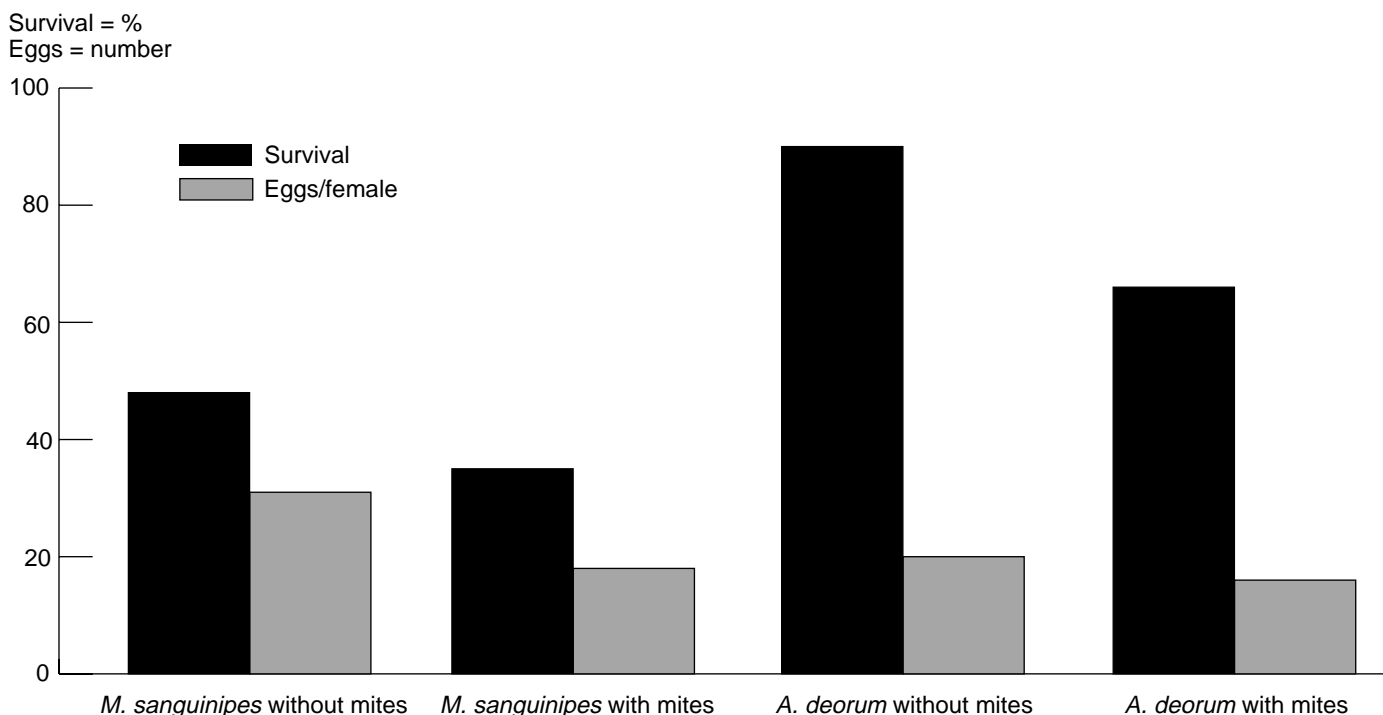
Little is known about the egg predation by mites because this occurs in the soil. However, based upon the mites' consumption needs (Rees 1973), their predatory depression of grasshopper egg survival could be substantial. Each mite nymph requires more than two grasshopper eggs to become an adult. Adult males require three eggs to be able to reproduce and adult females require seven to eight eggs to reproduce. Furthermore, each female mite deposits up to 4,000 eggs (Rees 1973), providing mite populations the potential to increase rapidly and substantially as grasshopper population numbers increase.

When studied in the laboratory, the ectoparasitic effects of larval mites were thought to be of no consequence to grasshopper survival or reproduction (Huggans and Blickenstaff 1966). This conclusion is not unexpected because the grasshoppers had greater quantities of high-quality food than they could consume and were maintained at near optimal temperatures and humidities. Unlike the laboratory studies, our field investigations indicate that larval mites can reduce grasshopper survival and reproduction dramatically.

In western Montana, we have studied the survival and reproduction of *Melanoplus sanguinipes* in cages that were placed over field vegetation and that maintained field temperature and moisture conditions. We have found that the grasshopper densities attained in the cages were comparable to field densities and were food limited (Belovsky and Slade 1994). In another set of experiments conducted in the same fashion, we stocked cages with grasshoppers that either had no wing mites on them, or had one or more wing mites on them.

When we compared the survival of grasshoppers with and without mites in the cages, we found that mites reduced the survival of grasshopper nymphs and adults by an average of 29 percent, and female reproductive output was reduced by an average of 47 percent (fig. I.9-1). Rather than an inconsequential effect, the ectoparasitism by wing mites reduced the grasshopper population's overall egg production by 62 percent.

The effect of ectoparasites in reducing the grasshopper population's egg production becomes stronger when grasshoppers experience greater intraspecific competition for food (higher densities). For example, cages initially stocked with 4 adults exhibited only a 45-percent reduction in total egg production, while cages initially stocked with 10 adults exhibited a much greater reduction, 69 percent. Therefore, the loss of hemolymph to wing mites must be considered in the context of environmental conditions, and the judgment that mite ectoparasitism is unimportant from laboratory studies is of little value. Similar results for the leg mite and the grasshopper *Ageneotettix deorum* were observed with total egg production being reduced by 41 percent (fig. I.9-1).



**Figure I.9-1**—Comparison of the survival and reproduction for two grasshopper species with and without mite infections. Results are statistically significant, and the values represent the means of at least 10 caged populations for each treatment.

The importance of egg predation by nymphal and adult mites and ectoparasitism by larval mites in controlling grasshopper numbers depends upon the abundance of mites. Predation and ectoparasitism effects will be of little importance if there are not large enough numbers of mites relative to grasshopper numbers.

In our field experiments, the grasshoppers that were infected had an average of 3.5 mites. Samples from grasshopper populations in different habitats in western Montana showed that from 0 to 75 percent of the grasshoppers were infected (average = 20.5 percent) at a site, and the individuals that were infected had an average of 2.5 mites. Extending our experimental results on ectoparasitism to field grasshopper populations indicates that larval mites may reduce overall egg production on average by 9 percent, with the effect varying from 0 to 33 percent in different populations.

The predicted natural reductions in total egg production by mites are not adequate in many instances to serve as a viable control method. However, the impact of ectoparasitism by mites could potentially help control grasshopper numbers if the percentage of grasshoppers infected can be increased.

We compared the percentage of grasshoppers infected by mites at different sites in western Montana with environmental characteristics (average daily air temperature, average solar radiation, average soil surface temperature, average soil temperature at less than an inch to almost 2 inches (2–5 cm), average relative humidity, percent cover by vegetation, soil moisture, and the rate of water passing through the soil). We found that infection increased with the rate of water passing through the soil, indicating that mite abundance may be limited by the soil's drainage (the poorer the drainage the fewer the

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mites). Because the egg, nymphal, and adult stages of the mites live in the soil, we suspect that survival of these stages, rather than survival of the ectoparasitic larval stage, is reduced in soils with poor drainage.

Consequently, to take advantage of the mites' efficiency in controlling grasshopper egg production, a pest manager would need to counteract the local environmental conditions that lead to poor drainage. This type of habitat management may be difficult. Pest managers may be able to raise mites in large numbers and release them into the environment to overcome the poor survival of mite eggs, nymphs, and/or adults in the soil. Raising large numbers of mites in the laboratory is difficult because of the mites' complex life cycle and varied needs for survival and reproduction.

## Nematodes

Nematodes are parasites that live within the grasshopper's body (endoparasites), and they are even less well understood than mites. Two species, *Mermis nigrescens* and *Agamermis decaudata*, are important parasites of grasshoppers. These species are even more difficult to identify taxonomically than the mites. These roundworms have a 2- to 3-year life cycle. The larval stages live in the hemolymph of grasshoppers and are considered parasites because they obtain nourishment by absorbing nutrients from the hemolymph. Nematodes are considered parasites rather than parasitoids because parasitoids would consume the grasshopper's body and nematodes do not.

Grasshoppers become infected with *Mermis nigrescens* when they ingest the nematode's eggs, which have been deposited on vegetation. Grasshoppers become infected with *Agamermis decaudata* when the newly hatched larvae penetrate a grasshopper's body (Streett and McGuire 1990). The infection generally lasts for 1 to 3 months and usually results in the death of the grasshopper when the adult nematode(s) exits from the grasshopper's body. The remainder of the nematode's life is largely spent in the soil except when adult females emerge for egg deposition.

In western Montana, we have found, by dissecting large numbers of *M. sanguinipes* in different years and habi-

tats, that nematodes infected less than 10 percent of the grasshoppers at most sites in most years. The highest infestation level we observed at one site in a single year was more than 90 percent. We also found that nematode-infected female grasshoppers still produced eggs, but egg production was reduced by 85 percent.

Nematodes have the potential to be used as a biological control agent if pest managers could enhance nematode numbers by improving survival in the soil or by supplementing their numbers by releases. However, nematode ecology is even more poorly understood than that of mites, and in nature, nematode numbers are usually even lower than mite numbers.

## Future Prospects

Employing mites and nematodes actively as biological control agents will require a better understanding of these parasites' natural histories and their ecological impacts on grasshoppers. Also, nobody knows if these parasites can be raised economically in the laboratory. Scientists may be able to take advantage of these natural grasshopper enemies through habitat manipulation that increases their populations or by adding to their natural populations. Mites and nematodes are native enemies of our grasshoppers and may potentially provide an environmentally "friendly" control strategy that can be sustainable for longer periods of time with less attention by pest managers.

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## I.10 Birds and Wildlife as Grasshopper Predators

L. C. McEwen, B. E. Petersen, and C. M. Althouse

In the early years of this country's agriculture, birds were considered the first line of defense against insect damage. The first laws to protect birds were proposed in 1877 (U.S. Entomological Commission 1878). The act establishing the U.S. Department of Agriculture (USDA) in 1862 made reference to "the introduction and protection of insectivorous birds" (McAtee 1953). A Section of Economic Ornithology and Mammalogy was formed in USDA's Division of Entomology in 1885, and it was expanded into a Division of Food Habits Research in 1921. Much of the wildlife food-habits work was summarized in a book by Martin et al. (1951) in which the authors reported almost universal predation on grasshoppers by insectivorous and omnivorous birds, mammals, and reptiles.

It is interesting that most of the early studies in economic ornithology were not done by ornithologists (people studying birds) but rather by entomologists (those studying insects). For example, S. A. Forbes, an entomologist, founded the field of economic ornithology more than 100 years ago and defined many of the principles of integrated pest management (IPM) as we know them today (Metcalf 1980). The results of examination of more than 40,000 bird stomachs were reviewed by W. L. McAtee (1953). More than 200 species of birds were found to prey on grasshoppers (fig. I.10-1).



**Figure I.10-1**—More than 200 species of birds are known to feed on grasshoppers. Even seed-eating species, such as this Savannah sparrow, depend on insects for high-protein food during the breeding season. (Photograph by C. K. Miller, of the Nature Conservancy, Clear Lake, SD; used by permission.)

Some of the larger species, such as kestrels (sparrow hawks) (fig. I.10-2), gulls, and meadowlarks, could capture in excess of 100 grasshoppers per day. Swainson's hawks are known to gather in flocks of several hundred to feed on grasshoppers when they become abundant (Wakeland 1958). More recently Johnson et al. (1987) observed large flocks of these hawks capturing about 100 grasshoppers per bird per day in Idaho.

It is not surprising that grasshoppers are so important as food for wildlife because they (1) have high energy value and contain 50–70 percent crude protein (Ueckert et al. 1972, DeFoliart 1975), (2) are widely distributed and available in most western habitats, and (3) are large enough to easily exceed the energy cost of capture by foraging birds and wildlife. Grasshoppers are especially important for successful raising of young by the majority



**Figure I.10-2**—Research on kestrels has shown their high rate of predation on grasshoppers and their compatibility with grasshopper IPM control materials, such as dimilin, *Beauveria bassiana*, and Sevin 4-Oil®. (Photograph by B. E. Petersen; used by permission.)

of bird species (McEwen 1987) and for many mammals as well. Nestlings and chicks must go through a period of rapid development and growth to survive and perpetuate their species. Even many species that, as adults, eat mostly seeds and plant materials are completely insectivorous in early life (fig. I.10–3). Grasshoppers are highly preferred for feeding young of many kinds of songbirds, upland shore birds, game birds (quail, grouse, pheasants, and turkeys), and even certain hawks and owls (McAtee 1935, 1953).

Grasshoppers are beneficial to a healthy, vigorous, grassland ecosystem when they are at low to moderate (non-economic) densities. This family of insects preceded today's rangeland plant species and vertebrate animal life by millions of years (Carpenter 1953). Grasshoppers developed in the rangeland ecosystem during a long period of coevolution with other flora and fauna. Grasshoppers' ecologic role (Van Hook 1971) of providing food for wildlife, stimulating plant growth, creating plant litter for the soil, and cycling elements and nutrients was developed as a functional part of the whole ecosystem. Land managers should view grasshoppers as pests only when the insects increase to densities that are clearly damaging to the rangeland plant cover and ecosystem.



**Figure I.10–3**—Lark bunting with grasshopper captured to feed nestlings. Grasshoppers are a preferred food for young wildlife because of the high protein content and nutritional value. (Photograph by Lowell C. McEwen; used by permission. The shot was taken on the GHIPM Project's grasshopper spray area in Bighorn County, Wyoming.)

Although there is much evidence that birds and wildlife prey on grasshoppers, little research has been done to learn whether wildlife predators actually reduce grasshopper populations or prevent outbreaks. A few recent experiments determined the reduction in grasshopper densities attributed to birds on rangeland. Results show that bird predation commonly reduces grasshopper densities on rangeland by 30–50 percent (Joern 1986, Fowler et al. 1991, Bock et al. 1992). But predation is not so effective in some habitats (Belovsky et al. 1990). Studies of bird predation on other insect pest species also have found that birds significantly reduce pest numbers (McFarlane 1976, Takekawa et al. 1982, Crawford and Jennings 1989, Marquis and Whelan 1994).

Capture of grasshoppers for food by mammals has not received much attention as a suppressing force on grasshopper populations. Small mammals, such as shrews, ground squirrels, deer mice, and grasshopper mice, and larger species, including skunks, foxes, and young coyotes, all eat grasshoppers when available (Martin et al. 1951). Many reptiles and amphibians do the same (fig. I.10–4).

Most investigators agree that predation is more important before, rather than after, insect pests reach the outbreak stage. Bird and mammal predation on grasshoppers is considered a stabilizing force on grasshopper populations. Wildlife predation acts as a preventive factor to grasshopper outbreaks, rather than a means of quick reduction after a buildup to high pest densities. However, instances have been recorded (Wakeland 1958) of flocks of birds saving valuable forage from destruction by grasshopper outbreaks. Perhaps the best known example is the arrival of gulls to save crops in Utah from Mormon crickets (Forbush 1907).

The recognition of the value of birds in combating insect pests has led to efforts not only to protect insectivorous species but also to increase their numbers by providing nest boxes and improving habitat. Nest boxes have been successfully used for hundreds of years on a large scale in Europe to attract birds that control forest insect pests (Takekawa et al. 1982). In the United States, forest management effects on bird populations and relationships to insect outbreaks were reviewed by Thomas (1979) and Crawford and Jennings (1989). A study of insectivorous





**Figure I.10-4**—Over the summer, relatively low grasshopper densities on a rangeland area can be diminished further by natural biological factors, including bird, mammal, and reptile predation. But wildlife predation is effective only when grasshopper densities are low (noneconomic) and should be viewed as a preventive force against grasshopper outbreaks, not as a total control measure after outbreaks have occurred.

birds feeding on the insect pests of white oak (*Quercus alba*) concluded that bird predation reduced insect numbers by 50 percent and resulted in one-third greater growth of the oaks (Marquis and Whelan 1994).

Control of locusts that had been a chronic problem on 8,200 acres of grassland in China was achieved by using birds. This was done by creating nesting habitat, planting small shrubs, and digging water seeps to increase the number of insectivorous birds (Anonymous 1988, Yu 1988). Control was successful over many years. Predation on grasshoppers by birds was found in food-habit studies of rangeland birds foraging at edges of Montana wheat fields (McEwen et al. 1986).

Bird densities on the semiarid western rangelands of the United States are generally lower than in other ecosystems that receive higher precipitation. However, numbers of highly insectivorous birds, such as meadowlarks and grasshopper sparrows, can be increased by improving range condition and increasing perennial grass and forb cover. The wildlife associated with healthy stands of native grasses, forbs, and shrubs can contribute greatly to prevention of grasshopper outbreaks (McEwen 1982, McEwen 1987). Figure I.10-1 shows an example of grasshopper suppression by wildlife.

An investigation of bird numbers and range grasshopper densities on the North Dakota Grasshopper Integrated Pest Management Project Demonstration Area indicated a significant negative relationship (George and McEwen 1992). This relationship was a strong indication of possible effects of avian predation on grasshopper densities.

Although bird population densities vary on rangeland, most studies show a normal population range of 1 to 3 birds/acre in the late spring to summer breeding season. Models of predation (McEwen 1987) by birds at these densities show a grasshopper reduction potential of at least 50 percent. In a recent review of the role of birds in controlling insect pests, Kirk et al. (1996) developed a model that indicates even greater potential for regulation of grasshoppers—based on bird numbers, capture rates, and energetics.

Wildlife populations are an important biological control factor in natural suppression of rangeland grasshoppers. Management practices that improve range condition and habitat for insectivorous and omnivorous wildlife can dampen or prevent extreme grasshopper population fluctuations and help reduce damage to vegetation.

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## I.11 Enhancing Biological Control of Grasshoppers by Construction and Placement of Bird Nest Boxes

B. E. Petersen, L. C. McEwen, and C. M. Althouse

Wildlife can play a significant role in the regulation of grasshopper population dynamics (see chapter I.10). Placement of nest boxes for American kestrels (also known as sparrow hawks), bluebirds, and other insect-eating bird species can provide a strong and stabilizing factor to help control grasshopper populations and prevent outbreaks (fig. I.11–1). Kestrels take large numbers of grasshoppers and in some areas are called “grasshopper hawks.” Many other bird species that nest in cavities and nest boxes also feed on grasshoppers during the breeding season and feed them to their young. These insects are a very important source of protein and other nutrients for young birds during growth and development.

Because the birds listed in table I.11–1 are limited by the number of natural cavities available, their abundance can be increased significantly by the construction and placement of nest boxes. Each cavity-nesting bird species prefers nest boxes of a particular size but frequently will nest in larger boxes when smaller ones are not available. Placement of nest boxes on a large scale by land managers might reduce the need for, and cost of, chemical spraying and could be important in a grasshopper integrated pest management system.

Kestrels and bluebirds (eastern, western, and mountain bluebirds) are among the most common species attracted to properly placed nest boxes. Plans and directions for construction are shown in figures I.11–2 and I.11–3. Because most cavity-nesting species are territorial, placement of boxes should not be too close together so that birds avoid using them. Defended territories vary with the species of birds, food availability, and their other needs.

American kestrels have the largest territories compared to other cavity-nesting species. In open country, where the boxes are within direct line of sight, the distance between them should be at least 2,460 ft (750 m). When trees intervene, such as along a meandering river or irregular woodland edges, the boxes can be as close as 656 ft (200 m). Entrance holes should face south to southeast, away from prevailing winds and storms. Preferred height of boxes should be a minimum of 10 ft (3 m) but lower posts (7–9 ft) (2–2.7 m) also may be used. Boxes can be wired at top and bottom to posts, poles, or smaller trees

or nailed through the holes to large-diameter trees. Add 1 inch of wood chips or dried grass for nest material as kestrels do not bring in their own nesting material. Boxes should be cleaned out and fresh chips or grass added before each nesting season.

The three species of bluebirds defend smaller areas surrounding their nests than do kestrels; therefore, greater numbers of nest boxes can be provided per unit area. In open country, where bluebird boxes are within direct line



**Figure I.11–1**—Nest boxes placed on poles or trees bordering open rangeland readily attract birds, such as kestrels, that require cavities for nesting. Cavity-nesting birds are highly insectivorous and contribute to biological control of grasshopper populations. A young kestrel can be seen peeking out of the entrance hole. (Photograph by B. E. Petersen; used by permission.)

of sight, the distance between can be as short as 300 ft (92 m). Entrance holes also should face south to south-east, away from prevailing winds and storms. Boxes can be wired at top and bottom to posts, poles, or smaller trees or nailed through the holes to large-diameter trees at a height of about 5 ft (1.5 m) for ease in checking. No nesting material need be added to boxes because bluebirds bring in their own nesting material; but boxes should be cleaned out each year after the nesting season by removing debris and old material.

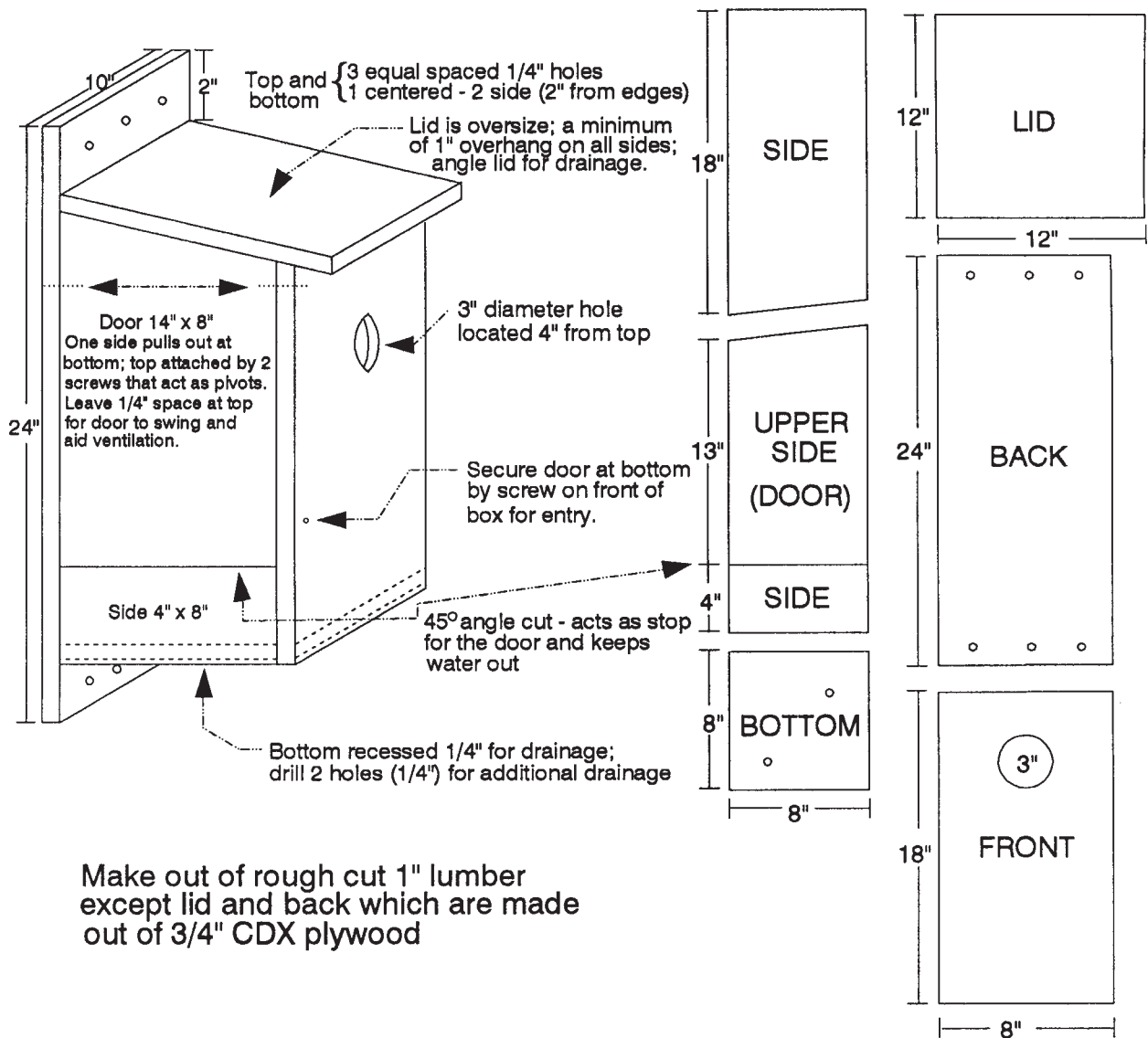
Information on construction and optimum placement of the various kinds of nest boxes can be obtained from State wildlife agencies or conservation organizations, such as the Bluebird Recovery Program, Box 566, Minneapolis, MN 55458; the North American Bluebird Society, Box 6295, Silver Spring, MD 20906-0295; or a local chapter of the Audubon Society.

**Table I.11-1—Dimensions (in inches) of nest boxes for several avian species**

Species	Diameter of entrance	Entrance height above bottom	Depth of cavity	Bottom of cavity
American kestrel	3.0	12 – 14	14 – 18	8 × 8
Downy woodpecker	1.25	6 – 8	8 – 10	4 × 4
Northern flicker	2.5	14 – 16	16 – 18	7 × 7
Red-headed woodpecker	2.0	9 – 12	12 – 15	6 × 6
House wren	1.0	5 – 6	6 – 8	4 × 4
Bluebird	1.5	7 – 8	8 – 10	5 × 5
Tree swallow	1.5	4 – 5	6	5 × 5
Chickadee	1.25	6 – 8	8 – 10	4 × 4

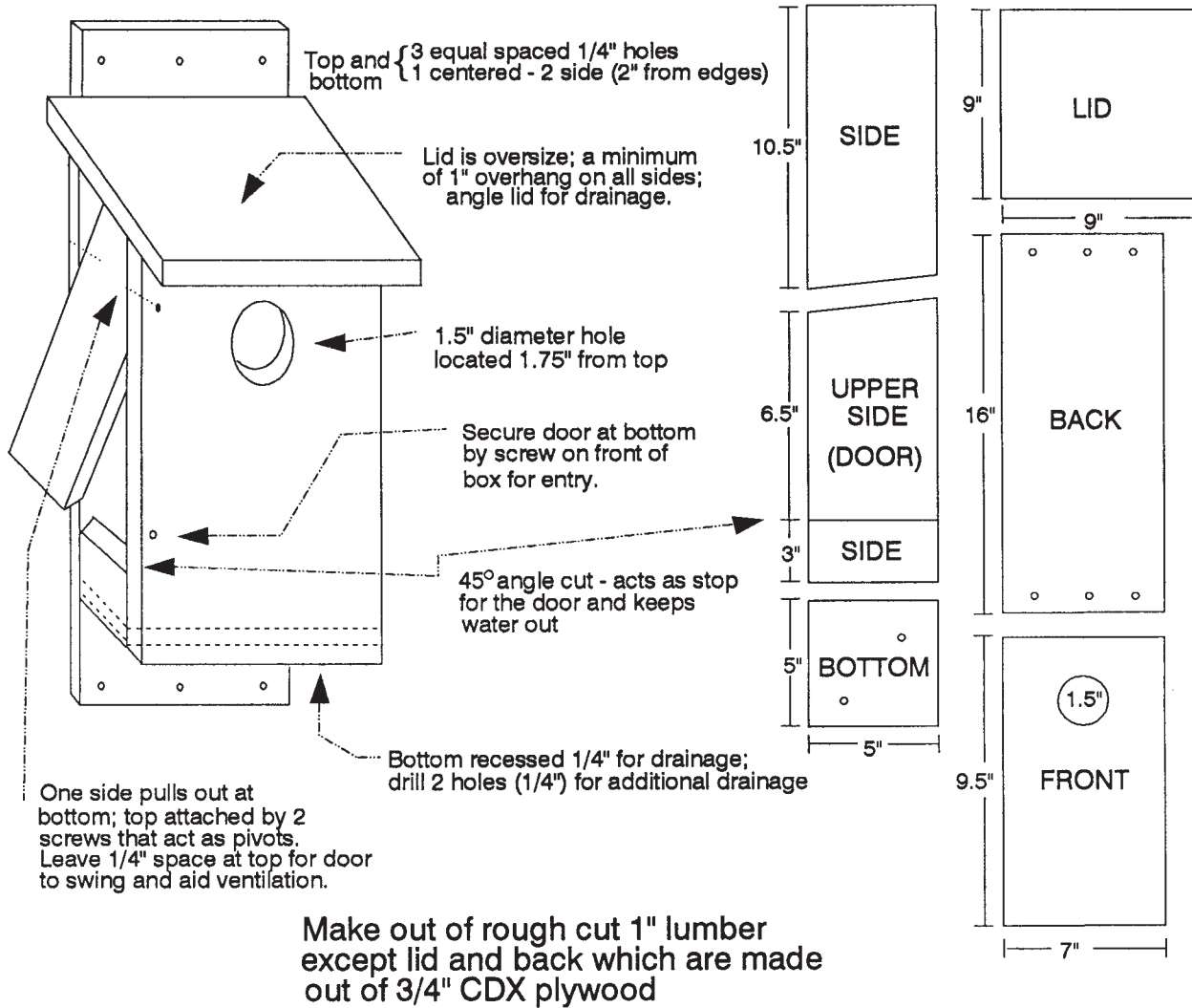
Note: Entrance should face south to southeast. Height of box is variable: larger birds prefer greater heights (about 10 feet or more), and smaller birds use lower boxes (about 5 feet or more above the ground).

# KESTREL NEST BOX



**Figure I.11-2**—American kestrel nesting box construction plan with dimensions and description of door mechanism. Entrance should face south to southeast, away from prevailing winds and storms. Boxes can be attached to trees, poles, or posts. Optimum height of boxes is a minimum of 10 ft (3 m), but lower attachments can sometimes be successfully used if taller ones are not available.

# BLUEBIRD NEST BOX



**Figure I.11-3**—Bluebird nesting box construction plan with dimensions and description of door mechanism. Entrance should face south to southeast, away from prevailing winds and storms. Boxes can be attached to fenceposts, small trees, or poles at preferred heights of 5 ft (1.5 m) or higher.

# I.12 The Biological Control Potential of Parasites, Predators, and Fungal Pathogens

D. L. Hostetter and R. J. Dysart

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## Introduction

Grasshoppers, like all other animals, are subject to a large number of parasites, predators, and pathogens, including fungi, protozoa, and viruses (Henry et al. 1985, Prior and Greathead 1989, Streett and McGuire 1990). Parasites, predators, and pathogens can be used as “classical” biological control agents. Classical biological control is defined as “the importation and release of an organism outside its natural range for the purpose of controlling a pest species” (Howarth 1991). Another approach, “augmentative” biological control, uses native or exotic organisms that are released periodically to enhance mortality in a targeted pest population. Insect pathogens generally fall in this category because many can be mass-multiplied and applied as biological pesticides (Prior and Greathead 1989).

## Insect Parasites and Predators

**Classical Introduction Approach.**—According to a review article by Prior and Greathead (1989), the classical biological control of a pest grasshopper using an insect parasite or predator as the beneficial agent has been attempted on nine occasions: there were two cases using bombyliids or bee flies, three cases using sarcophagid flies, two cases using meloid beetles, and two cases using scelionid wasps. Only two of these nine attempts resulted in the establishment of the introduced beneficial, a meloid beetle in Corsica and a scelionid wasp in Hawaii. However, the only project that has been claimed as a success was the introduction of a *Scelio* sp. from Malaysia, released against the rice grasshopper in Hawaii.

As suggested by Greathead (1992) and by Siddiqui et al. (1986), the possibilities for classical work certainly have not been exhausted, particularly with any scelionid egg parasites having an acceptable degree of host specificity. A controversy surrounding the request by Richard J. Dysart for permission to release a species of *Scelio* from Australia against pest grasshoppers in the United States seemed to pivot around the issue of host specificity. In spite of the constraints involved in the classical biological control approach, there are even more problems to consider in the augmentative approach.

**Augmentative Approach.**—Using insect parasites or predators as substitutes for chemical insecticides is not considered feasible for the control of grasshoppers. In his recent review of biological control options for tropical locusts and grasshoppers, Greathead (1992) expressed the same sentiments. In order for this approach to be workable, the natural enemy to be used must have a number of attributes:

- An acceptable level of host specificity, assuring some degree of safety to nontarget organisms,
- The ability to be easily reared in a laboratory situation and be produced in large quantities, and
- Costs of production and delivery to the target areas low enough so that the cost of using the biocontrol organism is competitive with the cost of using chemicals.

Concerns about host specificity would eliminate several groups of natural enemies, for example, the meloid and carabid beetles, whose larvae wander through the soil in search of a wide range of hosts. Similarly, certain beneficial groups can be eliminated from consideration because they are not amenable to handling in captivity, for example, the egg predators (Bombyliidae, Meloidae) and the nemestrinid parasites (Greathead 1992).

Although certain scelionid egg parasites can be reared easily in the laboratory, the rearing process is dependent on a constant supply of grasshopper eggs of a certain age. Considering the immense areas that would require release of parasites, plus the logistics of rearing and delivery, it is certain that the costs of using *Scelio* sp. parasites in an augmentative approach would be unacceptable.

## Classical Introduction Approach to the Use of Fungi

One of the first documented reports of attempting to use *Entomophaga* (= *Empusa*) *grylli* Fresenius (Batko) as a classical biological agent occurred in South Africa in 1896 (Howard 1902). A man named Arnold Cooper, of Richmond, Natal (South Africa), noticed grasshoppers dying apparently from a fungous disease. He took specimens to the Bacteriological Institute at Grahamstown, where a fungus capable of infecting healthy grasshoppers was isolated. Subcultures of the isolate were made, and vials containing them were distributed to planters in areas

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where grasshoppers were abundant. Planters such as H. H. Wells chronicled the situation in 1899: "I dipped captured adult grasshoppers into fluid containing the fungus then released them into the swarm over a period of two to three days...to my profound astonishment I found grasshoppers hanging in clusters all over my farm...millions of them." Many other equally favorable reports were received by the Bacteriological Institute, and distribution of the culture tubes continued.

Questions concerning the precise "nature" of the fungus were raised in 1899 and 1900. Specimens sent to the Royal Botanic Gardens, Kew, England, were identified as a *Mucor* sp. The same determination had been made simultaneously in Victoria, Australia, from similar specimens received from Natal. Circumstantial evidence suggests that perhaps two different fungi were in fact distributed. *Mucor* sp., which is easily cultivated and was readily identified by the authorities of the day, could have been contaminated with resting spores of *Entomophaga* sp. This scenario would support the reports of "clusters of diseased grasshoppers" by planters such as H. H. Wells and early photographs showing dead grasshoppers hanging from the tops of foliage. That phenomenon provides strong evidence of infection by *Entomophaga* sp. It is also apparent that "mixtures of fungal cultures" originating in South Africa were freely distributed to Australia and North America during the period 1899–1901 (Howard 1902).

Documents indicate that fungus cultures were obtained from South Africa by Dr. L. O. Howard in 1900 for subculture and release against grasshoppers in Colorado. A total of 223 "probable releases" were made in 24 States plus the Philippine Islands and Cuba during the period 1901–02 (Howard 1902). Howard further states that "No effort was made to determine the exact nature of the fungus contained in the culture tubes received from South Africa in the spring of 1900, but subsequent events indicate plainly that the Bacteriological Institute at Grahams-town is sending out more than one kind of fungus."

Professor L. Bruner (1901) also reported on a fungus, *Sporotrichum* sp. (= *Beauveria* sp.) he discovered infecting locusts in Argentina in 1897–98. He noted that "it is quite similar to the fungus which is used in destroying chinch-bugs in some portions of the United States."

Bruner also stated "that [although] considerable time has been spent in experimenting with this South American fungus upon our North American grasshoppers, thus far the results have all been negative since not a single insect has died from the disease."

These early attempts to use entomopathogenic fungi as "classical" biological control agents set the precedent for introduction and distribution of exotic pathogens in North America. It is apparent that numerous releases of unknown species from a wide variety of locations were made with little concern for environmental consequences beyond reduction of the pest species of the day.

For more than 100 years, the literature on grasshopper fungi has documented the evolution of a wide range of biological facts and observations. Habitat and climatic requirements are most often alluded to as dampening factors for the expression of fungus disease. The initial association between cool, wet, spring weather and an ensuing fungus epizootic plus other observations led to the current data base.

Many entomologists have reported the importance of microhabitats and macrohabitats for the development and expression of fungus epizootic among grasshopper populations. Reports indicate that fungus-infected grasshoppers are often restricted to roadside ditches; perimeters of cropland; low-lying, moist swales and intermittent waterways in pastures and hayfields; and various other noncultivated habitats (Hostetter et al. 1992 unpubl., Packham et al. 1993, McDaniel 1987).

A review of the accumulated information suggests that perhaps entomopathogenic fungi can be exploited in a "classical" sense through novel manipulations and applications already existing in North American agroecosystems.

The theoretical basis for the use of pathogens in biological control has been thoroughly discussed by many authors; most notably by Anderson (1980, 1982) and Hochberg (1989).

A mathematical model derived by Hochberg (1989) shows that host populations may be regulated to low and relatively constant densities if sufficient numbers of



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pathogens are translocated from reservoirs to habitats where transmission can occur. The model accounts for host–pathogen interactions based on heterogeneity; pathogen populations are not uniform. Transmissibility and lifespan of the pathogen differ among individuals or life stages in the environment. Pathogens are considered as two distinct subpopulations; one as transmissible and short lived, and one as nontransmissible and long lived (e.g., *Entomophaga macleodii* and *E. grylli* pathotype 3, conidia and resting spores).

Infective entities of the pathogen can cause infection only when they are translocated (abiotically or biotically) from the reservoir to the susceptible host. Hochberg suggests that, to increase the efficacy of indigenous pathogens of insects, the focus should be on the identification and manipulation of pathogen reservoirs between nontransmissible and transmissible subpopulations.

The model suggests that for the introduction of exotic pathogens as classical biological control agents, the conditions for the likelihood of success are (1) long lifespan of pathogen stages residing in reservoirs and (2) the propensity of these stages to be translocated to the habitat of the host for transmission.

Two practical applications of this model would be the use of existing Conservation Reserve Program (CRP) land and Federal and State highway rights-of-way as reservoirs or “refugia” for hosts, pathogens, parasites, and predators (Parker 1971).

The CRP program, which was devised in accordance with Title XII of the Food Security Act of 1985 (P.L. 99–198), provides for farmers to enter voluntarily into multiyear (10-year minimum) contracts with USDA to take specified highly erodible cropland out of annual production and put it into some other permanent vegetation. CRP acreage has been identified, quantified, and mapped for each county in each State by personnel of USDA’s Agricultural Stabilization and Conservation Service. Blocks of land most often occur in multiples of 40 acres and will be available as a stabilized system (for a minimum of 10 years).

It may be feasible to isolate grasshopper populations on CRP acreage with timely applications of chemical agents or mechanical barriers followed by inoculation/suppression with biological agents utilized in concert with naturally occurring parasites. Geographical imaging systems (GIS) are in place and could be used to delineate graphically and link strategic release areas based on ecological requirements of natural enemies across vast acreages. Host–pathogen reservoirs could be maintained and manipulated by augmentative releases of pathogens, parasites, and predators.

Manipulation of the habitat could be effected in a variety of ways: (1) clearcutting or stripcutting of foliage, which forces susceptible stages of the target species to concentrate in an area favorable to pathogens and arthropod natural enemies; (2) regulation of irrigation practices to create optimum habitat (cover crops) within the reservoir; (3) timely use of disruptive techniques (cultivation, spring-tooth harrow, mowers) to facilitate movement of pathogens from the soil (reservoir) to the host habitat (transmission–infection arena).

The current soil conservation program under the aegis of P. L. 99–198 will probably be succeeded by another “idle acres” program that may provide an exceptional opportunity for demonstrating the principles of IPM.

Federal and State highway rights-of-way could be manipulated to become “beltway reservoirs” for beneficial organisms across entire States. Millions of dollars are spent each year throughout the rangeland States for highway beautification and maintenance programs (e.g., landscaping, mowing, spraying). Monies diverted into development and conservation of habitat may be a wise investment toward long-term stability in the agrosystem. Perhaps a highly visible program of conservation and manipulation of “reservoirs of natural enemies” along the Nation’s roadways would pique public interest and support.

**Augmentative Approach.**—Presently, entomopathogenic fungi have the greatest probability of exploitation as microbial control agents for managing grasshopper populations. The wide range of orthopteran hosts and environments from which fungi have been isolated has revived interest in this group over the last decade.

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Worldwide, at least 10 genera of fungi are known to be entomopathogens of grasshoppers and locusts (Prior and Greathead 1989). Use in the initial phase will be “augmentative”: “insecticidal” formulations and applications will be used to augment natural enemies in the target area (Foster et al. 1991–94 unpubl.).

The most promising candidates are found among the *Beauveria* spp., *Metarhizium* spp., and *Entomophaga* spp. *Beauveria* spp. and *Metarhizium* spp. have host-specific strains and are purported to be nonhazardous to nontarget organisms (Prior and Greathead 1989). Conidia, or spores (the infective entity), are easily produced on commercially available solid substrates or in fermentation processes and can be formulated and applied similarly to other contact chemical pesticides (Foster et al. 1991–94a and b unpubl.).

Because they are lipophilic, the conidia of *Beauveria* spp. and *Metarhizium* spp. can be formulated with oil carriers and applied via ultralow-volume techniques. Oil droplets have the advantage in that droplets of smaller volume (mean diameter) can be generated at the nozzle (time of release), and the oil prevents evaporation during travel to impact on the target (grasshopper cuticle). Oil formulations have the advantage of spreading over the also lipophilic insect cuticle, thereby carrying conidia to intersegmental membranes and joints. Delivery to those areas increases the probability of penetration and infection of the insect (Prior and Greathead 1989).

Vegetable, soybean, or corn oils produced within or near insecticide-application areas could provide sustainable, nontoxic, environmentally safe formulation bases. The use of vegetable oils could decrease reliance on petroleum-based carriers.

The augmentative application of *Entomophaga grylli*, pathotype 1 (= *E. calopteni* [Bessey] Humber), was attempted in South Dakota (McDaniel 1987). McDaniel noticed the presence of *E. grylli* while conducting grasshopper surveys in 1979–80. Among other observations, he noted that the majority of grasshoppers dying from the fungus were found in areas not subject to cultivation (e.g., field borders, roadside ditches, alfalfa fields) and from the edges of corn and soybean fields.

McDaniel reported that he “triggered two fungus outbreaks in the spring of 1982 in plots in Hughes county near Blunt, SD and at a location 21 miles west on the Bad River road in Stanley county.” The triggering was accomplished by collecting 4,468 plant sections, each of which had a fungus-killed grasshopper attached; taking them to an area known to be free of the fungus disease; and taping them to the tops of tall grasses and alfalfa plants.

Fungus-killed grasshoppers were observed 15 days after inoculation and a 53-percent reduction of the population occurred within 45 days. McDaniel also reported that the fungus continued to kill grasshoppers at these plots through 1986 with no additional inoculum of spores.

McDaniel developed a method of extracting resting spores from cadavers for inoculation of field plots. He extracted 2 gal of pure spores from 38 gal of hand-picked, dead, fungus-killed grasshoppers. He was able to effect disease in release plots using infected grasshoppers or by applying with a grass-seed spreader ground-up bodies of *Melanoplus differentialis* (Thomas), *M. bivittatus* (Say), and *M. sanguinipes* (F.) that had been treated with fungal spores.

McDaniel (1987) attributed the unsuccessful inoculations done with pure resting spores to the fact that they had been stored for several months at room temperature between collection in late fall and application in early spring.

*Entomophaga* spp.—particularly the Australian isolate, *Entomophaga grylli* pathotype 3—may be best utilized as “classical biological control agents.” Members of this complex cannot be produced easily on axenic substrates or in large enough quantities to be used as insecticidal treatments. Current ideology views this as a limitation of the present state of technology; however, perhaps not all entomopathogenic fungi or other microbial agents are best used as insecticides.

The best utilization of entomopathogens will evolve over time along with increased understanding of the ecology and the systems that regulate it. The many avenues of availability are just beginning to be explored. Exploitation will require long-term commitment, innovative

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approaches, and the willingness to tailor management practices within the principles of ecology.

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