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Evaluation of *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces fumosoroseus* as Entomopathogens of the Cactus Moth, *Cactoblastis cactorum* (Lepidoptera:Pyralidae)

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ABSTRACT

The three fungal pathogens *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae), *Paecilomyces fumosoroseus* (Wize) Brown & Smith (Deuteromycotina: Hyphomycetes), and *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Clavicipitaceae) were evaluated as potential biological control agents against the cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae). The entomopathogens, *M. anisopliae* and *P. fumosoroseus*, tested against the cactus moth eggs did not infect the eggs. The chorion may serve as protective covering for the eggs that prevents infection. However, *C. cactorum* was found to be a suitable host for both *M. anisopliae* and *B. bassiana*. Mean (± SE) conidial germination was 95.6 ± 0.5% for *M. anisopliae* and 91.6 ± 0.7% for *B. bassiana*. The fungus *M. anisopliae* was highly pathogenic to 1st instar larvae of cactus moth. The relative virulence at LC_{50} of *M. anisopliae* as compared to *B. bassiana* was over 1,000-fold greater at 7-, 14-, and 21-d post -treatments. A total of 289 dead cactus moths collected from the treatment groups were investigated for fungal infection, and 98% of them showed mycosis at the end of 21 d of the experiments. Cadavers from the controls showed no fungal growth at the end of the experimental period. The greater pathogenicity found for *M. anisopliae* suggests this fungus could provide new avenues for the biological control of the cactus moth, targeting mainly the 1st instar larvae, and may complement current control strategies.

Additional Key Words: biological control agent, pathogenicity, fungal pathogens, cactus, *Opuntia* spp.

The cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) continues to be of major concern because of its unabated expansion into the southeastern United States, thereby threatening the cactus industries in Mexico and the southwestern United States (Irish 2001, Soberón et al. 2001, Hight et al. 2002; Solis et al. 2004; Zimmermann et al. 2004). Since the initial reports in south Florida in 1989 (Mahr 2001), the cactus moth has expanded its geographical distribution along both the Gulf and Atlantic Coasts. *C. cactorum* has been reported as far west as Louisiana (LSU 2009), and as far north as Bull Island, South Carolina (Hight and Carpenter 2009). Genetic analysis suggests the moth has been introduced into the United States at least twice (Simonsen et al. 2008). *C. cactorum* has also been found in Isla Mujeres, Mexico (Legaspi and Legaspi 2008, LSU 2009). Recent studies on the biology and pheonology of *C. cactorum* were reported by Legaspi and Legaspi 2007, Legaspi et al. 2009a, 2009b, Hight and Carpenter 2009, and Raghu and Walton 2007.

Pest control strategies against *C. cactorum* have centered on the removal of egg sticks (Zimmermann et al. 2000), the use of insecticides (Leibee and Osborne 2001; Bloem et al. 2005), pheromone traps (Heath et al. 2006), the sterile insect technique (Carpenter et al.

2001a, 2001b; Hight et al. 2005; Tate et al. 2007) and the use of sterile insects to trap males (Bloem et al. 2003). Biological control is currently being considered as a possible control option (Stiling 2002; Legaspi and Legaspi 2008). Herein, we evaluated the fungal pathogens *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae), *Paecilomyces fumosoroseus* (Wize) Brown & Smith and *Beauveria bassiana* (Bals.-Criv.) Vuill. as biological control agents against *C. cactorum* eggs and 1st instars through laboratory bioassays.

MATERIALS AND METHODS

Eggs and first-intar larvae of the cactus moth were obtained from a colony reared on fresh cactus pads, sp. *Opuntia ficus-indica* (L.) Miller at USDA, ARS, CMAVE in Tallahassee, FL. These stages of the cactus moth were used in the laboratory bioassay because they are found outside the cactus pad in natural conditions (JCL, personal observations; Legaspi et al. 2009b). These are stages of the cactus moth that are likely to be exposed and most vulnerable to entomopathogens (Zimmermann et al. 2004; Lozano and España 2008; Legaspi and Legaspi 2008). We used 10 eggs (8-10 day old) per egg stick or three 1st-instar larvae (1-d old) in each clear plastic cup (30 ml) covered with a cardboard lid (Solo, Inc., Highland Park, IL). Each cup represented a replicate and there were 5-10 replicates per fungal concentration. The experiments were repeated on 3-4 different dates. *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* were sprayed on the egg sticks while *Metarhizium anisopliae* and *Beauveria bassiana* were sprayed on the larvae.

To determine the pathogenicity of *Metarhizium anisopliae, Paecilomyces fumosoroseus,* and *Beauveria bassiana* against immatures of the cactus moth, we cultured the fungi on Petri plates (10.0 cm x 1.5 cm) containing Sabouraud maltose agar (Difco, Detroit, MI) supplemented with 1% yeast (SMAY), and incubated at 27 ± 1°C, 85 % RH, and 13:11 (L:D) h photoperiod. Conidia from 10-14-day-old cultures were harvested with a camel-hair brush, and spore concentrations were determined using a hemocytometer (Kanga et al. 2002). The fungi were serially diluted in sterile deionized water containing 0.01 % Silwet L-77 (Loveland Industries, Greely, CO) to provide the concentrations needed for the bioassays.

Isolates of the fungi were tested at different concentrations of 10^4, 10^5, 10^6, 10^7, and 10^8 conidia per ml. For each concentration, fifteen 1st-instar cactus moth larvae were transferred to a glass Petri plate lined with wet Whatman filter paper (90 mm diameter). The glass Petri plates containing the larvae were placed on top of ice cubes just before spraying. The insects were sprayed with 1 ml of the conidial suspension using a Potter Precision Spray Tower (Burkhard Manufacturing, Rickmansworth, England) with 0.7 kg cm-2 pressure and a 0.25 mm orifice diameter nozzle. Larvae treated with deionized water containing 0.02% Silwet L-77® served as controls.

After the treatments, the larvae were transferred to individual plastic cups (30 ml) and covered with a cardboard lid. A slice of fresh cactus pad was provided as a food source for the larvae and were replaced every 2-3 days. The cups were held in 27 ± 2 ºC, 85% RH, and 13:11 (L:D) h photoperiod in a Percival Scientific Incubator (auto-regulated relative humidity and lighting) (Percival Manufacturing Company, Boone, Iowa). Mortality was recorded daily for 21 d and the data were subjected to Probit analysis to generate dose -mortality regression lines, and the LC_{50} values using POLO-PC software (LeOra Software, Petaluma, CA) (LeOra Software 1987; Russell et al. 1977).

To determine conidia viability at the time of each experimental run, each concentration of fungal suspension was sprayed onto 3 Petri dishes containing SMAY (Kanga et al. 2004). The conidia were incubated for 20 h at 27 ± 1 ºC, 85 % RH. After incubation, 3 droplets of lactophenol cotton blue stain (0.5% cotton blue) were added to each Petri dish to fix and stain the conidia, preventing any further germination from occurring in the sample. The droplets were covered with a glass slide and evaluated using 400X phase-contrast magnification. The number of conidia that germinated in the first 100 conidia observed under the microscope was determined for each of the 3 droplets on each slide.

Dead cactus moth larvae were collected daily from the fungal treatments and the controls, and tested in the following way to determine if mortality was due to infection. The cadavers were surface-sterilized by dipping them successively in 65-70% ethanol (10-15 min), 2% sodium hypochlorite solution (2-3 min), and sterile water (20-40 s). They were then transferred with a camel-hair brush to Petri dishes containing SMAY and incubated at 27 ± 1 ºC, 85 % RH for 7-14 d. The Petri dishes were sealed with parafilm before incubation and the dead larvae were observed daily for the presence of external fungal hyphae. Numbers of dead cactus moth larvae with external hyphae were counted, and to reduce the possibility of cross contamination, these insects were removed from the Petri dishes. Only cactus moth larvae that showed fungal growth were considered to have died from infection and used to compute the pathogenicity of the fungal pathogens.

RESULTS

Cactus moth eggs were not susceptible to *M. anisopliae* and *P. fumosoroseus* entomopathogens because all the eggs hatched 21 d after the start of the experiment. However, *C. cactorum* was found to be a suitable host for both *M. anisopliae* (Fig. 1), and *B. bassiana* (Fig. 2). Mean (± SE) conidial germination was 95.6 ± 0.5% for *M. anisopliae* and 91.6 ± 0.7% for *B. bassiana*. The fungus *M. anisopliae* was highly pathogenic to 1st instar larvae of cactus moth larvae. The relative virulence at LC_{50} of *M. anisopliae* as compared to *B. bassiana* was over 1,000-fold greater at 7-, 14-, and 21-d post-treatments (Table 1). A total of 289 dead cactus moths collected from the treatment groups were investigated for fungal infection, and 98% of them showed mycosis at the end of 21 d of the experiments. Cadavers from the controls showed no fungal growth at the end of the experimental period.

DISCUSSION

The use of insect pathogens as biological control agents against *C. cactorum* was summarized by Pemberton and Cordo (2001a). High levels of insect mortality by fungal pathogens *Beauveria* spp. (Hypocreales: Clavicipitaceae) were reported in Australia (Dodd 1940), but only low levels in South Africa (Pettey 1948). Two species of the microsporidian *Nosema* spp. (Microsporida: Nosematidae) were described from *C. cactorum* in South Africa (Fantham 1939). One of these species, *N. cactoblastis* Fantham, caused up to 100% mortality in some areas of South Africa (Pettey 1948). Pemberton and Cordo (2001b) conducted surveys for *Nosema* spp. in South Africa and Argentina; however, no *Nosema* were collected from South Africa and only low levels of infection were found in larvae from Argentina (0 – 6%). The authors attributed low infection levels to time of collection and low host abundance. The cactus moth also has been found to be susceptible to nuclear polyhedrosis virus isolated from *Autographa californica* (Speyer) (Lepidoptera: Noctuidae) (Vail et al. 1984). The ineffectiveness of both *M. anisopliae* and *P. fumosoroseus* against *C. cactorum* eggs may be at least partially attributed to the chorion surrounding the egg that may serve as protective covering that prevented infection. Nevertheless, the egg stage may be more successfully attacked through predation by ants or parastism by *Trichogramma* spp. (Robertson 1988; Legaspi and Legaspi 2008).

Beauveria bassiana was demonstrated to cause 100% mortality in the white grub, *Laniifera cyclades* Druce (Lepidoptera: Pyralidae) in greenhouse and *Opuntia* cactus field experiments in Mexico (Lozano and España 2008). The fungus was applied by introducing infected *Galleria mellonella* L. (Lepidoptera: Pyralidae) cadavers through orifices in

Table 1. Virulence of *Metarhizium anisopliae* and *Beauveria bassiana* against *Cactoblastis cactorum* larvae.

Fungal isolate	N[3]	Slope SE	LC_{50} (95% CL)[4]	LC_{90} (95% CL)[4]	χ^2
After 7 d					
M. anisopliae	300	1.18 ± 0.15	0.36 (0.16 – 0.66)	25.69 (11.15 – 91.07)	15.63
B. bassiana	150	0.59 ± 0.33	19.54 (9.03 – 31.71)	107.478 (21.67 – 373.30)	2.43
After 14 d					
M. anisopliae	300	1.03 ± 0.15	0.17 (0.06 – 0.37)	23.04 (9.19 – 98.06)	11.98
B. bassiana	150	0.69 ± 0.20	3.89 (0.01 – 13.06)	54.49 (11.96 – 149.82)	4.97
After 21 d					
M. anisopliae	150	0.66 ± 0.18	0.03 (0.002 – 0.194)	61.16 (10.50 – 104.21)	4.59
B. bassiana	150	0.61 ± 0.19	0.04 (0.004 – 0.389)	177.89 (36.66 – 297.15)	7.41

[3] Number of insects tested.
[4] Concentrations are expressed in conidia per ml X 0^5 for *M. anisopliae* and conidia per ml X 0^{10} for *B. bassiana.*

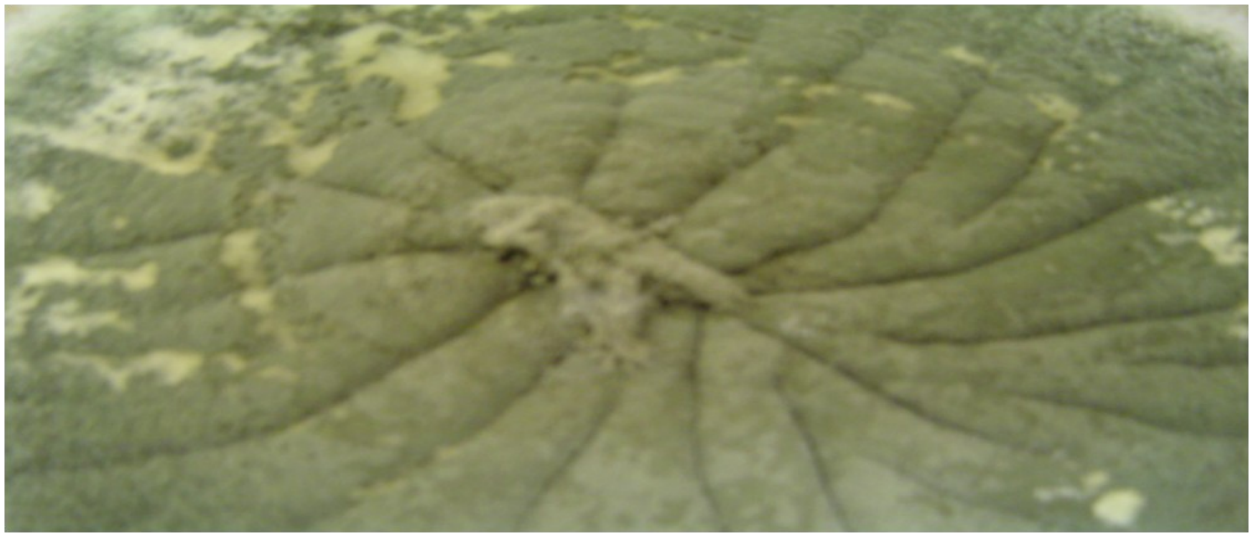

Fig. 1. Mycelia of *Metarhizium anisopliae* emerging from dead cactus moth larvae collected from the treated samples after 10 d incubation at 27 ± 1 ºC , 85 % RH. Larvae were surface-sterilized and plated on SMAY to investigate the recovery of the fungus.

Fig. 2. Cactus moth larvae collected from treated samples is covered with mycelia and conidia of *Beauveria bassiana* after 14 d incubation at 27 ± 1 ºC, 85 % RH. Larvae were surface-sterilized and plated on SMAY to investigate the recovery of the fungus.

the cactus stem pads. Although labor-intensive, the authors suggested the method may be effective against cactus pests, including *C. cactorum. Beauveria bassiana* was also found effective against adult cactus weevils, *Metamasius* (= *Cactophagus*) *spinolae* Gyllenhal (Coleoptera: Curculionidae) in *Opuntia* cactus in the laboratory (Tafoya et al. 2004).

Pemberton and Cordo (2001a) speculated that the fungal pathogen *Entomophaga maimaiga* Humber, Shimazu & Soper (Zygomycetes: Entomophthorales) might be effective against *C. cactorum* based on its success against the Gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). Furthermore, they speculated that *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) and its products also may be effective control agents, as they are commonly employed against Lepidopteran pests.

In summary, *C. cactorum* eggs and 1st instars were targeted for biological control using entomopathogens because these stages have been identified as possible vulnerable life stages due to their protracted durations and exposed environment. *Cactoblastis cactorum* eggs were not infected by *M. anisopliae* nor *P. fumosoroseus.* However, 1st instars were found to be suitable hosts for both *M. anisopliae* and *B. bassiana*. The greater pathogenicity found for *M. anisopliae* suggests this fungus could provide new avenues for the biological control of the cactus moth, targeting mainly the 1st instar larvae, and may complement current control strategies. Future studies on evaluating these entomopathogens against third- and fourth-instar *C. cactorum* larvae are warranted because the latter stages have been observed outside the cactus pads from surveys in its natural habitat (see Legaspi et al. 2009b) as well as in potted cactus plants in the laboratory colony. A comprehensive study of virulence of the fungi has yet to be investigated as well as the assessment of these fungal pathogens for potential biological control of the cactus moth.

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