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Laboratory and field efficacy of entomopathogenic fungi for the management of the sweetpotato weevil, *Cylas formicarius* (Coleoptera: Brentidae)

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ABSTRACT

The sweetpotato weevil, *Cylas formicarius* (F.) (Coleoptera: Brentidae), is one of the most important pests of sweet potatoes in the world. With free trade between the United States and the U.S.-controlled Mariana Islands, *C. formicarius* has spread along with this commodity. Because of the cryptic nature of the larvae and nocturnal activity of the adults, and the cancellation of long-residual pesticides, this pest has become increasingly difficult to control. Therefore, the present study sought to explore and to compare the effectiveness of *Metarhizium brunneum* F52 (90 ml a.i./ha), *Beauveria bassiana* GHA (40 ml a.i./ha), spinosad (90 g a.i./ha), azadirachtin (1484 ml a.i./ha), *B. bassiana* + *M. brunneum* (20 ml a.i./ha + 45 ml a.i./ha), *B. bassiana* + azadirachtin (20 ml a.i./ha + 742 ml a.i./ha), *B. bassiana* + spinosad (20 ml a.i./ha + 45 ml a.i./ha), *M. brunneum* + azadirachtin (45 ml a.i./ha + 742 ml a.i./ha) and *M. brunneum* + spinosad (45 ml a.i./ha + 45 grams a.i./ha) in controlling this pest in both the laboratory and the field. The treatment with *B. bassiana* + *M. brunneum* was the most effective in reducing tuber damage by *C. formicarius*, producing the highest yields. The most adult cadavers were found in plots treated with the combination of two fungi. This combined fungal formulation appears to be appropriate for the practical control of *C. formicarius* on sweet potatoes.

1. Introduction

The sweetpotato weevil, *Cylas formicarius* (F.) (Coleoptera: Brentidae), is the most destructive insect affecting tropical and subtropical production of sweet potato (*Ipomoea batatas* (L.) Lam., Convolvulaceae) (Chalfant et al., 1990), attacking sweet potatoes both in the field and in storage (Sherman and Tamashiro, 1954). The production of terpene in the stored roots in response to tunneling by *C. formicarius* larvae imparts a bad odor, a bitter taste and leaves the sweet potatoes ranging from unpalatable to inedible (Ray and Ravi, 2005; Uritani et al., 1975). The infestation normally spreads from old sweet potato gardens, through the cuttings used for planting (Sutherland, 1986). The weevil population is greatest at the start of the dry season as high temperatures crack the surface of the soil, thereby exposing the tubers (Talekar, 1982). Larvae generally cannot move through the soil but can easily enter into the soil cracks to reach the tubers (Cockerham et al., 1954).

In addition to *I. batatas*, the major host plant of *C. formicarius* (Chalfant et al., 1990), at least 49 other members of the Convolvulaceae have been recorded as hosts for *C. formicarius*, which has been recorded feeding on seven genera in six tribes within this plant family (Austin et al., 1991). In Guam and other Micronesian Islands, the Aiea Morning Glory, *Ipomoea triloba* L. (Convolvulaceae), is widespread and serves as an alternative host for *C. formicarius* (Reddy et al., 2012b). Because of the cryptic nature of the larvae and the nocturnal activity of the *C. formicarius* adults, it is becoming difficult to control this pest using chemicals. Additionally, the life history of *C. formicarius* make the pest easiest to control with long residual pesticides that are now out of favor and often unavailable. Recently, Leng and Reddy (2012) reported several low-risk insecticides such as spinosad and azadirachtin to be effective against *C. formicarius* in a laboratory study, but their effectiveness was not tested in the field. Our previous studies dealing with pheromone-baited traps have also shown promise for monitoring this pest (Reddy et al., 2012a), and mass trapping techniques

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have been shown to reduce damage caused by *C. formicarius* (Reddy et al., 2014). Sweet potatoes are mainly grown on the island of Rota and exported to other neighboring islands. Since there are no quarantine restrictions to the movements of sweet potatoes among the Mariana Islands (Guam and Northern Mariana Islands of Saipan, Rota and Saipan), *C. formicarius* is spreading to new areas.

The larvae and adults of *C. formicarius* are susceptible to many natural enemies such as parasitoids, predators, and pathogens (Jansson, 1991). In particular, the fungal pathogens *Beauveria bassiana* and *Metarhizium brunneum* (a taxon in the *Metarhizium anisopliae* species complex) (Ascomycota: Hypocreales) have commonly been observed to attack *C. formicarius* (Jansson, 1991) and other *Cylas* species (Ondiaka et al., 2008). Entomopathogenic fungi such as those from the *M. anisopliae* and *B. bassiana* species complexes are currently being used to control agricultural and forest pests worldwide (Butt et al., 2001). These fungi are registered in the USA, as well as in many other countries, as biopesticides (Kabaluk et al., 2010). Such microbial biopesticides are sustainable in IPM programs because of their active relationship with insects. In some cases, compatible products may be combined with entomopathogenic fungi to increase control, to decrease the amount of insecticides required, and to minimize the risks of environmental pollution and pest resistance (Quintela and McCoy, 1998). Nonetheless, the efficacy of some fungi as a biological control agents can be reduced by unfavorable temperature and humidity (Yasuda et al., 1997). However, the hot and humid conditions of sweet potato fields in Guam and other Micronesian Islands are favorable for the use of *B. bassiana* and *M. anisopliae*.

In this study, various fungal entomopathogens were tested individually and in combination along with several effective, low risk insecticides such as azadirachtin and spinosad (Leng and Reddy, 2012), for their laboratory and field efficacy against *C. formicarius*.

2. Materials and methods

2.1. Insect rearing

Pheromone lures consisting of rubber septa loaded with *Z*3-dodecenyl-*E*2-butenoate, sealed in an impermeable bag for shipping and storage, were obtained from Chem Tica Internacional S.A. (San José, Costa Rica). Pherocon unitraps (Trécé Incorporated, Adair, Oklahoma, USA) baited with these lures were used to trap adult *C. formicarius* in sweet potato fields in Latte Heights (Guam, USA) during 2010. The trapped adults were taken to the laboratory, placed in batches in collapsible cages (12 × 10 × 10 cm), fed leaves and pieces of the sweet potato, and maintained at 22 ± 2 °C, 70–80% relative humidity and a 16:8 h L:D photoperiod. Approximately 5–6 generations were completed before using the offspring for experiments. For all experiments, 3–4 week old adults were obtained from these laboratory colonies (Gadi and Reddy, 2014).

2.2. Fungi and other chemicals

Conidia of *B. bassiana* strain GHA were supplied as an unformulated technical grade powder by Laverlam International (Butte, Montana, USA). The conidial titer was 1.6×10^{11} conidia/g and viability was 98%, based on conidial germination in the laboratory on potato dextrose yeast extract agar after incubation for 18 h at 27 °C. Cultures of *M. brunneum* F52 (a commercialized isolate previously identified as *M. anisopliae*) were obtained from Novozymes Biologicals Inc. (Salem, Virginia, USA). Conidial powders were stored dry at 4–5 °C until formulation and use. The chemicals used in the present study – azadirachtin (Aza-Direct) and spinosad – were obtained as shown in Table 1.

2.3. Laboratory tests

Laboratory tests were carried out from 12 September to 15 October 2013 with the hypothesis that the chemicals we tested, when topically applied, would exhibit contact toxicity to *C. formicarius* adults (Table 1). For each replicate, 10 adults were transferred to a disk of Whatman No. 1 filter paper (9 cm diam, Whatman® quantitative filter paper, ashless, Sigma–Aldrich, St. Louis, Missouri, USA) in a 9 cm disposable Petri dish.

Each dish received a 10-g piece of sweet potato and two 7 cm sweet potato branches with leaves (4–8) as food for the insects. Five replicate (prepared at separate times using different cultures and batches of insects) Petri dishes of 10 adults were sprayed (Household Sprayer, Do It Best Corp., Ft. Wayne, Indiana, USA) with 0.5 mL of its assigned treatment (Leng and Reddy, 2012). Two control treatments were maintained; in one, the dishes were sprayed with 0.5 mL of tap water, and in the other, no treatment was applied. Following applications, dishes were maintained under laboratory conditions (previously described), and adult mortality was assessed at 24, 48, 72–96, 120–144, and 168–192 h after treatment.

2.4. Field experiments

Identical trials were conducted at the University of Guam Agricultural Experiment Stations at Yigo (N13°31.930′ E144°52.351′) in northern Guam and at the Inarajan Experiment Station (N13°61.963′ E144°45.353′) in southern Guam from October 01, 2013 to January 30, 2014.

2.4.1. Plot design and treatment procedures

Treatment plots measuring 6 × 6 m were arranged in a randomized block design and separated from other plots by 1 m buffer zones to prevent any treatment effect. Sweet potato cuttings of the variety IB 195 (Kuma 2) known to be highly susceptible to *C. formicarius* damage (Nandawani and Tudela, 2010) were transplanted into rows 80 cm apart with 30 cm between plants within each row. Each treatment was replicated three times, for a total of 33 individual plots. Each plot consisted of 12 rows of 15 sweet

Table 1
Material and rate of application in each treatment. Spray volume 94 L/ha.

Treatment	Material	Rate (active ingredient)	Source
C	Control (water spray)	–	–
T1	*Metarhizium brunneum* F52 emulsifiable concentrate (Met 52 EC)	90 ml/ha	Novozymes Biologicals (Salem, VA).
T2	*Beauveria bassiana* GHA emulsifiable concentrate (BotaniGard ES)	40 ml/ha	Laverlam International Corporation, Butte, MT
T3	spinosad (Conserve SC®)	90 g/ha	Dow Agro Science LLC, Indianapolis, IN
T4	azadirachtin/Aza-Direct®	1484 ml/ha	Gowan Company, Yuma, AZ
T5	*B. bassiana* + *M. brunneum*	20 ml/ha + 45 ml /ha	As stated above
T6	*B. bassiana* + azadirachtin	20 ml/ha + 742 ml/ha	As stated above
T7	*B. bassiana* + spinosad	20 ml/ha + 45 g/ha	As stated above
T8	*M. brunneum* + azadirachtin	45 ml/ha + 742 ml/ha	As stated above
T9	*M. brunneum* + spinosad	45 ml/ha + 45 g/ha	As stated above