BIOCONTROL OF GRASSHOPPERS WITH *BEAUVERIA BASSIANA* IN CAPE VERDE

SUMMARY OF 1992 RESEARCH

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

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EXECUTIVE SUMMARY

This report summarizes the 1992 results of an ongoing research project designed to develop the fungus *Beauveria bassiana* for safe, effective, and environmentally sensitive grasshopper and locust control in Africa. Funding for this project was provided by the U.S. Agency for International Development, Washington, D.C., through a grant entitled "Integrated Pest Management Strategies for Sustained Control of Grasshoppers and Locusts in Africa. 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 Instituto Nacional de Investigaçao e Desenvolvimento Agraria (INIDA) in Cape Verde. Major objectives of the project in 1992 included the following: (1) to continue lab and field evaluations of *B. bassiana* against economically significant African grasshoppers and locusts; and (2) to improve formulations and application methods of *B. bassiana* for grasshopper and locust control in Africa. Research achieved a major advance in biocontrol of grasshoppers and locusts. Results of this research are summarized in the following two paragraphs.

In controlled small-scale field trials in Cape Verde in late summer 1992, *Beauveria* spores were applied to replicated 2-hectare plots on the island of São Tiago. Two different formulations were tested: an ultra-low volume (ULV) oil formulation and a high volume emulsifiable suspension (ES) formulation. Grasshoppers (primarily *Oedaleus senegalensis*) from the treated plots were collected, reared in laboratory cages, and observed for mortality. Both formulations achieved greater than 98% mortality within 7 days after treatment. In addition, daily counts in the field plots measured grasshopper population-density reductions. Plots treated with ES and oil-formulated spores exhibited 46% and 43% population-density reductions, respectively. This represents the first time that significant grasshopper population-density reduction has been demonstrated with a bioinsecticide in Africa. The difference between mortality levels in the caged grasshoppers and those remaining in the treated plots is probably largely due to migration of adults. In order to obtain more accurate density-reduction data, relatively large (> 25 hectare) plots should be treated, with density measured near the center of the plots to minimize the effect of migration. Such larger trials are planned for the 1993 and 1994 field seasons.

In laboratory evaluations, topical bioassays with oil and ES-formulated *B. bassiana* spores were conducted against African migratory locusts (*Locusta migratoria*) at INIDA in Cape Verde. Both formulations exhibited near 100% mortality in 5-6 days at the highest dosages. However, spores in oil exhibited significantly greater virulence (LD_{50} = 2,900 spores/insect) compared to the ES-formulated spores (LD_{50} = 11,000 spores /insect). The reasons for this difference are poorly understood and are the subject of further investigation. However, field evaluations demonstrated that, at the economically feasible dose rates used in these trials, there is little practical difference between the effectiveness of the two formulations in either grasshopper mortality or population-density reduction levels.

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1.0 INTRODUCTION

This report provides a synopsis of results from applied research in 1992 to develop the fungus *Beauveria bassiana* for safe, effective, and environmentally sensitive grasshopper and locust control in Africa. Funding for this project was provided by the U.S. Agency for International Development, Washington, D.C., through a grant entitled "Integrated Pest Management Strategies for Sustained Control of Grasshoppers and Locusts in Africa. This ongoing project involves 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 Instituto Nacional de Investigaçao e Desenvolvimento Agraria (INIDA) in Cape Verde.

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 suggest that *B. bassiana* can be an effective biocontrol agent in Africa. However, previous small-scale (1 hectare) field trials in 1990 revealed difficulties in formulation and coverage.[5] Furthermore, grasshopper population-density reduction with *B. bassiana* had not previously been demonstrated in Africa. Therefore, a major goal of the 1992 field trials was to demonstrate both high levels of mortality in the field as well as significant reductions in field population density. Field trials were also intended to compare the efficacy of two different spore delivery formulations, oil and an emulsifiable suspension (ES).

Many different grasshopper and locust species are responsible for crop loss in Africa. The virulence of the Mycotech *Beauveria bassiana* strain against *Oedaleus senegalensis* is well established.[5,8] In addition, 1992 field trials in Mali provided strong evidence that other African species are susceptible.[8] It is important to evaluate *Beauveria*'s general virulence against other economically significant African acridians. To the degree that this virulence can be demonstrated, *Beauveria*'s potential usefulness as a broad-spectrum grasshopper/locust biocontrol agent in Africa will be confirmed. The first step in determining this virulence is to perform bioassays with various different species. Topical bioassays were carried out in Cape Verde in fall 1992 with *B. bassiana* against *Locusta migratoria*, an insect capable of causing major economic damage in the Sahel. The following sections summarize the results of the 1992 lab and field evaluations in Cape Verde.

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 lab and field evaluations in Cape Verde, 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. Although not used in this trial, clays of African origin could potentially be used in the future.

2.2 Field Research Site

INIDA personnel chose a field site 3 km east of the town of Tarrafal, which is located on the north end of São Tiago island. This area has been sparsely planted with acacia trees as part of a reforestation project. The ground was very rocky, with limited ground vegetation. Trees, rocks, and uneven terrain made this site less than ideal for conducting field trials. However, it was the only site available with grasshoppers at the time. Because of the late date that funding for the project was approved, the grasshopper population was composed almost entirely of fifth instar larvae and adult *O. senegalensis*. Ideally, field trials would be conducted against younger populations to reduce migration.

2.3 Field Trial

Treatment

A total of nine treatment plots, 100 x 200 meters (2 hectares) each, were laid out with a compass and flagging tape. Three plots were treated with oil-formulated spores, three were treated with emulsifiable suspension-formulated spores, and three were left untreated. The ULV oil formulation was sprayed at a rate of 2.5×10^{13} spores per hectare in a volume of five liters per hectare with hand-held Microulva sprayers (Micron Sprayers, Ltd.). This rate and volume were used successfully in the 1991 small-scale trials in Cape Verde.[5]

Monitoring

Approximately one hour after treatment, 250 grasshoppers were collected from each plot with sweep nets. These were transported back to the INIDA complex, separated into five cages of 50 grasshoppers each, and maintained in a laboratory. With three plots per treatment, this yielded a total sample of 750 grasshoppers per treatment, or 2,250 grasshoppers for the three treatments (oil, emulsifiable suspension, and untreated). Every day, INIDA personnel placed fresh food (grass) in the cages and checked cages for mortality.

Daily grasshopper population-density counts were conducted in the treated plots by a team of Cape Verde Plant Protection Service technicians. This team used the PRIFAS "visual square meter" method. Each technician walked across a plot, pausing periodically to visually estimate the number of grasshoppers in a square meter. One hundred square meters were counted per plot per day. From this, the number of grasshoppers per hectare was calculated.

2.4 Laboratory Bioassays

Laboratory topical bioassays are commonly employed to establish the virulence of a particular strain of fungus to a particular species of grasshopper. Bioassay data can be 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.

INIDA personnel in Cape Verde performed topical bioassays to compare the respective effectiveness of oil-formulated *B. bassiana* spores and emulsifiable suspension-formulated spores. African migratory locusts (*Locusta migratoria*) were selected as the target species--both because they are responsible for often severe economic damage in Africa and because a thriving source colony of *L. migratoria* has been established at INIDA. Doses of 1,000, 5,000, 10,000, 50,000, or 100,000 spores per insect were applied in a volume of 0.2 microliters (oil formulation) or 1 microliter (ES formulation). A droplet was applied to the pronotum of each treated insect. Twenty-five insects were treated per dose. Untreated controls and controls treated with oil or ES alone (i.e. no spores) were also included.

3.0 RESEARCH RESULTS AND DISCUSSION

3.1 Field Trials

The most remarkable result in this trial was the reduction in grasshopper population density (Fig. 1). For the first time, significant population-density reduction was demonstrated with a fungal bioinsecticide against acridians in relatively large field plots in Africa. After one week, populations treated with oil-formulated spores were reduced by 43%. ES-treated populations were reduced by 46%. The seven day density reduction is statistically significant for both the oil and ES formulations ($p = 0.03$ and $p = 0.0204$, respectively).

Cumulative mortality of grasshoppers collected one hour post-treatment is shown in Fig. 2. After seven days, more than 98% of the grasshoppers treated with oil or ES treatments were dead, compared with just 12% of the untreated control populations. Most mortality occurred 4-5 days after treatment. Similar high levels and rapid mortality have been seen in previous trials in Cape Verde and Mali.[5,8] As was seen in the 1992 Mali field trials, there was virtually no difference in efficacy or timing between oil- and ES-formulations.

3.2 Laboratory Bioassays

Results of topical bioassays wth *B. bassiana* againts *L. migratoria* are shown in Fig. 3 and 4. The LD_{50} levels for the oil and ES formulations (corrected for oil alone and ES alone mortality), are as follows:

	LD_{50} (5 days)	95% confidence limits
Oil	2,900 spores/insect	1,600-5,000 spores/insect
ES	11,000 spores/insect	5,700-22,000 spores/insect

As can be seen, the oil formulation exhibited an LD_{50} almost 4 times lower than the emulsifiable suspension. That is, spores formulated in oil were roughly four times as lethal as spores formulated in ES. A number of explanations are possible for the oil formulation's enhanced virulence: (1) Oil may promote better contact or adhesion of spores to the cuticle. (2) Oil may more effectively invade the intersegmental joints where spores are thought to penetrate most easily. (3) The oil milieu may be more conducive to germination.

Figure 1. Densities of *Oedaleus senegalensis* in plots treated with *Beauveria bassiana* in oil or an emulsifiable suspension in Cape Verde. 1992.

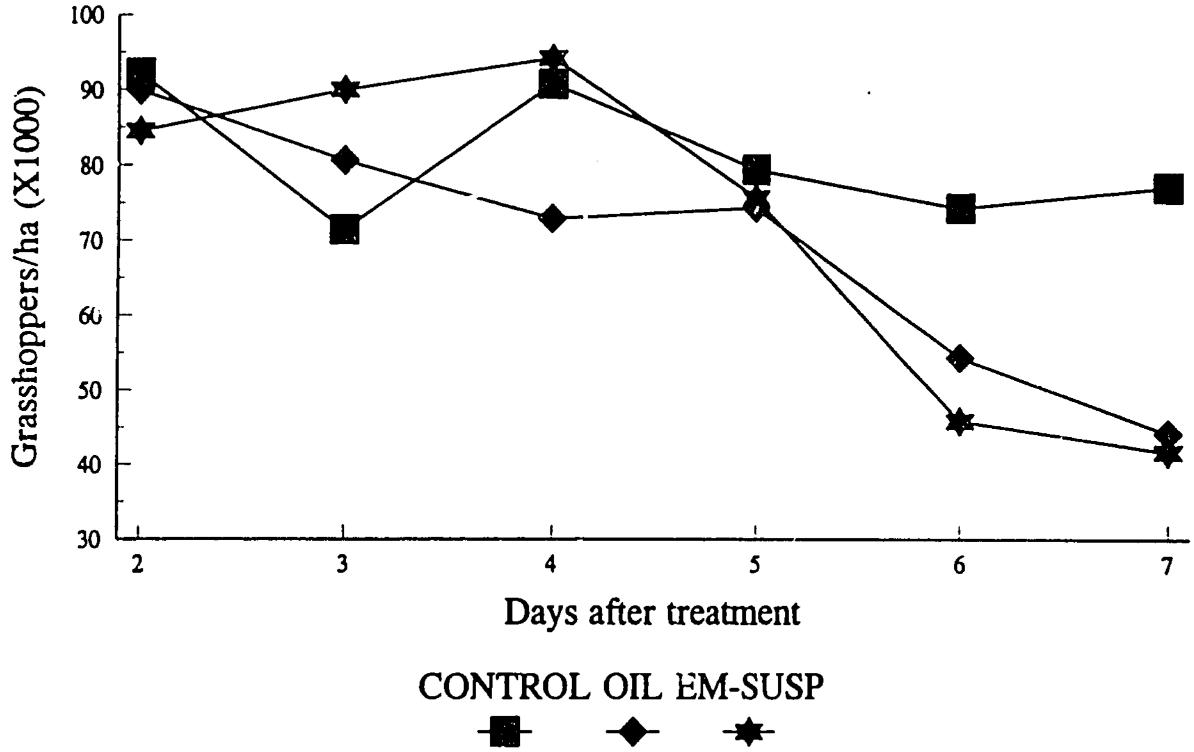


Figure 2. Cumulative mortality among *Oedaleus senegalensis* following treatment of field plots with *Beauveria bassiana* in oil or an emulsifiable suspension in Cape Verde. 1992.

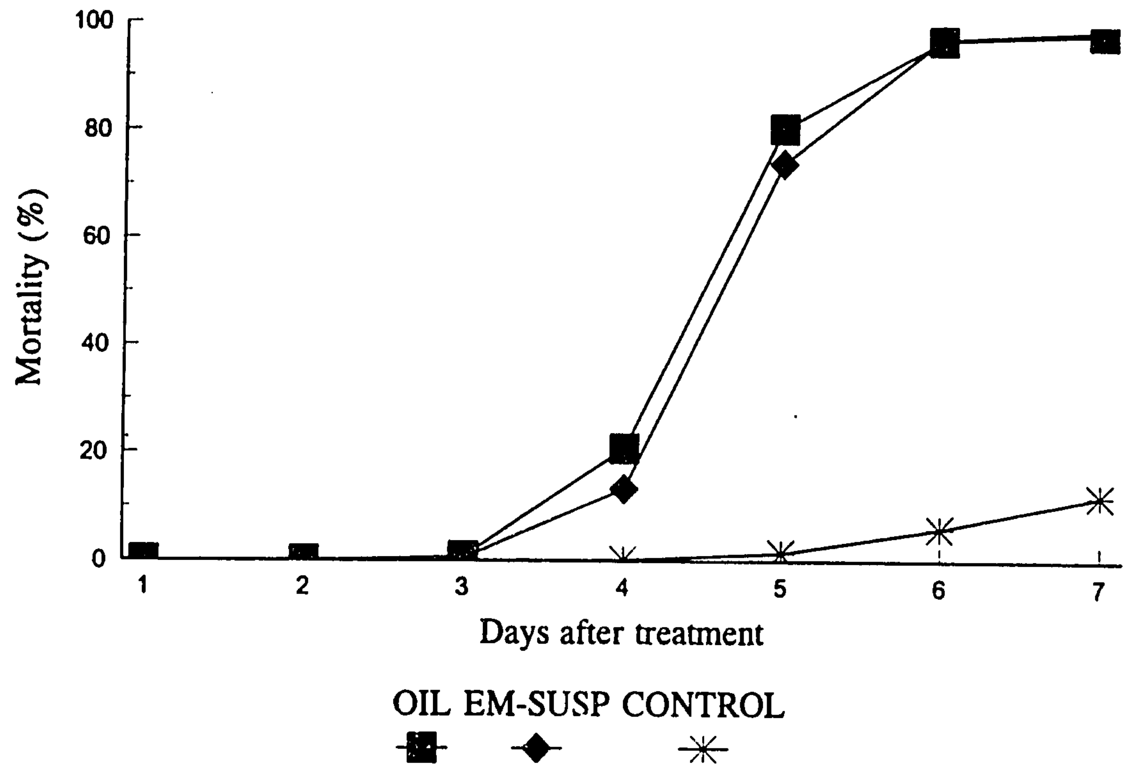


Figure 3. Bioassay of *Beauveria bassiana* formulated in oil against fourth instar migratory locusts, *Locusta migratoria migratorioides*, in Cape Verde. 1992.

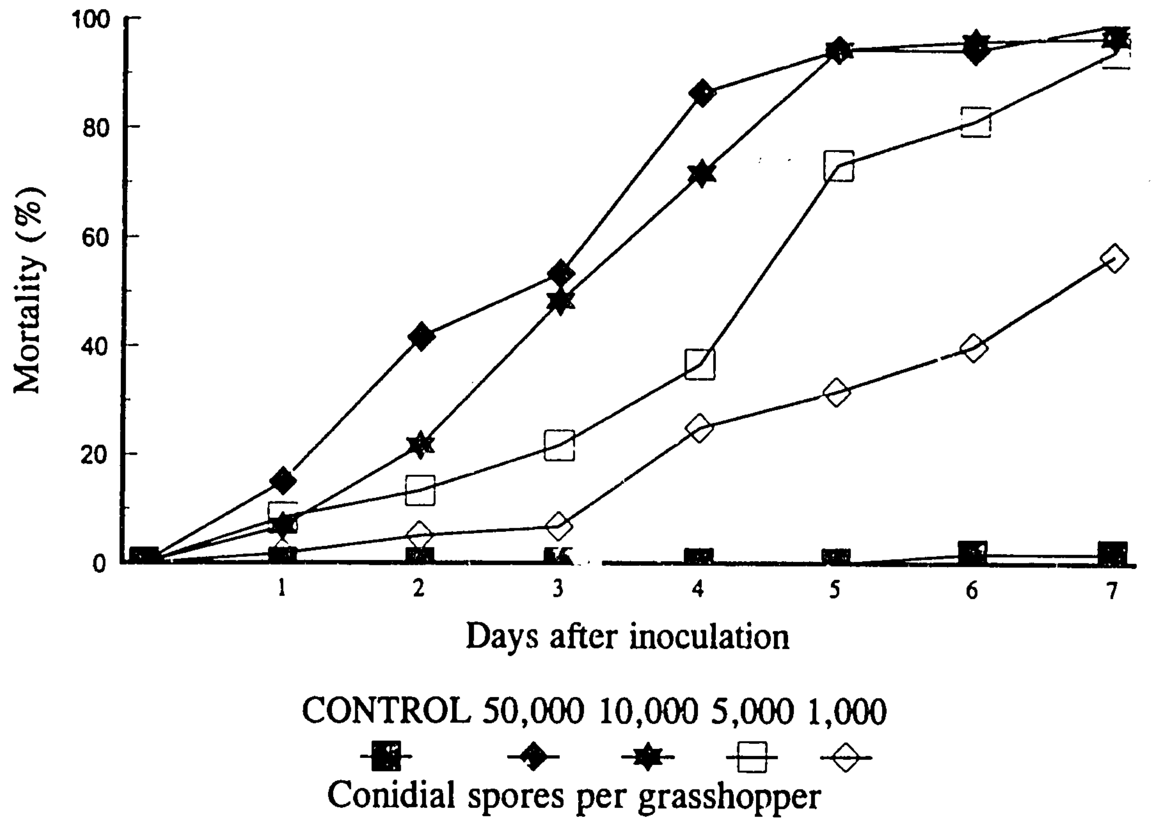
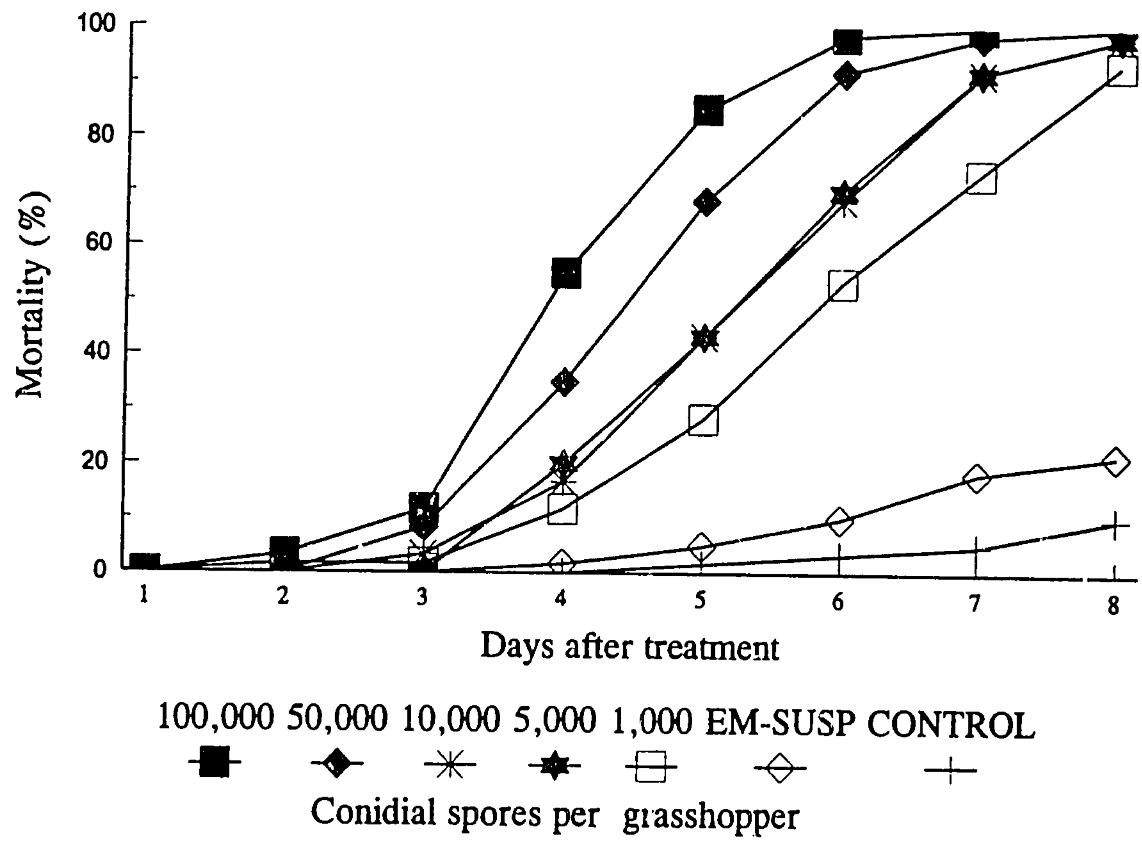


Figure 4. Cumulative mortality among fourth instar migratory locusts, *Locusta migratoria migratorioides,* inoculated with *Beauveria bassiana* formulated in an emulsifiable suspension in a laboratory bioassay in Cape Verde. 1992.



4.0 CONCLUSION

Research in Cape Verde in 1992 achieved a major advance in biocontrol of grasshoppers and locusts in Africa. For the first time, significant grasshopper population-density reduction was demonstrated in the field with a bioinsecticide. Although previous trials in Cape Verde and North America demonstrated high rates of mortality among grasshoppers treated in the field and then collected and held in cages, there remained concern that handling may have increased mortality. In the 1992 trials, the 43-46% density-reduction rates remained below the near-total mortality achieved with the caged grasshoppers. However, this is believed to be primarily due to grasshopper migration. Project researchers believe that much higher population-density reductions will be seen in larger-scale field trials.

Unfortunately, the two-hectare plot size used in the 1992 trials is not large enough to eliminate the influence of migration. Grasshoppers can easily move in and out of the plots after treatment. This means that grasshoppers counted on day 7 are not the same grasshoppers that were treated at day 0. Migration is especially problematic when the grasshopper population consists primarily of adults, which can fly and thus travel relatively long distances in a short period, as was observed during the 1992 trials. Migration lowered density reduction because the treated population remaining in the plots was "diluted" by untreated grasshoppers from outside the plot. Also, many treated grasshoppers escaped. In order to obtain more accurate density-reduction data, larger plots should be treated, with density measured near the center of the plots to minimize the effect of migration. Such trials are proposed for the 1993 and 1994 field seasons. In light of the success of the 1992 trials in demonstrating significant field population-density reduction, it seems likely that even greater reductions will be observed in larger-scale field trials.

Topical bioassays revealed that the LD_{50} of oil-formulated *Beauveria* vs. *L. migratoria* is 2,900 spores per insect. This is on the same order of magnitude as the LD_{50} measured in 1991 vs. *O. senegalensis* (approx. 1,000 spores/insect).[5] If *Beauveria* is effective in the field against *O. senegalensis*, as was shown in the 1992 trials, LD_{50} results suggest that *Beauveria* may be equally effective in the field against *L. migratoria*.

The LD_{50} levels determined by these bioassays reveal that the oil formulation is several times more lethal to *L. migratoria* than the emulsifiable suspension formulation. Oil apparently enhances the efficacy of the spores for reasons that are still poorly understood. (Research is continuing to address this issue). However, at the economically feasible dose rates used in these field trials, there was little practical difference between the two formulations in either grasshopper mortality or density reduction (Fig. 1 and 2).

5.0 ACKNOWLEDGEMENTS

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