The millipede of Santo Antao, Spinotarsus caboverdus : survey for pathogenic microorganisms, bioassay tests of fungal pathogens against S. caboverdus and Melanoplus sanguinipes

by Jorge Mendes Brito

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology
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Abstract:
The millipede of Santo Antao, Spinotarsus caboverdus (Pierrard), is an introduced pest of potatoes on two of the Cape Verde Islands: Santo Antao and S. Vicente. Surveys for microorganisms associated with S. caboverdus were conducted from the soil, live and dead millipedes, and other arthropods. Of the three isolates obtained from soil samples, two were identified as saprophytic species, while the other appeared to be related to a Paecilomyces lilacinus that exhibits some mammalian toxicity. No pathogenic fungi were found from the total of 2572 live and 2193 dead millipedes examined. Two nematode species were found. Caenorhabditis sp., a gut contaminant with no apparent effect on the millipedes, was found in the hindgut of 3.86% of the millipedes examined. Rhabdilis necromena, which is a parasitic nematode previously introduced from Australia against S. caboverdus, was also found in the hindgut of 1.14% of the millipedes examined. A total of 620 live millipedes were examined for the presence of other microorganisms. The only microorganisms found were some gregarine species which infected 12.4% of the millipedes examined. Two isolates of Beauveria bassiana (Balsamo) Vuillemin were obtained from the banana weevil, Cosmopolites sordidus (Germar), from two sites at S. Antao (Cha de Arroz and Joao Dias). Six strains of B. bassiana and two strains of Metarhizium flavoviride (W. Gams and Rozsypal) were included in laboratory tests against S. caboverdus using three different bioassay methods: application of fungal inocula to soil at different moisture levels; topical application of conidia to millipedes; and application of conidia to pieces of potato which were then fed to the millipedes. Results of these bioassays indicated that the fungi were not virulent to the millipedes. Mortality of the millipedes never surpassed 50%, except when soil moisture was very low, which occasionally led to 100% mortality. However, this occurred independent of fungal inocula levels used. A topical bioassay test was conducted against Melanoplus sanguinipes (F.) using a strain of B. bassiana 7A. Mortality ranged from 26.7% at dose 2.5 x 10^3 to 91.9% at dose 6.4 x 10^5 spores per grasshopper. The dose required to kill 50% of M. sangidnipes (LD50) was 1.2 x 10^4. It was concluded that this fungal strain has potential for grasshopper management in the future.

THE MILLIPEDE OF SANTO ANTAO, *Spinotarsus caboverdus*: SURVEY FOR PATHOGENIC MICROORGANISMS, BIOASSAY TESTS OF FUNGAL PATHOGENS AGAINST *S. caboverdus* AND *Melanoplus sanguinipes*

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ABSTRACT

The millipede of Santo Antao, *Spinotarsus caboverdus* (Pierrard), is an introduced pest of potatoes on two of the Cape Verde Islands: Santo Antao and S. Vicente. Surveys for microorganisms associated with *S. caboverdus* were conducted from the soil, live and dead millipedes, and other arthropods. Of the three isolates obtained from soil samples, two were identified as saprophytic species, while the other appeared to be related to a *Paecilomyces lilacinus* that exhibits some mammalian toxicity. No pathogenic fungi were found from the total of 2572 live and 2193 dead millipedes examined. Two nematode species were found. *Caenorhabditis sp.*, a gut contaminant with no apparent effect on the millipedes, was found in the hindgut of 3.86% of the millipedes examined. *Rhabditis necromena*, which is a parasitic nematode previously introduced from Australia against *S. caboverdus*, was also found in the hindgut of 1.14% of the millipedes examined. A total of 620 live millipedes were examined for the presence of other microorganisms. The only microorganisms found were some gregarine species which infected 12.4% of the millipedes examined. Two isolates of *Beauveria bassiana* (Balsamo) Vuillemin were obtained from the banana weevil, *Cosmopolites sordidus* (Germar), from two sites at S. Antao (Cha de Arroz and Joao Dias). Six strains of *B. bassiana* and two strains of *Metarhizium flavoviride* (W. Gams and Rozsypal) were included in laboratory tests against *S. caboverdus* using three different bioassay methods: application of fungal inocula to soil at different moisture levels; topical application of conidia to millipedes; and application of conidia to pieces of potato which were then fed to the millipedes. Results of these bioassays indicated that the fungi were not virulent to the millipedes. Mortality of the millipedes never surpassed 50%, except when soil moisture was very low, which occasionally led to 100% mortality. However, this occurred independent of fungal inocula levels used. A topical bioassay test was conducted against *Melanoplus sanguinipes* (F.) using a strain of *B. bassiana* 7A. Mortality ranged from 26.7% at dose 2.5×10^3 to 91.9% at dose 6.4×10^5 spores per grasshopper. The dose required to kill 50% of *M. sanguinipes* (LD_{50}) was 1.2×10^4. It was concluded that this fungal strain has potential for grasshopper management in the future.

I. INTRODUCTION

The millipede of Santo Antao, *Spinotarsus caboverdus* (Pierrard), is a polyphagous myriapod that was first described in 1987 (Pierrard 1987). Since its introduction into Cape Verde during the 1970s it has become the most serious pest of potatoes (Neves et al. 1993). This species is a problem in two of the Cape Verde Islands: Santo Antao and S. Vicente. Other crops attacked include sweet potatoes, young stems of cassava (after planting), and young seedlings of corn and beans.

The most economically serious consequence of this millipede in Santo Antao and S. Vicente is the fact that fruits, vegetables, and other commodities grown on these two islands are quarantined from the other islands in Cape Verde and some countries in the African mainland (McKillup et al. 1991). The low potential for profitability of potatoes due to quarantine considerations and the high cost of treatment with chemicals has made it nearly economically unfeasible to grow this crop (Neves et al. 1993).

Control measures developed to date have emphasized the use of pesticides. Other types of control measures attempted have included mechanical control, biological control, and quarantine of fruits and vegetables. Mechanical management of the Santo Antao millipede has included the use of peel of papaya,

mango, and banana to attract the millipedes and then collecting and burning them in the morning. Biological control efforts against the millipede to date have included the introduction of a nematode from Australia. However, no impact on field populations of the millipede has been observed (Neves et al. 1993).

Special geological features of Santo Antao and S. Vicente have led to concerns about the use of conventional control techniques against arthropod pests. Valleys bordered by relatively steep slopes, sandy-gravely soil, a shallow water table, and emphasis on chemical control strategies for pest management provide high potential for increasing groundwater contamination (McKillup et al. 1991). Hence, it is imperative that alternative millipede management strategies, such as biological control, be developed to achieve more sustainable levels of pest management for potatoes and other crops in the future.

Statement of the Hypotheses

The main objective of this study was to test the following hypotheses:

(1) Fungal pathogens are present in the soil and millipede populations that can infect and kill *S. caboverdus.*

(2) Fungal pathogens that occur on grasshoppers can infect and kill *S. caboverdus.*

II. LITERATURE REVIEW

Millipedes

Millipedes belong to the arthropod class Diplopoda (Eisner et al. 1978). Approximately 10,000 species of millipedes have been described in the world and their size ranges from as little as 2 mm to 30 cm in length (Hopkin and Read 1992). Millipedes are an old group and have been recorded as far back as Devonian times (Eisner et al. 1978).

Habitats and Ecology

Most millipedes are detritivores, preferring to eat decaying plant material instead of living vegetation (Wooten and Crawford 1975; Dzingov et al. 1982), although a few are known to cause economic damage to crops (Demange 1982). Primarily woodland animals, millipedes mainly occur in the surface regions of soil, especially in the litter, where they play an important role not only in plant decomposition, but also in microbial dissemination (Dzingov et al. 1982). Millipedes are normally found under leaf litter or stones, or beneath the soil surface (Wooten and Crawford 1975). Others have been found in trees or in dry environments such as desserts (Hopkin and Read 1985). Some species of millipedes are found in association with the remains of dead animals. However, it

is still unclear whether they feed on decaying tissue or use cadavers as a moist site for shelter (Hopkin and Read 1992).

Millipedes are relatively long-lived organisms compared to other terrestrial arthropods. For example, the pill millipede, *Glomeris marginata* (Villres), may take several years to gain maturity and can live for 11 years (Carrel 1990).

Millipedes deposit their eggs in hollow capsules of their own construction, within which the young spend part of their existence (Eisner et al. 1970). Egg capsules are covered primarily with soil and vegetative debris. The capsules are built with additional material of maternal enteric origin, and may contain defensive compounds which may afford protection against microorganisms (Eisner et al. 1970). The egg capsules also protect the eggs from rapid fluctuations in humidity and temperature (Hopkin and Read 1992).

All diplopods hatch from eggs (Blower and Gabbutt 1964). The larva which develops from the pupoid is considered to be the first larval stadium (Blower and Gabbutt 1964). Young millipedes are blind, but in many cases the ocelli may be present and visible through the cuticle (Causey 1943). The first stadium has fewer antennal segments than the adults and they possess one pair of legs on each of 2 to 4 segments (Causey 1943).

Millipedes molt several times throughout their life span, and the timing of molting is often correlated with climatic conditions (Hopkin and Read 1992). The progression in growth through various stadia is similar to that which occurs in insects. Certain morphological changes typically occur with each molt, including

the number of segments, defense glands, ocelli, size, and weight (Hopkin and Read 1992).

Several species of millipedes reproduce parthenogenetically. Some species of millipedes exhibit a very low ratio of males to females, and in others no males have been found at all (Hopkin and Read 1992).

Economic Importance

As stated earlier, millipedes play important roles in plant decomposition and in mixing and inoculating soils with microorganisms (Dzingov et al. 1982). Reports of millipedes attacking crops are less common. Kevan (1983) reported millipedes damaging several crops in Canada including strawberry fruit, potato tubers, and the roots of plants grown in greenhouses. In some Eastern European countries black millipedes (e.g. *Chromatoiulus unilineatus* C. L. Koch) have caused damage to sugar beet crops when no alternative sources of food were available (McKinlay 1993). In Africa, cotton and groundnut have been damaged by spirostreptid millipedes (Masses 1981). The black Portuguese millipede, *Ommatoiulus moreletti* (Lucas), an introduced pest in Australia, is known to create a nuisance by invading houses in large numbers at night during spring and autumn in South Australia (McKillup 1988).

Control Strategies

Methods recommended for millipede control include cultural, chemical, and biological control. Burning the soil, rather than removing the humus, was a

cultural method recommended by Thompson in 1950 (Hopkin and Read 1992). Another cultural control approach is the removal of all plant debris from areas infested with millipedes (Appel 1988).

A number of chemical pesticides have been used against millipedes including DDT, lindane, dieldrin, carbaryl, and propoxur. Edwards (1974) stated that most of the organophosphate pesticides that have been used against millipedes have had little effect. Although carbaryl reportedly can be effective in controlling migrations of millipedes, chemicals do not usually give satisfactory control (Hopkin and Read 1992).

Biological control of millipedes is a strategy whose potential has not been widely explored. Natural enemies known to attack millipedes include fungi, viruses, protozoa, nematodes, microsporidia, and Diptera (Hopkin and Read 1992). However, the only biocontrol agent released to date against *S. caboverdus* has been a parasitic nematode, *Rhabditis necromena* (Sudhaus and Schulte), released on the Islands of Santo Antao, Cape Verde in 1988 (Neves et al. 1993).

The Millipede of Santo Antao

Taxonomy

Although there has been disagreement among myriapodologists concerning the generic position of *S. caboverdus*, this millipede species has been most commonly placed in the order Spirostreptida, family Odontopygidae, and subfamily Odontopyginae (Neves et al. 1993). In 1982, Enghoff placed the

millipede of S. Antao in the genus *Tibiomus* and family Odontopygidae (Enghoff 1993). In 1987, Pierrard named the millipede of S. Antao, *Spinotarsus caboverdus* (Pierrard 1987). Hence, the current taxonomy of *S. caboverdus* is as follows: Order: Spirostreptida; Family: Odontopygidae; and Subfamily: Odontopyginae.

Geographic Origin

Spinotarsus caboverdus was supposedly introduced from the Africa mainland, perhaps from Angola, Guinea Bissau, S. Tome, or Mozambique, countries which maintained contact with one another in the past under Portuguese colonial domination. Other evidence that *S. caboverdus* is of African origin is the fact that millipedes in the subfamily Odontopyginae are frequently reported throughout tropical Africa, excluding Madagascar (Kraus 1966).

Distribution

Spinotarsus caboverdus is well distributed throughout the valleys of Santo Antao and, at present, infests most of the irrigated areas as well as some non-irrigated areas (Neves et al. 1993). Since 1984, it has been found in gardens and in some irrigated areas of S. Vicente Island (McKillup et al. 1991). It has also been found in non-irrigated humid areas and in dryland agricultural areas where rainfed crops are grown (Brito, unpub. data).

Biology and Ecology

Very little is known about the biology of the millipedes of S. Antao. *S. caboverdus* lays its eggs in soil, preferably in slightly moist areas rich in organic

matter, and protects them with a ball of soil debris (Delgado and Silva 1991). The number of stadia of this species is not known. Under laboratory conditions, 15 instars have been observed (Neves and Brito unpub. data). Adults as well as young millipedes are found throughout the year, which suggests that there is no diapause (Delgado and Silva 1991). *S. caboverdus* spends most of its life on the soil surface, but it can also migrate deeper into the soil if food resources become scarce (Neves et al. 1990). *S. caboverdus* is often found in patchy aggregations. During the day it concentrates in dump areas, under fallen fruit (papaya and banana), leaf mulch, and other debris (Neves et al. 1993). Adults are very active at night, which is when most of the damage occurs on the crops (Brito, unpub. data). *S. caboverdus* is extremely sensitive to desiccation. Thus, during the day this millipede is found in cool areas, under rocks, leaves, or inside buildings (Neves et al. 1993).

Economic Importance

Unlike most other millipedes, which are associated with decaying plant material, *S. caboverdus* damages potatoes by burrowing through the maturing tubers. Yield losses from this millipede are common and can be as high as 100% (McKillup et al. 1991). Other crops reportedly attacked by *S. caboverdus* include sweet potatoes, young stems of cassava (after planting), and young seedlings of corn and beans (Neves et al. 1990). *S. caboverdus* infests papaya fruit, squash, and cabbage and is becoming an increasing nuisance in houses and restaurants (Brito unpub. data). Farmers also occasionally report millipede damage to corn

and beans in rainfed agricultural areas. However, no damage has been reported for sugar cane or banana, which are considered to be the most important cash crops in Santo Antao (Neves et al. 1993).

Other millipede species known to cause economic losses to crops on the African mainland include odontopygid millipedes, which have been reported as pests of groundnut in west Africa (Demange 1975; Rossion 1976), and *Prionopetalum etiennei*, which has been reported to attack potatoes in southern Senegal (Demange 1982).

Control Strategies

Control measures developed against *S. caboverdus* have mostly emphasized the use of pesticides. Jolivet (1986) tried many different chemicals against *S. caboverdus*, but he never obtained 100% mortality. One of the chemicals, Unden 75% (propoxur), has been used extensively with baits. Although this pesticide offers effective control, its high toxicity and potential side effects on non-target species, beneficial natural enemies, field workers, and groundwater contamination make it less desirable than if safer alternatives control measures were available. Another problem associated with the use of baits is the fact that high densities of millipedes are found not only on potato crops, but also in neighboring fields of banana and sugar cane from which reinvasion can occur. Hence, baits impregnated with chemicals have to be repeatedly placed in the field, making this method very costly and labor intensive (Neves et al. 1993).

The sudden population outbreak of *S. caboverdus* in Santo Antao has all the characteristics of an introduced species (McKillup et al. 1991). Following its introduction, it quickly increased and spread rapidly, as do many organisms that are introduced into new environments in the absence of their natural enemies (McKillup et al. 1991). Four insect predators were initially considered for release against the millipede of S. Antao, including two species of Staphylinid beetles, *Staphylinus olens,* and *S. aethiops*, and two species of carabid beetles, *Steropus globosus* and *Carabus lusitanicus* (Neves et al. 1993). Even though these species were known natural enemies of the black portuguese millipedes *Ommatoiulus moreletti* (Lucas) (Baker 1985), the release of these beetles in S. Antao was not approved because of the concerns of releasing general predators (i.e., the consequences of releasing general predators in a new environment are unpredictable; Neves et al. 1993).

The first natural enemy approved and released against *S. caboverdus* was the parasitic nematode, *R. necromena*, which was known to kill other millipede species (Baker 1985). This nematode reportedly has been responsible for the decline of the introduced black portuguese millipede in South Australia (Baker 1978).

Laboratory studies of infective stages of *R. necromena* (obtained from Australia) were conducted in S. Antao in 1988 (McKillup et al. 1991). Approximately 50 million nematodes also were released at different sites in S. Antao in 1988 (Neves et al. 1990). However, no impact on field populations of

the millipede has been observed. McKillup and his collaborators (1991) concluded that *R. necromena* was not a good candidate against *S. caboverdus* for two main reasons: (1) the number of parasitic nematodes recovered from the gut and hemocoel were very low and (2) the infective stages of *R. necromena* appeared to be too large to be ingested by *S. caboverdus*.

Biological control efforts since the release of the nematode have focused on the search for endemic natural enemies that could conceivably be used for the biocontrol of the millipede. In summer 1993 a survey of fungal pathogens of *S. caboverdus* was conducted on the islands of S. Antao and S. Vicente for potential pathogens of the millipede. Bioassay tests were then conducted the following year to determine the relative virulence of the fungal pathogens that could potentially be used for millipede control.

Fungal Pathogens

Beauveria bassiana

Beauveria bassiana, also known as the white muscardine, was the first disease in animals reported to be caused by a fungus (Tanada and Kaya 1992). Bassi de Lodi demonstrated the contagious and pathogenic nature of this disease attacking silkworms, and developed measures to control the disease (Ainsworth 1956). *Beauveria bassiana* is characterized by globose and oval spores that occur in almost equal proportions (Tanada and Kaya 1992). The infection cycle of *B. bassiana* begins with the adhesion of the conidia to the host cuticle and is

followed by the production of germ tubes which penetrate the host cuticle and produce hyphal bodies in the host hemocoel (Hung and Boucias 1992). In fire ants, *Solenopsis invicta* (Buren), the conidia germinate in the digestive track within 72 hours and then the hyphae penetrate the gut wall between 60 and 72 hours after germination (Broome et al. 1976). This injury enables the digestive juices to enter the hemocoel and change the hemolymph pH, which can be detrimental to the host (Tanada and Kaya 1992). This entomopathogenic fungus may infect its host via the respiratory system and alimentary tract (Lomer and Prior 1991), although the most common means of infection is penetration through the external integument of the host (Pekrul and Grula 1979).

Beauveria bassiana, which infects a wide range of hosts mainly in the Lepidoptera, Coleoptera, Hemiptera, Diptera, and Hymenoptera orders, is also known to occur in soil as a saprophyte (Tanada and Kaya 1992).

Some isolates of *B. bassiana* produce toxins, namely beauvericin, beauverolides, bassianolide, and isarolides. The toxin that has been given the most attention is the depsipeptide beauvericin which is chemically related to the enniatins. No reports on LD_{50} doses have been published, but there is evidence that it is toxic to mosquito larvae, brine shrimp, housefly adults, and cockroach cardiac cells (Roberts 1981).

Beauveria bassiana has been used extensively in the past few years for controlling a wide array of hosts, including Colorado potato beetle, *Leptinotarsa decemlineata* (Say), in the former USSR; the pine caterpillar, *Dendrolimus*

punctata, in the People's Republic of China (Lomer and Prior 1991); the grasshopper, *Oedaleus senegalensis* (Kraus), in the Cape Verde Islands (Bradley et al. 1992); the red locust, *Nomadacris septemfasciata* (Seville), in South Africa (Schaefer 1936); the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), in Israel (Trefi 1984); and the fire ant, *S. invicta*, in Brazil (Setimac et al. 1993).

The use of *B. bassiana* for controlling soil inhabiting insects is also well known (Tanada and Kaya 1992). However, the use of this fungal species is largely dependent on the ability of this pathogen to survive for relatively long periods of time in soil (Lingg and Donaldson 1980). Survival of the conidia is dependent on temperature and soil water content; conidia half-lives were reported to range from 14 days at 25 °C and 75% water saturation to 276 days at 10 °C and 25% water saturation (Lingg and Donaldson 1980).

Bradley (1992) reported that *B. bassiana* is a promising alternative to pesticides because it does not infect humans, other animals, including leaf cutter bees, or plants. Therefore, the risk to public health and the environment are minimal. However, other studies have reported that certain strains of *B. bassiana* may infect the lungs of wild rodents and the nasal passages of humans and giant tortoises (McCoy et al. 1988). Another study also reported adverse side effects on several beneficial and predaceous insects, including the green lacewing, *Chrysoperla carnea* (Stephens) (Donegan and Lighthart 1989) and the lady beetle, *Hippodamia convergens* (Guerin and Meneville) (James and Lighthart 1992). Genthner and Middaugh (1992) also reported that developing fish embryos had

visible side effects and death occurred when exposed to the conidiospores of *B. bassiana.*

Metarhizium flavoviride

The genus *Metarhizium* is considered to include just three species: *Metarhizium anisopliae* (Metsch.) Sorokin, *M. flavoviride* and *M. album* Petch. (Bridge et al. 1993). *M. anisopliae* has been isolated most often from insects from a wide range of orders, while the other species have a more limited pattern of occurrence and distribution (Rombach et al. 1986). Isolates of *Metarhizium spp.* differ widely in their host ranges (Ferron et al. 1975). Separation of the populations of *M. anisopliae* has been based on cultural characteristics. However, individual isolates cannot always be distinguished by these methods (Yip et al. 1992). Conidial measurements have been used as a critical taxonomic character for species differentiation in the genus *Metarhizium* (Rombach et al. 1986). Because conidial dimensions are the same for *M. anisopliae* and *M. flavoviride*, spore measurements alone do not distinguish the two species (Bridge et al. 1993). The colony color also is not reliable even though *M. flavoviride* produces pale-green, greyish yellow-green, or olivaceous-buff colonies, while *M. anisopliae* produces yellowish green, olivaceous dark-herbaceous green, pink or vinaceous-buff colonies (Bridge et al. 1993).

Metarhizium anisopliae has a worldwide distribution and it has been used extensively in experimental systems for the biological control of a wide variety of insects (Gillespie 1988). In contrast, only a few studies have been reported

concerning *M. flavoviride*. Moore et al. (1992) reported *M. flavoviride* infecting and killing the desert locust, *Schistocerca gregaria* (Forskal). According to the authors, adults of the desert locust began to die after five days when inoculated with 5 microliters of inoculum of *M. flavoviride* at a dose of 1.6 x 10^8 conidia per locust (Moore et al. 1992). The black vine weevil, *Otiorhynchus sulcatus* (F.), which is a serious pest of strawberries, grapes, and azaleas, has been reported to be susceptible to *M. flavoviride*. Strains of *M. flavoviride* proved to be highly virulent when an aqueous suspension of conidia was spread directly on the larvae of black vine weevil at 20 °C. Mortality reached 95% or more at a concentration 4 x 10^5 conidia per insect (Soares et al. 1993). Oil-based ULV sprays containing conidia of *M. flavoviride* have readily killed locusts at humidities lower than those considered necessary for infection by other fungal pathogens (Bateman et al. 1992). Laboratory assays on desert locusts using formulations of *M. flavoviride* in cotton seed oil demonstrated superior performance over water-based suspensions. The results were especially pronounced at low humidities (35% RH) (Bateman et al. 1993).

Although *M. flavoviride* was evaluated in the current study, *M. anisopliae* has been studied more extensively. *M. anisopliae* is easily identified by the green cylindrical conidia which are produced in chains forming a dense compact layer of spores (Zimmermann 1993). The fungal colony at the beginning of formation has a white appearance; as the conidia mature the colony becomes dark green (Tanada and Kaya 1992). However, there are other strains of this fungus that

form different colored colonies; *M. a. album*, for example, forms white colonies, while *M. a. brunneum* appears yellow to brown. These strains not only differ in virulence, but they also infect different hosts (Diomande 1969).

Metarhizium anisopliae is known to be able to invade the hemocoel of insects 24 hours after application of the conidia to the host cuticle (Gunnarsson 1988). Death of the host normally occurs 5-10 days after application of this fungus (Moore et al. 1992).

Metarhizium anisopliae generally develops and sporulates better at higher temperatures than does *B. bassiana* under the same conditions (Zimmermann et al. 1994). Research has shown that for most *M. anisopliae* strains, the optimal temperature for infection is between 25 and 30 °C (Zimmermann et al. 1994). Some isolates from tropical areas are adapted to higher temperatures and can grow slowly at a constant temperature of 36 °C (Zimmermann et al. 1994).

Cultures of *M. anisopliae* produce cyclodepsipeptides, destruxins A, B, C, D, and E and also desmethyldestruxin B (Suzuki et al. 1970). Destruxins are considered a potential new generation of insecticides (Vey et al. 1987) and have been reported to cause tetanic paralysis when injected into larvae of *Galleria mellonella* (L.) (Roberts 1969). These toxins have the potential to kill not only lepidopteran but also dipteran larvae, including mosquitos (Vey et al. 1987).

Metarhizium anisopliae has been found worldwide as part of the natural soil flora (Zimmermann 1993). The current target pests of *M. anisopliae* include:

Isoptera; Orthoptera, *Locusta migratoria* (Reiche and Fairmarie) and *S. gregaria*; Homoptera (Splittlebugs); and Coleoptera (*Oryctes sulcatus*) (Zimmermann 1993).

Metarhizium anisopliae has been recognized as an effective agent for the biological control of insects since 1879, and today it is among the few microorganisms that have the potential to be commercialized (McCoy et al. 1988). One of the first successful uses of this fungus in a field application was reported in 1884 against the white grub (*Anisoplia austraca*) and beet root weevil (*Cleonus punctiventris*) (Zimmermann 1993). Among the most recent applications of *M. anisopliae* are field experiments carried out in 1992 in Sudan against *S. gregaria* (Zimmermann et al. 1994). The results of these experiments demonstrated that infection of locusts with blastosphores of *M. anisopliae* can be achieved under African climatic conditions (Zimmermann et al. 1994).

III. SURVEYS FOR PATHOGENS

A survey for fungal pathogens of *S. caboverdus* was conducted during spring and summer of 1993 on the Islands of S. Antao and S. Vicente. The survey concentrated on collecting and examining samples from the soil and live and dead millipedes for pathogenic fungi.

Soil Samples

Multiple samples of soil were taken at different potato-growing sites on S. Antao and S. Vicente. Two sites on the Islands of S. Vicente and 49 on S. Antao were surveyed for the presence of fungal pathogens.

Soil dilution plating techniques were used to isolate fungal pathogens from the soil samples. Approximately 200 g of soil per site was collected, placed in plastic bags, and brought to the laboratory in Ribeira Grande, S. Antao. The soil samples were thoroughly mixed, and then 10 1 g subsamples were drawn from the composite sample (from each site) for dilution plating. Each 1 g sample of soil was suspended in 9 ml of sterile 0.1% aqueous Tween 80 and shaken vigorously to dislodge the fungal spores from the soil particles. The solution was then subjected to two 10 fold serial dilutions (dilution A and dilution B, respectively), and a 0.2 ml aliquot was drawn from each solution and spread onto dodine oatmeal agar plates (Beilharz et al. 1982; Chase et al. 1986). This type of

agar usually excludes most non-entomopathogenic fungi (Jaronski per. comm.). The plates were incubated at room temperature for ten days. Samples of individual colonies of fungi growing on these plates were then extracted with a sterile needle and transferred to new petri dishes containing Sabouraud Dextrose Agar (SDAY). Cultures obtained during the survey were kept in the refrigerator and later brought to Mycotech, Inc., Butte, MT, and Montana State University for identification.

Live Millipede Samples

Live millipedes used in the fungal pathogen survey were collected from two sites on S. Vicente and 45 sites on S. Antao. Millipedes from each of the collection sites sampled on a particular sampling date were isolated individually in petri dishes and held at room temperature (ranging from 23 to 29 °C) for ten days. Millipedes were fed pieces of cut potatoes every two days and checked daily for mortality. Dead millipedes were transferred to another clean petri dish containing cotton balls moistened with distilled water to provide environmental conditions, particularly high humidity, suitable for fungal growth.

Dead Millipede Samples

Dead millipedes were collected from 44 sites and were processed as described above. Fungal conidia that developed within this period were isolated on dodine oatmeal agar. When the fungal colony reached 2 mm in diameter, a

portion of the fungal mycelia was transferred to a SDAY plate to allow for normal growth of the mycelia and sporulation.

Examination of Live Millipedes for Nematodes

Samples of 20 live millipedes were collected from each of 22 sites on S. Antao to determine levels of infection by the pathogenic nematode, *R. necromena*, using a protocol developed by McKillup (1988). Live millipedes were brought to the laboratory and washed in distilled water to remove any soil attached to the exoskeleton. The head and posterior 2nd and 3rd segments then were gently removed in order to extract the intestines. The head and the removed segments were placed in a petri dish containing 3 ml of distilled water. The hemocoel and the exoskeleton received a similar treatment. Petri dishes were covered and left for three days at room temperature to allow the nematodes to develop for later identification.

Examination of Live Millipedes for Other Microorganisms

Twenty live millipedes from each of 32 sites on Santo Antao were examined to determine if other pathogenic microorganisms were associated with the millipedes. The millipedes were ground individually in 2 ml of distilled water and checked under the microscope for the presence of bacteria, viruses, and protozoa.

Fungal Pathogens Associated with Other Arthropods

Cadavers of the banana weevil, *Cosmopolites sordidus* (Germar), with symptoms of fungal infection were collected within old banana leaf axils from two sites at S. Antao and brought to the laboratory where they were placed individually in petri dishes containing moistened cotton to induce sporulation. Following sporulation, the fungal isolates were cultured at room temperature on dodine oatmeal agar plates. After being transferred twice to the SDAY plates, conidiospores were harvested by scraping the mycelia using a sterile spatula, then freeze dried, and stored at -4°C. A subsample of spores was suspended at a concentration of 1 mg of spore powder per 1 ml of sterile 0.1% Tween 80. Two more dilution rates (1/1000, 1/10000 of the spore powder) were then prepared and spread onto the surface of the SDAY plates. Plates were incubated at 26°C. Densities of spores were determined by direct counts with a hemocytometer. The number of viable spores was estimated by plate count under the microscope.

Results of the Pathogen Survey

Fungal Pathogens from Soil

A list of the sites surveyed for entomopathogenic fungi inhabiting the soil on S. Antao and S. Vicente is presented in Table 1. Eighteen colonies of fungi were isolated from 51 infested areas in Santo Antao and S. Vicente. The fungal isolates were identified by Dr. Stefan Jaronski (Mycotech Inc., Butte, MT). Four of the isolates appeared to be saprophytic and therefore would not be expected to

Table 1. Fungal isolates obtained from soil samples from S. Antao and S. Vicente Islands in 1993 during a survey for pathogens of the millipede, *Spinotarsus caboverdus*.

			Dilution A			Dilution B		
No.	Site	Date	N	Colony	Contam.*	N	Colony	Contam.*
1	Afonso Martinho	2/6	4		4	4		1
2	Passagem I	3/6	4		4	4	1 *Penicillium*	-
3	Passagem II	3/6	4		4	4		-
4	Vicente	3/6	4		4	4		-
5	Pedra Grande	3/6	4	1 *Scopulariopsis*	3	4		-
6	Cova	4/6	4		4	4	1 *Aspergillus*	1
7	Cha de Arroz	4/6	4		4	4		1
8	Cha de Pedras	7/6	4		3	4		1
9	Pia	7/6	4		4	4		2
10	Cha de Igreja	7/6	4		1	4		-
11	Garca de Cima	7/6	4		4	4		-
12	Lajedos	9/6	4		4	4	1 *Fusarium*	-
13	Santa Barbara	11/6	4	1 *Paecilomyces*	3	4	1 *Paecilomyces*	1
14	Joao Dias	11/6	4		4	4		-
15	C. Pelingrina	14/6	4		-	4		-
16	Ribeira de Penede	22/6	4		-	4		-
17	Cabo da Ribeira	22/6	4		-	4		-
18	Cha Joao Vaz	22/6	4		-	4		-
19	Boca Coruja	22/6	4		3	4		-
20	Joao Afonso	24/6	4		1	4		-
21	Figuieral	24/6	4		-	4		-
22	Bica	24/6	4		-	4		-
23	Lajedos II	2/7	4		-	4		-
24	Caibros	2/7	4		4	4		-
25	Boca de Ambas Ribeiras	2/7	4		2	4	3 *Fusarium*	-
26	Ribeira das Patas	2/7	4		-	4		-
27	S. Silvestre	3/7	4		4	4	1 *Scopulariopsis* 1 *Fusarium*	-
28	Pia de Cima	3/7	4		4	4		4
29	Chocho	10/7	4	3 *Paecil.* 1 *Aspergillus*	-	4	1 *Aspergillus*	-
30	Faja de Matos	10/7	4		4	4		4
31	Cha de Igreja	10/7	4		4	4		-
32	Ribeira de Janela	15/7	4		4	4		4
33	Passagem III	15/7	4		4	4		1
34	Cabo da Ribeira II	15/7	4		4	4		-
35	Ponta do Sol	15/7	4		4	4		-
36	Salesianos	19/7	4		-	4		-
37	Jorge Luis	19/7	4		-	4		-
38	Ribeira da Cruz	19/7	4		-	4		-
39	Picoteiro	19/7	4		-	4		-
40	Campinho	26/7	4		4	4		4
41	Furnas	26/7	4		4	4	1 *Aspergillus*	2
42	Pinhao	26/7	4		4	4		4
43	Lombo Branco	26/7	4		4	4		4
44	Cova II	27/7	3		3	3		3
45	Ribeira de Vinha	27/7	3		3	3	2 *Aspergillus*	-
46	Jorge Luis II	27/7	3		3	3		3
47	Ribeira da Cruz	27/7	3		3	3		3
48	Picoteiro II	27/7	3		3	3		3
49	Fontainhas	31/7	3		-	3		-
50	Mao pra Traz	31/7	3		-	3		-
51	Lugar de Guene	31/7	4		-	4		-
	Total		197		123	197		46

*Contaminants.

have entomopathogenic properties. The other isolate was not readily identifiable, but appeared to be *Paecilomyces lilacinus*, which may have some mammalian toxicity (Jaronski pers. comm.).

Fungal Pathogens on Live and Dead Millipedes

A total of 2572 live millipedes from 47 sites and 2193 dead millipedes from 44 sites were evaluated for the presence of fungal pathogens. No pathogens were found on live millipedes (Table 2), although two isolates of saprophytic microorganisms were obtained from dead millipedes (Table 3).

Examination of Parasitic Nematodes on Live Millipedes

A total of 440 millipedes from 22 sites were examined for the presence of parasitic nematodes (Table 4). Two species of nematodes were found during the examination of the millipedes. The first was *Caenorhabditis sp.*, which was found in the hindgut of 3.9% of the millipedes examined. According to McKillup (1988), this nematode is simply a gut contaminant with no apparent impact or control potential for *S. caboverdus*. The second nematode found was *R. necromena*, which was introduced from Australia. This nematode was found in the hindgut of 1.14% of the millipedes examined from 24 sites in S. Antao.

Table 2. Results of surveys for potential pathogens of live millipedes, *Spinotarsus caboverdus*, collected at various sites in S. Antao and S. Vicente in 1993.

No.	Site	Date	No. Collected	No. Obs.	No. Sporulated*
1	Afonso Martinho	2/6	20	-	-
2	Passagem I	2/6	20	-	-
3	Passagem II	3/6	10	-	-
4	Vicente	3/6	20	1	-
5	Pedra Grande	3/6	20	1	-
6	Cova	4/6	14	1	-
7	Cha de Arroz	4/6	20	-	-
8	Cha de Pedras	5/6	20	3	-
9	Pia	5/6	20	-	-
10	Cha de Igreja	7/6	20	2	-
11	Garca de Cima	7/6	20	-	-
12	Lajedos	8/6	20	-	-
13	Santa Barbara	10/6	20	1	-
14	Joao Dias	10/6	20	2	-
15	Cabouco de Pelingrin	13/6	20	3	-
16	Ribeira de Penede	16/6	20	-	-
17	Cabo da Ribeira	16/6	20	-	-
18	Cha Joao Vaz	18/6	20	1	-
19	Chocho	18/6	37	2	-
20	Boca de Coruja	22/6	38	7	-
21	Joao Afonso	23/6	40	5	-
22	Figueiral (R. Grande)	23/6	40	2	-
23	Bica	24/6	40	2	-
24	Caibros	28/6	40	3	-
25	Boca de Ambas Ribeira	28/6	40	1	-
26	Ribeira das Patas	30/6	40	7	-
27	S. Silvestre	1/7	40	1	-
28	Pia de Cima	1/7	41	1	-
29	Chocho II	7/7	100	-	-
30	Cha de Arroz II	7/7	100	1	-
31	Santa Barbara	9/7	100	-	-
32	Faja de Matos	9/7	100	2	-
33	Cha de Igraja II	9/7	100	1	-
34	Ribeira de Janela	13/7	100	4	-
35	Passagem II	14/7	100	-	-
36	Cabo da Ribeira II	14/7	100	-	-
37	Ponta do Sol	15/7	100	5	-
38	Salesianos	17/7	100	4	-
39	Ribeira de Vinha	17/7	32	4	-
40	Picoteiro	19/7	100	6	-
41	Campinho	20/7	100	4	-
42	Furnas	20/7	100	3	-
43	Pinhao	22/7	100	-	-
44	Lombo Branco	22/7	100	3	-
45	Fontainhas	28/7	100	1	-
46	Mon pra Traz	28/7	100	1	-
47	Lugar de Guene	30/7	100	4	-
Total			2572	89	-

*Cadavers were transferred to petri dishes containing moistened cotton for fungal development.

Table 3. Results of surveys for potential pathogens of dead millipedes, *Spinotarsus caboverdus*, collected at various sites in S. Antao and S. Vicente in 1993.

No.	Site	Date	No. Exam.	No. Sporulated	Contam.*
1	Afonso Martinho	2/6	20	-	1
2	R. da Vinha	2/6	16	-	-
3	Passagem	3/6	10	-	2
4	Salesianos	3/6	20	-	-
5	Pedra Grande	3/6	20	-	3
6	Cova	4/6	4	-	3
7	Cha de Arroz	4/6	20	-	1
8	Cha de Pedras	5/6	20	-	6
9	Pia	5/6	10	-	4
10	Cha de Igreja	7/6	20	-	7
11	Garça de cima	7/6	4	-	-
12	Lajedos	8/6	18	-	2
13	Chocho	9/6	9	-	-
14	Santa Barbara	10/6	20	-	3
15	Joao Dias	10/6	20	-	-
16	Cabouco de Pelingrina	13/6	20	1 *Scopulariopsis*	3
17	Ribeira de Penede	16/6	20	-	18
18	Cabo da Ribeira	16/6	20	-	3
19	Cha Joao Vaz	16/6	20	-	12
20	Boca de Coruja	22/6	40	-	17
21	Joao Afonso	23/6	40	-	32
22	Figueiral (R. Grande)	26/6	40	-	23
23	Bica	24/6	40	-	36
24	Caibros	28/6	40	-	18
25	Boca de A. Ribeirasiras	28/6	14	-	13
26	Ribeira das Patas	30/6	40	-	4
27	S. SIlvestre	1/7	40	1 *Scopulariopsis*	6
28	Pia de Cima	1/7	40	-	2
29	Cha de Arroz II	9/7	100	-	30
30	Santa Barbara II	9/7	100	-	43
31	Faja de Matos	9/7	100	-	70
32	Cha de Igraja II	9/7	100	-	46
33	Ribeira de Janela	13/7	100	-	69
34	Passagem II	14/7	100	-	67
35	Cabo da Ribeira II	14/7	100	-	76
36	Ponta do Sol	15/7	100	-	71
37	Picoteiro	19/7	100	-	76
38	Campinho	20/7	100	-	27
39	Furnas	20/7	100	-	58
40	Pinhao	23/7	100	-	69
41	Lombo Branco	23/7	100	-	79
42	Fontainhas	28/7	48	--	20
43	Mon pra Traz	28/7	100	-	62
44	Lugar de Guene	30/7	100	-	35
Total			2193		1117

*Contaminants.

Table 4. Results of surveys of parasitic nematodes associated with live millipedes, *Spinotarsus caboverdus*, in S. Antao in 1993.

No.	Site	Date	No. Exam	Hemocoel			Hindgut		
				Caenorhab.	Rhabditis	Others	Caenorhab.	Rhabditis	Others
1	Caibros	30/6	20	-	-	-	-	-	-
2	Boca de A. Ribeiras	1/7	20	-	-	-	-	-	2
3	Chocho	10/7	20	-	-	-	1	-	-
4	Cha de Arroz	11/7	20	-	-	-	-	2	-
5	Santa Barbara	12/7	20	1	-	-	1	-	-
6	Cha de Igreja	14/7	20	-	-	1	-	-	-
7	Ribeira de Janela	17/7	20	-	-	-	-	-	-
8	Ponta do Sol	18/7	20	-	-	-	-	-	-
9	Cabo da Ribeira	18/7	20	-	-	-	1	-	-
10	Passagem	20/7	20	3	-	-	1	2	-
11	Joao Afonso	24/7	20	-	-	-	-	-	-
12	Furnas	24/7	20	-	-	-	1	-	-
13	Campinho	24/7	20	-	-	-	-	-	-
14	Pinhao	26/7	20	-	-	-	1	-	-
15	Lombo Branco	26/7	20	-	-	-	-	-	1
16	Pia	31/7	20	-	-	-	-	-	-
17	Boca de Coruja	31/7	20	-	-	-	-	-	1
18	Caibros II	31/7	20	1	-	-	-	-	-
19	S. Silvestre	1/8	20	1	-	-	2	1	-
20	Fontainhas	1/8	20	-	-	-	-	-	-
21	Mon pra Traz	2/8	20	2	-	-	1	-	-
22	Ribeira das Patas	2/8	20	-	-	-	-	-	-
Total			440	8	0	1	9	5	4

Other Microorganisms Associated with Live Millipedes

A total of 520 live millipedes from 26 areas were checked for other microorganisms including bacteria and gregarines (Table 5). Although none of the above microorganisms was found by the methods employed, a few gregarine species were found. Under the microscope the spores appeared to be ellipsoidal with truncate ends. The percentage of millipedes infected by these gregarines was 12.4%. These organisms had no apparent impact on *S. caboverdus*.

Table 5. Results of surveys for pathogens other than fungi on live millipedes, *Spinotarsus caboverdus*, in S. Antao in 1993.

No.	Site	Date	No. Exam.	Bacteria	Gregarines
1	Chocho	7/7	20	1	2
2	Cha de Arroz	8/7	20	-	-
3	Santa Barbara	9/7	20	-	-
4	Faja de Matos	10/7	20	-	-
5	Cha de Igreja	11/7	20	-	-
6	Ribeira de Janela	13/7	20	-	-
7	Passagem	15/7	20	1	5
8	Ponta do Sol	21/7	20	-	8
9	Picoteiro	21/7	20	-	3
10	Cabo da Ribeira	21/7	20	-	5
11	Campinho	22/7	20	-	3
12	Furnas	22/7	20	-	-
13	Pinhao	23/7	20	-	1
14	Lombo Branco	23/7	20	-	4
15	Afonso Martinho	27/7	20	-	3
16	Boca de Ambas Ribeiras	27/7	20	-	5
17	Boca de Coruja	28/7	20	-	6
18	S. Silvestre	28/7	20	-	5
19	Furnas	28/7	20	-	4
20	Mon pra Traz	29/7	20	-	4
21	Lajedos	29/7	20	-	4
22	Fontainhas	29/7	20	-	3
23	Ribeira das Patas	2/8	20	-	1
24	Salesianos	2/8	20	-	2
25	Cha Joao Vaz	3/8	20	-	5
26	Cha de Manuel Santos	4/8	20	-	4
Total			520	2	77

Fungi Isolated from Other Arthropods

Two isolates of entomopathogenic fungi were obtained from the banana weevil, *C. sordidus*, and were brought to the United States. These fungal isolates have been identified as *Beauveria bassiana* (Vuillemin) Balsamo by Dr. Stefan

Jaronski (Mycotech Inc.) and also by Dr. Richard Humber (USDA-ARS, Plant, Soil and Nutrition Laboratory, Ithaca, New York).

Discussion

The lack of entomopathogenic fungi found in the surveys to some extent might be attributed to conventional control practices that are commonly used. The pesticide Unden 75% has been extensively used with baits or applied directly to the soil surface (Neves and Brito unpub. data). It is possible that this pesticide may have had a detrimental effect on entomopathogenic fungi (Neves et al. 1993). However, studies conducted by Jaworska (1987) demonstrated that propoxur and *B. bassiana* were compatible.

Abiotic factors may also be responsible for the results obtained, including inactivation of microorganisms by sunlight, desiccation, and temperature, or humidity levels insufficient for germination and development of spores (Goettel 1991). Another explanation for not finding pathogens in the soil could be attributed to the presence of antagonistic microorganisms (Setimac et al. 1993).

Millipedes also are known to contain a number of compounds that are used in defense. The defensive compounds of certain Polydesmidae millipedes are known to have antifungal activity and are able to suppress mycelia growth and spore germination of fungi (Roncadori 1985). It is also known that extensive quantities of defensive compounds produced by millipedes might be sufficient to cover them completely and potentially protect the cuticle from attack by fungi

(Roncadori 1985). This may explain why no fungal isolates were obtained from live or dead millipedes that were pathogenic to *S. caboverdus*.

IV. BIOASSAY OF FUNGAL PATHOGENS AGAINST MILLIPEDES

Beauveria bassiana and *M. flavoviride* are two entomopathogenic fungal species belonging to the Deuteromycotina subdivision, class Hyphomycetes (Tanada and Kaya 1992; Lomer and Prior 1991). Although *B. bassiana* and *M. anisopliae* are able to infect 100 different insect species in several orders, some isolates are known to have a high degree of specificity (Ferron et al. 1975; Fargues 1976). Host specificity is supposedly influenced by a number of factors including the physiological state of the host, the properties of the insect's cuticle, the nutritional requirements of the fungus (Kerwin and Washino 1986), and the cellular defense of the host (Ferron 1978). Because of the known pathogenicity of these fungal pathogens to arthropods, bioassay tests were conducted on a number of fungal isolates to determine their potential for controlling *S. caboverdus*. These included strains of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium flavoviride* (W. Gams and Rozsypal).

Materials and Methods

Laboratory bioassays were conducted on the Island of Santo Antao in 1993. The three methods used in the bioassay tests on *S. caboverdus* were application of the fungal conidia to the soil at three moisture levels, topical application of fungal conidia to the second or third segment on the dorsum of the

millipedes, and application of fungal conidia to pieces of potato on which the millipedes fed. Dosages ranged from 10^3 to 10^8 spores per millipede or per gram of soil. The number of moisture levels used in bioassay trials was determined by the availability and quantity of the fungal conidia. Probit analysis was used only when mortality exceeded 50%, or to analyze trend data that was binomially distributed (i.e., to analyze the results of bioassay tests using inoculated potato).

Millipedes

The millipedes used in the bioassay tests were obtained from Cha de Arroz in Ribeira Grande, S. Antao. They were collected from the field and maintained in the laboratory on pieces of potato in plastic cups.

Fungal Isolates

Eight fungal isolates were used in the bioassay studies. These included three strains of *B. bassiana* (S36B6, S33B4, and S2B1) isolated from soil in Madagascar and two strains of *M. flavoviride* (SP8 and SP2) isolated from grasshopper cadavers (*L. migratoria*) from Madagascar which were provided by Dr. Jaronski, Mycotech Inc. Two other strains of *B. bassiana* (Bb2 and Bb4) were provided by Mr. Delgado, INIDA, Cape Verde which were previously isolated from grasshoppers at Mycotech Inc. in Butte, MT, and were being used in grasshopper trials in Cape Verde. A last strain of *B. bassiana* (7A) was isolated

from the banana weevil by the author from Cha de Arroz and Joao Dias, S. Antao in 1993.

Bioassays Using Inoculated Soil

Soil samples taken from sites from which the millipedes were obtained were sifted through a 200 mm mesh screen, dried in an electric oven for two hours, and then allowed to cool for 12 hours period. The water holding capacity was then determined by measuring the amount of distilled water that drained for a period of 24 hours from saturated soil into a glass funnel (Jaronski pers. comm.). Spore counts were conducted and viability was ascertained prior to the experiments.

Soil samples with moisture levels ranging from 10 to 30%, 10 to 40%, or 20 to 70% (in increments of 10%) were inoculated at the rate of 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 conidia per g of soil formulated in paraffinic superior crop oil supplied by Mycotech Inc. or Tween 80 at 0.1%. Twenty-five millipedes were used for each dosage, of which five millipedes were randomly assigned to a 1 oz. plastic cup with 20 g of inoculated soil. The experiment was replicated five times. The millipedes were fed every other day with fresh pieces of potato. Control groups consisted of millipedes exposed to non-treated soil and millipedes exposed to soil treated with oil or Tween 80 at 0.1% without conidia. Treatment and control cups were kept in the laboratory at room temperature for a ten day period (mean temperature $\pm$ SE = 23.71 $\pm$ 0.1568; mean RH = 50.55 $\pm$ 0.1568).

Any millipedes that died within that period were transferred to petri dishes containing moistened cotton and checked daily for fungal development. Data were analyzed using the General Linear Model (G.L.M.) procedure in SAS 6.08 for Windows (SAS/STAT 1988), and mean comparisons were made using the Student Newman Keul's (SNK) test. An arcsine transformation was performed on the percentage values prior to analysis.

Topical Bioassay

Fungal conidia were applied to the dorsal region of the second or third anterior segment of each millipede. Each millipede was inoculated with a dosage of 10^3, 10^4, 10^5, 10^6, or 10^7 spores formulated in paraffinic superior crop oil or Tween 80 at 0.1%. Three control groups consisted of millipedes treated with oil, millipedes treated with 0.1% Tween 80, and untreated millipedes. Five millipedes were randomly assigned to each petri dish. The experiment was replicated five times. Millipedes were supplied freshly-cut pieces of potato every other day, and kept at room temperature for 10 days. Mortality was recorded daily. Any dead millipedes found within this period were removed and transferred to petri dishes containing cotton moistened with distilled water and held for 10 days. Data were analyzed using procedures as outlined for the previous bioassay. An arcsine transformation was performed on the percentage values prior to analysis.

Bioassay Using Inoculated Potato

Fungal inocula were applied to a freshly punched potato disk (1.6 mm in diameter and 0.3 thick) and then placed inside a petri dish containing an individual millipede. Twenty-five millipedes, which consumed more than half of the potato disk (within a 24 four hour period), were used in the bioassay tests for each dose. The control groups were as in the previously described test. Millipedes were kept at room temperature. Any dead millipedes were removed and transferred to petri dishes containing cotton moistened with distilled water and held for ten days. Data were analyzed using logistic regression analysis for testing the upward trend effect of mortality as a function of fungal dose. An arcsine transformation was performed on the percentage values prior to analysis.

Results of Bioassay Tests

The results of bioassay tests of conidia of *B. bassiana* strains (7A, S2B1, S33B4, S36B6, Bb2, and Bb4) formulated in oil and applied to soil samples to which millipedes were exposed are presented in Tables 9-14, respectively, in the Appendix. In general, *B. bassiana* formulated at dosages specified appeared to have little influence on the levels of mortality observed among millipedes. Relatively high levels of mortality were occasionally observed in the low moisture ranges (10-20% RH), but this occurred in both the control and inoculated groups, suggesting that low moisture rather than fungal infection was responsible for millipede mortality.

The results of bioassay tests of conidia of the same *B. bassiana* strains formulated in Tween 80 at 0.1% and applied to the soil are presented in Tables 15-18, respectively, and are found in the Appendix. The highest levels of millipede mortality were again associated with low moisture levels (10-20% RH), with no apparent mortality from fungal infection.

The results of bioassay tests of conidia of *M. flavoviride* strains (SP8 and SP2) formulated in oil and applied to soil samples to which millipedes were exposed are presented in Tables 19 and 20, respectively, and are found in the Appendix. Mortality of the millipede was generally very low, except in a few instances where mortality levels occasionally reached 100%. However, this was observed both in the control groups and fungal treatment, indicating that fungal infection was likely not responsible for the observed mortality.

The results of bioassay tests of conidia of *M. flavoviride* strain SP8 formulated in Tween 80 and applied to soil samples to which the millipedes were exposed are presented in Table 21 in the Appendix. Mortality among the millipedes was very low in both the control group and fungal treatments.

The results of bioassay tests of conidia of *B. bassiana* strains (7A, S2B1, S33B4, S36B6, Bb2 and Bb4) formulated in oil and applied topically are presented in Tables 22-27, respectively, and are found in the Appendix. Again mortality of the millipede was generally very low for both control groups and fungal inocula treatments, suggesting that millipede mortality did not result from fungal infection.

The results of bioassay tests of *B. bassiana* strains formulated in Tween 80 and applied topically are presented in Tables 28-31, respectively, and are found in the Appendix. Mortality of the millipede was negligible in both the control group and fungal treatments, suggesting no impact by the fungi on the millipede.

The results of bioassay tests of conidia of *M. flavoviride* strains (SP8 and SP2) formulated in oil and applied topically are presented in Tables 32 and 33, respectively, and are found in the Appendix. In general, the fungal dose for the two strains appeared to have little impact on the levels of mortality observed.

The results of bioassay tests of conidia of the above *M. flavoviride* strains formulated in Tween 80 and applied topically are presented in Tables 34 and 35, respectively, and are found in the Appendix. As was found in the topical bioassay tests using fungal spores formulated in oil, the mortality of the millipedes was very low in both the control and the fungal treatments.

The results of bioassay tests of conidia of *B. bassiana* strains (7A, S2B1, S33B4, S36B6, Bb2 and Bb4) in oil formulation and applied to pieces of potato are presented in Tables 36-47, respectively, and are found in the Appendix. As was found in the soil and topical bioassay tests, mortality of the millipede was again very low, suggesting that the *B. bassiana* strains had little influence on the levels of millipede mortality observed. Probit analysis also showed a nonsignificant relationship between mortality and fungal dose for all of the *B. bassiana* strains examined ($P > 0.05$).

The results of bioassay tests of conidia of *M. flavoviride* strains (SP8 and SP2) formulated in Tween 80 applied to pieces of potato are presented in Tables 48-51, respectively, and are found in the Appendix. Millipede mortality followed the same trend as that observed in the other bioassay tests, as mortality was again very low for both the control groups and fungal treatments. Probit analysis showed a nonsignificant relationship between mortality and fungal dose for the two *M. flavoviride* strains examined ($P > 0.05$).

Discussion

Fungal pathogens used in bioassay tests caused no noticeable millipede mortality. A possible explanation could be that the fungal isolates used were either isolated from the soil or from other arthropods and these may not be adapted for attacking millipedes. Perhaps a more plausible explanation is that *S. caboverdus* is protected from fungal attack by defensive compounds, which has been demonstrated for other millipede species (Roncadori 1985).

For example, some species of *Cylindroiulus* millipedes are reportedly attacked by ectoparasitic fungi of the Laboulbeniales (Hopkin and Read, 1992). According to these authors the hyphae are able to infect the anterior-most three pairs of legs of females and anterior most seven pairs of legs of males. These authors suggest that the absence of defensive compounds in these regions may allow infection to occur. Some species of glomerid millipedes coil into a sphere when attacked and secrete from one to eight viscous drops from segmental

glandular openings, located along the dorsal middle line. The secreted fluid contains quinazolinone alkaloids, which are known to act as potent antifeedants and toxins for spiders, insects and vertebrates (Carrel 1984). Also, a wide variety of benzoquinones have been reported from different species of millipedes. The natural function of these compounds is not well understood, although they have been reported to play anti-microbial and/or antifeedant roles (Attygalle et al. 1993). The defensive secretions of certain species of polydesmid millipedes also are known to exhibit antifungal activities by deterring mycelia growth and spore germination of fungi (Roncadori et al. 1985).

As mentioned previously, the fungi used in the bioassay tests did not show any infectivity towards *S. caboverdus*, whether they were isolated from insects or the soil. These results suggest that *S. caboverdus* may also be protected by defensive compounds, which have general antibiotic activity towards fungi. In order to better understand these results, studies on potential defensive compounds of *S. caboverdus* should be conducted in the future.

V. BIOASSAY TESTS OF *Beauveria bassiana* 7A AGAINST *Melanoplus sanguinipes*

Some groups of entomopathogenic fungi are known to be pathogenic against a relatively broad range of insects (Zimmermann et al. 1993). In the course of searching for potential natural enemies of *S. caboverdus*, banana weevils were observed that exhibited symptoms of infection by *Beauveria*. These fungal isolates were later identified as *B. bassiana* (Humber pers. comm. 1993). Although subsequent bioassay tests with this fungus showed no pathogenicity against the millipede, it seemed appropriate to test this fungal strain against another organism to provide evidence that the lack of activity against millipedes was due to intrinsic factors. Grasshoppers were selected because these insects are important pests on rain-fed crops in Cape Verde, and because there is research in progress on the use of fungal pathogens against them.

Materials and Methods

The grasshopper, *M. sanguinipes*, used in this study came from rearing colonies at South Dakota State University, Brookings, SD. *B. bassiana* 7A, used in the bioassay tests was isolated from the banana weevil at S. Antao in the summer of 1993. Spore counts were conducted and viability was ascertained as described previously.

Five treatments and two controls were included in the protocol to evaluate the pathogenicity of *Beauveria* 7A to *M. sanguinipes*. Treatments were 2.5×10^3, 1.0×10^4, 4.0×10^4, 1.6×10^5, and 6.4×10^5 conidia per grasshopper. The controls included grasshoppers treated with oil without conidia and untreated grasshoppers. Each treatment and control group was replicated three times involving 25 grasshopper nymphs per replicate. Inoculation of the fungal formulation and the control oil were applied to the pronotum as 0.2 microliters of suspension. Following inoculation, the 25 grasshoppers from each replicate were randomly dispersed into five separate wire mesh cylinders (5.1 cm dia., length 10.2 cm), five grasshoppers per cage. Grasshoppers were fed wheat bran and washed lettuce daily and were maintained in a growth chamber at 27 °C (mean temperature ± SE = 26.97 ± 0.342). Mortality was assessed daily for 12 days. Probit analysis (PROC PROBIT in GLM of SAS) was used to analyze the relationship between grasshopper mortality and fungal dose. An arcsine transformation was performed on the percentage values prior to analysis.

Results

The percentage mortality of *M. sanguinipes* for the untreated control (C) and for the control treated with oil only (CO) was very low, 2.67 and 6.67, respectively. Percentage mortality increased from 26.67% at a dose of 2.5×10^3 spores per grasshopper to 91.89% at a dose of 6.4×10^5 spores per grasshopper (Table 6). There was an increase in mortality from 82.35% to 91.89% when the

Table 6. Mean cumulative percent mortality of *Melanoplus sanguinipes* inoculated with *Beauveria bassiana* 7A formulated in oil.

Dose	Rep.*	Mean**	SE
C	15	2.67 a	2.67
CO	15	6.67 a	2.42
2.5×10^3	15	26.67 b	4.65
1.0×10^4	15	37.50 b	4.10
4.6×10^4	15	54.06 c	5.04
1.6×10^5	15	82.35 d	5.55
6.4×10^5	15	91.89 d	3.37

* Each replication is comprised of five grasshoppers per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

dose was increased from 1.6×10^5 to 6.4×10^5. Analysis of the results showed that the mortality estimates resulting from all fungal dosages were significantly higher than those levels observed for the untreated control group and the control group treated with oil ($P < 0.05$, Table 6). The Anova results showed that *B. bassiana* strain 7A had a significant effect on the mortality of *M. sanguinipes* ($F = 61.55$, $df = 6$, and $P = 0.0001$) (Table 7). The results of probit analysis of the mortality of *M. sanguinipes* as a function of the dose of *Beauveria* strain 7A produced a strong positive relationship between mortality and the dosages used (Log Dose = 0.4748 and $P = 0.0001$) (Table 8). The LD_{50} for *M. sanguinipes* mortality was estimated to be 1.2×10^4 spores per grasshopper (fiducial limits were estimated to be 3.2×10^3 to 3.6×10^4).

Table 7. ANOVA results of *Beauveria bassiana* 7A effects on *Melanoplus sanguinipes*.

Source	DF	Type III SS	F Value	p
Treat	6	77544.10	61.55	0.0001
Block	2	5.10	0.01	0.9879
Treat*Block	12	2588.91	1.03	0.4319

Table 8. Probit analysis of *Beauveria bassiana* 7A effects on *Melanoplus sanguinipes*.

Analysis of Maximum Likelihood Estimates				
Variable	Parameter Estimates	Standard Error	χ^2	p
Intercept	-2.09	0.51	17.23	0.0001
Log(dose)	0.52	0.09	32.15	0.0001
Block	-0.02	0.14	0.01	0.9040

Discussion

Beauveria bassiana strain 7A can infect and cause mortality to *M. sanguinipes*. Unlike the millipedes, mortality of the grasshoppers from *B. bassiana* was found to follow a dose-dependent response. Hence, it appears that this entomopathogenic fungus has the potential for controlling this and other grasshopper species.

Delgado and Lobo Lima (unpub. data) demonstrated that *B. bassiana* can be used effectively against the grasshopper, *O. senegalensis*. Various formulations

of the fungal conidia in emulsifiable oil were found to be very effective against *O. senegalensis* in laboratory bioassay and field tests.

Results of cage trials also showed that when grasshoppers were sprayed directly they became infected by *B. bassiana*. Johnson et al. (1992) reported that over the 19 days following treatment mortality of grasshoppers sprayed with spores was approximately double the mortality observed in the untreated control.

In the present study, significantly higher levels of mortality were found in the *M. sanguinipes* treated with fungal inocula of *B. bassiana* strain 7A than in control groups. The high virulence and ease of production of this fungal strain suggest that this pathogen has high potential for commercial production. Conceivably, this fungal strain could become commercially available for grasshopper control, not only in Cape Verde, but in other parts of the world where grasshoppers still pose problems to crops and rangeland.

VI. SUMMARY

Soil samples were taken from a total of 51 sites on the Islands of S. Vicente and S. Antao to determine if any microorganisms were present that may be pathogenic to *S. caboverdus.*

Five species of fungi were isolated. Four of the isolates appeared to be saprophytic, while the other isolate appeared to be related to *Paecilomyces lilacinus*, which are known to have mammalian toxicity (Jaronski per. comm.).

No effective and safe pathogenic fungi were isolated on either live or dead millipedes surveyed. Live millipedes were also examined for parasitic nematodes, two species of which were observed. *Caenorhabditis sp.*, which is a gut contaminant, was found in the hindgut of 3.86% of millipedes examined, and *R. necromena*, which was introduced from Australia against *S. caboverdus*, was found in the hindgut of 1.14% of the millipedes. Another examination included a search for pathogenic microorganisms (bacteria and protozoa), which resulted in detection of gregarines in 12.4% of the millipedes examined. A significant result of this study is that it lends support to the hypothesis that millipedes are not susceptible to fungi. When searching for pathogenic microorganisms on banana weevil (C. sordidus), two isolates of *B. bassiana* were obtained from two locations at Santo Antao.

Six strains of *B. bassiana* and two strains of *M. flavoviride* were tested against *S. caboverdus*, using three different bioassay methods. No noticeable mortality of the millipede resulted from the fungal pathogens tested regardless of fungal dose and the type of application of inocula.

Beauveria bassiana strain 7A was tested against 4th instar nymphs of *M. sanguinipes*. When nymphs were inoculated with a dose of 6.4×10^5 spores per grasshopper, mortality levels reached 91.9%. The LD_{50} for *M. sanguinipes* mortality was estimated to be 1.2×10^4 spores per grasshopper.

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APPENDIX

Table 9. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Beauveria bassiana* 7A formulated in oil.

	Treatments												
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Rep.	10^8	Mean
10	-	-	5	88.00 ± 12.00	5	96.00 ± 4.00	5	80.00 ± 20.00	5	100.00 ± 0.00	-	-	91.00 a
20	-	-	10	30.00 ± 10.77	5	84.00 ± 11.66	10	20.00 ± 10.33	10	6.00 ± 4.27	5	16.00 ± 11.66	31.20 b
30	-	-	10	10.00 ± 5.37	5	24.00 ± 9.79	10	10.00 ± 4.47	10	6.00 ± 4.27	5	0.00 ± 0.00	10.00 c
40	-	-	5	4.00 ± 4.00	-	-	5	4.00 ± 4.00	5	8.00 ± 4.90	5	0.00 ± 0.00	4.00 c
Mean**		-		33.00 b		68.00 a		28.50 b		30.00 b		5.33 c	

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 10. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Beauveria bassiana* S2B1 formulated in oil.

	Treatments												
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Rep.	10^8	Mean
10	5	40.00 ± 16.73	-	-	5	8.00 ± 8.00	5	4.00 ± 4.00	5	8.00 ± 8.00	-	-	15.00 b
20	5	32.00 ± 13.56	5	76.00 ± 11.66	5	28.00 ± 10.20	10	46.00 ± 9.91	10	12.00 ± 5.33	5	24.00 ± 7.80	36.33 a
30	5	12.00 ± 4.90	5	16.00 ± 9.8	5	12.00 ± 8.00	10	12.00 ± 6.11	10	12.00 ± 5.33	5	20.00 ± 6.32	14.00 b
40	-	-	5	8.00 ± 8.00	-	-	5	12.00 ± 8.00	5	8.00 ± 4.90	5	0.00 ± 0.00	7.00 b
Mean**		28.00 a		33.33 a		16.00 a		17.00 a		10.00 a		14.67 a	

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 11. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Beauveria bassiana* S33B4 formulated in oil.

	Treatments																
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Rep.	10^8	Mean
10	5	80.00 ± 12.65	-	-	5	40.00 ± 14.14	5	28.00 ± 18.55	5	24.00 ± 19.39	-	-	43.00 a
20	5	8.00 ± 8.00	5	20.00 ± 6.32	5	28.00 ± 13.56	10	16.00 ± 7.77	10	12.00 ± 6.80	5	44.00 ± 16.00	21.33 b
30	5	4.00 ± 4.00	5	20.00 ± 6.32	5	8.00 ± 4.90	10	4.00 ± 2.67	10	4.00 ± 2.67	5	8.00 ± 8.00	8.00 bc
40	-	-	5	8.00 ± 8.00	-	-	5	12.00 ± 12.00	5	0.00 ± 0.00	5	4.00 ± 4.00	6.00 c
Mean**		30.67 a		16.00 a		25.33 a		15.00 a		10.00 a		18.67 a	

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 12. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Beauveria bassiana* S36B6 formulated in oil.

	Treatments																
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Rep.	10^8	Mean
10	5	0.00 ± 0.00	-	-	5	36.00 ± 14.70	5	60.00 ± 16.73	5	80.00 ± 15.49	-	-	44.00 a
20	10	4.00 ± 2.67	5	60.00 ± 14.14	5	8.00 ± 8.00	5	20.00 ± 7.30	5	42.00 ± 13.48	5	32.00 ± 13.56	27.67 b
30	10	6.00 ± 3.05	5	4.00 ± 4.00	5	0.00 ± 0.00	5	12.00 ± 5.33	5	10.00 ± 6.15	5	28.00 ± 14.97	10.00 c
40	5	8.00 ± 8.00	5	12.00 ± 12.00	-	-	5	8.00 ± 4.90	5	0.00 ± 0.00	5	20.00 ± 8.94	9.60 c
Mean**		4.50 a		25.33 a		14.67 a		25.00 a		33.00 a		26.67 a	

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 13. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Beauveria bassiana* Bb2 formulated in oil.

	Treatments											
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Rep.	10^8	Mean	
20	5	4.00 ± 4.00	5	16.00 ±11.67	5	0.00 ± 0.00	5	28.00 ±17.44	5	28.00 ± 4.90			15.20	a
30	5	0.00 ± 0.00	5	4.00 ± 4.00	5	4.00 ± 4.00	5	12.00 ± 8.00	5	16.00 ± 7.48			7.20	ab
40	10	4.00 ± 2.67	10	8.00 ± 8.00	10	6.00 ± 3.05	10	8.00 ± 6.11	10	6.00 ± 3.75			6.40	ab
50	10	4.00 ± 2.67	10	8.00 ± 3.06	10	6.00 ± 4.27	10	0.00 ± 0.00	10	4.00 ± 2.67			4.40	b
60	10	4.00 ± 2.67	10	6.00 ± 3.06	10	6.00 ± 3.05	10	10.00 ± 4.47	10	10.00 ± 4.47			7.20	ab
70	5	4.00 ± 4.00	5	0.00 ± 0.00	5	0.00 ± 0.00	5	4.00 ± 4.00	5	8.00 ± 4.90			3.20	b
Mean**		3.33 a		7.67 a		3.67 a		10.33 a		12.00 a				

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 14. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Beauveria bassiana* Bb4 formulated in oil.

	Treatments										
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Mean	
20	10	22.00 ± 8.14	10	30.00 ± 8.56	10	24.00 ±12.93	10	4.00 ± 4.00	10	24.00 ±10.24	20.80	a
30	10	10.00 ± 3.33	10	14.00 ± 9.91	10	6.00 ± 4.27	10	6.00 ± 4.27	10	2.00 ± 2.00	7.60	b
40	10	16.00 ± 7.18	5	12.00 ± 4.42	10	4.00 ± 2.67	10	6.00 ± 4.27	10	2.00 ± 2.00	8.00	b
50	5	0.00 ± 0.00	5	4.00 ± 4.00	5	8.00 ± 4.90	5	12.00 ± 8.00	5	4.00 ± 4.00	5.60	b
60	5	8.00 ± 4.90	5	4.00 ± 4.00	5	4.00 ± 4.00	5	8.00 ± 8.00	5	0.00 ± 0.00	4.80	b
70	5	4.00 ± 4.00	5	0.00 ± 0.00	5	8.00 ± 4.90	5	4.00 ± 4.00	5	8.00 ± 8.00	4.80	b
Mean**		10.00 a		10.67 a		9.00 a		6.67 a		6.67 a		

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 15. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Beauveria bassiana* 7A formulated in Tween 80 at 0.1%.

	Treatments																
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Rep.	10^8	Mean
10	-	-	5	68.00 ± 18.55	5	44.00 ± 13.26	5	12.00 ± 4.90	5	48.00 ± 8.00	-	-	43.00 a
20	-	-	10	10.00 ± 8.03	5	28.00 ± 18.55	10	12.00 ± 8.00	10	8.00 ± 4.42	5	0.00 ± 0.00	11.60 b
30	-	-	10	0.00 ± 0.00	5	16.00 ± 11.67	10	0.00 ± 0.00	10	12.00 ± 5.33	5	0.00 ± 0.00	5.60 b
40	-	-	5	4.00 ± 4.00	-	-	5	0.00 ± 0.00	5	0.00 ± 0.00	5	0.00 ± 0.00	1.00 b
Mean**		-		20.50 b		29.33 a		6.00 bc		17.00 b		0.00 c	

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 16. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Beauveria bassiana* S2B1 formulated in Tween 80 at 0.1%.

	Treatments										
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Mean
10	-	-	5	0.00 ± 0.00	5	16.00 ± 16.00	5	20.00 ± 10.95	5	0.00 ± 0.00	9.00 a
20	-	-	5	0.00 ± 0.00	5	4.00 ± 4.00	5	4.00 ± 4.00	5	4.00 ± 4.00	3.00 a
30	-	-	5	4.00 ± 4.00	5	12.00 ± 8.00	5	0.00 ± 0.00	5	0.00 ± 0.00	4.00 a
Mean**		-		1.33 a		10.67 a		8.00 a		1.33 a	

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 17. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Beauveria bassiana* S33B4 formulated in Tween 80 at 0.1%.

	Treatments								
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Mean
10	-	-	5	16.00 ± 7.48	5	28.00 ±14.70	5	40.00 ±17.88	5	16.00 ±11.66	25.00 a
20	-	-	5	36.00 ±14.70	5	24.00 ±11.66	5	12.00 ± 8.00	5	12.00 ± 4.90	21.00 a
30	-	-	5	0.00 ± 0.00	5	8.00 ± 8.00	5	8.00 ± 4.90	5	0.00 ± 0.0	4.00 b
Mean**		-		14.14 a		2.83 a		19.80 a		2.83 a	

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 18. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Beauveria bassiana* S36B6 formulated in Tween 80 at 0.1%.

	Treatments								
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Mean
10	-	-	5	0.00 ± 0.00	5	20.00 ± 8.94	5	16.00 ±16.00	5	28.00 ±12.00	16.00 a
20	-	-	5	4.00 ± 4.00	5	0.00 ± 0.00	5	8.00 ± 4.90	5	4.00 ± 4.00	4.00 b
30	-	-	5	0.00 ± 0.00	5	0.00 ± 0.00	5	4.00 ± 4.00	5	0.00 ± 0.00	1.00 b
Mean**		-		1.33 a		6.67 a		9.33 a		10.67 a	

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 19. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Metarhizium flavoviride* SP8 formulated in oil.

	Treatments																
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Rep.	10^8	Mean
10	5	24.00 ± 7.48	-	-	5	8.00 ± 4.90	5	24.00 ± 11.66	5	28.00 ± 10.20	-	-	21.00 a
20	10	10.00 ± 4.47	5	8.00 ± 8.00	5	8.00 ± 4.90	10	20.00 ± 10.33	10	8.00 ± 6.11	5	0.00 ± 0.00	9.00 b
30	10	2.00 ± 2.00	5	4.00 ± 4.00	5	4.00 ± 4.00	10	2.00 ± 2.00	10	0.00 ± 0.00	5	0.00 ± 0.00	2.00 b
40	5	0.00 ± 0.00	5	4.00 ± 4.00	-	-	5	0.00 ± 0.00	5	8.00 ± 4.90	5	0.00 ± 0.00	2.40 b
Mean**		9.00 a		5.33 a		6.67 a		11.50 a		11.00 a		0.00 a	

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 20. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Metarhizium flavoviride* SP2 formulated in oil.

	Treatments									
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^6	Rep.	10^7	Rep.	10^8	Mean
20	-	-	5	36.00 ± 7.48	5	20.00 ±12.65	5	48.00 ±17.44	5	48.00 ±14.97	39.20 a
30	-	-	5	4.00 ± 4.00	5	20.00 ± 8.94	5	8.00 ± 4.90	5	8.00 ± 4.90	12.00 b
40	-	-	5	4.00 ± 4.00	5	8.00 ± 4.90	5	0.00 ± 0.00	5	4.00 ± 4.00	3.20 b
Mean**		-		14.67a		16.00 a		18.67 a		15.00 a	

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 21. Mean cumulative percent mortality (± SE) of *Spinotarsus caboverdus* exposed to various moisture levels in soil inoculated with various dosages of conidia of *Metarhizium flavoviride* SP8 formulated in Tween 80 at 0.1%.

	Treatments										
Moist.	Rep.*	UNTR	Rep.	CO	Rep.	10^5	Rep.	10^6	Rep.	10^7	Mean
10	-	-	5	4.00 ± 4.00	5	8.00 ± 4.90	5	8.00 ± 4.90	5	4.00 ± 4.00	6.00 a
20	-	-	5	4.00 ± 4.00	5	0.00 ± 0.00	5	0.00 ± 0.00	5	12.00 ± 4.90	4.00 a
30	-	-	5	4.00 ± 4.00	5	0.00 ± 0.00	5	0.00 ± 0.00	5	4.00 ± 4.00	2.00 a
Mean**		-		4.00 a		2.67 a		2.67 a		6.67 a	

* Each replication is comprised of five millipedes per cup.

** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 22. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Beauveria bassiana* 7A formulated in oil.

Dose	Rep*	Mean**	SE
C	5	0.00 a	0.00
CO	15	2.70 a	1.82
10^3	5	4.00 a	4.00
10^4	5	0.00 a	0.00
10^5	15	6.70 a	2.52
10^6	10	0.00 a	0.00
10^7	0	4.00 a	2.67

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 23. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Beauveria bassiana* S2B1 formulated in oil.

Dose	Rep*	Mean**	SE
C	5	0.00 a	0.00
CO	15	1.33 a	1.33
10^3	5	0.00 a	0.00
10^4	5	4.00 a	4.00
10^5	15	8.00 a	3.27
10^6	10	8.00 a	4.42
10^7	10	2.00 a	2.00

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 24. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Beauveria bassiana* S33B4 formulated in oil.

Dose	Rep*	Mean**	SE
C	5	4.00 a	4.00
CO	15	2.66 a	1.82
10^3	5	0.00 a	0.00
10^4	5	0.00 a	0.00
10^5	15	1.33 a	1.33
10^6	10	0.00 a	0.00
10^7	10	6.00 a	3.05

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 25. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Beauveria bassiana* S36B6 formulated in oil.

Dose	Rep*	Mean**	SE
C	5	4.00 a	4.00
CO	10	8.00 a	4.42
10^3	5	0.00 a	0.00
10^4	5	0.00 a	0.00
10^5	10	10.00 a	6.15
10^6	5	12.00 a	4.90
10^7	5	0.00 a	0.00

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 26. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Beauveria bassiana* Bb2 formulated in oil.

Dose	Rep*	Mean**	SE
C	5	0.00 a	0.00
CO	5	16.00 a	11.66
10^4	5	8.00 a	4.90
10^5	5	8.00 a	4.90
10^6	5	4.00 a	4.00

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 27. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Beauveria bassiana* Bb4 formulated in oil.

Dose	Rep*	Mean**	SE
C	10	10.00 a	4.27
CO	10	8.00 a	4.47
10^4	10	6.00 a	6.15
10^5	10	10.00 a	6.83
10^6	10	10.00 a	4.42

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 28. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Beauveria bassiana* 7A formulated in Tween 80 at 0.1%.

Dose	Rep*	Mean**	SE
CT	10	0.00 a	0.00
10^3	5	0.00 a	0.00
10^4	5	8.00 a	4.90
10^5	10	2.00 a	2.00
10^6	5	0.00 a	0.00
10^7	5	8.00 a	8.00

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 29. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Beauveria bassiana* S2B1 formulated in Tween 80 at 0.1%.

Dose	Rep*	Mean**	SE
CT	5	4.00 a	4.00
10^3	5	0.00 a	0.00
10^4	5	0.00 a	0.00
10^5	5	4.00 a	4.00

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 30. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Beauveria bassiana* S33B4 formulated in Tween 80 at 0.1%.

Dose	Rep*	Mean**	SE
CT	25	0.00 a	0.00
10^3	25	4.00 a	4.00
10^4	25	0.00 a	0.00
10^5	25	0.00 a	0.00

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 31. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Beauveria bassiana* S36B6 formulated in Tween 80 at 0.1%.

Dose	Rep*	Mean**	SE
CT	5	0.00 a	0.00
10^3	5	0.00 a	0.00
104	5	4.00 a	4.00
10^5	5	0.00 a	0.00

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 32. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Metarhizium flavoviride* SP8 formulated in oil.

Dose	Rep*	Mean**	SE
C	5	4.00 a	4.00
CO	10	10.00 a	3.33
10^3	5	0.00 a	0.00
10^4	5	4.00 a	4.00
10^5	10	10.00 a	4.47
10^6	5	0.00 a	0.00
10^7	5	0.00 a	0.00

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 33. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Metarhizium flavoviride* SP2 formulated in oil.

Dose	Rep*	Mean**	SE
C	10	4.00 a	2.66
CO	10	14.00 a	6.00
10^3	5	4.00 a	4.00
10^4	5	4.00 a	4.00
10^5	10	4.00 a	4.00
10^6	5	12.00 a	8.00
10^7	5	4.00 a	4.00

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 34. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Metarhizium flavoviride* SP8 formulated in Tween 80 at 0.1%.

Dose	Rep*	Mean**	SE
CT	5	0.00 a	0.00
10^3	5	4.00 a	4.00
10^4	5	4.00 a	4.00
10^5	5	0.00 a	0.00

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 35. Mean cumulative percent mortality of *Spinotarsus caboverdus* inoculated with *Metarhizium flavoviride* SP2 formulated in Tween 80 at 0.1%.

Dose	Rep*	Mean**	SE
C	5	0.00 a	0.00
CT	5	0.00 a	0.00
10^3	5	8.00 a	8.00
10^4	5	4.00 a	4.00
10^5	5	0.00 a	0.00

* Each replication is comprised of five millipedes per cup.
** Means followed by the same letters are not significantly different at the 0.05 level (based on Student Newman Keul's Test).

Table 36. Mean cumulative percent mortality of *Spinotarsus caboverdus* following exposure to inoculated potato baits with *Beauveria bassiana* 7A formulated in oil.

Dose	N	Mean	SE
C	25	4.00	3.92
CO	25	16.00	7.33
10^5	25	8.00	5.43
10^6	25	20.00	8.00
10^7	25	12.00	6.50

Tablel 37. Probit analysis of *Beauveria bassiana* 7A effects on *Spinotarsus caboverdus*.

Analysis of Maximum Likelihood Estimates				
Variable	Parameter Estimates	Standard Error	χ^2	p
Intercept	-1.03	0.30	11.96	0.0005
Log(dose)	-0.01	0.06	0.05	0.8318

Table 38. Mean cumulative percent mortality of *Spinotarsus caboverdus* following exposure to inoculated potato baits with *Beauveria bassiana* S2B1 formulated in oil.

Dose	N	Mean	SE
CO	25	24.00	8.54
10^5	25	36.00	9.60
10^6	25	32.00	9.33
10^7	25	52.00	9.99

Table 39. Probit analysis of *Beauveria bassiana* S2B1 effects on *Spinotarsus caboverdus*.

Analysis of Maximum Likelihood Estimates				
Variable	Parameter Estimates	Standard Error	χ^2	p
Intercept	-1.24	0.46	7.18	0.0074
Log(dose)	-0.14	0.10	2.81	0.0938

Table 40. Mean cumulative percent mortality of *Spinotarsus caboverdus* following exposure to inoculated potato baits with *Beauveria bassiana* S33B4 formulated in oil.

Dose	N	Mean	SE
CO	25	12.00	6.50
10^5	25	32.00	9.33
10^6	25	28.00	8.98
10^7	25	4.00	3.92

Table 41. Probit analysis of *Beauveria bassiana* S33B4 effects on *Spinotarsus caboverdus*.

Analysis of Maximum Likelihood Estimates				
Variable	Parameter Estimates	Standard Error	χ^2	p
Intercept	-1.60	0.52	9.48	0.0021
Log(dose)	0.03	0.10	0.11	0.7406

Table 42. Mean cumulative percent mortality of *Spinotarsus caboverdus* following exposure to inoculated potato baits with *Beauveria bassiana* S36B6 formulated in oil.

Dose	N	Mean	SE
C	25	4.00	3.92
CO	25	8.00	5.43
10^5	25	12.00	6.50
10^6	25	12.00	6.50
10^7	25	20.00	8.00

Table 43. Probit analysis of *Beauveria bassiana* S36B6 effects on *Spinotarsus caboverdus*.

Analysis of Maximum Likelihood Estimates				
Variable	Parameter Estimates	Standard Error	χ^2	p
Intercept	-2.55	0.74	12.02	0.0005
Log(dose)	0.13	0.13	1.10	0.3031

Table 44. Mean cumulative percent mortality of *Spinotarsus caboverdus* following exposure to inoculated potato baits with *Beauveria bassiana* Bb2 formulated in oil.

Dose	N	Mean	SE
C	25	4.00	3.92
CO	25	4.00	3.92
10^4	25	20.00	8.00
10^5	25	24.00	8.54
10^6	25	16.00	7.33

Table 45. Probit analysis of *Beauveria bassiana* Bb2 effects on *Spinotarsus caboverdus*.

Analysis of Maximum Likelihood Estimates				
Variable	Parameter Estimates	Standard Error	χ^2	p
Intercept	-2.70	0.75	12.83	0.0003
Log(dose)	-0.25	0.15	2.60	0.1073

Table 46. Mean cumulative percent mortality of *Spinotarsus caboverdus* following exposure to inoculated potato baits with *Beauveria bassiana* Bb4 formulated in oil.

Dose	N	Mean	SE
C	25	8.00	5.43
CO	50	10.00	4.24
10^4	25	0.00	0.00
10^5	50	12.00	4.60
10^6	50	6.00	3.66
10^7	25	12.00	6.50

Table 47. Probit analysis of *Beauveria bassiana* Bb4 effects on *Spinotarsus caboverdus*.

Analysis of Maximum Likelihood Estimates				
Variable	Parameter Estimates	Standard Error	χ^2	p
Intercept	-2.33	0.48	23.78	0.0001
Log(dose)	-0.01	0.10	0.01	0.9100

Table 48. Mean cumulative percent mortality of *Spinotarsus caboverdus* following exposure to inoculated potato baits with *Metarhizium flavoviride* SP8 formulated in oil.

Dose	N	Mean	SE
C	25	0.00	0.00
CO	25	20.00	8.00
10^5	25	8.00	5.43
10^6	25	8.00	5.43
10^7	25	8.00	5.43

Table 49. Probit analysis of *Metarhizium flavoviride* SP8 effects on *Spinotarsus caboverdus*.

Analysis of Maximum Likelihood Estimates				
Variable	Parameter Estimates	Standard Error	χ^2	p
Intercept	-1.41	0.50	8.10	0.0045
Log(dose)	-0.17	0.11	2.43	0.1190

Table 50. Mean cumulative percent mortality of *Spinotarsus caboverdus* following exposure to inoculated potato baits with *Metarhizium flavoviride* SP8 formulated in oil.

Dose	N	Mean	SE
C	25	0.00	0.00
CO	25	20.00	8.00
10^5	25	8.00	5.43
10^6	25	12.00	6.50
10^7	25	4.00	3.92

Table 51. Probit analysis of *Metarhizium flavoviride* SP2 on *Spinotarsus caboverdus*.

Analysis of Maximum Likelihood Estimates				
Variable	Parameter Estimates	Standard Error	χ^2	p
Intercept	-1.37	0.49	7.79	0.0053
Log(dose)	-0.18	0.11	2.78	0.0953