VII.7 Effects of the Fungus *Beauveria bassiana* on Nontarget Arthropods

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Introduction

Beauveria bassiana is currently being developed as a potential bioinsecticide alternative to traditional chemical pesticides for controlling grasshopper populations. Currently, *Nosema locustae* is the only other nonchemical treatment registered for control of grasshoppers on rangeland. *B. bassiana* offers at least two major advantages over *N. locustae*: (1) *B. bassiana* appears to kill grasshoppers more rapidly than does *N. locustae* (see VII.5 and I.3), and (2) *Beauveria* does not rely on the ingestion of its spores in a bait formulation by grasshoppers but is capable of directly penetrating through their exoskeleton (Goettel 1992).

Unfortunately, *B. bassiana* may possess at least one potential disadvantage. Unlike the narrow specificity of *N. locustae* for orthopterans (i.e., grasshoppers, locusts and crickets), *B. bassiana* is known to infect a wide variety of insects (Goettel 1992). The wide specificity of *Beauveria* is of concern because distribution of its conidia into the environment also might diminish beneficial insect populations. Attempts have been made to select strains of *B. bassiana* with increased specificity for grasshoppers by selecting stains isolated from grasshoppers (Prior 1992).

Mycotech Corporation (Butte, MT) has mass-produced a strain of *B. bassiana* isolated from an infected grasshopper found in Montana. Laboratory and field studies have indicated that this strain is infectious and lethal in confined populations of several species of grasshoppers (see VII.5). However, no information existed on its virulence in nontarget insects.

In 1993, South Dakota State University (SDSU) assisted the Animal and Plant Health Inspection Service (APHIS) by monitoring the population levels of nontarget arthropods in a *B. bassiana* field study located near Amidon, ND (Brinkman 1995). The grasshopper control data for this study are described in chapter VII.5. Important nontarget arthropods on rangeland include beneficial pollinators (flies and bees), predators (spiders, ants, ground beetles, robber flies, green lacewings, brown lacewings, antlions, ladybird beetles, blister beetles, and wasps), parasites or parasitoids (flies and several hymenopterans) and general scavengers (ants and darkling beetles).

Spray-tower laboratory bioassays as developed by Foster and Reuter (1991) also were used at SDSU to determine the effects of *B. bassiana* on nontarget insects. A spray tower consist of a small airbrush, such as artists use, mounted on a stand and connected to an air pump. A solution of fungal conidia (sporelike stage) can then be injected into the airstream and sprayed onto the insects. This method of conidia application should more closely simulate the field aerial application of conidia than would applying the conidia in a large single drop or by submerging the insects in a solution of conidia (Foster and Reuter 1991).

Adult yellow mealworm beetles *(Tenebrio molitor)* were evaluated with the bioassay because they are easily acquired commercially and have therefore served as research models in many laboratory studies. The species *T. molitor* belongs to the family Tenebrionidae, which is an important group of beetles on western rangeland. This beetle was selected also to represent the many species of beetles evaluated in the field study whose population levels appeared unaffected by the release of *B. bassiana* conidia into their locality.

According to Goerzen et al. (1990), alfalfa leafcutting bees *(Megachile rotundata)* should be considered in evaluations of potential microbial agents. Unfortunately, the low numbers of alfalfa leafcutting bees recovered in field plots prior to the North Dakota study made it impossible to evaluate the effects of *B. bassiana* on this species. Therefore, *M. rotundata* was evaluated in the laboratory bioassay. Spray tower bioassays were first conducted with fourth-instar *Melanoplus sanguinipes* grasshoppers in order to standardize our results with those reported in VII.5.

Field Studies

Methods.—Thirteen days prior to aerial treatments, sampling traps were placed in 4 control plots, 4 carbaryl plots, and 4 plots that were to receive *B. bassiana* at the rate of 9.9 trillion spores/64 oz/acre in oil formulation. Ground-dwelling arthropods were sampled with the use of pitfall traps. Pitfall traps are widemouth quart canning jars placed in the ground with the opening level with the soil surface. Ground-dwelling arthropods were captured, killed, and preserved as they fell into the jars, which contained 70 percent alcohol.

Aerial insects were sampled using malaise traps. Insects were captured by malaise traps as they flew into the netting, and instinctively crawled or flew up into jars at the top. Sampling traps were left in plots for 5 days, and then jars and samples were retrieved. Immediately after treatments, jars were replaced in plots and retrieved every 6 days for the duration of the summer season. Arthropod samples were taken to SDSU to be sorted and identified.

Results.—During the study period, an abnormally high level of precipitation fell on the study plots. The resulting high moisture level was favorable for the natural outbreak of *Beauveria* infections identified in the control grasshoppers from the untreated plots. This natural *Beauveria* outbreak may then have been at least partially responsible for the unexpected erratic results seen in this study in both the treated and untreated plots.

Ant and spider abundance declined in all plots following treatment but rebounded the next week. The sporadic heavy precipitation that occurred following treatment may have resulted in decreased activity of those ground-dwelling arthropods, and thus diminished their chances of falling in the pitfall traps. Therefore, the temporary decrease in ant and spider abundance did not appear to be due to *B. bassiana* or carbaryl treatments. Ground beetle (Carabidae) densities remained stable throughout the summer season.

Flies (Diptera) were the most prevalent aerial insects captured in malaise traps. Abundance of flying Diptera, Hymenoptera, Lepidoptera, Neuroptera, and Coleoptera increased in all plots following treatments. *B. bassiana* and carbaryl applications did not result in any noticeable declines in aerial insect abundance.

Alfalfa leafcutting bees were very rare at the study site. Only three individual Megachilidae were collected in malaise traps during the sampling season. The study site was dominated by mixed grasses, so there was little attraction for pollinating bees. Consequently, we were not able to determine if field applications of *B. bassiana* affected alfalfa leafcutting bees.

Laboratory Studies

Methods.—Fungal conidia (spores) and an oil carrier solution were supplied by Mycotech Corp. Aerial application of *B. bassiana* was simulated in the laboratory with the use of a spray tower. A favorable spray pattern was established in practice tests with the oil solution and the aid of oil-sensitive paper. Procedures, equipment and *B. bassiana* dosages were similar to those described in VII.5 and were selected based on recommendations by Foster and Reuter (1991).

A total of 360 individuals of each species were tested in the laboratory experiments. Prior to each spray event, clean newsprint was placed on the floor of the spray room. In addition, test insects (in groups of 10) were slowed by cooling to 35 °F (1.7 °C). Thirty individuals were sprayed with air for approximately 15 seconds first and were kept as controls. Thirty insects were sprayed with 0.09 mL of the oil carrier. Thirty insects were sprayed with 0.09 mL of oil containing 2.64 billion conidia/mL. Treatments were replicated four times. Insects were then observed for 10 days after treatment.

Results.—Grasshoppers treated with *B. bassiana* began expiring on day 5. After 10 days, more than 73 percent of treated grasshoppers had died. Mortality of beetles treated with *B. bassiana* was extremely low, and beetles did not appear to be susceptible to infection.

B. bassiana was extremely virulent to alfalfa leafcutting bees. Alfalfa leafcutting bees sprayed with *B. bassiana* began expiring on day 4. After 10 days, more than 87 percent of alfalfa leafcutting bees had died. However, mortality of alfalfa leafcutting bees sprayed with oil and air (control) was low. Dead alfalfa leafcutting bees were individually placed in glass vials with a moist cotton ball and were observed for evidence of infection. After approximately 7 days, external sporulation of hyphae (filaments of the vegetative structure of the fungus) was observed on 99 percent of alfalfa leafcutting bees treated with *B. bassiana*.

Conclusions

Treatment of the study sites with *B. bassiana* caused no measurable permanent decrease in populations of any of the monitored beneficial insects. This lack of effect occurred during a time period when moisture levels in the fields were abnormally high, and thus, environmental conditions should have been very good for the spread of the infection into beneficial insects. In fact, even some of the grasshoppers recovered from the control sites also were infected with *Beauveria*, but at low levels and most likely from a natural outbreak.

Spray-tower results on lab-reared grasshoppers were similar to those described in VII.5. The nonsusceptibility of the *Tenebrio molitor* to *B. bassiana* in the spray-tower bioassay was consistent with *Beauveria*'s apparent lack of effect on beetles in the field study. The effects of *B. bassiana* on alfalfa leafcutting bees were evaluated only with the spray-tower bioassay because few bees were recovered in the field. Existing bioassay data indicate that these insects are very susceptible to this strain of *B. bassiana*. Injury to the entire population of alfalfa leafcutting bees might be reduced through management.

B. bassiana conidia can persist if protected from environmental extremes (soil is the natural reservoir for conidia), but become nonviable after only a few hours of exposure to sunlight (Gaugler et al. 1989, see VII.5). Alfalfa leafcutting bees readily accept artificial nesting structures, which could be moved during spray operations and returned later.

References Cited

Brinkman, M. A. 1995. Non-target arthropod impacts and rangeland grasshopper management using *Beauveria bassiana* (Bals.) Vuillemin. Doctoral dissertation. Brookings, SD: South Dakota State University. 62 p.

Foster, R. N.; Reuter, K. C. 1991. Using a spraytower to simulate aerially applied liquid sprays for bioassaying insects in the laboratory: standard operating procedure. Phoenix, AZ: U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Methods Development, Rangeland Section. 5 p.

Gaugler, R.; Costa, S. D.; Lashomb, J. 1989. Stability and efficacy of *Beauveria bassiana* soil inoculations. Environmental Entomology 21(6): 1239–1247.

Goerzen, D. W.; Erlandson, M. A.; Moore, K. C. 1990. Effect of two insect viruses and two entomopathogenic fungi on larval and pupal development in the alfalfa leafcutting bee, *Megachile rotundata* (Fab.) (Hymenoptera: Megachilidae). The Canadian Entomologist 122: 1039–1040.

Goettel, M. S. 1992. Fungal agents for biocontrol. In: Lomer, C. J.; Prior, C., eds. Biological control of locusts and grasshoppers. Wallingford, UK: C.A.B. International: 122–130.

Prior, C. 1992. Discovery and characterization of fungal pathogens for locust and grasshopper control. In: Lomer, C. J.; Prior, C., eds. Biological control of locusts and grasshoppers. Wallingford, UK: C.A.B. International: 159–180.