sleeves are ca 50 cm long and the upper and lower panel sleeves ca 165 cm long. These sleeves hold the external front bag frame (Figs. 2.3 and 3G) of 1.6 cm O.D. polyvinyl chloride (PVC) pipe. The upper and lower parts of each of 2 pieces 79–83 cm long, and the side parts are 59–63 cm long. The lengths of the pieces are adjusted in the sleeves for bag rigidity and snapped together with PVC elbows and connectors without adhesive.

A 20 cm length of 0.3 cm thick Plexiglas® pipe 10 cm in diam. with the forward edge beveled inward, is inserted 15 cm into the rear canvas cylinder of the trap bag, and these are fastened together with a size 64 circular metal screw clamp (Fig. 3H). A small flange may be glued to the outside lip of the protruding end of the Plexiglas pipe to prevent the insect collecting bags from slipping off during use.

The insect collecting bags are made of the same nylon netting as is used for the trap bag. They are 30 cm long and 10 cm in diam. and are fastened over the flange of the trap cylinder either with a string tie or a Velcro® closure.

Direct costs associated with this insect trap were: outer framework, $38 for front bumper supports, $38.50 for the rear mount, $25 for tubing, and $25 for nuts, bolts, connectors and other hardware; trap bag, $21 for netting and canvas, $180 for sewing and tailoring, and $14 for tubing and Plexiglas pipe; and insect collecting bags, $2 each. The indirect cost of the labor of all those involved in the development of this trap could not be accurately estimated.

When the trap is used at intervals over an indefinite period of time, the 2 front bumper supports and the rear mount above the passenger compartment may be left on the vehicle. The trap bag with its internal and external front frames and the rear Plexiglas cylinder may also be kept assembled. One person can mount the trap for use in 15 min or less, operate it as needed, and dismount it in 10 min or less.

We have found it advisable to equip each of our vehicles used for trapping with a kit containing meteorological equipment; a ratchet wrench and sockets; an assortment of nuts, bolts and cotter pins; extra tube holders; and aluminum duct tape for temporary repairs to the netting or canvas. This vehicle-mounted trap, as shown in place in Fig. 3, has been operated 1–5 days weekly at 48 km/hr from April to October over a 3-year period with only minor, repairable damage to the nylon netting.

In one study, the trap was operated over the same set of 10 vehicle runs of 4 km each weekly during the fly seasons of 1981, 1982 and 1983. Average catches per run per year have been 24.5, 10.8 and 25.4 *C. variipennis* respectively. Comparisons of this trap with other vector surveillance methods will be published elsewhere.

References Cited

Barnard, D. R. 1979. A vehicle-mounted insect trap. Can. Entomol. 111:851–854.

Bidlingmayer, W. L. 1961. Field activity studies of adult *Culicoides furens*. Ann. Entomol. Soc. Am. 54:150–156.

Bidlingmayer, W. L. 1966. Use of the truck trap for evaluating adult mosquito populations. Mosq. News 26:139–143.

RAPID KILL OF MOSQUITO LARVAE BY HIGH CONCENTRATIONS OF *CULICINOMYCES CLAVISPORUS* CONIDIA

C. PANTER AND R. C. RUSSELL

Commonwealth Institute of Health, University of Sydney, N.S.W., 2006, Australia

Conidia of *Culicinomyces clavisporus* Couch, Romney, and Rao, a virulent fungal pathogen of mosquito larvae (Sweeney et al. 1973, Couch et al. 1974), are normally ingested during feeding, germinate in the foregut or hindgut, and cause death four or more days after ingestion when invading hyphae fill the haemocoel (Sweeney 1975). However, with high doses (10^4–10^6 conidia/ml) death can occur within 18 hr to 2 days without extensive growth of the fungus in the larvae, which has led to suggestions that a toxin may be involved (Couch et al. 1974, Sweeney 1983). This note reports on the rapid death of *Aedes aegypti* (Linnaeus) larvae exposed to conidia of the Australian strain of *C. clavisporus*.

Initial observation of rapid death was made during an experiment to determine whether dead conidia were toxic to larvae. Boiled conidia at concentrations of 10^5–10^7 conidia/ml did not kill first instar *Ae. aegypti* larvae, but live conidia at the highest concentration caused substantial kills within only 5 hr.

For further trials, the fungus was grown in shake culture at laboratory temperature (approximately 25°C) either in Nutrient Broth (Lab-Lemco, Oxoid) with 0.01% streptomycin and 0.002% neomycin or in Corn Steep Liquor Broth (1.5% or 2.0% w/v adjusted to pH7 with 10% KOH) with streptomycin and neomycin. Conidia were harvested by filtration through a milk filter to remove hyphae, then centrifuged and washed twice in distilled water prior to storage in distilled water at −70°C. Viability before

use was estimated by determining percentage of a total of 400 conidia successfully germinating within 24 hr at approximately 25°C on duplicate plates of NUTRANS Agar, (2/3 BBL Nutrient Agar, 1/3 Oxoid Lab-Lemco Broth, with 0.01% streptomycin and 0.002% neomycin).

Tests against larvae were conducted by inoculating known concentrations of live or heat-inactivated conidia into 250 ml plastic dental cups each containing 10 larvae and larval food, both as specified below, in 100 ml distilled water. Cups were incubated at approximately 25°C.

In the first trial Nutrient Broth-grown conidia were inoculated in 4 dosages to yield 6.6×10^3, 6.6×10^4, 6.6×10^5, and 6.6×10^6 viable conidia/ml in duplicate cups with 10 one-day *Ae. aegypti* larvae and 0.1 ml of a 5% suspension of dried yeast as larval food. Five cups were set up as uninoculated controls. The experiment was repeated with Corn Steep Liquor (1.5% w/v) Broth-grown conidia at concentrations of 1.3×10^3, 1.3×10^4, 1.3×10^5 and 1.3×10^6 conidia/ml. To ensure that a heat labile toxin was not involved, conidia killed by gentle heating at 37°C for 48 hr were tested at the same concentrations.

In the first trial all 20 larvae exposed to 6.6×10^6 conidia/ml and 19 of 20 larvae exposed to 6.6×10^5 conidia/ml died within only 4 hr post-inoculation (1 of 50 larvae died in uninoculated controls). Complete kills with 6.6×10^4 and 6.6×10^3 conidia/ml were obtained within 17 hr (5 of 50 control larvae died). In the second trial all larvae exposed to concentrations of 1.3×10^6, 1.3×10^5, or 1.3×10^4 conidia/ml died within 17 hr post-inoculation. Sixteen of 20 larvae exposed to 1.3×10^3 conidia/ml died within 17 hr. The remaining 4 larvae were moribund and died within 22 hr. Very few larvae died in uninoculated controls (2 of 50) or in cups inoculated with conidia inactivated by heating at 37°C (2 of 20 exposed to 1.3×10^4 heated conidia/ml, and none at the other concentrations).

In a larger trial, Corn Steep Liquor (2% w/v) Broth-grown conidia were inoculated at concentrations of 5×10^2, 5×10^3, 5×10^4 and 5×10^5 viable conidia/ml. There were 25 cups with 10 one-day first instar *Ae. aegypti* larvae for each concentration. Larval food consisted of 0.3 ml/cup of a suspension of 5% dried yeast and 1% powdered liver. Five cups with 5×10^5 boiled conidia/ml served as controls.

Boiled conidia and the 5×10^2 conidia/ml concentration had no effect within 9 hr. Rapid death occurred with the three highest concentrations of conidia, with time required to kill decreasing with greater dose (Fig. 1). Most larvae died within only 3.5 hr with 5×10^5 conidia/ml and within 6 hr at 5×10^4 conidia/ml. The time at which deaths began to occur at the latter concentration was not observed. Appreciable death (28% and 42%) occurred within 8 hr and 9 hr at the relatively low concentration of 5×10^3 conidia/ml.

The same batch of conidia was used under the same conditions to determine the susceptibility of different larval stages to rapid kill. Fifty of each of first, second, third, and fourth instar *Ae. aegypti* larvae were challenged with 10^6 conidia/ml. Boiled conidia were used for controls. Within 4.5 hr post-inoculation all 50 first instar larvae and 56% of second instar larvae were dead, but none of the third or fourth instar larvae. At 7 hr post-inoculation the remaining second instars had died, but only 2% of the third instars. Within 27 hr 88% of the third instars and 42% of fourth instars were dead. Boiled conidia did not kill larvae. The greater susceptibility of younger larvae to the fungus has been noted by Sweeney (1983).

Penetration of the foregut by conidia from highly virulent batches of *C. clavisporus* can be completed in as little as 6 hr (Sweeney et al. 1980, Sweeney et al. 1983). Sweeney (1981) has suggested that death within 24 hr may be caused by a lethal titer of toxins from penetrating hyphae that is attained only when the host is being invaded by large numbers of hyphae. However microscopic examination (phase contrast) of dissected cadavers from rapid kills has revealed germination of some conidia in the foregut but not hyphal outgrowth or invasion. Although limited hyphal outgrowth of some of the conidia attaching to external surfaces of larvae has been seen, there has been no evidence of invasion. No obstruction of the siphon has been observed. The di-

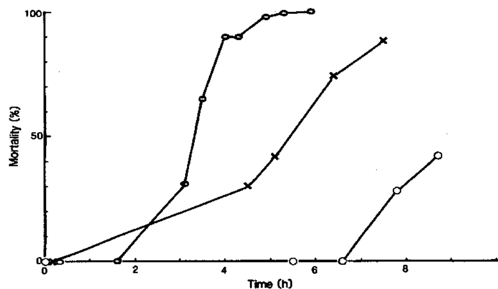

Fig. 1. Death of first instar *Ae. aegypti* larvae over time with different doses of *C. clavisporus* conidia.
⬭ 5×10^5 conidia/ml vs. 250 larvae
× 5×10^4 conidia/ml vs. 250 larvae
○ 5×10^3 conidia/ml vs. 250 larvae

gestive tracts contained large clumps of fungal conidia mixed with the larval food, but the possibility that physical blockage caused death in so short a time is unlikely. The fact that this rapid death has not been observed before may be attributable to altered procedures for growing and maintaining this particular culture of *C. clavisporus* yielding more potent batches, as consistently low LC_{50} values would appear to indicate.

This phenomenon of rapid death is under further investigation, with a toxin being suspected as the likely cause. This may have an important bearing on its potential application to the field. Presently one of the limitations of *Culicinomyces* as a biocontrol agent is its apparent inability to demonstrate significant persistence after application to the field (Sweeney 1981). If *Culicinomyces* must eventually be considered for use as a bio-insecticide with short-term effect, then further development of more potent, rapid killing preparations will be one direction which research on this fungus should take.

The study was funded by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. The able assistance of Messrs. K. Hall and S. Mather is acknowledged, and we thank Dr. A. W. Sweeney for helpful discussion.

References Cited

Couch, J. N., S. V. Romney and B. Rao. 1974. A new fungus which attacks mosquitoes and related Diptera. Mycologia 66:374–379.

Sweeney, A. W. 1975. The mode of infection of the insect pathogenic fungus *Culicinomyces* in larvae of the mosquito *Culex fatigans*. Aust. J. Zool. 23:49–57.

Sweeney, A. W. 1981. Prospects for the use of *Culicinomyces* fungi for control of mosquitoes, pp. 105–121. *In:* M. Laird (Ed.), Biocontrol of medical and veterinary pests. Praeger Scientific Pub., New York.

Sweeney, A. W. 1983. The time mortality response of mosquito larvae infected with the fungus *Culicinomyces* J. Invertebr. Pathol. 42:162–166.

Sweeney, A. W., R. G. Wright and L. van der Lubbe. 1980. Ultrastructural observations on the invasion of mosquito larvae by the fungus *Culicinomyces* Micron 11:487–488.

Sweeney, A. W., A. O. Inman, C. E. Bland, and R. G. Wright. 1983. The fine structure of *Culicinomyces clavisporus* invading mosquito larvae. J. Invertebr. Pathol. 42:224–243.

Sweeney, A. W., D. J. Lee, C. Panter and L. W. Burgess. 1973. A fungal pathogen for mosquito larvae with potential as a microbial insecticide. Search 4:344–345.

CLIMATIC EFFECTS ON *COQUILLETTIDIA PERTURBANS* IN BARRINGTON, RHODE ISLAND

PAUL M. CAPOTOSTO AND DAVID BOYES

Department of Public Works, 84 Upland Way, Barrington, RI 02806

The freshwater mosquito, *Coquillettidia perturbans* (Walker), is an important pest in the community and breeds in Echo Lake, which is large and mostly overgrown with emergent aquatic vegetation. The larvae of this species attach themselves with a modified siphon to the roots of these plants to develop. The pupae remain attached until the adult emergence in the spring and summer. The females bite actively during the early part of the night, are strong fliers, and are readily attracted to light traps (Carpenter and LaCasse 1974).

In 1975 the Barrington Mosquito Control Project was started and the primary species of concern was *Aedes sollicitans* (Walker), a salt marsh mosquito. Residents in Barrington, RI felt that adulticiding alone was ineffective and undesirable. The mosquito control program therefore sought to identify breeding areas, develop a larviciding project, and institute a program of open marsh water management (Ferrigno et al. 1975).

The data presented are part of the breeding area identification program. All adult populations of mosquitoes were monitored by New Jersey light traps model #1:w, equipped with a 40-watt bulb and a 24-hr timer. The light traps were operated from 8:00 p.m. to 12:00 midnight, 7 days a week, from June 1 to September 10 each year. The mosquitoes were collected each Monday, sorted and identified. The traps were located in seven areas in Barrington. Two traps were located north of Echo Lake.

Initial trapping indicated that as much as 70% of the mosquito population came from salt marsh, so a basic program of open marsh water