EVALUATION OF THE NEMATODE *ROMANOMERMIS CULICIVORAX* AGAINST CEMETERY MOSQUITOES[1]

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ABSTRACT. *Romanomermis culicivorax* Ross and Smith was tested against *Culex quinquefasciatus* Say and *Culiseta incidens* (Thomson) in both laboratory and field at 5, 10, and 20:1 inoculation ratios (preparasitic nematodes:mosquito larvae). In the laboratory, the preparasitic larvae infected 100% of the larvae of both species of mosquitoes at all inoculation ratios with the exception of *Cx. quinquefasciatus* at the lowest inoculation ratio (72% parasitism). In field applications in cemetery vases, only a 30% infection of *Cx. quinquefasciatus* was obtained at the highest inoculation ratio (20:1). Few *Cs. incidens* larvae (11%) were infected in the field experiment. Recycling of the nematodes did not occur in the cemetery vases. The low level of inoculation and lack of recycling in the cemetery vases were probably due to the low oxygen tension and high conductivity of the water in the vases.

The effectiveness of the parasitic nematode *Romanomermis culicivorax* Ross and Smith in the laboratory and field has been studied extensively in the last decade. Most of these studies have been conducted either in rice fields, ponds or other bodies of water (Petersen 1973, Galloway and Brust 1976, Levy and Miller 1977, Brown *et al.* 1977, Petersen *et al.* 1978) and the mosquito control obtained under certain conditions has encouraged further studies on this nematode.

Cemetery flower vases, albeit containing small reservoirs of water, are highly productive sources of mosquitoes (Kelley 1941, Aaron 1948, Mulla *et al.* 1978). Rose Hills Memorial Park in Whittier is one of the largest cemeteries in California containing over 100,000 vases at the pre-

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sent time and many more are added annually. Heavy populations of mosquitoes are produced in these vases which invade adjacent subdivisions and industrial complexes if no control or preventive measures are practiced. Several control measures against cemetery mosquitoes breeding in flower vases have been evaluated. In this study, an experimental inoculation of *R. culicivorax* in the vases was carried out and its potential as a biocontrol agent against mosquito larvae was assessed.

METHODS AND MATERIALS

Preparasites of *R. culicivorax* were obtained by flooding moist sand cultures with dechlorinated tap water (Brown and Platzer 1978a). The density of preparasitic nematodes per unit volume was determined by three counts of diluted samples of the preparasite suspensions. Both laboratory and field studies were conducted with preparasites obtained 8 hr after flooding of the sand cultures.

LABORATORY TEST. Vases (1 liter capacity, 10 cm diam) in the Sky Church lawn area of Rose Hills Memorial Park, were filled with tap water. Two weeks later larvae of *Culex quinquefasciatus* Say and *Culiseta incidens* (Thomson) naturally breeding in the vases were collected and brought to the laboratory. From these 50 larvae of each species were transferred into separate 1-liter glass jars containing 800 ml of cemetery tap water. The incidence of parasitism was determined at three inoculation ratios, 5:1, 10:1 and 20:1 (infective nematode larvae:mosquito larva) by adding known numbers of preparasites to the glass jars. Each test was replicated three times. Field-collected larvae of each species without added nematodes served as checks. Twenty mosquito larvae were dissected from each replicate 48 hr after exposure to the infective larvae.

FIELD TEST. At Sky Church Lawn, 20 vases containing mostly 1st- and 2nd-stage mosquito larvae were selected randomly and marked for each inoculation ratio. The density of mosquito larvae in each vase was determined by pouring all the water out of the vase into a white enamel pan. Preparasites of *R. culicivorax* were applied at the 3 ratios (5:1, 10:1 and 20:1) as tested in the laboratory; known numbers of preparasites were inoculated according to the total numbers of both species of mosquito larvae in each vase. In addition, 20 vases were inoculated with 2500 preparasites irrespective of the mosquito larval density.

Assessment of parasitism was made 6 and 13 days post-inoculation by pouring the contents of the vase in an enamel pan (36 × 24 cm). Ten 1st & 2nd stage larvae of *Cx. quinquefasciatus* were collected from each vase and pooled as one sample for each treatment level; similarly ten 3rd and 4th instars of the same species were also collected. After these samplings, only 3rd and 4th instars were collected and dissected from the test vases every 2 weeks for a total observation period of 105 days. A similar sampling procedure was used for *Cs. incidens*. One third of the available larvae in each vase were taken for assessment of parasitism. All mosquito larvae were dissected within 2 days of each sampling.

Conductivity and pH of the water in vases were measured on the day of inoculation and subsequent sampling time. Water temperature was recorded and averaged from 25 vases. At each observation time, 5 ml water samples (from enamel pan) collected from each test vase were pooled together. The samples were inspected microscopically for the presence or absence of pre- or post-parasitic stages of *R. culicivorax*. The test vases were filled with cemetery tap water after each observation.

RESULTS AND DISCUSSION

LABORATORY TEST. The two highest inoculation ratios produced 100% parasitism of *Cx. quinquefasciatus* larvae from the cemetery vases (Table 1) whereas 72% of the larvae were infected at the lowest inoculation ratio. Peterson

Table 1. Parasitism of *Cx. quinquefasciatus* and *Cs. incidens*[a] by *R. culicivorax* in laboratory experiments.

Treatment rate preparasites/ larva	% Parasitism[b]	
	Cx. quinquefasciatus	*Cs. incidens*
5	72b (17)a	100b (87)a
10	100c (92)c	100b (95)b
20	100c (100)c	100b (98)b
Check	0	0

[a] First stage larvae were collected from cemetery vases.

[b] Three replicates of 50 larvae each. Values in parentheses represents % superparasitism.

Percentages followed by different letters in vertical columns are significantly different ($P<.01$) by Duncan's multiple-range test.

(1973) reported that 100% of *Cx. quinquefasciatus* could be infected with an inoculation ratio of 3.5:1. All inoculation ratios caused 100% infections in *Cs. incidens* indicating that this species was more susceptible than *Cx. quinquefasciatus*. Superparasitism has been observed in many species of mosquitoes (Petersen 1973, Brown *et al.* 1977) and was also observed in this study (Table 1). Naturally occurring infections of the mosquito larvae by nematodes were not observed in the control mosquitoes.

The distribution of nematodes parasitizing mosquito larvae at 3 different rates is presented in Table 2. The frequency of 2 nemas per mosquito larva was found to be constant in *Cs. incidens* in spite of the increase in the inoculation rate. In *Cx. quinquefasciatus* the frequency increased from 1 to 3 per host with the increase of inoculation rate. In this study maximum of 9 nematodes per mosquito larva were recorded.

FIELD TEST. The density of mosquito larvae varied from 45 to 300 (average = 142) per vase. The level of parasitism achieved under field conditions was substantially lower than that in laboratory tests. The highest inoculation ratio produced 30% infection in *Cx. quinquefasciatus* and 11% in *Cx. incidens* (Table 3). Superparasitism was not observed in *Cs. incidens* but a small percentage of *Cx. quinquefasciatus* were superparasitized. No mosquito larva was superparasitized by more than 2 nematodes.

In subsequent samples after 13 days post-infection only one infected 4th instar of *Cx. quinquefasciatus* was recovered on the 56th day, and this provided some evidence of very low level of recycling. However, no additional mosquito larvae with infections were recovered, and it appeared that conditions in the cemetery vases were inimical for the hatching of preparasites and/or development of post-parasitic stages of *R. culicivorax*.

Table 2. Nematode burden in laboratory infections of *Cx. quinquefasciatus* and *Cs. incidens* with *R. culicivorax*.

No. of nemas/ host larva	% parasitism[a, b] at 3 infection ratios (nematodes/larva)					
	5:1		10:1		20:1	
	C. q.	*C. i.*	*C. q.*	*C. i.*	*C. q.*	*C. i.*
0	28	0	0	0	0	0
1	55	13	10	2	0	8
2	15	65	62	65	28	72
3	2	22	28	19	49	15
4	0	0	0	7	13	3
5	0	0	0	5	8	2
6	0	0	0	0	2	0
9	0	0	0	2	0	0

[a] See Table 1 for source of larvae.

[b] Three replicates of 50 larvae each.

Table 3. Parasitism[a] in larvae of *Cx. quinquefasciatus* in cemetery vases by *R. culicivorax* at three application rates.

Application rate preparasites/larva	% parasitism[b] in different stages (postinoculation days)			
	6		13	
	1–2 instars	3–4 instars	1–2 instars	3–4 instars
5	2	7 (1)	0	0
10	6 (0.5)	11 (2)	0	0
20	8	30 (9)	0	0.5
2500 preps/vase[c]	4 (1)	12 (5)	0	1

[a] At highest rate of inoculation only 2 *Cs. incidens* larvae were parasitized out of 18 larvae collected (11% infection).

[b] % in parentheses = % superparasitism.

[c] This application rate was equivalent to 316,000 preparasites/m^2.

Maximum water temperature in vases taken during observation was 30 ± 2°C. However, water temperature in southern California can show marked diurnal changes of 10°C. Water conductivity in the test vases varied from 1100 to 2600 μmhos/cm average = 1570 μmhos). Water pH was relatively constant (pH 6.2 to 7.0, average = 6.5). Conductivity and pH did not change significantly during the study period. Petersen and Willis (1970) did not find *R. culicivorax* in natural habitats where the water conductivity exceeded 400 μmhos/cm. However, under laboratory conditions, both infections and recycling took place at higher water conductivities (Brown and Platzer 1978). In addition, Levy and Miller (1977) found that *R. culicivorax* was infective for *Cx. quinquefasciatus* larvae in abandoned sewage settling tanks at conductivities of 1190 to 1300 μmhos/cm; no studies on recycling were reported in this situation. Neither pre- nor post-parasites were detected in the water collected from vases on any observation day in our studies.

Subsequent to the poor field performance of *R. culicivorax* the oxygen tension was determined in cemetery vases that had been filled with water and left in place in the cemetery lawn for 2 weeks. The initial oxygen content (measured with a YSI-51B 0_2 meter) was 7.6 mg/l but after 2 weeks the average was 0.6 ± 0.6 mg/l. The great decline in oxygen tension was due to the accumulation of organic debris, e.g., lawn cuttings and leaves. Infective larvae of *R. culicivorax* stopped moving after 8 hr in water from such vases. Brown & Platzer (1978b) reported that transient exposure to low levels of oxygen showed loss of infectivity in *R. culicivorax*. However, in the present study, the nematodes were exposed to constant oxygen deprivation and presumably were unable to penetrate the mosquito larvae.

In conclusion, it appears that *R. culicivorax* cannot be used effectively as a biological control agent in cemetery vases found in southern California. The oxygen deficiency and high water conductivity inherent in such mosquito breeding sites appear to be the major factors preventing the use of this nematode in cemetery vases.

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