MONTANA STATE UNIVERSITY

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
Montana State University
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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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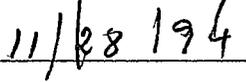
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ABSTRACT

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*.

No.	Site	Date	Dilution A			Dilution B		
			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	Figueiral	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.