EFFECTS OF SAND FORMULATED *METARHIZIUM ANISOPLIAE* SPORES ON LARVAE OF THREE MOSQUITO SPECIES[1]

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ABSTRACT. Larvae of mosquito species displaying different feeding behaviors were bioassayed with 2 formulations, floating and sand, of the spores of the fungus, *Metarhizium anisopliae*. Although both formulations worked equally well after 96 hours of incubation, more rapid mortality was obtained using the sand formulation with below surface feeding larvae while the floating preparation worked faster on surface feeders.

The fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin has received scrutiny as an insect pathogen for over a century, and recently as a possible mosquito control agent (Roberts, 1970, 1974; Roberts and Yendol 1971, Al-Aldroos and Roberts 1978). Limited field trials have shown promise (Ramoska 1980, unpublished; Roberts 1970, Daoust 1980, personal communication).

If we are to realize the full potential of any of the biological insecticides of mosquitoes, the influence of the target species' habits upon the pathogen must be understood and considered in application programs. This paper deals with the interaction of host species displaying different feeding habits with *M. anisopliae* preparations that deliver the fungus to different areas of the host habitat.

METHODS AND MATERIALS

Laboratory colonies of the 3 mosquito species used in this study, *Aedes aegypti* Linn., *Anopheles albimanus* Wiedemann and *Culex quinquefasciatus* Say were reared in the insectary at Kansas State University. Second instar larvae of these species were utilized in all experiments.

The strain of *Metarhizium anisopliae* var. *anisopliae* used was 'E_9' isolated in Brazil and was supplied on silica gel agar by Donald W. Roberts (Boyce Thompson Institute for Plant Research at Cornell University). It was grown on YPSS (Yeast extract, potassium phosphate and soluble starch, Emerson 1941) agar in enameled baking pans. The agar pans were seeded with *M. anisopliae* spores, foil covered and incubated for 2 weeks at 23°C. Spores were harvested by brushing across the agar surface with a small paint brush. Collected spores were dried out for 24 hr on filter paper in darkness and then transferred to glass containers and held at 4.0°C until needed. Prior to use the spores were filtered through a 147 μ mesh screen to reduce clumping.

Bioassays were carried out in disposable plastic cups containing 100 ml demineralized/dechlorinated water. The surface area of the water was 19.6 cm^2 and it was 4 cm deep. Twenty larvae were placed into each cup and the weighed dosage level of *M. anisopliae* was sprinkled onto the surface. Spores were added in a pure form (unformulated) or mixed, without an adhesive, with a sand carrier (sand formulated). The sand formulated mixture was prepared at a rate of 1 gm spores to 100 gm aquarium sand.

The choice of sand over surfactants or other methods of sinking the spores was made because it was felt that this method had the least chance of affecting the surface properties of the spore. The sand formulation insured that the sunken spores were identical in every way to the floating spores. Appropriate control cups were set up and mortality for the experiments was monitored daily for 5 days.

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Larvae were fed 5 drops of 10% ground rabbit chow (Universal Food Corp., Milwaukee, WI) in water immediately after the start of the experiment and daily thereafter. Morbid larvae were examined under phase contrast microscopy at the conclusion of each experiment.

'Skimming' the surface to remove floating spores was accomplished by applying the appropriate treatment prior to adding larvae to the cup, after which a filter paper disc was placed onto the water surface and the floating spores adhered to it. The paper was removed and the side walls of the cup were cleaned with new filter paper. The larvae were then added. This was repeated if spores were still seen on the water surface.

RESULTS AND DISCUSSION

The amount of *M. anisopliae* required to achieve high mortality (> 50%) for *An. albimanus* was considerably less than that required for either the *Culex* or the *Aedes* mosquito (Fig. 1). Because *An. albimanus* is a surface feeder while both *Cx. quinquefasciatus* and *Ae. aegypti* are depth feeders and are usually found well beneath the water-air interface, the difference in susceptibility between the species might be attributable to the fact that *M. anisopliae* spores are hydrophobic by nature and present very high dosage levels at the water's surface where *An. albimanus* are found. The linear dose-response curves for *Cx. quinquefasciatus* and *Ae. aegypti* are the result of either more chance contact with the surface spores as their density increases or due to the relative numbers of submerged spores increasing thus raising the number of spores which could be consumed by the larvae beneath the surface.

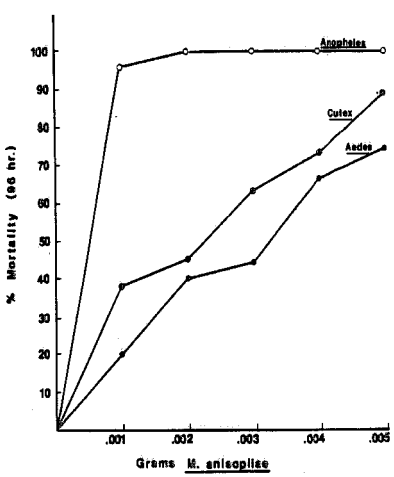

Fig. 1. Dose response curves for 3 species of 2nd instar mosquito larvae (*An. albimanus, Ae. aegypti* and *Cx. quinquefasciatus*) when inoculated with varying dosage levels of unformulated *M. anisopliae* spores.

In order to ascertain if any mortality could be attributed to the latter hypothesis, an experiment involving 'skimming' the water surface of spores was designed. Removal of floating spores from both formulated and unformulated treatments reduced mortality in both *Culex* and *Aedes*, indicating that the dose-response curves as shown in Fig. 1 are a result of both larval contact with floating spores and spores that become submerged.

Removing floating spores from the formulated spore treatments had less effect on the *Culex* and *Aedes* than removing them from unformulated treatments (Table 1). This was due to the greater

Table 1. Influence of sand on *Metarhizium anisopliae* efficacy toward 2nd instar *An. albimanus, Cx. quinquefasciatus* and *Ae. aegypti* larvae.

Treatment (0.5 mg/cm²)	% Mortality* 96 hr posttreatment		
	An. albimanus	*Cx. quinquefasciatus*	*Ae. aegypti*
Unformulated *M. anisopliae*			
unskimmed	100	70	57
skimmed	15	40	7
M. anisopliae in 1 gm sand			
unskimmed	98	75	59
skimmed	31	50	45

* Corrected using Abbott's formula (Finney, 1952).

amount of submerged spores available to the larvae in the formulated treatments, decreasing the effects of the floating spores. Surface material accounted for about 20% mortality in these species. Removal of floating spores accounted for a 67% and 85% decrease in mortality in formulated and unformulated treatments, respectively, in *Anopheles* (Table 1). The differences in mortality exhibited by the skimmed treatments of *Anopheles* would likely have been even greater had the method of surface spore removal been more complete. It is likely that few spores remaining after skimming were responsible for the *Anopheles* mortality observed in the skimmed treatments.

No differences appear in the efficacy between sand formulated and pure spore preparation for the *Culex* and *Aedes* species after 96 hr (Fig. 2). Predictably, *An. albimanus* did exhibit a much higher mortality for the unformulated (floating) preparation than it did for the sand formulation.

One distinct difference did appear between formulations (Fig. 1). Both *Aedes* and *Culex* larvae died faster when subjected to the sand formulation than to the unformulated material although both treatments contained identical dosage levels of *M. anisopliae* spores. All larvae that had died on the first day were examined at the end of the experiment and compared to those that died later in the experiment. The differences are shown in Fig. 3 A, B. Larvae that rapidly suc-

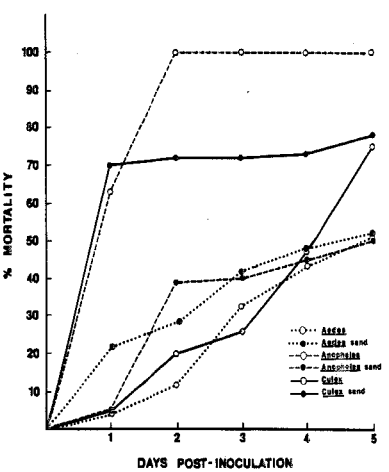

Fig. 2. Differences in response time among 3 species of 2nd instar mosquito larvae fed either a sand formulated preparation of *M. anisopliae* (sand) or unformulated *M. anisopliae* (0.5 mg conidia/cm^2).

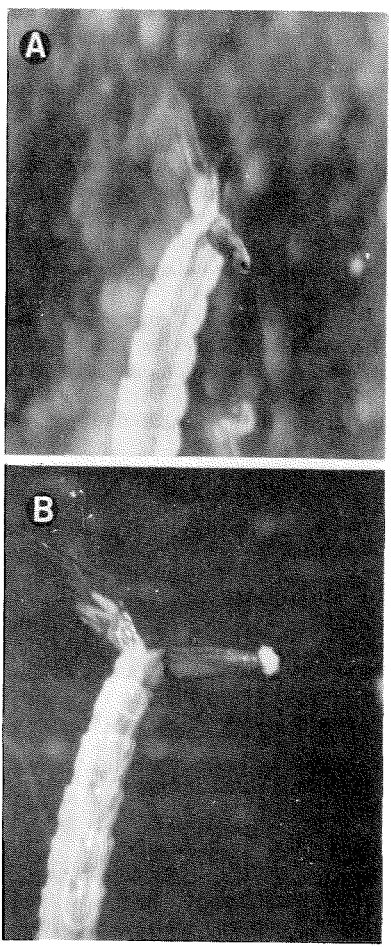

Fig. 3. A. *Culex quinquefasciatus* siphon tube 4 days after death from submerged *M. anisopliae* spores. B. Same species after 4 days incubation with floating spores. Insect died on day 3. (Photo by R. Elzinga.)

cumbed to the fungus did not exhibit mycelial growth at the mouth of the siphon tube (Fig. 3A) while those that died on the second through fifth day did (Fig. 3B). This agrees with Roberts (1970, 1977) who indicated that the fungus can kill larvae by a toxin when consumed or by mechanical action by attaching to the perispiracular valves and growing into the siphon tube. In our experiments those larvae feeding below the surface of the water were likely to consume a greater amount of spores from the sand formulated treatments than those incubated with floating spores. This explains the rapid mortality evident in the *Culex* and *Aedes* tests with formulated *M. anisopliae.* Since *Anopheles* are surface feeders, it follows that they would exhibit rapid mortality when they were incubated with the floating, unformulated spores as indicated in Fig. 2.

In field trials or future commercial utilization of *M. anisopliae,* faster control of the target population may be achieved if the fungus preparation is formulated to match the feeding habits of the target population.

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