I.6 Grasshopper Pathogens and Integrated Pest Management

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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-muscardine 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.

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.

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

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 sporeformers, 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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Availability note: Copies of the annual reports from the Grasshopper Integrated Pest Management Project are available from USDA, APHIS, PPQ, 4700 River Road, Riverdale, MD 20737.

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