THE COMPATIBILITY OF METHOPRENE WITH THE MOSQUITO FUNGUS *CULICINOMYCES CLAVISPORUS*

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Infection of mosquito larvae by *Culicinomyces clavisporus* Couch, Romney, and Rao normally occurs after ingestion of conidia (Sweeney 1975). Laboratory studies on the dose-recycling response of *Culicinomyces* suggest that high concentrations of conidia produce rapid kill of the target population, but little external sporulation on the cadavers (Sweeney 1983). With lower concentrations of conidia, death of larvae is slower and the majority of infected specimens develop external sporulation. Thus, application of the fungus at a lower dose rate may provide enhanced recycling and give a longer period of larval control. However, when the fungus is applied to produce maximum external sporulation a number of test larvae usually survive. It would be useful if another complementary agent was available to kill these survivors.

The insect growth regulator methoprene disrupts mosquito metamorphosis causing mortality in the pupal stage (Schaefer and Wilder 1972, Busvine et al. 1976). Some laboratory studies have combined methoprene with mosquito pathogens and demonstrated an additive effect on larval mortality. These include Finney et al. (1977) with *Romanomermis culicivorax* against *Aedes aegypti* (Linn.) and Spencer and Olson (1982) with *Ascogregarina culicis* against *Aedes epactius* (Dyar and Knab) larvae. Merriam and Axtell (1983) showed that, at recommended application rates, methoprene was compatible with the mosquito fungus *Lagenidium giganteum*. This paper describes laboratory experiments to investigate whether a combination of methoprene and *C. clavisporus* might provide effective initial mortality of the target population and permit recycling of the fungus for long-term larval control.

An isolate of the Australian strain of *C. clavisporus* was used as the source of inoculum for these experiments. It was grown in submerged culture, harvested and stored at −70°C until use by the method of Cooper and Sweeney (1982). Inoculum concentrations were estimated using an "Improved Neubauer" hemocytometer. Viability was measured by 24 hr germination counts of conidia on nutrient agar plates.

The effect of methoprene on germination of *Culicinomyces* conidia was examined using nutrient agar plates containing 5, 50 or 500 ppb methoprene. These were prepared by adding aqueous suspensions of Altosid® (Zoecon experimental formulation FZ–515–2) to media when it had cooled, after autoclaving, to 50°C. Bioassays using 4th instar *Ae. aegypti* larvae demonstrated that prior heating of methoprene to 50°C had no adverse effect on its toxicity. Two plates of each concentration of methoprene and two untreated control plates were inoculated with 5×10^6 conidia of *Culicinomyces*. Germination counts (based on at least 400 spores) were made after 24 hr.

Preliminary bioassays to determine the LC50's for methoprene and *C. clavisporus* were undertaken using early 4th instar larvae of *Ae. aegypti*. Four doses of methoprene (3, 5, 8 and 20 ppb) and one dose of fungus (5×10^3 conidia/ml) were selected for further experiments. The concentrations of methoprene used, although low when compared to field application rates, were chosen to investigate the interaction of the two agents on mortality. For these assays 20 early fourth-instar *Ae. aegypti* larvae were placed in 750 ml white plastic trays ($18 \times 12 \times 5$ cm) containing 200 ml of deionized water. All *Ae. aegypti* used in each assay were randomly selected from a pooled batch of larvae. Serial dilutions with distilled water were made of the thawed fungus concentrate and of the Altosid to give the required concentrations of both agents in the test trays. There were nine treatments per assay; one of fungus alone (5×10^3 conidia/ml); four of methoprene alone (3, 5, 8 and 20 ppb); and four combining the fungal dose of 5×10^3 conidia/ml with each of the methoprene doses used above. Three replicate trays per treatment and five control trays were set up for each assay. All trays were covered with lids to prevent evaporation and held at 25 ± 1°C. The larvae were fed powdered laboratory rat food on day 0 and on every second day thereafter.

During each assay the trays were examined daily. The number of dead larvae, dead pupae and dead pupal-adults (those which died during emergence from the pupal exuvium) were counted and then removed from each tray. The dead larvae and dead pupae were transferred to plastic containers (4.5 cm diam × 6 cm) containing 50 ml of deionized water for 24 hr at 25 ± 1°C. Then they were examined under the light microscope for presence of *C. clavisporus* and recorded for sporulation on the exterior cuticle. Assays were terminated when all test larvae had either died or emerged as adults. Sixteen assays with an average duration of 10–11 days were conducted. All mortalities were corrected for control mortality by Abbott's formula.

Germination counts of *Culicinomyces* conidia on nutrient agar plates impregnated with

methoprene were 98.8% (5 ppb methoprene), 84.0% (50 ppb methoprene) and 96.4% (500 ppb methoprene) compared to 97.8% on control plates of nutrient agar alone. It is clear from these results that methoprene did not inhibit germination of the fungus at the concentrations used in this experiment. Merriam and Axtell (1983) found that methoprene was relatively innocuous to mycelial growth of *Lagenidium* but that it did have an adverse effect on zoospore production at concentrations higher than those used for field application.

For many assays mortalities below 10% or above 90% were recorded with a single fungal dose of 5×10^3 conidia/ml. Similar variations in susceptibility between different batches of test larvae have been reported in previous bioassays of *Culicinomyces* (Sweeney 1981, Cooper and Sweeney 1982). Results in which fungal mortalities were at either extreme were difficult to interpret as any additive, synergistic, or antagonistic effects due to the addition of methoprene would be masked. Consequently only 3 of the 16 assays, where mortality due to the fungal treatment alone was approximately 50%, were used for the final analysis of results.

The assay results were pooled together and the observed frequencies, depending on sample size, were analysed by either the Fisher exact test or 2 × 2 contingency tables with Yates' correction factor (Zar 1974). The test mortality of the treatment with fungus alone (71.4%) was significantly less than those of the fungus/methoprene treatments and the mortality of test mosquitoes exposed to 3 ppb methoprene was also significantly less than that in which this methoprene dose was applied with the fungus (Table 1). Thus, when methoprene and

Culicinomyces were combined, mortality of test insects was greater than when either agent was applied separately. The fungus principally killed larvae while methoprene killed pupae and pupal-adults. More than 70% of mortality due to the fungus occurred in the larval stage whereas less than 10% larval mortality occurred when methoprene was used alone (Table 1). Georghiou and Lin (1974) found the period of maximum sensitivity to Altosid for two culicine species to be between 10 and 30 hr prior to pupation. Conversely, although the fungus is lethal to all larval instars, young larvae are more susceptible (Sweeney 1983). Thus a temporal killing pattern occurred when methoprene and *Culicinomyces* were applied in combination. *Culicinomyces* acted first, killing some of the target population as larvae. The remainder of this population pupated and then more died—the majority due to methoprene. Finally the survivors eclosed and became adults. Since the fungal dose was constant, approximately the same number of larvae died in each fungal treatment within an assay, so that the number of adult survivors was largely determined by the concentration of methoprene added.

External sporulation of *Culicinomyces* on cadavers was not adversely affected by methoprene. The percentage of dead larvae with sporulation was 60.7% when the fungus was applied alone at 5×10^3 conidia/ml. When methoprene was added to this fungal dose external sporulation was: 59.2% (3 ppb methoprene), 74.4% (5 ppb methoprene), 58.6% (8 ppb methoprene), and 46.3% (20 ppb methoprene).

These experiments suggest that a combination of insect growth regulators and *C. clavisporus* may be capable of development as an in-

Table 1. Effects of methoprene and *Culicinomyces* alone and in combination against early fourth instar *Aedes aegypti* larvae. 180 Larvae/treatment and mortality corrected by Abbott's formula.

Total dose added		Percent of test mosquitoes dying as:			
Fungal dose conidia/ml	Methoprene dose (ppb)	Larvae	Pupae	Pupal-Adults	% Total mortality ($\bar{x} \pm$ S.E.)
5×10^3	0	51.3	20.1	0	71.4 ± 5.6
0	3	6.4	33.7	4.5	44.6 ± 5.1
0	5	0.0	77.6	9.7	87.3 ± 4.3
0	8	0.6	90.6	5.0	96.2 ± 1.9
0	20	0.0	92.2	3.8	96.0 ± 2.5
5×10^3	3	60.0	35.0	0.7	95.7 ± 1.7[ab]
5×10^3	5	47.4	42.4	3.9	93.7 ± 3.2[a]
5×10^3	8	41.6	56.6	0.6	98.8 ± 1.2[a]
5×10^3	20	37.8	62.2	0.0	100.0 ± 0.0[a]

[1] Larval, pupal and pupal-adult mortality combined. Significant differences between these mortalities were tested using either the Fisher exact test or 2 × 2 contingency tables with Yates' correction factor at the 0.05 significance level (Zar, 1974).

[a] Significantly greater than mortality in treatment using fungus alone.

[b] Significantly greater than mortality in corresponding methoprene treatment without fungus.

tegrated control option for mosquito larvae. If used in this way the fungus may be induced to kill part of the target larval population with the remainder being eliminated by methoprene. Sporulation on dead larvae killed by the fungus might then produce significant recycling against subsequent larval generations. However, before this objective could be realized, problems of storage of this fungus must be solved (Sweeney 1985) and formulations developed to give consistent results against mosquito populations in the field.

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A NEW METHOD FOR APPLYING AROSURF MSF (MONOMOLECULAR SURFACE FILM FORMULATIONS)

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The use of water as a carrier/diluent to facilitate application of low technical levels (i.e., 0.2–0.5 gal/acre) of the mosquito larvicide and pupicide Arosurf® MSF[1] to enhance vegetative/canopy penetration and/or prevent overdosing with conventional ground and aerial spray systems has been discussed by Levy et al. (1982). Levy et al. (1984a, 1984b) have also addressed the operational feasibility and techniques, as well as the difficulties associated with mixing Arosurf MSF, or formulations of Arosurf MSF and *Bacillus thuringiensis* var. *israelensis,* or Abate® 4–E (Temephos) in water for application of homogeneous water-base formulations.

Since Arosurf MSF is essentially insoluble in water (solubility < 2.5 ppm), tests were conducted to determine if an application system could be developed that would eliminate the need for high shear agitation while allowing effective ground and aerial application of label rates of the product in water. Preliminary studies by Hertlein et al. (unpublished data) and Levy et al. (1982) indicated that several Dema Liquid Chemical Injectors[2] could be used to meter precise quantities of chemical larvicides, biological control agents and monomolecular surface films into a stream of water for final application at recommended rates at high spray pressures and volumes without the need for tank agitation. In this system the main spray tank would contain only water, therefore eliminating major spray tank cleaning problems.