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The Florida Entomologist, Vol. 55, No. 2. (Jun., 1972), pp. 117-120.

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## TWO MUSCARDINE FUNGI PATHOGENIC TO DIAPREPES ABBREVIATUS

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#### ABSTRACT

In the laboratory, the fungus Metarrhizium anisopliae (Metschnikoff) Sorokin infected 6.7% of the adults and none of the larvae of the so-called sugarcane rootstalk borer weevil, Diaprepres abbreviatus (L.). In contrast, Beauveria bassiana (Balsamo) Vuillemin infected 92.7% of the adults within 7 days and 76.9% of the larvae within 12 days.

The so-called sugarcane rootstalk borer weevil, Diaprepes abbreviatus (L.), an important pest of citrus and sugar cane in the West Indies, was recently found infesting about 2500 acres of citrus in the vicinity of Apopka, Florida (Woodruff 1968). Federal and state agencies immediately began measures to eradicate the weevil from the area with insecticides. Also, a laboratory was set up at Apopka (a satellite of the Humid Areas Citrus Insects Investigations Laboratory at Orlando, Fla.) to investigate alternative methods of controlling this weevil and especially to investigate possible biological control agents.

Jones (1915) and Wolcott (1936, 1948) reported the presence of the green muscardine fungus Metarrhizium anisopliae (Metschnikoff) Sorokin on all stages of D. abbreviatus held in confinement in Puerto Rico, and Wolcott (1948) suggested that the fungus might be effective in the field if conditions were favorable. Also, reports exist of infection of species of Curculionidae by the white muscardine fungus Beauveria bassiana (Balsamo) Vuillemin and by M. anisopliae (Gayer and Benjamin 1971, Crow et al. 1968, Cothran and Gyrisco 1966). In addition, Walstad et al. (1970) and Bell and Hamalle (1970) all studied the effects of these fungi on several species in the laboratory. Tests were therefore made at the Apopka laboratory in 1971 to determine the pathogenicity of the muscardine fungi M. anisopliae and B. bassiana to adults and larvae of D. abbreviatus. The results are reported here.

#### METHODS AND MATERIALS

Adult weevils were collected in the field and held in laboratory cages 2-3 weeks before the tests were made. Larvae (6-8 months old) were reared on potted citrus seedlings, and removed from the soil around the plants 1-2 days before testing. (Before the tests, the larvae were washed in sterile water to remove particles of soil.) The fungi B. bassiana (A-6511) and M. anisopliae (California isolate) were obtained from J. V. Bell, microbiologist, USDA, Charleston, S. Carolina, and cultured on Sabouraud maltose plus 10% yeast extract agar.

The tests were made by inoculating 15 adults and 13-14 larvae with the conidia of each fungus by rolling them in sporulating cultures for 2-3 min; no attempt was made to apply a constant amount of inoclum. Then the

TABLE 1. Percentage Accumulated Infection of Adult and Larval D. abbreviatus INOCULATED WITH SPORES OF B. bassiana (A-6511) OR M. anisopliae (CALIF, ISOLATE) AND HELD AT 100% RH AND 25-27°C.

Treatment	Stage*	% Infected at indicated day posttreatment					
		3	6	7	8	9	12
B. bassiana	Adult Larval	0	46.3	92.7	92.7	92.7 69.3	92.7 76.9
M. anisopliae	Adult Larval	0	6.7	6.7	6.7	6.7 0	6.7 0
Control	Adult Larval	0	0	0	0	0	0

<sup>\*</sup> Larvae examined at 9 and 12 days only

insects exposed to each treatment (and the 15 control adults and 8 control larvae) were placed in separate chambers similar to those described by Doane and Allan (1968). Relative humidity was maintained at 100% by passing air through sterile water (Johnson 1940). Temperature was maintained at 26-27°C. During the holding period, the adults were fed sterilized citrus leaves, and the larvae were held without food in sterile moistened sand in 1/2-pint ice cream cartons. Dead adult weevils were removed from the holding chambers each day, and dead larvae were removed from the sand at 9 and 12 days posttreatment. All dead insects were surface sterilized and reisolation of the fungus was used as a criterion of infection.

#### RESULTS AND DISCUSSION

At 7 days post-inoculation, all adults treated with B. bassiana, but only 20% of those inoculated with M. anisopliae and none of the controls were dead. Also, 92.7% of the adults exposed to B. bassiana demonstrated mycosis vs. only 6.7% of those exposed to M. anisopliae (Table 1). Likewise, larvae inoculated with B. bassiana were 76.9% infected at 12 days posttreatment but no mycosis occurred in larvae treated with M. anisopliae or in the untreated controls.

Fig. 1 shows the vegetative growth that appeared outside the body within 48 hr after death of B. bassiana inoculated adults.

All adult weevils treated with fungus were highly active immediately after inoculation and did not feed for at least 24 hr; the untreated controls began feeding within a short time after they were placed in the holding

The parasitic relationship between B. bassiana isolate A-6511 and the larval and adult stages of D. abbreviatus therefore appears to be well advanced, and this strain may be a good candidate for use in field studies for possible control of the weevil. In contrast, the California isolate of M. anisopliae is apparently only weakly virulent. This strain then is not the same one recorded from D. abbreviatus by Jones (1915) and by Wolcott



Fig. 1. A healthy adult *D. abbreviatus* (left) and an adult infected with *B. bassiana* (right).

(1936, 1948). Pathogenic studies with other strains of *M. anisopliae* may provide a virulent strain that can be considered for use as a biological control agent against *D. abbreviatus*.

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The Florida Entomologist 55(2) 1972

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