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FIELD TRIALS WITH THE MERMITHID NEMATODE, *ROMANOMERMIS CULICIVORAX*, IN CALIFORNIA

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ABSTRACT. Field tests were conducted with *Romanomermis culicivorax* Ross and Smith, against 4 species of mosquito larvae in 3 natural and 2 artificial sites. Infective nematodes were disseminated at 706 to 25,000 per m² surface area. All species of mosquito larvae were infected and the percentage infection was dependent on the mosquito subfamily, application rate, and test site. In mixed mosquito populations anophelines were more susceptible to parasitism than culicines. Infections in culicine mosquitoes did not exceed 62% at the highest exposure rates. Control of mosquito larvae was reduced in sites with dense vegetation or algal mats.

INTRODUCTION

The broad host range of *Romanomermis culicivorax* Ross and Smith (= *Reesimermis nielseni* Tsai & Grundman of authors, in part) suggests it as a promising biological control agent for mosquitoes—at least 52 species of mosquitoes are known to be infected (Petersen 1973). This study was conducted to determine the feasibility of using *R. culicivorax* as a control agent in mosquito producing habitats in California. Field tests were conducted in 2 artificial and 3 natural sites against 4 species of mosquitoes.

MATERIALS AND METHODS

Romanomermis culicivorax was propagated in *Culex pipiens* Linnaeus following the procedures of Petersen and Willis (1972a). Preparasites were introduced into treatment areas with an 8 liter Hudson® sprayer to give a coarse spray (Petersen and Willis 1972 b). In all studies, preparasites were from 3 to 6 hr old at the time of application. Ponds were sampled 48 hr after treatment. Native mosquito larvae were recovered from treated areas with either a 400 ml dipper or a sweep net. Parasitism was determined by dissecting mosquito larvae in the laboratory within 48 hr after collection. Levels of parasitism were indicative of the number of mosquito larvae killed by the treatment as infected larvae never progressed to the pupal stage.

Water temperature was measured and in some studies, pH, conductivity and oxygen concentrations were determined.

RESULTS

ARTIFICIAL POND STUDIES. Two field trials were conducted in 30 m^2 ponds at Oasis, Riverside County. In the first trial, 2 mosquito species, *Anopheles franciscanus* McCracken and *Culex tarsalis* Coquillett were present. Three ponds were treated at the rate of 1,000 preparasites/m^2 of surface area and a 4th pond served as a control (Table 1). A sparse growth of nut-grass (*Cyperus* sp.) was present in the bottoms of the ponds. An average of 87.5% of *An. franciscanus* and 9.5% of *Cx. tarsalis* larvae were infected.

were redistributed, resulting in 250±50 mosquito larvae/pond. Three treatment levels were applied (1,000, 5,000 and 10,000 preparasites/m^2 of surface area) to duplicate ponds at each treatment level. Two types of treatment applications were made. In the 1st type, nematodes were applied to the surface of 1 set of ponds from a polyethylene squeeze bottle. In the 2nd, a Hudson® sprayer was used and the wand was moved throughout the pond to distribute the preparasites homogeneously. After 48 hr, 20 larvae were examined from each pond and an average of 20, 23.5 and 41.5% of the larvae were found parasitized at the 1,000, 5,000 and 10,000 preparasite/m^2 treatments, respectively. No differences were evident in the 2 methods of application (Table 2).

The 2nd field trial at Oasis consisted of 4 treatment rates with each treatment applied to 2 ponds. During this study, larvae of *An. franciscanus* were present in small numbers, while large numbers of *Cx.*

Table 1. Parasitism in larvae of *An. franciscanus* and *Cx. tarsalis* after *R. culicivorax* was disseminated from a hand sprayer at 1,000 larvae/m^2 in large ponds at Oasis.[a]

Mosquito	Number of larval mosquitoes	Average per treated pond (3)	Control pond
An. franciscanus	examined	16	6
	parasitized	14	0
	% parasitized	87.5	0
Cx. tarsalis	examined	46	55
	parasitized	4	0
	% parasitized	9.5	0

[a] Ponds were sprayed 11/5/75. Mosquitoes were collected 11/8/75 (10 dips per pond). Diurnal fluctuations in temperature of air and water between 11/5 and 11/8 were 4–34C and 16–22C, respectively.

The large differences observed in the field infections of *An. franciscanus* and *Cx. tarsalis* prompted a further study of the infections of *Cx. tarsalis* which were present in large numbers in 1 m^2 ponds in Riverside. These ponds had redwood sides, clay bottoms, were 30 cm deep and lacked vegetation (Legner and Medved 1973). Prior to treatment, all mosquito larvae were collected from the ponds with sweep nets and

tarsalis and *Culiseta inornata* (Williston) were present. A heavy growth of nut-grass covered the sides and bottom of all ponds. Again a high level of infection was obtained in *An. franciscanus*, with lower infection levels occurring in *Cx. tarsalis* and *Cs. inornata* which were found to be about equally susceptible (Table 3). The infection level in all species increased as dosage was increased.

Table 2. Parasitism of *Cx. tarsalis* after *R. culicivorax* was disseminated at 3 rates at the surface and throughout small ponds at Riverside.[a]

Treatment Rate (preparasites/m²)	Type of Appli-cation	Percent *Culex tarsalis* parasitized
1,000	surface	17
	overall	23
5,000	surface	20
	overall	27
10,000	surface	43
	overall	40

[a] Ponds were treated 9/12/76. Mosquitoes were collected 9/14/76. 20 larvae per pond were dissected. 250 ± 50 *Cx. tarsalis* were present in each pond at the time of treatment. Temperature fluctuated between 15 and 23 C during study.

NATURAL STUDY SITES. A 59 m^2 pool at the bottom of Villa Park Dam in Orange County which contained *An. franciscanus* was treated at the rate of 950 preparasites/m^2. Thick growths of tules (*Scirpus* sp.) were present along the banks, the pool, 60 cm deep, was shaded by overhanging trees, and 50% of the water surface was covered with algae. Thirty-five larvae were collected from the site post-treatment and 28.6% of these were infected (Table 4). Multiple infections occurred in several larvae.

A 1,400 m^2 (20x70m) plot in a rice check (field) was treated at the rate of 706 preparasites/m^2 surface area in Sutter County. Prior to treatment, 10 sentinel cages (Case and Washino 1976), each containing twenty 2nd and 3rd instar *An. freeborni* Aitken larvae, were placed in the field. At the time of treatment, a fairly thick growth of rice was present and heavy algal growth occurred where rice growth was less dense. The rice plants were 60 cm high at the time of treatment. Water depth was from 30–56 cm. On the average, 11 mosquitoes per sentinel cage survived in the treated plot and 12 in the untreated plot. Treated mosquitoes were highly infected (84.5%) with an average of 3.1 nematodes per infected larva (Table 5).

A 1,400 m^2 plot in a rice check was treated in Colusa County at the rate of 1,280 preparasites/m^2. The water depth in the field was 45±5 cm. Two bands of rice

Table 3. Parasitism in larvae of *An. franciscanus, Cx. tarsalis,* and *Cs. inornata* after *R. culicivorax* was disseminated from a hand sprayer at 3 rates in large ponds at Oasis.[a]

Treatment Rate (preparasites/m²)	Number of larval Mosquitoes	Mosquito larvae *An. franciscanus*	*Cx. tarsalis*	*Cs. inornata*
0	examined	5	31	29
	parasitized	0	0	0
	% parasitized	0	0	0
1,000	examined	6	30	27
	parasitized	4	4	6
	% parasitized	67	13	22
10,000	examined	12	35	36
	parasitized	10	20	20
	% parasitized	83	57	56
25,000	examined	2	26	38
	parasitized	2	16	22
	% parasitized	100	62	58

[a] Ponds were sprayed 11/4/76. Mosquitoes were collected 11/6/76. Diurnal water temperature range was 15–24C, pH was 7.1, oxygen was 4.2 mg/liter, and conductance was 276 micromhos/cm in the 8 ponds at the time of treatment.

Table 4. Parasitism in larvae of *An. franciscanus* after *R. culicivorax* was disseminated from a hand sprayer at 1,070 larvae/m^2 of surface area in a pond below Villa Park Dam, Orange Co.[a]

Number of Mosquito Larvae		
Before Treatment		After Treatment
examined	10	35
parasitized	0	10
% parasitized	0	28.6

[a] Pond was sprayed 7/12/76. Mosquitoes were collected 7/14/76. Pretreatment collection made 7/8/76. Pond size was 59 m^2 and water temperature was 23 C at time of application.

Table 5. Parasitism in larvae of *An. freeborni* after *R. culicivorax* was disseminated from a hand sprayer at 706 larvae/m^2 of surface area in a rice field in Sutter Co.[a]

Number of Mosquitoes[b]		
Treated Plot		Untreated Plot
examined	110	118
parasitized	93	0
% parasitized	84.5	0

[a] Plot was sprayed 7/20/76. Mosquitoes were collected 7/22/76. Treated and untreated plots were 1,400 m^2 each. Diurnal water temperature range was 17–35C during the infection period.

[b] Total recovered from 10 sentinel cages per plot. Twenty 2nd to 3rd instar larvae were placed in each cage.

of differing heights ran lengthwise in the field. Tall rice (80±5 cm) was present in one area, and shorter rice (50±5 cm) was present elsewhere. The rice was very dense as the treatment was applied just one week prior to draining of the rice check for preharvest drying of the soil. Preparasites for this study were obtained from 2 sand cultures of *R. culicivorax* which were applied separately in the plot which was sprayed while walking the plot lengthwise. After treatment, 10 sentinel cages, each containing twenty 2nd and 3rd instar *An. freeborni* were placed in the high rice area and 5 in the low rice portion. Ten additional cages were placed in an adjacent 1,400 m^2 area which was not treated. An average of 8.7 mosquito larvae survived in the treated sentinel cages with an average of 15.7 larvae surviving in the untreated sentinels. Overall, 49% of the treated sentinel mosquitoes were infected, with infection ranging from 15–100% per cage. The infected mosquitoes contained an average of 1.6 nematodes each. Infection in the containers placed in the low rice ranged from 15–44% and averaged 25% with an average of 1.3 nematodes/infected mosquito. Infection in the high rice containers ranged from 33–100% and averaged 77.5%, with an average of 1.8 nematodes/infected mosquito.

In addition to the sentinel cages, 204 native *A. freeborni* were collected randomly from the field by dipping. These consisted of 16, 41, 60 and 87 1st, 2nd, 3rd, and 4th instars, respectively. Of these, 31, 39, 58 and 21% of the 1st through 4th instars respectively were infected for an overall average of 36% infection. An average of 2.2 preparasites/infected mosquito larva was found.

DISCUSSION

The infection of *An. franciscanus* by *R. culicivorax* has not been previously reported. Like other anophelines, it appears to be as highly susceptible to infection. Of 18 species studied by Petersen (1975) *Cs. inornata* was found to be the most susceptible to parasitism by *R. culicivorax* and was 9.5 times more susceptible than *Culex pipiens quinquefasciatus* Say. *Cx. tarsalis* was 3.5 times more susceptible than *Cx. p. quinquefasciatus* and *An. freeborni* was 6 times more susceptible. In addition, 2 of these species, *Cs. inornata* and *An. freeborni,* were also found to be attractive hosts in the field (Petersen et al. 1973). When water temperatures were suitable for nematode activity (air temperature above 18C), 43% of native *Cs. inornata* were found to be infected in Louisiana (Petersen and Willis 1971). In preliminary field tests in California rice fields, Petersen et al. (1972) found 50% and 80–85% of *An. freeborni* became infected when preparasites were

Table 6. Parasitism in larvae of *An. freeborni* after *R. culicivorax* was disseminated from a hand sprayer at 1,280 larvae/m² of surface area in a rice field in Colusa Co.[a]

		Number of Mosquitoes	
		Treated Plot	Untreated Plot
An. freeborni[b] (Sentinels))	examined	87	157
	parasitized	43	0
	% parasitized	49	0
An. freeborni[c]	examined	204	41
	parasitized	74	0
	% parasitized	36	0

[a] Plots were sprayed 9/7/76. Mosquitoes collected 9/9/76. Treated and untreated plots were 1,400 m² each. Diurnal water temperature range was 14–24 C during the infection period. Rice plants were from 50 cm to 80 cm above water level.

At the time larvae were collected, oxygen was 4.5 mg/liter, conductance was 590 micromhos/cm and the pH was 7.2.

[b] Totals recovered from 10 sentinel cages per plot. Twenty 2nd to 3rd instar larvae were originally placed in each cage.

[c] Naturally occurring larvae which were collected by dipping.

applied at 500 and 1,000/sq yd, respectively.

In the 2 rice field trials conducted during this study, *An. freeborni* was highly susceptible to infection by *R. culicivorax* at treatment rates of approximately 1,000 preparasites/m². In the Colusa County study, the differences in infectivity between the sentinel cages placed in high and low rice areas (25 versus 77.5%) were thought to be due to differences in infectivity of the 2 nematode cultures used, as the high rice was sprayed with preparasites obtained from one culture and the low rice with preparasites from a 2nd culture. This difference in infectivity of the 2 cultures also partially accounted for the low infectivity in the native mosquitoes (36%). These were collected randomly from the treated plot and many of the larvae had multiple infections which indicated that these were infected in an area which contained numerous infective larvae. The dense vegetation present in the natural sites probably contributed to the lower levels of infectivity in the anopheline species present in these sites than in those found in the artificial sites which had less vegetation during spraying and the vegetation may have decreased the chance of contact between preparasites and mosquitoes.

In the 2 studies conducted in 30 m² ponds at Oasis, *Cx. tarsalis* was found to be less susceptible to infection by *R. culicivorax* than was *An. franciscanus* even at high treatment levels. *Cs. inornata* was found to be about as susceptible under field conditions as was *Cx. tarsalis*. Since many anopheline species, *Cx. tarsalis* and *Cs. inornata* are all highly susceptible in the laboratory, the reason for this difference in field susceptibility is not known. We suspect that the difference arises through differences in larval habits. Since anopheline larvae tend to spend more time at the surface than larvae of culicines, it is reasonable to propose that possibility of infection by preparasites is greatly enhanced since the preparasites are located mainly in surface waters (Petersen 1973).

We attempted to test this hypothesis by the study at Riverside in 1 m² ponds through comparison of efficacy of surface versus overall application of preparasites. However, no differences in levels of infections in *Cx. tarsalis* due to the 2 treatments were evident in this study. Petersen and Willis (1974) have speculated that differences in susceptibility of anophelines might be related to the thickness of the host cuticle.

Levels of temperature, pH, oxygen and conductivity which were measured during these studies were all within the acceptable ranges for use of this parasite (Brown and Platzer 1977a and b) and did not adversely affect the infection levels obtained in these studies.

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BOOK REVIEW

Controlled Release Pesticide Formulations. By N. Cardarelli. CRC Press, Inc. 210 p., illus. $51.95

Those involved in pesticide formulation will find interest in this book. After a brief introduction, 32 pages deal with antifouling formulations, 50 pages with molluscicides and schistosomacides, 30 pages with herbicides, 23 pages with insecticides and 3 pages cover rodenticides, nematocides and vertebrate control. An impressive number (970) of references are cited.

Approximately 11 pages deal specifically with mosquito control and this includes sections on larvicides, adulticides and repellents. The book is written to provide general background information on formulation but it is not a "cookbook" on how to formulate. Those directly involved in formulating pesticides will find that this book is worth the price. As other CRC books, the quality of the printing and illustrations is very good.

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