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BIOCONTROL OF GRASSHOPPERS AND LOCUSTS IN MALI

SUMMARY OF

1992 RESEARCH AND TRAINING

Project Title: Integrated Pest Management Strategies for Sustained Control of Grasshoppers and Locusts in Mali

Cooperative Agreement No. 688-0517-A-00-2255-00

USAID Mali
Bamako, Mali

Montana State University

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EXECUTIVE SUMMARY

This report summarizes the results of a 1992 training and research project designed to introduce safe, effective, and environmentally sensitive methods of grasshopper and locust control to Mali. Funding for this project was provided by the U.S. Agency for International Development's mission in Bamako--USAID/Mali--through a cooperative agreement entitled "Integrated Pest Management Strategies for Sustained Control of Grasshoppers and Locusts in Mali"; also by Ciba-Geigy AG. The project involved collaboration between several different organizations, including the Entomology Research Laboratory, Montana State University, Bozeman; Mycotech Corporation, a research and development firm in Butte, Montana; and the Institut d'Economie Rurale (IER) and Service Nationale de la Protection des Végétaux (SNPV) in Bamako. Major objectives of the project included the following: (1) to provide intensive technical training to key Malian scientists and technicians in lab and field procedures for control of grasshoppers and locusts using natural entomopathogens; (2) to undertake lab and field evaluations of the fungal pathogen, *Beauveria bassiana*, against economically significant grasshoppers in Mali; and (3) to conduct research to adapt *B. bassiana* formulations and application methods to grasshopper and locust control in Mali.

Results of the 1992 training and research project were generally regarded as highly promising. Intensive training in lab procedures for use of natural pathogens was provided for IER personnel at IER facilities in Bamako and for two IER scientists and two SNPV technicians at the Entomology Research Laboratory in Bozeman. General training focused on microscopy, grasshopper/locust anatomy, histopathology, pathogen detection and isolation, and pathogen analysis. In addition, trainees received extensive specialized training in topical bioassay techniques and protocols for handling and analyzing *B. bassiana* bioinsecticides. Topical bioassays comparing fungal strains and their effect on different grasshopper species were carried out at IER and the Entomology Research Laboratory. Field trials with *B. bassiana* were conducted in conjunction with SNPV personnel and the assistance of Ciba-Geigy near a base camp in Mourdiah, Mali. Spores were applied using both an ultra-low volume (ULV) oil formulation and an emulsifiable concentrate formulation to replicated 50 m^2 enclosed plots. The delivery rate for the oil formulation--2 liters/ha--was significantly lower than the 5 liters/ha rate used in previous field trials with *B. bassiana* in Africa. Grasshoppers in the treated plots were collected, held in cages, and observed for mortality. More than 88% of the grasshoppers were dead within 7 days. Mortality levels were approximately the same as that seen in previous trials in Cape Verde and North America (80-100%). Population density counts in the enclosed plots showed significant initial density reduction. However, after a week, treated and untreated population densities were nearly equal owing to migration of adult grasshoppers. In order to obtain more conclusive density reduction data, relatively large plots (> 25 ha) will have to be treated. Untreated grasshoppers placed on treated vegetation one hour after spraying exhibited significantly higher mortality than controls. However, heavy rainfall subsequently washed off spores and grasshoppers placed on treated vegetation two days after spraying showed no increase in mortality over controls. The 1992 project results suggest that effective biocontrol methods for grasshopper/locust management can be successfully introduced to Mali.

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1.0 INTRODUCTION

This report provides a synopsis of results from a training and research project in 1992 designed to introduce safe, effective, and environmentally sensitive methods of grasshopper and locust control to Mali. Funding for this project was provided by the U.S. Agency for International Development's mission in Bamako, USAID/Mali, through a cooperative agreement entitled "Integrated Pest Management Strategies for Sustained Control of Grasshoppers and Locusts in Mali"; also by Ciba-Geigy AG, Basel, Switzerland. Several different organizations collaborated on this project, including the Entomology Research Laboratory, Montana State University, Bozeman; Mycotech Corporation, a research and development firm in Butte, Montana; and the Institut d'Economie Rurale (IER) and Service Nationale de la Protection des Végétaux (SNPV) in Bamako.

Environmental and public-health concerns raised by massive spraying of chemical insecticides in Africa during the 1980s locust plague have led to the search for safer, more environmentally compatible insect control methods. Research during the past four years suggests that the fungus *Beauveria bassiana* offers exceptional potential for grasshopper/locust control in Africa. *B. bassiana* is an attractive alternative to chemical insecticides for several reasons. It does not infect humans, animals, or plants and therefore poses minimal health and environmental risks. Research has shown that this strain does not infect leaf cutter bees, an important crop pollinator. Similarly, toxicology studies with rats and baby birds demonstrated no adverse effects. *B. bassiana* can infect hundreds of different insect species, but many strains are adapted to attack only certain insects.[1] This characteristic further minimizes potential impacts on non-target organisms. Insects are infected both by direct contact with spores as well as by contact with spores on vegetation. Infection is rapid, causing reduced feeding and mobility within 48 hours with mass mortality beginning soon afterward. Spores are economical to produce, using low-cost, readily available materials. Spores are stable for several years under proper storage conditions and can be formulated in a variety of liquid and solid carriers for application with conventional equipment.

A strain of *B. bassiana* developed and produced by Mycotech Corporation has been shown to be highly infective to grasshoppers and locusts in the United States, Canada, and Cape Verde.[2,3,4,5,6,7] Field trials conducted in Cape Verde and North America in 1990, 1991, and 1992 demonstrated 80 to 100% mortality within a week in grasshoppers collected from treated plots and grasshoppers held in field cages.[3,5] These results have attracted considerable interest in African countries.

The overriding purpose of the 1992 training and research project was to initiate biocontrol methods for grasshopper and locust management in Mali. Specific objectives included the following: (1) to provide intensive technical training to key Malian scientists and technicians in lab and field procedures for control of grasshoppers and locusts using natural entomopathogens; (2) to undertake lab and field evaluations of the fungal pathogen,

Beauveria bassiana, against economically significant grasshoppers in Mali; and (3) to conduct research to adapt *B. bassiana* formulations and application methods to grasshopper and locust control in Mali. The following sections summarize these activities.

2.0 DESCRIPTION OF RESEARCH PREPARATIONS AND PROCEDURES

2.1 Formulations

Beauveria bassiana conidia for use in the project were produced in Butte, Montana, by Mycotech Corporation using a solid substrate system. The spores were grown in a series of bench-scale batch cultures with dry weight substrate capacities of 1.5 to 3.0 kg. Following processing, spores were recovered as a dry powder. Spore concentrations ranged from 5×10^{10} to 1.5×10^{11} viable spores per gram dry powder. Spores were later checked for viability just prior to application. For the trials in Mali, conidia were formulated in both oil and clay-based emulsifiable concentrate carriers. Each formulation has certain distinct advantages. Spores are stable in the oil for at least a year, and oil suspensions can be applied with unmodified spray equipment. Clay allows the hydrophobic spores to be suspended in water, and it also provides some shading and protection from ultraviolet light. Because of their low cost, clay carriers make possible high volume, low cost application. Previous work in Cape Verde has shown that both formulations provide good spray coverage.

2.2 Field Research Site

The test location was approximately 5 km east of Mourdiah, a town 250 km north of Bamako, the capital of Mali. Mourdiah was selected as the study site because there is an existing infrastructure there to support grasshopper control research, and because the terrain is very suitable for this type of field trial. A base camp in Mourdiah, operated by Ciba-Geigy, provided food, water, living accommodations, storage and laboratory space, and equipment. The landscape was flat, with fairly uniform grass cover and sparse tree growth, providing evenly distributed grasshopper populations, and facilitating plot layout. Daytime temperatures over the course of the trial ranged from 25 to 35 degrees C.

2.3 Species Composition and Age Structure

Species and age composition of the area was determined by collecting grasshoppers in cloth sweep nets. Identification was made by senior SNPV personnel. To avoid altering the density within the enclosures, pretreatment collections were made in areas adjacent to the enclosures. Post-treatment collections were made within the enclosures.

2.4 Field Enclosure Trial

Enclosures

Grasshoppers were treated in 50 m^2 enclosures. The enclosures were intended to limit the movement of grasshoppers in and out of the treated areas. Enclosures were 5 x 10 m, constructed of standard cloth mosquito netting 0.5 m high supported by wooden stakes. Stakes and netting were purchased in Bamako markets. The enclosures were left uncovered, exposing the treated area to natural weather conditions. As a consequence, grasshopper migration was limited, but not completely prevented. This method, using 50 m^2 enclosures, was used previously in Cape Verde by the MSU/Cape Verde Project, with good results.[5]

Both formulations, oil and emulsifiable concentrate, were applied to four replicated 50 m^2 enclosures. With four untreated controls, this yielded 12 enclosures. Prior to treatment, grasshoppers were collected nearby in sweep nets and added to the enclosures to increase the population density to a visually estimated 10-15 per square meter.

The oil formulation was sprayed at a rate equivalent to 2.5×10^{13} spores per hectare in a volume of 2 liters per hectare, from a Microulva sprayer (Micron Sprayers). The oil carrier was used in Cape Verde in 1991[5], but at 5 liters per hectare. The 1992 Mali trials marked the first time that the oil formulation had been sprayed at only 2 liters per hectare. A decreased spray volume is desirable because it would reduce costs for materials and shipping. It would also allow less frequent refilling of the spray apparatus, which is especially important if spraying large areas from an aircraft.

The emulsifiable concentrate formulation was sprayed from a manual pump backpack sprayer (Berthoud) at the equivalent of 2.5×10^{13} spores per hectare in 100 liters. The emulsion formulation had never before been used in Africa. The clay powder aids the suspension of normally hydrophobic fungal spores in water. This allows water to be substituted for oil in the formulation. Water and clay are far less expensive than oil, which makes possible low cost, high volume application. However, the effectiveness of water suspensions in promoting contact and adherence of fungal spores to the insect cuticle needs to be further evaluated. Spray coverage was monitored by the use of oil/water-sensitive cards (Ciba-Geigy).

Monitoring

To monitor infection, 75 grasshoppers (3 cages of 25) from each enclosure were collected approximately one hour post-treatment and held at the Ciba-Geigy camp. With four enclosures per treatment, this yielded a total sample of 300 grasshoppers per treatment, or 900 grasshoppers for the 3 treatments (oil, emulsifiable concentrate, and untreated control). Daily observations were made to count dead and live grasshoppers. Dead grasshoppers were collected and 25-47 of them were held individually in high humidity vials to check for

sporulation of the fungus from the cadaver. The purpose of checking for sporulation was to confirm that mortality was caused by *Beauveria* infection.

Two methods were employed to follow changes in population density within the enclosures: (1) In the first method, five 0.25 m^2 rings were placed in the enclosure prior to treatment. SNPV personnel counted grasshoppers in these rings to determine the number of grasshoppers per square meter. (2) The second method consisted of walking through the enclosure and visually estimating the number of grasshoppers in a square meter. Using this method, five square meters were counted per enclosure at each timepoint.

2.5 Field Cages

Experiments in Cape Verde and the United States have demonstrated that grasshoppers can be infected with *Beauveria* by being exposed to sprayed vegetation.[2] This is important because it shows that *Beauveria* spores remain viable in the field after spraying and can infect grasshoppers not directly hit with the bioinsecticide. This includes grasshoppers that were shielded from the spray and grasshoppers that migrated into the area after spraying. To obtain further data on this issue, each formulation (oil and emulsifiable concentrate) was sprayed on an additional 50 m^2 plot. One hour after spraying, four bottomless 1 m^2 cages were set up on each sprayed plot and a control plot (a total of 12 cages). Twenty-five (25) grasshoppers were placed in each cage. Another set of four cages was placed on each of the three plots two days after spraying. The cages were checked daily for live and dead grasshoppers. Unlike the 50 m^2 enclosures, these cages are enclosed on both the sides and the top. The bottomless cage design allows captive grasshoppers to live in conditions closely approximating their natural environment. Field cages were constructed of wood frames and mosquito netting, purchased and constructed in Bamako.

2.6 Training Bioassays

Laboratory topical bioassays are very commonly used to establish the virulence of a particular strain of fungus to a particular species of grasshopper. Bioassay data often are used to determine the LD_{50} (the dose that kills 50% of the population) and the LT_{50} (the time at which 50% of the population is dead). In a topical bioassay, a carefully controlled dose of fungus is applied directly to the skin of the insect, and the insects are then monitored for mortality.

For the purposes of training Malian personnel and determining the virulence of *B. bassiana* against several important African grasshopper species, topical bioassays were conducted as part of this project.

At the IER laboratory in Bamako, senior IER personnel were instructed in topical bioassay techniques by a Mycotech scientist. Topical bioassays were then performed using an oil formulation of *B. bassiana*, strain GHA (Mycotech) against three economically important African grasshopper species: *Oedaleus senegalensis*, *Hieroglyphus daganensis*,

and *Kraussaria angulifera*. Insects were field collected. Doses of 0, 1000, 10,000, or 100,000 *Beauveria* spores per insect were administered in a volume of 0.2 microliters from a Hamilton syringe. Droplets were applied to the pronotum of the insects. Twenty-five insects were treated per dose (100 grasshoppers of each species). Treated insects were caged in groups of 5-10 per cage. Daily feedings and mortality checks were made for two weeks.

In November 1992, two IER and two SNPV personnel travelled to Bozeman, Montana, to participate in a short course on biological insect control methods at Montana State University. As part of the course, the Malians performed topical bioassays of two oil-formulated *B. bassiana* strains, GHA and BF49, on two North American grasshopper species, *Melanoplus sanguinipes* and *Melanoplus differentialis*. Grasshoppers were from colonies at the Rangeland Insect Laboratory at Montana State University. Insects received doses of 100, 390, 1560, 6250, 25,000, or 100,000 spores in 0.2 microliters from a Hamilton syringe. Untreated controls and controls treated with oil alone were also included. 18-24 insects were treated, then caged in groups of 4-6 for feeding and monitoring. At the conclusion of the test, the Malians were introduced to basic methods of statistical analysis, including calculation of the LD_{50} and LT_{50}, using their data. The Malian scientists were also taught essential techniques, such as spore viability assays, staining procedures, and dose calculations--all necessary for *Beauveria* work.

3.0 RESEARCH RESULTS AND DISCUSSION

3.1 Species Composition and Age Structure

Table 1 lists the species and age composition of grasshoppers in the test plots. *Oedaleus senegalensis* was the dominant species, comprising 88% of the population at the beginning and 93-94% of the population at the end of the trial. *Oedaleus senegalensis* is one of the most economically damaging grasshopper species in West Africa. No other species accounted for more than 3% of the population. The age structure of the population changed significantly over the course of the trial. Only 4% of grasshoppers were adults prior to treatment, while 35-41% were adults eight days after treatment. Because adult grasshoppers can fly, they can easily migrate in and out of the test enclosures. This fact contributed to difficulty in measuring population density changes due to mortality.

3.2 Formulation Characteristics

The oil formulation, at 1.25×10^{13} spores per liter, was viscous, but flowed well through the largest (pink) nozzle of the Microulva sprayer. Oil-sensitive spray cards indicated an even spray distribution throughout the enclosure. The emulsifiable concentrate formulation was not viscous, and it sprayed well from the backpack sprayer. However, the reservoir required frequent agitation to prevent the clay from settling out. When the tank was not shaken, occasional clogging resulted. This formulation was visible on vegetation, so water-sensitive spray cards were not required to monitor coverage.

TABLE 1

Species Composition and Age Structure

DAY OF TREATMENT

Untreated (Sum of 4 samples from area near enclosures)

INSTAR

Species	L1	L2	L3	L4	L5	Adult
OSE	2	31	96	136	180	11
KAM	4	5	1			
AGL		11	1	1		
ABL			1	1		
KAN	1	2	5	1		
PCO		1			1	2
SEP						1
ACL		1				
ASI					1	
HDA		1	1			
DAX		3	2			
ONI			1	3	3	5

Total Counted = 516

TABLE 1 (cont.)

8 DAYS POST-TREATMENT

Untreated (Collected from 2 enclosures)

INSTAR

Species	L1	L2	L3	L4	L5	Adult
OSE		8	8	23	21	46
AGL				1		
ABL			1	1		
SEP						1
HDA		2				
DAX		1	1			

Total counted = 114

Oil Treatment (Collected from 2 enclosures)

INSTAR

Species	L1	L2	L3	L4	L5	Adult
OSE		8	8	23	30	46
AGL				1		
ABL		2		1		
KAN		1				
HDA			1			
DAX		1				

Total counted = 122

TABLE 1 (cont.)

<u>Emulsifiable Concentrate Treatment</u> (Collected from 2 enclosures)

INSTAR

Species	L1	L2	L3	L4	L5	Adult
OSE		8	12	28	20	37
DAX		3				
SEP				1		1
PCO						1
ABL					2	

Total counted = 113

<u>Key to Abbreviations</u>

ABL = *Acrotylus blondeli*
ACL = *Acorypha clara*
AGL = *Acorypha glaucopsis*
ASI = *Aiolopus simulatrix*
DAX = *Diabolocatantops axillaris*
HDA = *Hieroglyphus daganensis*
KAM = *Kraussela amabile*
KAN = *Kraussaria angulifera*
ONI = *Oedaleus nigeriensis*
OSE = *Oedaleus senegalensis*
PCO = *Pyrgomorpha cognata*
SEP = *Stenohippus epacromioides*

3.3 Mortality Following Treatment of Field Enclosures

Mortality of treated grasshoppers held in cages at the camp is shown in Figures 1 and 2. Grasshopper mortality was assessed in two different ways: (1) by counting dead grasshoppers; and (2) by counting the remaining live grasshoppers. In the first case, the cumulative dead were compared to the initial cage population of 75 grasshoppers to obtain a mortality level. For example, a cumulative total of 30 dead grasshoppers would represent 40% mortality (30/75 = 40%). In the second case, the remaining live grasshoppers were subtracted from the initial cage population. For example, 45 live grasshoppers would also represent 40 % mortality (75 - 45 = 30; 30/75 = 40%). The first method helped avoid skewing of results from grasshopper escape through small holes that developed in the cage walls. The second method helped counter the effect of the "missing dead": On many occasions, small fragments of dead grasshoppers were found in the cages. This suggested that grasshopper cadavers were being eaten by scavenger insects and/or other grasshoppers.

The first method (counting dead grasshoppers) would have under-enumerated mortality. Even so, both oil and clay formulations yielded around 80% mortality by day 6 (Fig. 1). The second method (counting the remaining live grasshoppers), which showed mortality levels nearing 100% by day six (Fig. 2), probably over-enumerated mortality. The actual mortality level was probably around 90%. Results closely match those obtained in previous trials in 1990 and 1991 in Cape Verde and North America (80-100% mortality). The Abbott's adjusted percent mortality, based on the remaining live grasshoppers, is presented in Table 2. It shows that, by the sixth day, 97% mortality was obtained with the oil formulation and 94 percent with the emulsifiable concentrate formulation. Mortality for both formulations was statistically significant compared with untreated controls ($p < 0.0001$) at days 5 and 6 post-treatment. Interestingly, there was no significant difference in mortality between the oil and emulsifiable concentrate formulations. The apparent high control mortality in the "initial minus live" assessment method (Fig. 2) probably represents skewing caused by grasshopper escape from small holes in the cage walls.

TABLE 2

Abbott's Adjusted % Mortality, Based on Live Insects Remaining

Days Post-treatment	Oil	Emulsifiable Conc.
0	0 %	0 %
4	54 %	48 %
5	85 %	86 %
6	97 %	94 %

Figure 1. Cumulative mortality among grasshoppers in cages after treatment of enclosures with *Beauveria bassiana*. Mali. 1992. (Based on counts of dead grasshoppers.)

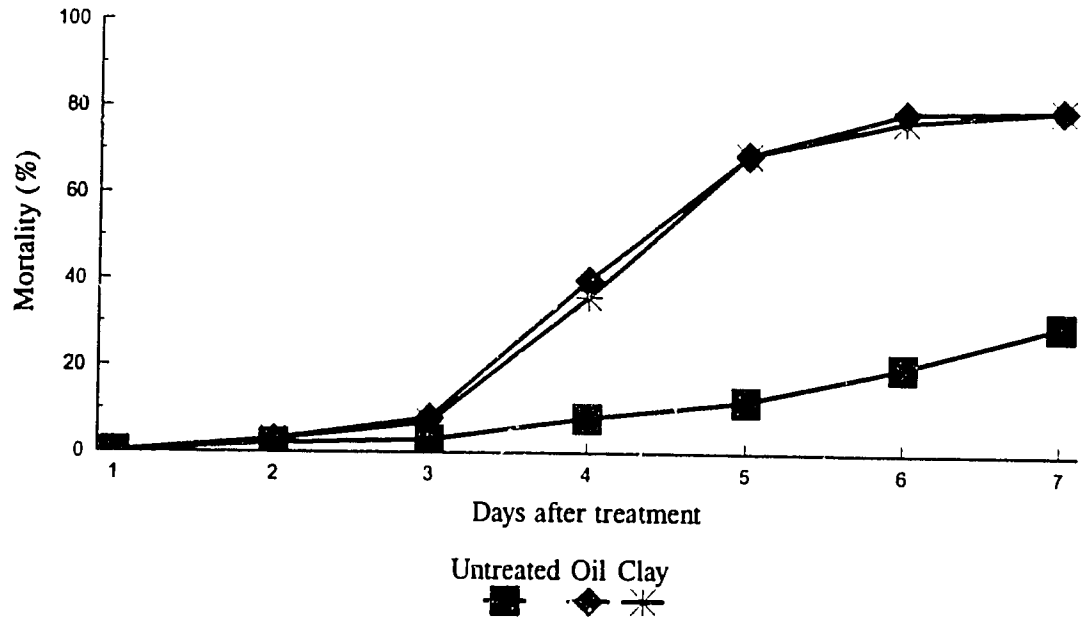
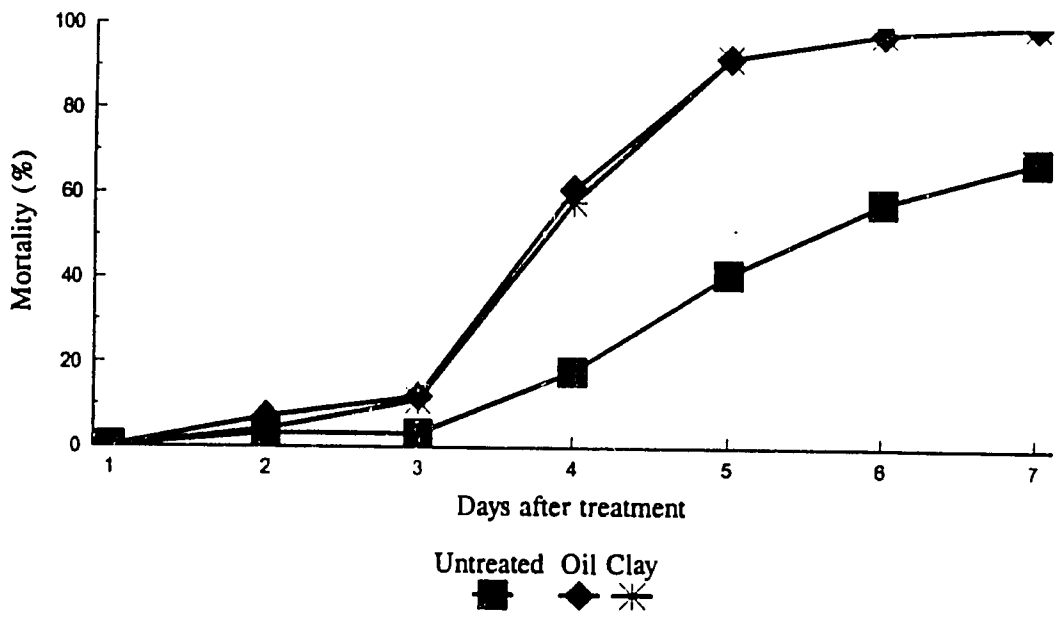


Figure 2. Cumulative mortality among grasshoppers in cages after treatment of enclosures with *Beauveria bassiana*. Mali. 1992. (Based on counts of live grashoppers.)

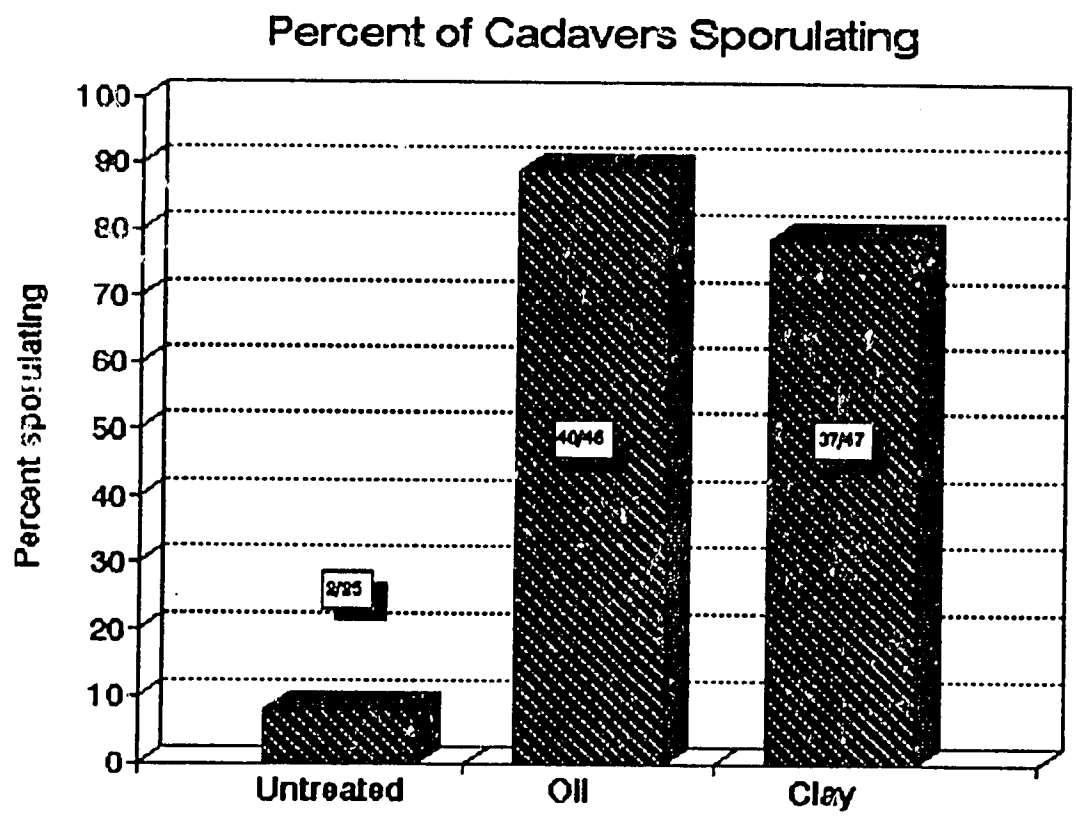


Of dead grasshoppers recovered from the oil treatment cages, 40 of 45 (89%) were observed to sporulate after 5 days. From the emulsifiable concentrate treatment cages, 37 of 47 (79%) sporulated, and from the control, 2 of 25 (8%) sporulated (Fig. 3). Sporulation figures for the treated grasshoppers match the mortality figures fairly closely. As expected, control sporulation is much lower than control mortality. This suggests that most of the treated grasshopper deaths are due to induced *Beauveria* infection, while almost all control deaths are the result of other causes. Sporulating cadavers from the control group were probably infected by fungal entomopathogens existing naturally.

3.4 Population Density Changes

Results of ring counts and visual square meter counts were inconclusive. For 1 to 4 days after treatment, treated populations were significantly reduced relative to controls (Fig. 4 and 5). However, at the end of the trial (7 days), treated and untreated population densities were nearly identical. This is probably explained by the fact that age structure data (Table 1) reveal a jump of 31-36% in the number of adults in the enclosures. Because adult grasshoppers can fly, they were able to move in and out of the enclosures easily. In fact, this movement was observed frequently, particularly near the end of the trial. Migration in

Figure 3



and out of the plots would clearly make accurate density reduction measurements impossible, and this is a likely explanation for the indeterminate results. In order to obtain more conclusive density reduction data, relatively large plots (> 25 ha) will have to be treated, with density measured near the middle of the plot to minimize the effect of migration.

3.5 Field Cages

The purpose of the field cage trials was to assess infectivity of sprayed vegetation at different periods after spraying. Mortality was measured using the "initial cage population minus live grasshoppers" method because dead grasshoppers quickly disappear in the field owing to scavenging and cannibalism. Grasshoppers that were exposed to treated vegetation one hour after spraying exhibited relatively high mortality by 7 days post-treatment (Fig. 6). Oil-treated vegetation yielded 64% dead and emulsifiable concentrate-treated cages yielded 72% dead. Mortality levels would probably have been significantly higher; however, heavy rainfall on the second and third days after spraying undoubtedly washed spores off the vegetation. The apparent high control mortality is probably an artifact of the method used, the result of skewing from grasshopper escape.

Grasshoppers exposed to treated vegetation 2 days after spraying died at the about the same rate as controls (Fig. 7). This was almost certainly the result of the heavy rainfall, which had already washed most of the spores off the vegetation. Remaining levels of *Beauveria* were apparently not sufficient for infection.

3.6 Training Bioassays

Malian scientists and technicians were given extensive specialized training in topical bioassay techniques and protocols for handling and analyzing *Beauveria*. Results of training bioassays at IER facilities in Mali and at the Entomology Research Laboratory at Montana State University are presented below.

Mali

Results of training bioassays with African grasshoppers conducted by personnel at IER are shown in Figures 8, 9, and 10. Approximate LD_{50} and LT_{50} for *Kraussaria* and *Hieroglyphus* are as follows:

	LD_{50} @ 10 days	LT_{50} @ 10^4 spores/insect
Kraussaria	12,000 spores	12 days
Hieroglyphus	9,000 spores	5 days

Figure 4. Densities of grasshoppers in enclosures treated with *Beauveria bassiana*. Mali. 1992.
(Determined by the ring count method.)

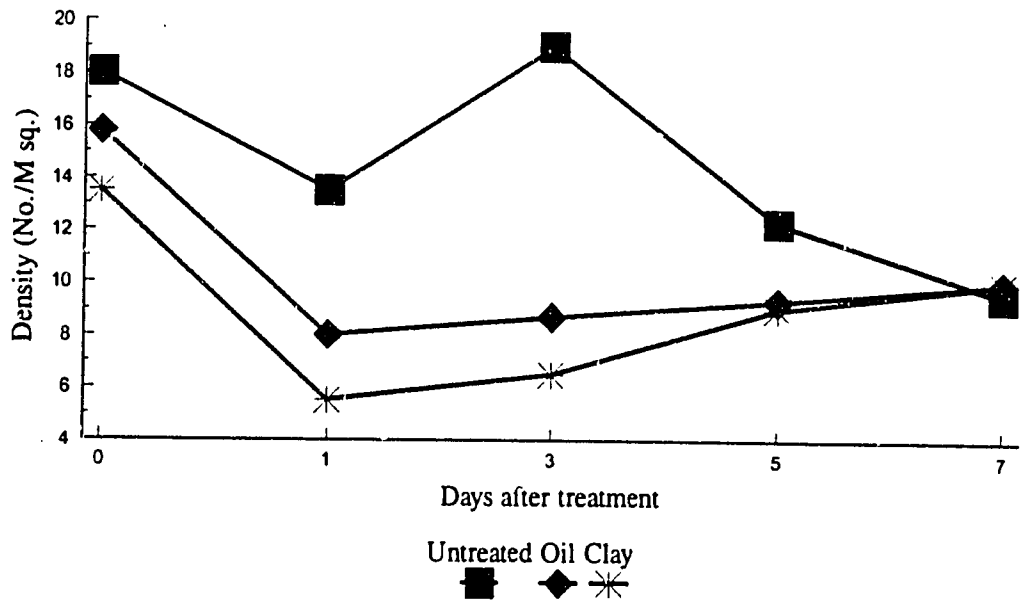


Figure 5. Densities of grasshoppers in enclosures treated with *Beauveria bassiana*. Mali. 1992.
(Determined by the visual square meter method.)

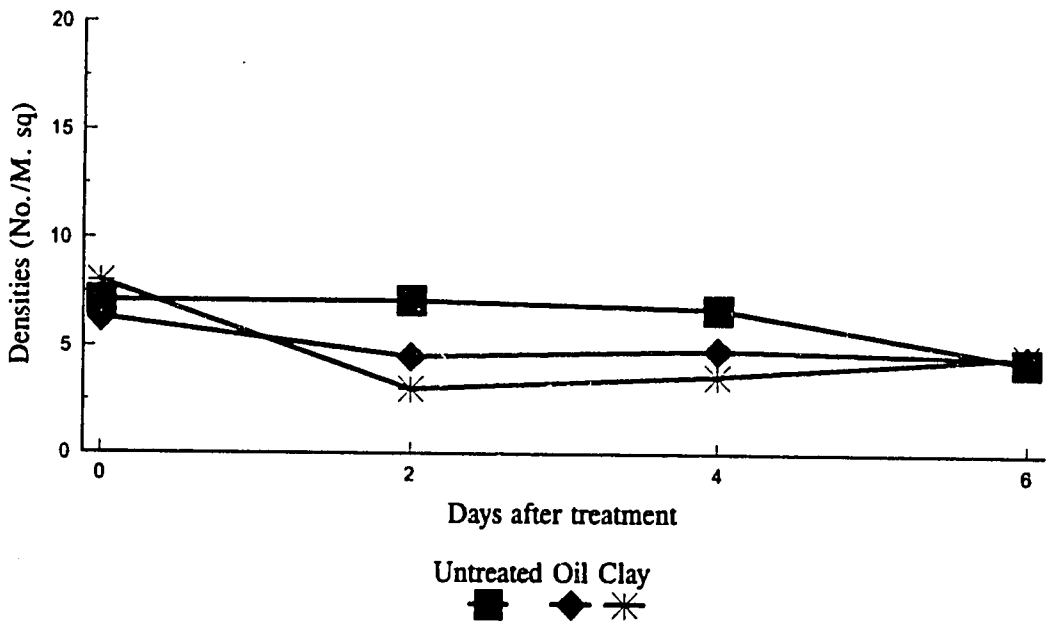


Figure 6. Mortality among grasshoppers in field cages following collection 1 hour after treatment of enclosures with *Beauveria bassiana*. Mali. 1992.

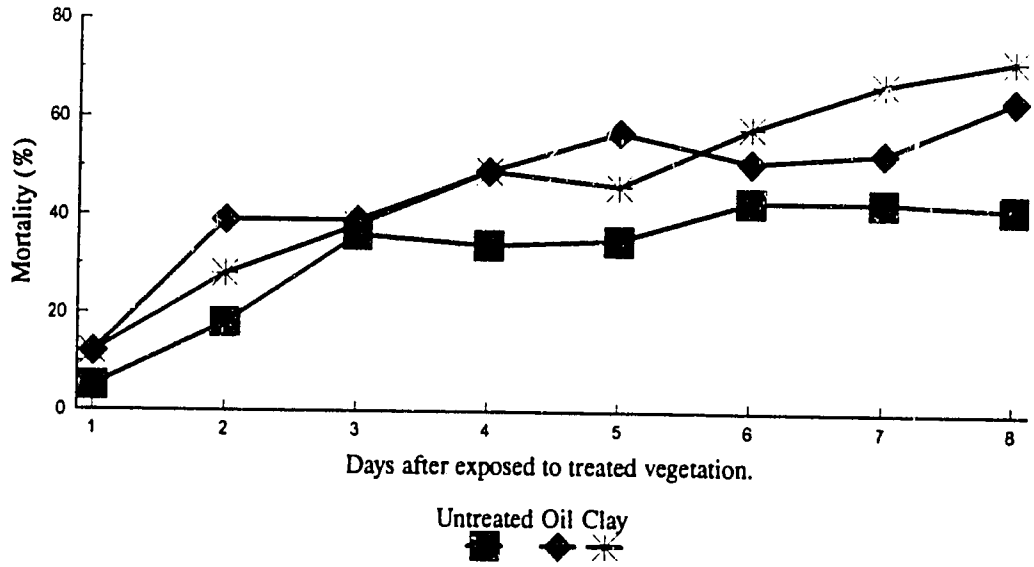
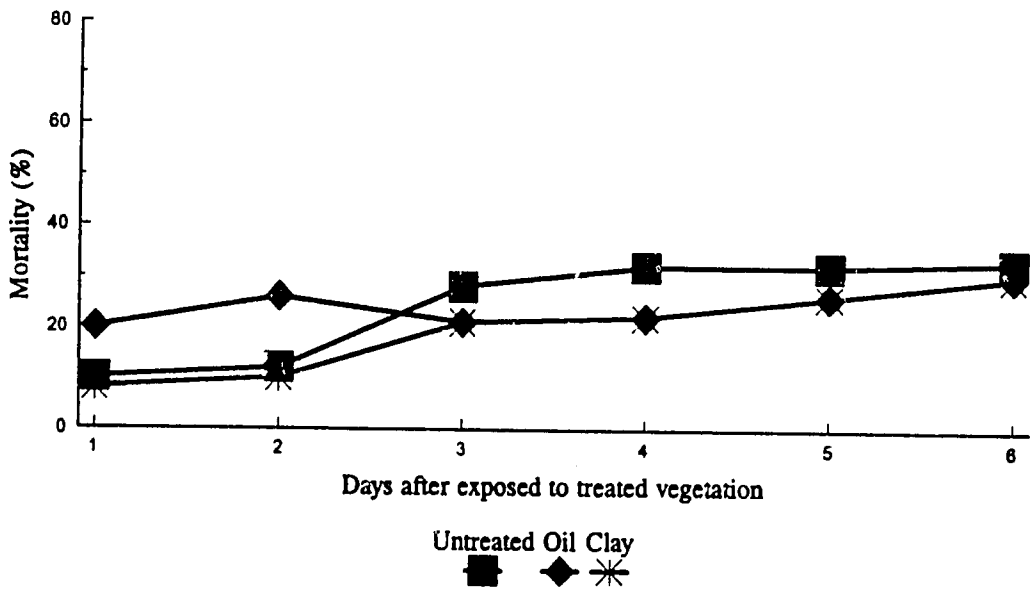


Figure 7. Mortality among grasshoppers in field cages following collection 48 hours after treatment of enclosures with *Beauveria bassiana*. Mali. 1992.



Kraussaria showed a higher LD_{50} than *Hieroglyphus*. That is, it took more spores to kill 50% of the *Kraussaria* than to kill 50% of the *Hieroglyphus*. This suggests that *Kraussaria* is more resistant to this *Beauveria* strain than *Hieroglyphus*. The higher LT_{50} of *Kraussaria* confirms its superior resistance.

The *Oedaleus* experiment showed exceptionally high control mortality (Fig. 10), higher even than the two lowest doses, making a valid LD_{50} and LT_{50} impossible to determine. This may indicate problems with storage or handling of the untreated grasshoppers, or their unintentional contamination with *Beauveria*. These problems are to be expected with personnel inexperienced in bioassay techniques, and should disappear with practice.

Bozeman

Timecourse mortality data from training bioassays conducted at Montana State University are shown in Figures 11-14. LD_{50} and LT_{50} data for different strains and different grasshoppers species are as follows:

	LD_{50} (10 day)	LT_{50} (10^5/insect)
M. sang. GHA	13000 spores	6.5 days
M. sang. BF49	7600 spores	6.5 days
M. diff. GHA	97000 spores	6.8 days
M. diff. BF49	98000 spores	8.8 days

For *M. sanguinipes*, the GHA LD_{50} was about twice the BF49 LD_{50}, indicating that BF49 is more virulent toward *M. sanguinipes* than GHA. GHA and BF49 had nearly identical LD_{50}s versus *M. differentialis*, and those LD_{50}s were nearly 10 times greater than the LD_{50}s versus *M. sanguinipes*. Thus, *M. differentialis* shows greater resistance to both GHA and BF49 than does *M. sanguinipes*. The LT_{50} of both fungal strains is higher versus *M. differentialis*, which also demonstrates the greater resistance of that grasshopper species.

3.7 Other Training

In addition to specialized training in use of *Beauveria*, 2 IER scientists and 2 SNPV technicians were given three weeks of intensive general training in lab procedures for use of natural entomopathogens at the Entomology Research Laboratory at Montana State University. Topics covered included microscopy, grasshopper/locust anatomy, histopathology, pathogen detection and isolation, and pathogen analysis. This training established a solid foundation for future applied use of natural entomopathogens for grasshopper and locust control in Mali.

Figure 8. Cumulative mortality among *Kraussaria angulifera* inoculated with *Beauveria bassiana* (strain GHA) in a laboratory bioassay. Mali. 1992.

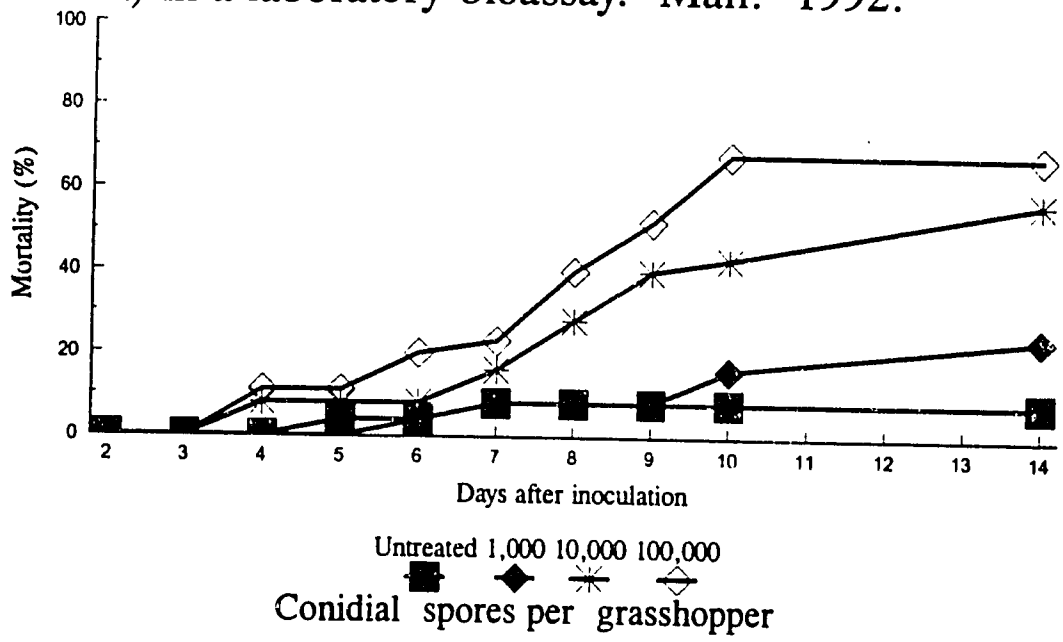


Figure 9. Cumulative mortality among *Hieroglyphus daganensis* following inoculation with *Beauveria bassiana* (GHA) in a laboratory bioassay. Mali. 1992.

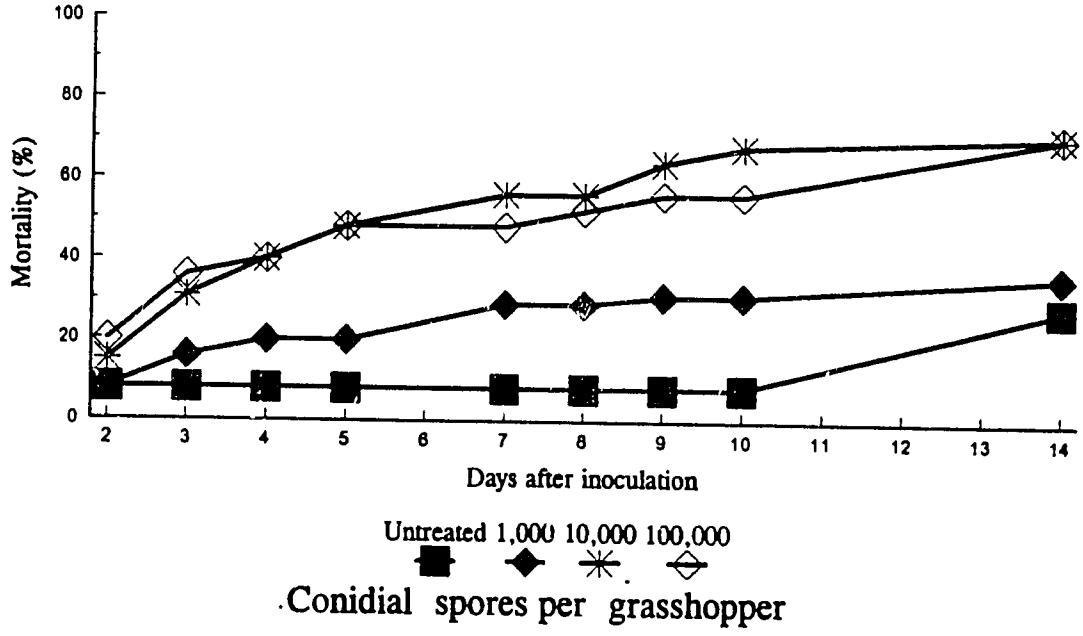


Figure 10. Cumulative mortality among *Oedaleus senegalensis* inoculated with *Beauveria bassiana* in a laboratory bioassay. Mali. 1992.

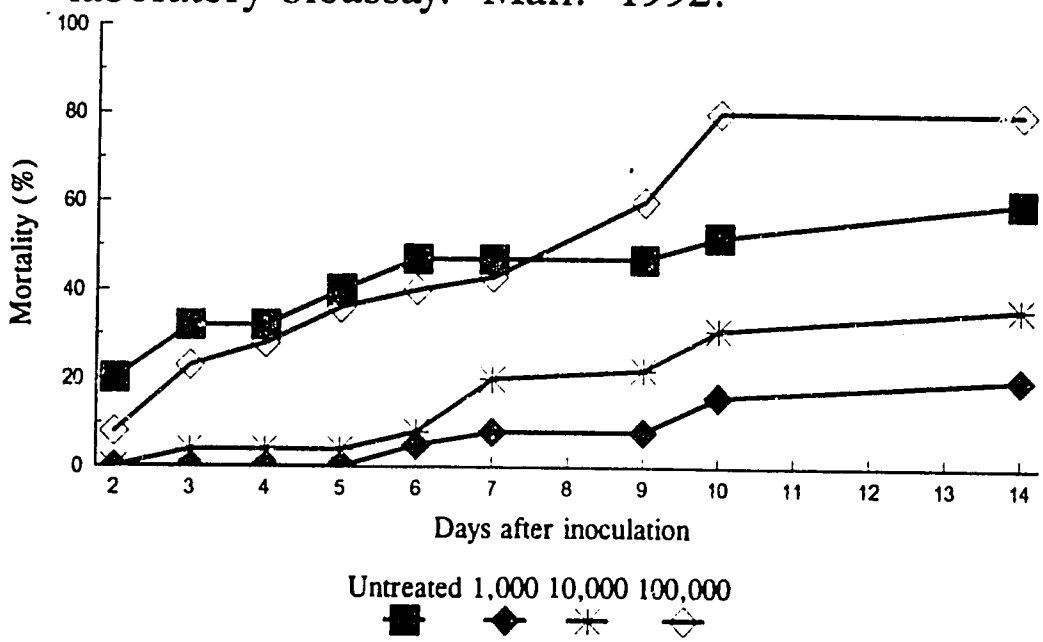


Figure 11. Cumulative mortality among nymphs of *Melanoplus sanguinipes* inoculated with *Beauveria bassiana* (strain GHA) in a laboratory training exercise for Malian personnel. MSU. 1992.

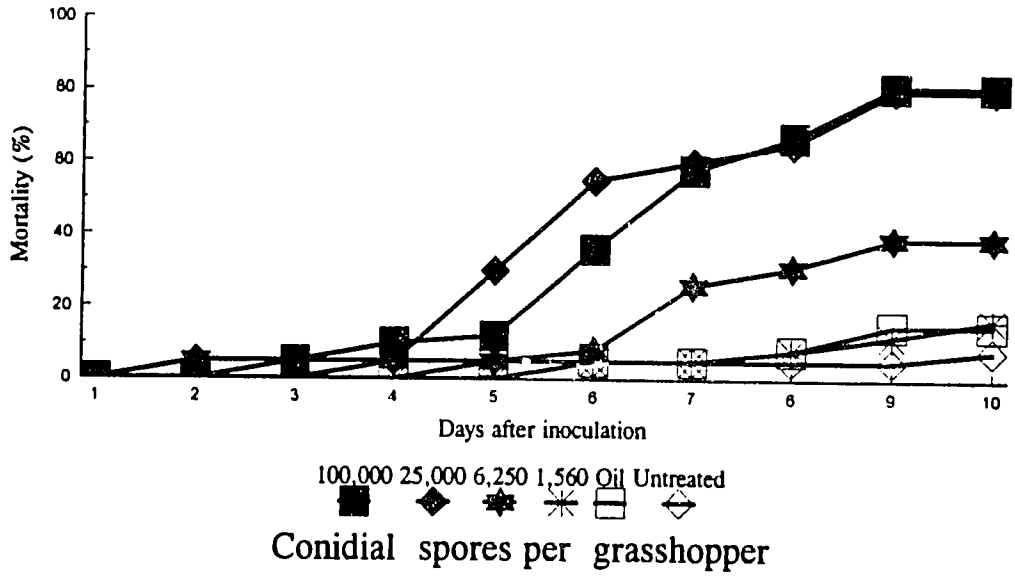


Figure 12. Cumulative mortality among nymphs of *Melanoplus sanguinipes* inoculated with *Beauveria bassiana* (strain BF49) in a laboratory training exercise for Malian personnel. MSU. 1992.

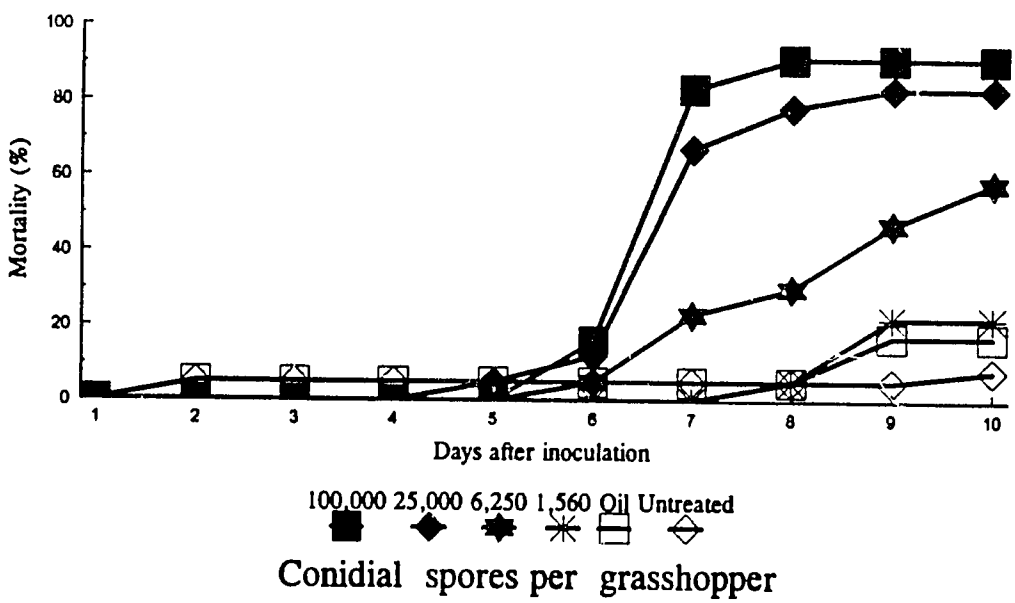


Figure 13. Cumulative mortality among nymphs of *Melanoplus differentialis* inoculated with *Beauveria bassiana* (strain GHA) in a laboratory training exercise for Malian personnel. MSU. 1992.

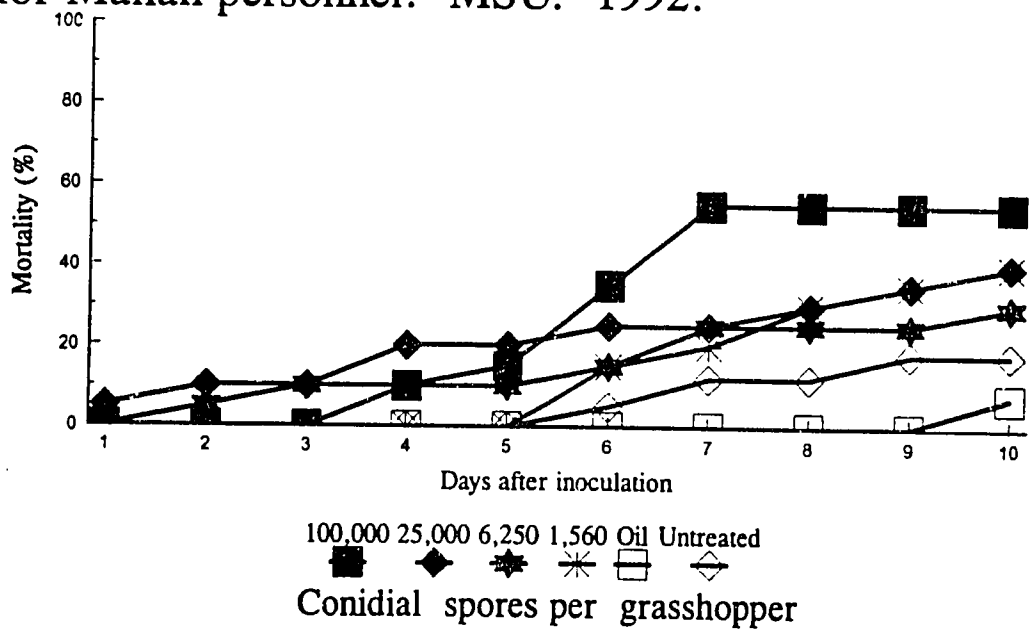
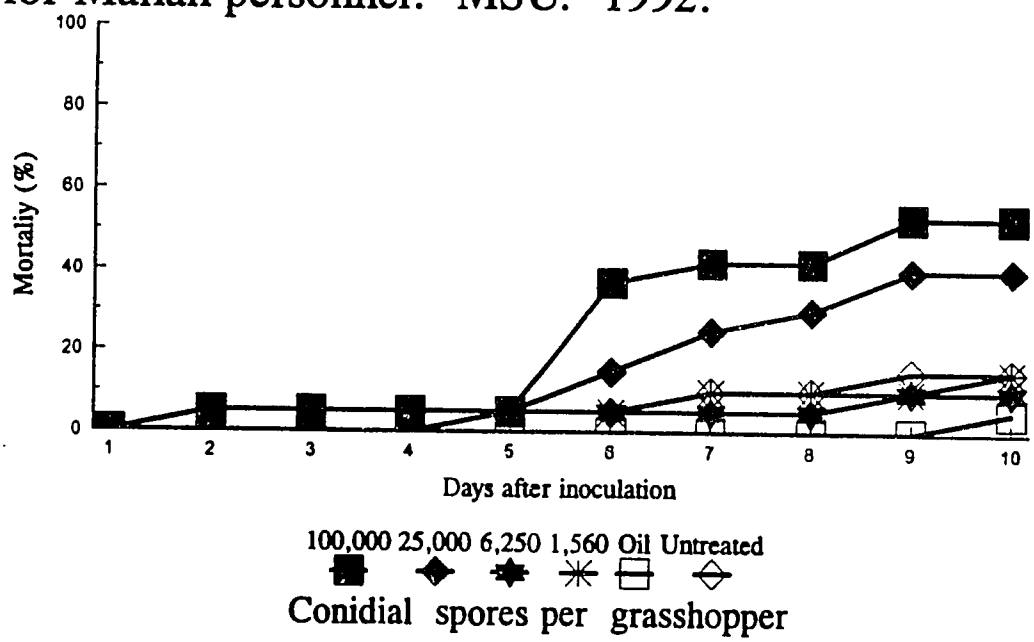


Figure 14. Cumulative mortality among nymphs of *Melanoplus differentialis* inoculated with *Beauveria bassiana* (stain BF49) in a laboratory training exercise for Malian personnel. MSU. 1992.



4.0 CONCLUSION

Results of the 1992 training and research project in Mali can generally be regarded as highly promising. Field trials with *Beauveria bassiana*, conducted in conjunction with SNPV personnel at Mourdiah, Mali, achieved high levels of grasshopper mortality within a week. Depending on the assessment method used, mortality varied from around 80% to nearly 100% by day 6, with actual mortality probably around 90%. Two problems prevented more precise assessment: (1) disappearance of dead grasshoppers from scavenging and cannibalism; and (2) disappearance of live grasshoppers through small holes that developed in the enclosure walls. These holes were a result of the relatively flimsy nature of the mosquito netting used to construct the enclosures. In previous field trials in Cape Verde, more substantial mesh from the United States has been used, and grasshopper escape has not been a problem. For future trials in Mali, more substantial materials are recommended.

The field trials in Mali demonstrated that a much lower delivery rate can be used for the oil-based *B. bassiana* bioinsecticide. The delivery rate used in previous trials in Africa was 5 liters/ha. This time, the rate was reduced to only 2 liters/ha, without any discernible effect on infectivity. Although the oil formulation was considerably more viscous than in previous trials, it flowed well through the largest nozzle of the Microulva sprayer, making it easy to apply. A more concentrated formulation is advantageous in that it allows larger areas to be sprayed without refilling the sprayer, and it substantially reduces shipping and materials costs.

A new clay-based emulsifiable concentrate formulation was tested for the first time in Africa. This formulation proved to be as effective as the oil formulation: there was virtually no difference in either grasshopper mortality or sporulation. The major advantage of the emulsifiable concentrate is its low cost relative to the oil formulation. Water and clay are much cheaper than oil. The emulsifiable concentrate was easy to apply with the backpack sprayers used. However, it required frequent agitation to prevent settling of the clay and subsequent clogging of the spray nozzle. Work is in progress to modify this formulation so that settling is minimized.

Mortality among grasshoppers exposed to treated vegetation was lower than in previous field trials in Africa. However, heavy rainfall on the second and third days after spraying washed spores off the vegetation, making conclusive analysis impossible. To achieve valid results, this part of the trial should be repeated. Likewise, because of adult migration in and out of the 50 m^2 enclosures, new attempts to conclusively measure density reduction are necessary. If enclosures are to be used, grasshopper populations to be treated should be second to fourth instar, so that migration will be lessened. However, in order to obtain truly accurate population density data, relatively large (> 25 hectare) plots should be treated, with density measured near the center of these plots to minimize the effects of migration. Such trials are proposed for the 1993 and 1994 seasons. The success of the 1992 trials under Malian environmental conditions argues for expanded field trials to gain the information and experience that is unobtainable with small-scale trials.

The bioassays conducted during this project were most valuable for the training they provided to the Malian participants. Bioassay and other techniques taught will enable these scientists and technicians to perform the quality control tasks necessary for the use of natural entomopathogens for safe, effective, and environmentally sensitive crop protection in Mali. The variable results of the bioassays reflect the inexperience that has begun to be remedied by this project. Through this training and research project, the Malian participants self-sufficiency and general level of understanding ot biocontrol techniques has been significantly increased. This project has established a solid foundation for future operational use of natural pathogens for pest control in Mali.

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