Table 1. Effect of cyromazine on mosquito development in a wastewater ditch (treatment on March 18, 1985).

Days after treatment	No. of larvae per dip (Average of 5 dips) Instar I	II	III	IV	Total no. of larvae/dip	No. of pupae/dip
0-time*	18	12	8	6	44	5
3	10	5	3	0	18	0
7	9	4	2	0	15	0
11	8	5	2	0	15	0
18	12	5	3	0	20	0
24	14	8	7	1	30	0
31	12	6	5	2	25	0
38	10	7	5	3	25	0
41	9	8	7	5	29	1
42	11	11	9	6	37	3
44	10	10	9	8	37	6

* 0-time = average larvae/dip before treatment (control).

laboratory observations was sufficient to determine larval mortality or adult emergence (Table 2) Twelve pupae and an equal number of adults were obtained 42 and 44 days, respectively after treatment.

It is anticipated that effective control of larvae for more than a month can be accomplished in other areas of the country under conditions similar to the present trial. Ordinarily, four treatments are necessary to accomplish good larval control during this period (about 5–6 weeks). However, in the present trial, only one treatment with cyromazine yielded the same results.

Table 2. Effect of cyromazine on mosquito development under laboratory conditions. Specimens brought from the field before and after treatment (treatment on March 18, 1985) counted in laboratory 10 days after sampling.

Days after treatment	No. of larvae/jar	No. of pupae	No. of adults
0-time*	245	42	30
3	90	2	2
7	75	0	0
11	75	0	0
18	100	2	0
24	150	2	0
31	125	5	2
38	125	3	0
41	150	4	2
42	200	12	3
44	215	20	12

* 0-time = No. of larvae/jar before treatment (control).

No deleterious effect on other aquatic organisms was noted. An abundance of water bugs, chironomids, tadpoles, *Daphnia* and various beetles were present in the water after treatment with cyromazine.

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FIELD EXPERIMENTS ON PERSISTENCE OF *CULICINOMYCES CLAVISPORUS*

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The fungus *Culicinomyces clavisporus* Couch, Romney and Rao has the potential to recycle in mosquito populations by sporulating on the external cuticle of dead larvae; however, larval control in field tests has not been obtained beyond 1–2 weeks after application (Sweeney 1981, Sweeney et al. 1983). It has been suggested that the application of doses lower than those used in previous tests may increase the proportion of larvae which develop postmortem sporulation and enhance the possibility of recycling. Evidence for this was obtained in laboratory observations which showed that exposure of larvae to 10^3 conidia/ml produced greater external sporulation on larval cadavers than did exposure to 10^6 conidia/ml (Cooper and Sweeney, 1986). This paper reports investigations of the persistence of *C. clavisporus* after field application.

All experiments were undertaken adjacent to the Army Malaria Research Unit, Ingleburn in 1 m^2 artificial ponds containing 100 liters of water, similar to those used by Schaefer et al. (1974) but lined with plastic sheeting rather than turf. A plastic container (28 × 28 × 58 cm) with 250 μm stainless steel screens, fitted to 11 × 11 cm holes in each side and to a 14 × 14 cm hole in the center of the bottom, was placed in the middle of each pond. The volume of pond water within each enclosure was approximately 10 liters.

The in vitro inoculum was produced in 20 liter laboratory fermenters as described previously (Sweeney 1981). Dead fourth-instar larvae bearing external conidia were used as in vivo inoculum. The numbers of conidia on dead larvae were estimated by haemocytometer counts of a sample of 20 specimens homoge-

nized in a tissue grinder with 5 ml of water. Both inocula were assayed prior to application with 1-day-old *Aedes aegypti* (Linn.) larvae by the method of Cooper and Sweeney (1982). These assays indicated that the conidia were virulent and viable with LC_{50}s between 10^2–10^3 conidia/ml and 24 hr germination percentages greater than 80%. The in vivo inoculum was applied by adding a known number of dead, spore-covered larvae to each pond enclosure.

Evaluation of previous trials has been complicated by the appearance of newly hatched larvae before death or emergence of all the larvae present at the time of treatment. In order to avoid this problem in the present tests oviposition was excluded by screening the enclosures with mosquito netting. The experimental ponds were designed to simulate the rockpool breeding sites of the test insect *Aedes rupestris* Dobrotworsky which is a natural host of *C. clavisporus* (Frances et al. 1985a). One hundred field collected second-instar *Ae. rupestris* larvae were added to each enclosure one day before application of the fungus. The recycling potential of the fungus was investigated by adding a second similar group of *Ae. rupestris* larvae to the enclosures 4 wk after treatment by which time the first group of test larvae had died or emerged. The larval mortality of both groups was determined indirectly by collecting the adults which emerged each day from underneath the nets.

Persistence of the fungus following application to the test ponds was also determined by an indirect method. During each week of the trial 100 ml samples of water and sediment were aspirated from the bottom of each enclosure. Twenty second-instar larvae of *Ae. aegypti* were exposed to these samples in the laboratory at 25 ± 1°C. Larvae were fed 0.1 ml of 5% w.v. yeast suspension every second day until all had either died or emerged as adults. Sporulating *C. clavisporus* on the external cuticle of dead specimens was considered to be evidence that the sample contained viable conidia. Air temperature and water temperature within the ponds were continually monitored by a recording thermograph.

The first trial was performed from October 1983 to January 1984 (over 16 wk during spring and summer) and the second from March to July 1984 (over 20 wk during autumn and winter). Prior to both trials water from the ponds was passed through 850 μm and 300 μm sieves 1 wk before the fungus was applied. The sediment held on the 300 μm sieve was returned together with 100 liters of fresh tap water. In the first trial in vitro inoculum was sprayed uniformly over the water surface of two ponds (both inside and outside the enclosures) to give application rates of 10^5 and 5 x 10^3 conidia/ml; one pond was treated with in vivo inoculum by placing 50 dead infected larvae inside the pond enclosure to give an application rate of 25 conidia/ml, and one pond was left untreated as a control. In the second trial four ponds were treated with in vitro inoculum (two at a rate of 5 × 10^4 conidia/ml, and two at 10^3 conidia/ml); three ponds were treated with in vivo inoculum by adding 200, 100 and 50 dead larvae inside the enclosures (yielding estimated dose rates of 8 × 10^3, 4 × 10^3 and 2 × 10^3 conidia/ml respectively), and one pond was left untreated as a control.

All of the first group of larvae died in ponds treated with fungus produced in vitro at application rates of 5 × 10^3, 5 × 10^4 and 10^5 conidia/ml but mortality was only 17% and 4% in the ponds treated with 10^3 conidia/ml (Table 1). Mortality of the second group of larvae in the ponds treated with in vitro inoculum was 10% or less. Test mortality in the pond with in vivo inoculum during the first trial was 47% for the first group of larvae and 30% for the second group of larvae. In the second trial the mortality of the first group of larvae was directly proportional to the quantity of in vivo conidia applied with 75% mortality at the highest dose, 59% at the intermediate dose and 34% at the lowest dose. The only significant effect on the second group of larvae in this trial was 25% mortality recorded for the high dose treatment with in vivo inoculum.

During the first trial the fungus was recovered from bottom sediment after 4 wk and 6 wk in the ponds with in vitro inoculum; up to 9 wk after treatment in the pond with in vivo inoculum; and several times in the control pond (Table 2). Native parrots and other birds, which are prevalent in the Ingleburn area, were observed to bathe in the ponds on some occasions and they may have been responsible for dissemination of the fungus to the untreated pond. Water temperature within the ponds ranged from 13 to 26°C for the first 9 wk of the trial but exceeded 30°C for a total of 7 hr in wk 10 and for 34 hr in wk 11. Existing strains of *C. clavisporus* are adversely affected by water temperatures above 30°C (Sweeney 1978) which may account for the lack of persistence for the latter part of this trial. Water temperatures were cooler during the second trial (ranging from 4 to 24°C) and under these conditions the fungus was recovered from all treated ponds for a minimum of 10 wk. In 4 of the ponds it was recovered on wk 20 following application after which time the trial was terminated. These latter results are similar to the labora-

Table 1. Mortalities of *Aedes rupestris* larvae exposed to *Culicinomyces clavisporus* within artificial ponds.

Pond no.	Inoculum	Dose (conidia/ml)	% mortality* of test larvae§	
			1st batch	2nd batch
First trial				
1	in vitro	10^5	100.0	4.4
2	in vitro	5×10^3	100.0	8.8
3	in vivo (50 dead larvae)	25	47.3	30.0
Second trial				
1	in vitro	5×10^4	100.0	10.3
2	in vitro	5×10^4	100.0	1.1
3	in vitro	10^3	17.4	0.0
4	in vitro	10^3	4.3	1.1
5	in vivo (200 dead larvae)	8×10^3	75.0	25.3
6	in vivo (100 dead larvae)	4×10^3	58.7	0.0
7	in vivo (50 dead larvae)	2×10^3	33.7	0.0

* Adjusted for control mortality by Abbott's formula. (All control mortalities less than 15%).
§ Based on survival to adults.

tory observations of Frances et al. (1984) who found that conidia remained active against mosquito larvae for up to 112 days in containers maintained at 14°C. However, in tests conducted by the same authors in a natural rockpool breeding site of *Aedes rubithorax* (Macquart), conidia were only recovered up to 4 days after application of the fungus when water temperatures ranged from 16.5 to 34.5°C and up to 14 days after application when temperatures were continuously below 10°C (Frances et al. 1985b).

In the present trials results obtained from the ponds treated with in vivo inoculum indicated that sporulation on dead larvae can be responsible for significant larval mortality in the field under favorable conditions. The fungus was recovered in bottom samples from the ponds treated with in vitro inoculum for between 4 and 20 wk after treatment but there

Table 2. Recovery of *Culicinomyces clavisporus* from bottom of enclosures within artificial ponds.

Pond no.	Inoculum	Dose (conidia/ml)	Number of weeks after application of fungus																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
*First trial**																						
1	in vitro	10^5	+	+		+																
2	in vitro	5×10^3	+	+		+	+	+														
3	in vivo (50 dead larvae)	25	+	+	+	+	+			+	+											
4	—	control			+	+	+	+			+											
Second trial																						
1	in vitro	5×10^4	+			+	+	+											+			+
2	in vitro	5×10^4	+	+	+	+	+	+	+							+		+	+			+
3	in vitro	10^3	+	+	+	+		+	+										+			+
4	in vitro	10^3	+	+	+	+	+	+			+			+			+					
5	in vivo (200 dead larvae)	8×10^3	+		+		+	+				+	+	+	+	+	+					
6	in vivo (100 dead larvae)	4×10^3			+	+	+	+	+	+	+		+	+	+	+		+		+		+
7	in vivo (50 dead larvae)	2×10^3	+			+	+	+		+		+										
8	—	control																				

\+ = *Culicinomyces* observed on exterior cuticle of *Ae. aegypti* larvae exposed to sediment.
* = Observations terminated after 16 wk.

was no effect against larvae added 4 wk after treatment. These larvae did not pick up a lethal dose of fungus even though it was present at the bottom of the sites. Laboratory bioassays of water samples from top and bottom of test sites in previous trials indicated that the conidia sink to the bottom within 48 hr after treatment (Sweeney et al. 1983) which suggests that rapid sinking of spores may be a serious impediment to effective recycling. More consistent results may be obtained if formulations could be developed which are stable on prolonged storage and which permit the conidia to remain for a longer time within the feeding zone of mosquito larvae.

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FAILURE OF MOSQUITOES TO COLONIZE TEASEL AXILS IN ILLINOIS

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Small volumes of water in natural and artificial receptacles are occasional oviposition sites for many mosquito species and frequent larval sites for a few species (Horsfall 1955). Worldwide, at least 400 mosquito species from 15 genera develop in 1,500 plant species which impound water (Fish 1983).

The cut-leaved teasel plant, *Dipsacus laciniatus* Linn., (Dipsacaceae) has received little attention in regard to mosquito production. Its pinnately lobed leaves are fused at their bases, forming a prominent cup at each node in which rain water and organic debris accumulate. An introduction from Europe and escapee from flower gardens and florists, it ranges from Massachusetts to Michigan (Fernald 1950) and is known from at least 25 counties in Illinois (Mohlenbrock and Ladd 1978), where it is apparently spreading. In Russia, larvae of the malarial mosquito, *Anopheles maculipennis* Meigen, are common in the axil water of these plants, found near human habitations (Borob'ev 1960). In New York, *Aedes triseriatus* (Say) and *Anopheles punctipennis* (Say) have also been infrequently recovered from teasels (Means 1973). This study was undertaken: (1) to assess the distribution, stand size and density, and water retention of these plants in northwestern Cook County, Illinois and; (2) to determine the incidence of mosquito breeding in the axils and species involved.

During the summer of 1985, 33 teasel stands were mapped within a 116 sq mile suburban area (pop. 404,800, 1980 census) in northwestern Cook County; the majority (72%) of stands located in southern Elk Grove and Maine townships. The size and density of these stands varied from 14 to 1500 m^2 ($\bar{x}=251$ m^2) and from 0.5 to 13.1 ($\bar{x}=5.4$) mature plants per m^2. Stands were found along roadsides, railroad tracks, in cemeteries (waste ground), behind buildings, and in other disturbed areas, proliferating in open, sunny fields. Leaves browned and withered towards the end of August with about 90% wilted by early September.

The number of microreservoirs per plant varied from 4 to 8, a few branching teasels possessing up to 10, on a plant 1.5–2.1 m high. The coneshaped cups at the leaf axils were largest at the bottom of each plant and decreased in size apically. Lowermost axils were on the average 10–14 cm diam and 8 cm deep, containing 150–350 ml of water, while