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Reduction of Grasshopper Populations following Field Application of the Fungus *Beauveria bassiana*

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Conidia of the Hyphomycete fungus Beauveria bassiana *(Bals.) were applied in an attempt to reduce field populations of grasshoppers, primarily the migratory grasshopper* Melanoplus sanguinipes *(Fabricius). Dry spores were applied with wheat bran carrier to three fallow fields at a rate of* 2.0×10^{13} *spores* ha^{-1} *in 10 kg bait* ha^{-1}. *Examination of culture plates that had been placed in the field to capture spores and of bran carrier with scanning electron microscopy indicated that a substantial portion of the* B. bassiana *colony-forming units (spores and clumps of spores) did not adhere to the bran and were applied in the field as free particles. Grasshoppers collected from the treated plots at intervals after application were assayed for infection by* B. bassiana. *The observed rate of mycosis in the treated populations was 70% of those collected after 2 days, declining to 41% by 13 days and 5% by 19 days after application. Analysis of reductions in population density gave results in agreement with the infection data. Treated populations declined 60% and 33% by 9 and 15 days after application respectively. The reductions were significant on both post-treatment sampling dates* ($p < 0.05$) *and the three replicated fields gave comparable results. This is the first field demonstration of effectiveness of this fungus as a microbial control agent of grasshoppers.*

Keywords: *microbial control,* Beauveria bassiana, *grasshoppers, Acrididae, insect population reduction, biological control*

INTRODUCTION

Grasshoppers and locusts (Acrididae) cause extensive damage to crops in the Americas, Africa and Asia. The development and use of naturally occurring pathogens offers the possibility of environmentally sound management of these pests. Hyphomycete fungi are promising candidates because, in addition to causing mortal diseases of certain insect species (e.g. Ferron, 1981; Zimmermann, 1986), they are usually inexpensive to produce and are available from commercial sources in quantities large enough for field testing. These fungi also appear to be innocuous to most non-target organisms (Goettel *et al.*, 1990). There are several records of the Hyphomycete, *Beauveria bassiana* (Bals.), infecting Orthoptera (MacLeod, 1954; Humber & Soper, 1986; Li,

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1987; Moore & Erlandson, 1988), including some detailed studies of infections of acridids as long ago as 1896 (Cooper, 1899; cited in Schaefer, 1936). Rare cases of epizootics involving this and related fungi have been reported, including a case in 1934 in which a large swarm of the red locust, *Nomadacris semptemfasciata* (Serville), was killed by *B. bassiana* (Schaefer, 1936). A search in Africa for fungi pathogenic to locusts and grasshoppers is presently being conducted (Prior *et al.*, 1992).

Pathogenicity of *B. bassiana* to grasshoppers under laboratory conditions has been demonstrated (Marcandier & Khachatourians, 1987; Moore & Erlandson, 1988; Bidochka & Khachatourians, 1990). In general, fungal spores infect insects either through penetration of the integument or via ingestion and subsequent penetration of the gut wall (Charnley, 1984). Death of the affected insects results from toxaemia or nutrient depletion. Although integumental penetration is the most common method of entry, it has been demonstrated that *B. bassiana* penetrates several non-acridid insect species via the gut after the insect has ingested spores (Bao & Yendol, 1971; Broome *et al.*, 1976). In the Colorado potato beetle, spores were shown to penetrate the integument after being consumed and excreted in the faeces (Allee *et al.*, 1990). However, there is little information on the exact mode of pathogenesis of this fungus to grasshoppers.

In our initial tests with a strain of *B. bassiana* received from Mycotech Corp. (Butte, MT, USA), grasshoppers fed wheat leaves that had been sprayed with moderate rates of the spores died within 10 days (Johnson *et al.*, 1988a). In subsequent, more detailed, laboratory tests, spores of *B. bassiana* sprayed in water or applied on food (leaf lettuce and wheat bran) were found to infect and kill grasshoppers of several species (Goettel & Johnson, 1992).

Information on the efficacy of *B. bassiana* against grasshoppers in field situations is lacking. In this study, we determine to what degree a dry spore–bait application reduces a grasshopper field population density, and estimate the prevalence of infections in the survivors. We used wheat bran as a carrier because it is readily consumed by all stages of grasshoppers, and such baiting is a convenient way of effecting contact between the spores and the target insects.

MATERIALS AND METHODS

Conidia Production and Formulation

The *B. bassiana* isolate used in this study was obtained from a migratory grasshopper collected near Three Forks, MT, USA and has been designated by Mycotech as strain GHA (C. Bradley, personal communication). Conidia were produced by Mycotech using solid culture technology (Bradley *et al.*, 1992), using a series of batches containing 1–2 kg of starch-based growth substrate. Conidia yields were from $1–3 \times 10^{13}$ conidia kg^{-1} of dry substrate, with final spore densities of $2–7 \times 10^{10}$ conidia g^{-1} in the milled product. The conidia were transported to Alberta as a dry powder and were stored at 4°C prior to use.

Conidia Viability

To determine germinability of the raw spore product, conidia powder was suspended and diluted in sterile water. Aliquots of 0.1 ml containing approximately 10^4 conidia were spread with a sterile glass rod on to the surface of Sabouraud's dextrose agar supplemented with 2% yeast extract, streptomycin (50 µl ml^{-1}) and penicillin (25 IU ml^{-1}) in 10 cm Petri dishes, and incubated at room temperature. After 7–10 h, spores producing a germ tube and non-germinated spores were counted using a phase-contrast microscope. The effective viability of the spores used in this study was determined to be 85%.

Formulation

The bait–spore mixture was formulated in a stainless steel mixer (Marion Mixer model 6041, Rapids Machinery Co, Marion, IA, USA) at the Lethbridge Research Station. The wheat bran was moistened slightly, the dry spore concentrate was added and the mixture was tumbled for

2–3 min. While mixing was in progress, the bait was sprayed with a solution of water and molasses, applied through three nozzles (Delavan LF1 65°; 250 kPa) spaced 38 cm apart on a spray boom mounted in the mixing tank. The mixer shaft and four 45 cm blades attached to it turned at 40 rpm during spraying and mixing. During preparation and mixing, the temperature of the bran bait remained below 24°C. The final bait constituents were 60 kg wheat bran, 3.1 kg dry conidia powder, and enough water and molasses to bring the final wet weight up to 70 kg, with a content of 2% molasses by weight.

Field Application

The experiment was conducted near Vulcan, Alberta, Canada. All plots were contained in fallow cereal fields with a sparse cover crop of clover naturally infested with pest species of grasshoppers. Three randomized complete blocks were delineated, each with one treated and one untreated plot of 150×150 m (2.25 ha). Blocks were separated by 100–300 m. Bait was evenly applied up to the edges of treated plots, including a 10 m border, but sampling for the impact on the grasshoppers was restricted to a 50×50 m central zone within each of the six plots.

The bait mixture was applied at a rate of 10 kg ha^{-1}, using a motor-driven bran blower modified to deliver bait directly into the path of a blower duct. This rate of bait application provided 2.0×10^{13} spores ha^{-1}. Bait application was controlled with a hydraulically driven rubber belt at the bottom of a hopper. The bran applicator was mounted on a four-wheel-drive pickup truck, driven at 15 km h^{-1}. The practical swath width for application purposes was 6–10 m, but the finer components were observed drifting up to 30 m. Three plots were treated on the afternoons of 19 June (Block 1) and 24 June (Blocks 2 and 3), 1991, and only enough bait to treat one plot was loaded at a time. Control plots were not treated with bran because previous experience with field trials indicated that neither the small positive effect of bran as a nutritive source, nor the small negative effect of tyres driving over the field, would affect the populations in a detectable way.

Grasshopper Population Monitoring and Mortality Assessment

Samples of approximately 500 grasshoppers were collected with sweepnets from each plot just before bait application so that grasshopper species and age composition at the site could be determined. Taxonomic keys and descriptions of Brooks (1958) and Vickery and Kevan (1983) were used for species identification. Grasshopper population density was monitored by counting the living grasshoppers observed in 300 permanent 0.25 m^2 quadrats just before and on two dates following treatment application. The quadrats were simple open squares of 0.6 cm diameter steel rods painted orange, and did not restrict grasshopper movement. They were placed in the central sampling zones of the plots at the beginning of the experiment (50 per plot, in five marked transects of 10 quadrats each).

The uniquely identified quadrats at permanent locations allowed adjustment for the initial population density. Analysis of variance (SAS Institute Inc., Cary, NC, USA, general linear model procedure) (SAS Institute, 1989) was used to assess the significance of the change in grasshopper field density, estimated by the difference at each quadrat location between the observations on the pre- and post-treatment sampling dates. Both block and treatment effects were taken into account.

Application Rate and Infection Measurements

Bait was collected at the time of field application by placing a plastic bag in the applicator path. The captured bait was returned to the laboratory and fed to 30 individually confined third-instar *Melanoplus bivittatus* (Say) at rates of one flake per hopper, two flakes per hopper or *ad libitum*, as a simple check of effectiveness. At death, the grasshopper cadavers were transferred to high humidity and checked daily for 2 weeks for evidence of *B. bassiana* mycosis.

In order to assess spore application, a culture plate containing a selective medium (oatmeal

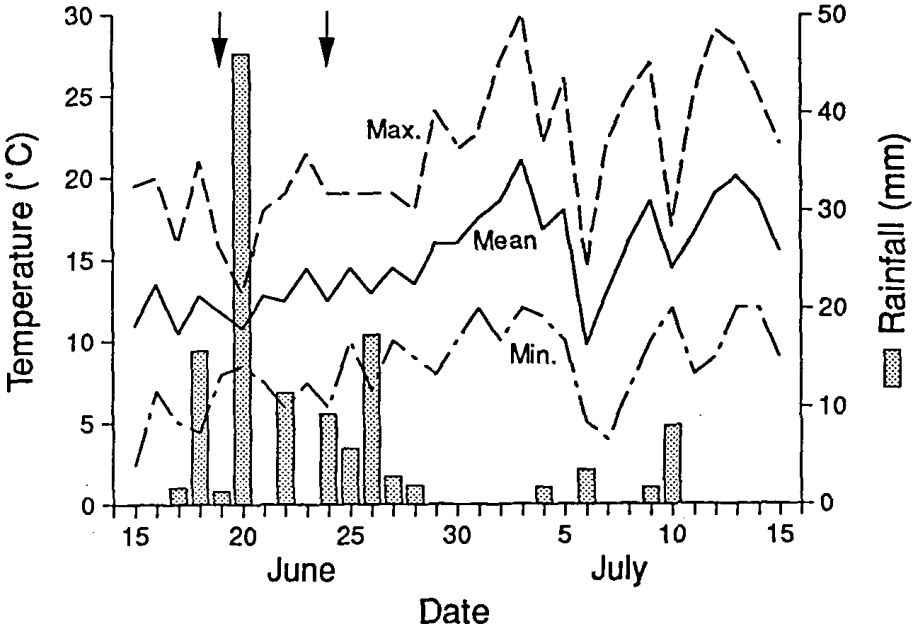

FIGURE 1. Weather parameters during the study period as measured at the Vulcan Airport by Environment Canada. Arrows indicate treatment times.

agar, Cyprex (dodine), crystal violet, penicillin and streptomycin sulphate; Chase *et al.*, 1986) was placed 1, 2, 3, 4 or 5 m from the bait applicator during a single pass. Ten cm plates, 10 in total, from two replications of the test in Block 1 were returned to the laboratory and incubated at room temperature. The number of bait particles on each plate was recorded immediately, and the number of *B. bassiana* colony-forming units (CFU) was recorded after 7 days. Samples of bran bait collected from the blower were fixed in osmium vapour, air dried, sputter coated with gold and observed with a Hitachi S-570 scanning electron microscope at an accelerating voltage of 10 kV.

The infection rate of grasshoppers in the fields was estimated by collecting grasshoppers with sweepnets from the treated plots and confining them in cages in the laboratory. The grasshoppers were collected at intervals up to 19 days post-treatment, and observed daily for up to 31 days. Dead grasshoppers were transferred to incubation under high humidity. Mycosis was confirmed if a sporulating layer of *B. bassiana* appeared on the cadaver.

Post-treatment records of grasshopper population density were made approximately 9 and 15 days after application (paired treated and untreated plots were always sampled the same day, but block collection dates were selected to minimize slight differences in heat accumulated between application dates).

Field Conditions

Weather during the week of application was cool and moist, with: 20–60% daytime cloud cover; maximum temperatures 16–22°C; 54–65% relative humidity; wind 1–3m s^{-1} gusting to 6 m s^{-1}; rain 50 mm (Figure 1). The field was not cultivated or sprayed during the experiment, and no vegetation was cut. High densities of grasshoppers and warmer weather near the end of the experiment resulted in loss of vegetation from some plots, especially Block 3.

RESULTS AND DISCUSSION

Conidia Condition, Quantity and Capture

We suspect that a significant portion of the spores was effectively applied as a dust rather than on the bran bait. The laboratory bioassays of bran flakes that had been blown out of the applicator resulted in no mortality attributable to the fungus. The lack of activity in the collected bran, despite the demonstrable virulence of the spore powder (Goettel & Johnson, 1992) indicated that the spores did not adhere well to the bran flakes, and were probably separated from the carrier during the violent propulsion through the applicator fan and ducts. Examination of bran particles with a scanning electron microscope substantiated this hypothesis. The spores did not appear to be strongly adhered to the bran, and the molasses may have served only to clump the spores together, thus reducing the effective number of CFU, rather than gluing the spores to the bran (Figure 2). Furthermore, examination of the culture plates indicated that numerous *B. bassiana* spores were deposited, although few bran flakes were captured on the same plates (Table 1). The number of CFUs captured on the plates did not differ significantly among the positions or between the two replications of the test (Anova, $p < 0.3$).

Infection and Sporulation

Of 1080 grasshoppers collected over a period of 19 days and confined for 31 days, 575 showed clear evidence of *B. bassiana* infection (Table 2). Because only living, active grasshoppers were collected, and because this entomopathogen is not transmitted among grasshoppers on which a sporulating layer of the fungus has not yet developed, these figures provide a conservative estimate of the proportion of the grasshopper population that was either carrying inoculum or exhibiting early stages of the disease. The lag observed between collection date and the time for death due to mycosis indicates that insects in an advanced state of infection were not collected with sweepnets, suggesting that such insects became cryptic. Such a phenomenon was shown in previous studies with *Nosema locustae* (Johnson, 1989) and deltamethrin (Johnson *et al.*, 1986).

Within 2 days of application, sufficient contact with the spores had occurred to cause death by *B. bassiana* mycosis of 70% of the grasshoppers that were collected and confined in laboratory cages. The results from the remaining five collection dates indicated significant and declining levels of infection in the field (Figure 3). In the present study, much higher infection rates were obtained than in previous field tests of this fungus against grasshoppers in Africa (Johnson *et al.*, 1992) and Alberta (Johnson, unpublished data) using different formulations of the same fungal strain.

Population Reductions

Pretreatment population densities averaged 8.8 grasshoppers per 0.25 m^2 ($n = 300$, SEM = 0.33). The grasshopper population at the time of treatment had a species composition typical for Canadian prairies, and consisted primarily of the immature stages of *M. sanguinipes*, and smaller numbers of *M. bivittatus* (Say), *M. infantilis* Scudder, *M. packardii* Scudder and *Camnula pellucida* (Scudder). All stages were present for some species, and less than 11% of the grasshoppers collected for species identification were in the adult stage (Table 3).

Analysis of variance of the change in grasshopper counts confirmed that application of dry spores reduced significantly grasshopper field densities by 9 days after treatment ($p < 0.05$) and 15 days after treatment ($p < 0.01$). By chance, the treated plots began with a higher population density than did the untreated plots, but by the first post-treatment sampling date, the numbers of grasshoppers in the treated plots had been reduced to about half that of those in the untreated areas (Table 4). The treatment effect occurred throughout the treated area, as evidenced by the general shift in the distribution of changes in density (initial count minus subsequent count) after treatment with *B. bassiana* (Figure 4). At 9 days after application, 53% of the quadrats in untreated plots showed decreases in grasshopper numbers over initial counts, while 83% of those in the treated quadrats showed decreases. The magnitude of the shift was less by the 15th day

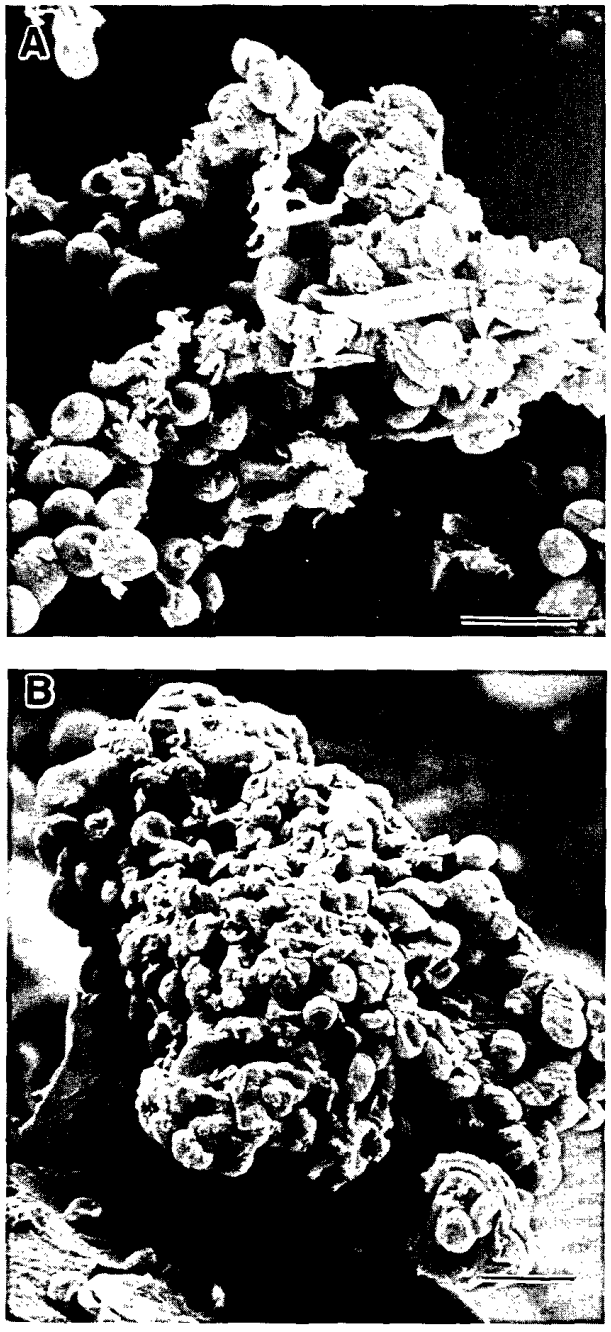

FIGURE 2. Scanning electron micrographs of *B. bassiana* conidia used in the field test: clump of unformulated spores, as supplied by Mycotech Corp. (A); spore clump after formulation with 2% molasses on bran (B). Bar = 5 μm.

after application, when the grasshopper counts had decreased from pretreatment counts at 61% of the untreated quadrats, as opposed to 77% in the treated.

Although less effective than what is generally considered to be acceptable performance when evaluating chemical insecticides, *B. bassiana* provided significant reductions in grasshopper population density in all three experimental blocks, as evidenced by the adjusted block mortali-

TABLE 1. Numbers of CFU of *B. bassiana* and bran bait particles on 10 cm dodine oatmeal agar plates placed at 1 m intervals from the bran applicator during field treatment in Block 1

Distance from applicator (m)	Rep. 1		Rep. 2	
	CFU	Bran	CFU	Bran
1.0	682	3	> 1500	2
2.0	1026	1	1167	1
3.0	959	0	579	0
4.0	554	0	757	0
5.0	861	0	644	0

ties of 54–64% (Table 4). The common Abbott's ratio indicated a substantive overall reduction in grasshopper abundance that persisted to at least 15 days after application, and approximately corresponded to the pattern of infections (compare Tables 2 and 4).

Conditions during this experiment were apparently conducive to survival and activity of the fungal agent, and it may be claimed that the results are atypical because they represent activity that could be expected only under moist conditions. It may also be argued that the general requirement of some fungi for high moisture is incompatible with the dryness common in grassland ecoregions. Fungi have terminated some grasshopper outbreaks during moist periods, even across extensive areas of rangeland. For example, the dominant role of infection by *Entomophaga grylli* (Fres.) in the crash of the extensive outbreak of the clear-winged grasshopper, *C. pellucida*, in 1963, is well documented (Pickford & Riegert, 1964) and smaller epizootics have occurred since then. More recent observations on the activity of two pathotypes document the increase in the prevalence of infection that is observed following the arrival of above-average rainfall (e.g. Carruthers *et al.*, 1986; Erlandson *et al.*, 1988), but this is not universally true of all fungal entomopathogens. Although moisture is required for conidiation of Hyphomycetes, it has not been demonstrated that it is a limiting factor in penetration and pathogenesis by *B. bassiana* (Marcandier & Khachatourians, 1987). For example, field cage tests with *Verticillium lecanii* (Zimm.) applied to grasshoppers with and without simulated rainfall showed that the lack of environmental moisture does not preclude infection, although it may impede subsequent transmission of infection from one insect to another (Johnson *et al.*, 1988b). Activity of a microbial control agent requires favourable environmental conditions, but also a host population and proximal inoculum. Natural fungal epizootics, and also successful microbial control, are not prevented only by the lack of conducive weather. We think it more likely that the quantity and

TABLE 2. The decline in *B. bassiana* mycosis of confined grasshoppers collected from treated plots following field application of conidia

Collection date (days after application)	Number collected	Percentage with mycosis[a]
2	583	69.8
7	156	51.3
8	108	48.1
13	51	41.2
14	87	11.5
19	95	5.3

[a] Grasshoppers were considered to have died of mycosis if a sporulating layer of *B. bassiana* developed on the cadaver under conditions of high humidity.

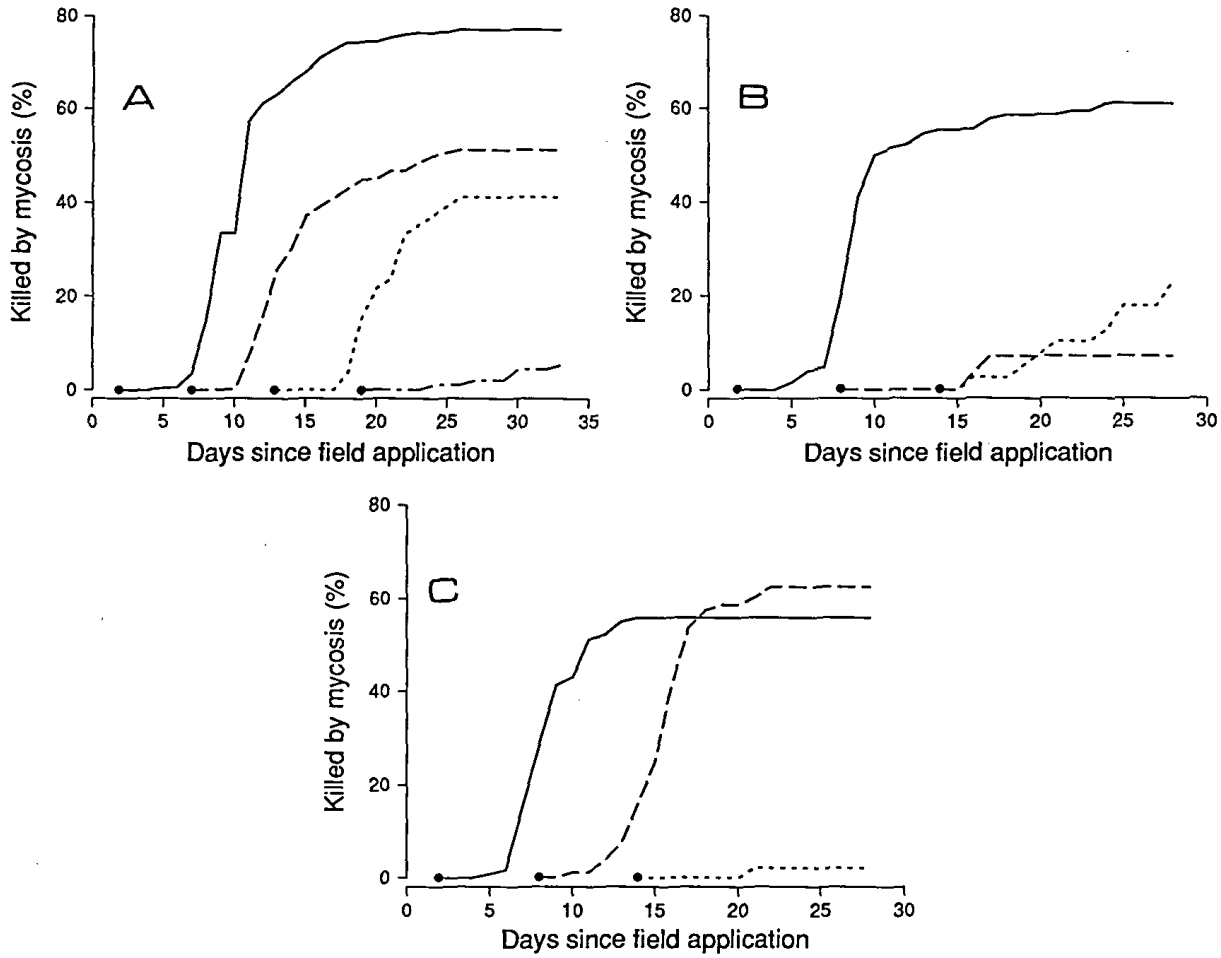

FIGURE 3. The proportions of the caged grasshoppers that died of *B. bassiana* mycosis (cumulative mortality): block 1 (A); block 2 (B); block 3 (C). Collections were made 2 to 19 days after application, and the confined grasshoppers were observed daily for mortality and mycosis. Grasshoppers were considered to have died of mycosis if a sporulating layer of *B. bassiana* developed on the cadaver within 2 weeks in high humidity. Solid circles indicate times of field collections.

quality of inoculum are the usual limiting factors. The inadvertent and effective application of dry conidia deserves further research, especially for use in arid and semi-arid environments.

This is the first field demonstration of effectiveness of this fungus as a microbial control agent of grasshoppers. The significant reductions in population density and the prevalence of infection in field collections confirms the potential of dry *B. bassiana* spores to infect and kill grasshoppers for crop protection. The beauveriosis disease may also have sublethal effects that contribute to crop protection through reduced feeding and reproduction, so our results present a conservative estimate of the overall impact of the treatment. Economic analysis by Mycotech Corp. indicates that an application rate of 10^{13} conidia ha^{-1} can be competitive in price with chemicals currently used for grasshopper control (C. Bradley, personal communication). The next steps towards a practical application of these findings will be determination of the effect of environmental variables on efficacy, elucidation of the mode of penetration and pathogenesis, a search for new isolates in Canada and in Africa, and improvement of pathogen formulation and targeting methodology.

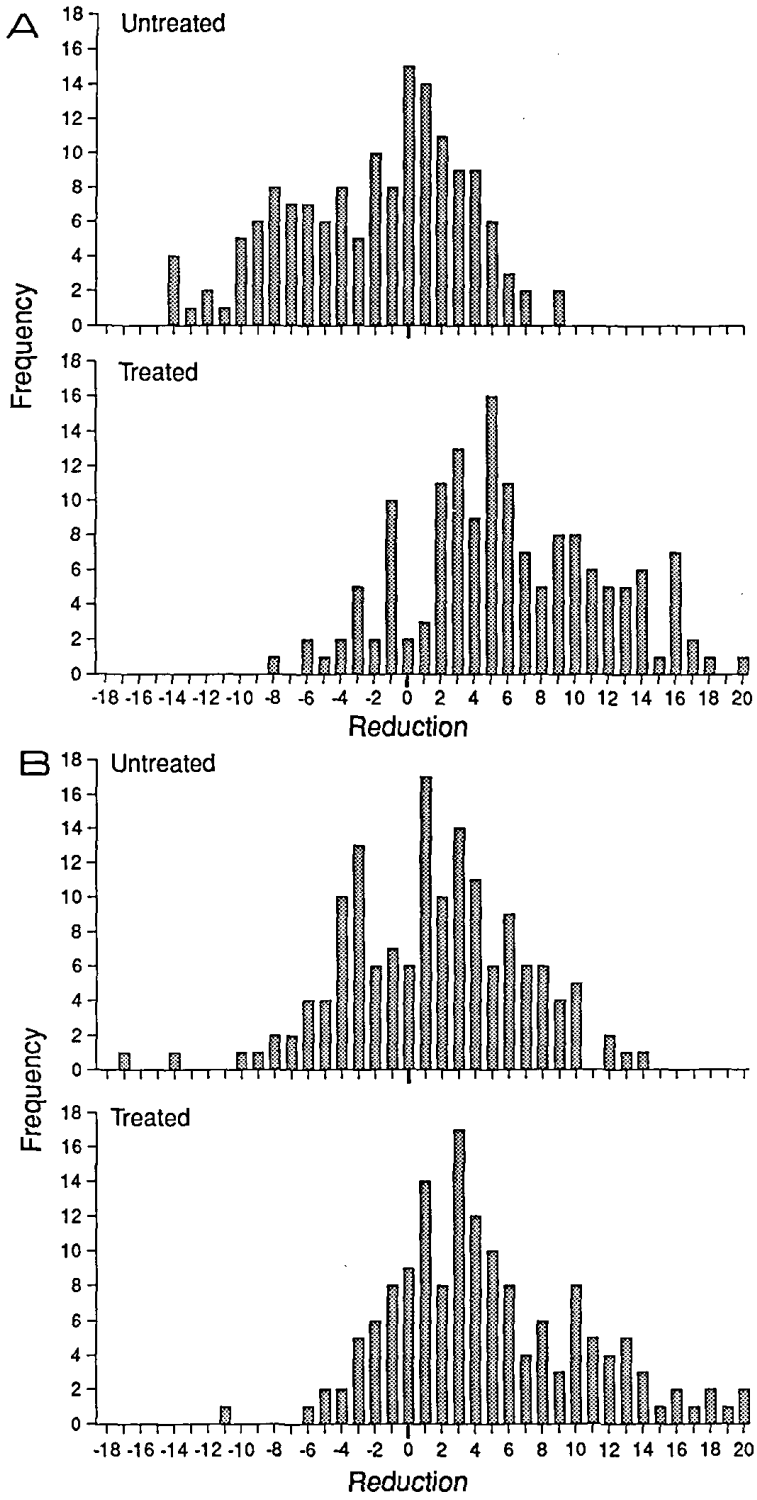

FIGURE 4. The distribution of changes in grasshopper density in the sampling quadrats. The drop in the number of grasshoppers per 0.25 m^2 is shown. Negative numbers indicate a net increase. Nine days after application of the inoculum (A); 15 days after application of the inoculum (B).

TABLE 3. The percentages of the target insects in each stage of development for each species

Species	Instar 1	2	3	4	5	Adult	Species[a]
M. sanguinipes	2.5	6.7	16.9	41.9	20.4	11.7	82.8
M. packardii	3.9	5.8	28.5	35.3	21.7	4.8	13.2
M. bivittatus	0.0	17.7	23.5	27.5	25.5	5.9	3.3
M. infantilis	0.0	14.3	0.0	28.6	57.1	0.0	0.5
C. pellucida	0.0	20.0	0.0	0.0	20.0	60.0	0.3

[a]The overall percentage representation of each species, including all developmental stages, at the time of field application ($n = 1570$).

TABLE 4. Changes in mean grasshopper population density after field treatment with spores of *B. bassiana*

Days	n[a]	Mean density Untreated	Treated	Abbot's adjusted mortality (= Block) Common[b]	1	2	3
0	150	7.56 (0.41)[c]	10.05 (0.51)				
9	150	7.59 (0.43)	4.06 (0.26)	60	64	54	57
15	150	6.15 (0.41)	5.31 (0.35)	33	43	24	38

[a]n is the number of 0.25 m^2 quadrat counts.
[b]Calculation and properties of the common odds ratio as an evaluator of insect control efficacy as described by Schaalje *et al.*, 1986.
[c]SEM.

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