ESTABLISHMENT AND RECYCLING OF *ROMANOMERMIS CULICIVORAX* (NEMATODA: MERMITHIDAE) IN LOUISIANA RICELANDS[1]

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ABSTRACT. The postparasitic stage of *Romanomermis culicivorax*, applied at dosage rates of 0.025 and 0.05 g/m^2, was able to establish, recycle and overwinter in a riceland habitat in southern Louisiana. Percent parasitism ranged from 0 to 54.1% during the 22-month study. Parasitism of *Psorophora columbiae* larvae was significantly greater ($P<0.05$) along levee ditches than in the central portions of the fallow riceland plots on 3 of 4 sampling dates in 1983. As percent parasitism increased within the population of *Ps. columbiae* located in bioassay containers, the degree of superparasitism also increased.

INTRODUCTION

Petersen and Willis (1972), in Louisiana, were the first to experimentally release preparasites and postparasites of *Romanomermis culicivorax* Ross and Smith against natural populations of mosquito larvae. Subsequent research emphasized the use of preparasites because of their ease of application. However, this technique required proper timing of application, especially for floodwater mosquitoes, to coincide with the short duration of the early instars of mosquitoes, which are the most susceptible to infection, and the ephemeral preparasitic stage of *R. culicivorax* (Petersen and Willis 1970, Petersen et al. 1978).

The potential of postparasites applied to ricelands became apparent when Westerdahl et al. (1979, 1982) reported successful infection of mosquito larvae in a California rice field. Their study showed that *R. culicivorax* recycled, overwintered and survived through the following rice growing season.

There are several advantages to using postparasites to inoculate mosquito breeding habits of floodwater species. The first and primary advantage is that the habitat does not have to be flooded at the time of nematode application as long as the soil is moist. The nematodes subsequently bury into the soil, mature to adults, mate and deposit eggs. After an appropriate incubation period, the eggs of *R. culicivorax* remain viable for several months and readily hatch with the onset of the next several floodings. The eggs of *R. culicivorax* are capable of "installment hatching" (J. J. Petersen, USDA-ARS, University of Nebraska, Lincoln, NE, personal communication); a characteristic that has been observed in the laboratory. Many floodwater mosquitoes also possess this characteristic (Schwardt 1939). Installment hatching is defined as the nonsynchronous hatching of mosquito eggs when subjected to a periodic inundation. This characteristic allows newly hatched preparasites to invade freshly emerged mosquito larvae within a common habitat after such successive flooding.

Secondly, postparasites can be applied to standing water without the presence of early instar mosquitoes and still continue with their life cycle. In contrast, preparasites must be applied to standing water and have susceptible mosquito instars available at the time of application if success is to be achieved. The application of preparasites must be accomplished within the first 28 hr after hatch of *Psorophora columbiae* (Dyar and Knab) eggs to be effective (T. W. Walker and C. L. Meek, Louisiana State University, Baton Rouge, LA, unpublished data).

This study was conducted to evaluate the use of *R. culicivorax* as a component of an integrated pest management program in the ricelands of the southern region of the United States, with *Ps. columbiae* as the target species.

MATERIALS AND METHODS

The study site was located in Jefferson Davis Parish, LA in a previously harvested rice field. The soil type was a Crowley silt loam (fine, montmorillonitic, thermic, Typic Albaqualf). Five adjacent 20 × 20 m plots were constructed with typical rice field levees surrounding the periphery of each plot.

On September 9, 1982, 4 of the 5 unflooded plots in the fallow rice field were treated with *R. culicivorax* postparasites at a male:female ratio of about 1:1. Two plots were treated each with 0.025 g/m^2 of postparasites and two each with 0.05 g/m^2. This is approximately equal to 50

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and 100 nematodes /m^2 (J. J. Petersen, personal communication). At these dosages, the treatments were equivalent to 10 and 20 g/plot, respectively. The treatments were conducted by burying the aliquots of postparasites approximately 2 cm deep in each square meter of soil for protection and adding about 5 ml of well water to initially prevent desiccation.

The plots were designated A through E. Plots A and C were treated with 20 g of nematodes each and plots B and D with 10 g each. Plot E was used as a control and was not treated with nematodes; however, it was subjected to the same cultural and water management practices as the treated plots.

All plots were flooded with well water 33 days posttreatment to allow sufficient time for the previously inoculated postparasites to mature, mate and produce eggs. This flooding not only was an attempt to stimulate late seasonal hatching of native mosquito populations but also marked the beginning of the bioassay phase to evaluate *R. culicivorax* parasitism of natural and sentinel mosquito larvae.

Each plot had 8 sampling sites with 4 of the sites equally distributed in the levee ditch that surrounded the inner edges of the plot (i.e., one site per side). The remaining 4 sites were located near the center of the plot for a total of 40 sampling sites within the 5 plots.

Two bioassay methods were used to determine parasite levels in mosquito larvae. The primary method involved using a predetermined number of sentinel mosquito larvae placed in 8 floating bioassay containers which were located in each of the sampling sites within the plots. The bioassay containers were constructed of polyvinyl-chloride pipe equipped with screen-covered openings (Walker and Meek 1985). Sixty first and early second instars of *Ps. columbiae* were placed in each bioassay container. Previous laboratory data indicated the early larval age classes (i.e., first and second instars <28 hr old) were most susceptible to infection by *R. culicivorax*. After 3 days of exposure, the larvae were removed from the bioassay containers, transported to the laboratory and examined for *R. culicivorax* infection. Periodically, parasitism of the native larval population of mosquitoes at each site was also sampled by taking 10 dips/site using a standard 465 ml dipper.

The plots were flooded 11 times and drained 4 times during the 22 month period to provide water levels sufficiently high (10.2 cm) to use the bioassay containers, to simulate heavy rainfall patterns and agronomic practices common to southwestern Louisiana. The fallow rice field plots were flooded on October 12 and 25, 1982 and March 10, May 13, July 15, August 31 and November 1, 1983. Following each flood, the water levels were allowed to subside through natural soil percolation and evaporation. The plots were not flooded with irrigation water for 100 days during the winter months of 1982–83 and 131 days during the winter months of 1983–84. During these periods, percent available soil, moisture was periodically monitored with a Beckman® Bouryovces Model EN-2B moisture meter (Beckman Instruments, Inc., Cedar Grove, NJ). In 1984, the plots were planted in rice and subjected to the normal cultural activities. These activities included: seedbed preparation by disking the soil, vehicular grading the land while the plots were subjected to a flooded condition, aerial application of germinated seed rice (LaBelle variety), and aerial applications of the herbicide molinate at 3.4 kg AI/ha, the herbicide 2,4-D at 0.76 kg AI/ha, and the fertilizers 21% ammonium sulfate [$(NH_4)_2SO_4$] at 14.7 kg AI/ha and 45% urea [$CO(NH_2)_2$] at 31.5 kg AI/ha. In conjunction with these cultural activities, the plots were flooded on April 27, May 8 and June 19, 1984.

Selected climatological conditions, such as rainfall and ambient temperature, were monitored by on site recording instruments and compared with data collected by a substation of the Louisiana Office of State Climatology in nearby Jennings, LA. Additionally, water temperature, dissolved oxygen, conductivity, and total salinity were recorded at the time of sampling.

Data from bioassay containers were subjected to statistical analyses based on a model utilizing 5 plots, 2 treatments, 2 regions (levee ditch and center of plot) and 8 sample sites. An analysis of variance of these data used the SAS general linear models procedure for testing the hypothesis that the means obtained from the plots were the same (SAS 1982).

RESULTS AND DISCUSSION

The percent parasitism of *Ps. columbiae* by *R. culicivorax* in riceland research plots over a 22-month period is shown in Table 1. Parasitized *Ps. columbiae* larvae were recorded only once in bioassay containers during the fall months of 1982. Plot A had one infected larva of *Culex salinarius* Coquillett on October 18 (39 days posttreatment). Following the November 1 flooding, the plots remained free of irrigation water for 100 days during the winter months of 1982–83. Two early season samplings in March 10 and April 19, 1983 yielded no parasitism of *Ps. columbiae* larvae. Beginning May 13 and continuing for the remainder of the sampling dates in 1983, parasitism ranged from 3.9 to

Table 1. Percent parasitism of *Psorophora columbiae* resulting from a single application of *Romanomermis culicivorax* postparasites in plots located in a Louisiana rice field, 1982–84.

Date	% parasitism at indicated postparasite application rate				
	20 g/plot		10 g/plot		0 g/plot
	A	C	B	D	E
1982	*Postharvest rice field*				
October 12	0	0	0	0	0
October 18	0.4	0	0	0	0
October 25	0	0	0	0	0
November 1	0	0	0	0	0
November 8	0	0	0	0	0
1983	*Fallow rice field*				
March 10[1]	0	0	0	0	0
April 19[2]	0	0	0	0	0
May 13	28.0	25.2	25.5	13.2	0
May 27	22.8	15.1	9.5	5.4	0
July 15	20.2	3.9	15.7	26.7	0
November 1	39.1	54.1	43.3	46.4	44.5[3]
1984	*Active rice field*				
May 18	4.8	0	0	0	0
June 19	0	0.8	0.2	2.9	0.6

[1] Plots A and C had 3 bioassay containers each; plot B, D, and E had 2 containers each.

[2] Each plot had 4 containers each.

[3] Due to levee break and interchange of water between plots D and E.

54.1% among all treatment plots with an end of the season high ranging from 39.1 to 54.1.

Parasitism in the untreated control plot was first recorded on November 1. This infiltration of nematodes was believed due to a levee break and movement of irrigation water from the treated plot to the control plot. The infection rate within the control plot was comparable to rates for the treatment plots. During the sampling periods of May and June 1984, the percentages of parasitism decreased markedly compared with the late season infection rates of 1983.

Table 2 shows the percent parasitism of native mosquito larval populations by *R. culicivorax* in the riceland plots. Eight species of mosquitoes were collected. In order of larval abundance, the species were: *Anopheles crucians* Wiedemann (249); *Ps. columbiae* (219); *Cx. salinarius* (164); *An. quadrimaculatus* Say (103); *Uranotaenia sapphirina* (Osten-Sacken) (32); *Ur. lowii* Theobald (21); *Aedes vexans* (Meigen) (1); and *Culiseta inornata* (Williston) (1). Parasitism occurred in all species except *Ae. vexans, Cs. inornata* and *Ur. sapphirina.* One larva each of *Cx. salinarius* and *Ur. lowii* were parasitized by *R. culicivorax.*

Unlike *Ps. columbiae* larvae located in bioassay containers during the fall of 1982, a percentage of the native larval populations were infected within the treatment plots. Of the 524 larvae collected in 1982 in plots A through D, an average of 4.4% (23) were infected with *R. culicivorax.* During 1983, the percentage increased to an average of 22.1% (46/208) infection. This also corresponded to an increase in the parasite level of *Ps. columbiae* located in bioassay containers (Table 1).

Overall native larval populations within all plots for the 22-month study were low. Dipping samples for 1982 averaged 0.12 larva/dip during mid-October and 1.39 larvae/dip in early November. For 1983, samples averaged 0.16 larva/dip on May 16, 0.38 larva/dip on July 18, and 0.03 larva/dip on November 4.

Climatological parameters recorded during the 22-month study did not appear to cause any measurable adverse effects on populations of *R. culicivorax* in the riceland plots. Selected aquatic parameters that were monitored during the study period also did not appear to adversely affect the nematode population within the plots. Water temperature, dissolved oxygen, conductivity and total salinity remained within the optimal limits for *R. culicivorax* during the entire study for each flooding sequence as defined by Petersen and Willis (1970, 1972), Levy and Miller (1977), and Brown and Platzer (1977, 1978).

Percent parasitism of *Ps. columbiae* larvae by *R. culicivorax* within peripheral ditches and central areas of pans of riceland plots is provided in Table 3. From late spring through late fall of 1983, when parasitism was most pronounced, significant differences ($P<0.05$) occurred between peripheral ditches and central areas on 3 of 4 sampling dates. These differences indicated that greater percentages of parasitism occurred in levee ditches than in the central portions of pans. Previous research indicated that rice field levee ditches provided suitable habitats for significantly higher numbers ($P<0.01$) of *Ps. columbiae* larvae (Andis and Meek 1984) and populations of aquatic arthropod predators (M. D. Andis and C. L. Meek, Louisiana State University, Baton Bouge, LA, unpublished data). In May and June 1984, levels of infection from the bioassay containers located in ditches again accounted for most of the parasitism; however, no significant differences ($P>0.05$) existed between the ditches and the pans.

During 1983 and 1984, there were no significant differences ($P>0.05$) between the postparasitic application rates of 0.025 and 0.05 g/m^2 and resultant parasitism in riceland test plots for 7 of the 8 sampling dates. A significant

Table 2. Percent parasitism of natural mosquito populations resulting from a single application of the postparasitic stage of *Romanomermis culicivorax* in plots located in a Louisiana rice field, 1982–83.[1]

		Postparasite application rate (g/plot)									
		20				10				0	
		A		C		B		D		E	
Date	Mosquito Species	No. larvae	% parasitism	No. larvae	% parasitism	No. larvae	% parasitism	No. larvae	% parasitism	No. larvae	% parasitism
1982											
Oct. 15	*Ps. columbiae*	24	4.2	0	—	5	40.0	4	0	3	0
	An. crucians	0	—	0	—	3	0	1	0	1	0
	Cx. salinarius	0	—	1	100	0	—	0	—	6	0
Nov. 1	*An. crucians*	70	1.4	48	22.9	48	6.3	28	3.6	37	0
	An. quadrimaculatus	28	3.6	18	0	28	3.6	8	0	12	0
	Cx. salinarius	19	0	10	0	16	0	114	0	16	0
	Uranotaenia spp.	13	7.7	10	0	12	0	15	0	2	0
	Cs. inornata	0	—	0	—	0	—	1	0	0	—
1983											
Mar. 13		0	—	0	—	0		0	—	0	—
Apr. 12		0	—	0	—	0	—	0	—	0	—
May 16	*Ps. columbiae*	0	—	13	0	11	0	21	0	1	0
Jul. 18	*Ps. columbiae*	17	23.5	54	24.1	59	30.5	3	33.3	0	—
	An. crucians	0	—	3	100	0	—	8	25.0	1	0
	An. quadrimaculatus	0	—	5	20.0	0	—	2	50.0	0	—
Nov. 4	*Ps. columbiae*	1	100	0	—	2	100	1	0	0	—
	An. crucians	1	0	0	—	0	—	0	—	0	—
	An. quadrimaculatus	1	0	0	—	1	0	0	—	0	—
	Cx. salinarius	2	0	0	—	2	0	0	—	1	—
	Ae. vexans	0	—	0	—	0	—	1	0	0	—

[1] Each plot was sampled by taking 10 dips at each 8 sampling sites (80 dips/plot).

difference ($P<0.01$) did occur on May 27, 1983, in which the percent parasitism of *Ps. columbiae* larvae in bioassay containers was greater in plots treated with the higher postparasitic rate. However, it generally appears that in field applications, postparasitic dosage rates of 0.025 g/m² (approximately 50 postparasites/m²) are sufficient to establish parasitism equivalent to levels obtained in this study.

Superparasitism of sentinel mosquito larvae

Table 3. Percent parasitism of *Psorophora columbiae* larvae by *Romanomermis culicivorax* in peripheral levee ditches and pans of riceland plots.[1]

		% parasitism at indicated postparasite application rate				
		20 g/plot		10 g/plot		0 g/plot
Date		A	C	B	D	E
1983						
May 13	Ditch	36.8	39.5	36.1	14.3	0
	Pan	19.1	11.0	14.9	12.1	0
May 27	Ditch	12.9	12.5	3.2	6.7	0
	Pan	32.7	17.7	15.9	4.1	0
July 15	Ditch	15.5	7.7	24.2	44.7	0
	Pan	25.0	0	9.3	2.6	0
November 1	Ditch	67.0	88.4	82.0	75.3	84.1[2]
	Pan	11.1	19.8	4.6	17.6	5.0
1984						
May 18	Ditch	8.3	0	0	0	0
	Pan	0	0	0	0	0
June 19	Ditch	0	0	0	5.8	1.2
	Pan	0	1.6	0.5	0	0

[1] Values are means for 60 *Ps. columbiae*/bioassay container; 4 containers/ditch/plot and 4/pan/plot.
[2] Due to levee break and interchange of water between lots D and E.

within the bioassay containers occurred on 4 of the 6 sampling dates in 1983. As defined, superparasitism is the infection of a single host by ≧2 parasites of the same species. Data indicate that the percent parasitism increased within a population of mosquito larvae, the number of parasites per host also increased (Table 4). At parasitism levels of <11% the infections ranged from 1 to 3 nematodes/host with the greatest number of infected larvae having 1 nematode. When percent parasitism was ≧91%, the infection ranged from 1 to > 10 nematodes/host and the number of nematodes per host became more evenly distributed. In addition, as parasitism levels of larval populations increased, a large proportion of the host population was superparasitized. When < 11% of the mosquito larval population was infected, 0.5% of the larvae was superparasitized; and when parasitism was ≧91%, superparasitism increased to 83.9%.

Table 4. Percent superparasitism of *Psorophora columbiae* by *Romanomermis culicivorax* in plots located in a Louisiana rice field, 1983–84.[1]

No. sample populations	Percent parasitism	Percentage of hosts containing the indicated number of nematodes										
		0	1	2	3	4	5	6	7	8	9	10+
40	1–10	95.0	4.5	0.3	0.2	0	0	0	0	0	0	0
20	11–20	84.6	12.0	3.0	0.4	0	0	0	0	0	0	0
14	21–30	75.8	17.6	4.3	2.3	0	0	0	0	0	0	0
7	31–40	64.2	22.5	5.8	2.9	4.0	0	0.6	0	0	0	0
3	41–50	51.7	27.0	9.0	4.4	3.4	3.4	0	1.1	0	0	0
5	51–60	43.0	29.0	15.0	9.0	2.0	1.0	1.0	0	0	0	0
4	61–70	34.5	26.4	21.8	5.7	9.2	1.2	0	0	1.2	0	0
2	71–80	24.1	9.2	24.2	18.5	13.0	5.6	3.7	1.8	0	0	0
2	81–90	14.0	20.9	20.9	4.7	30.2	2.3	7.0	0	0	0	0
10[2]	91–100	4.0	12.1	15.5	18.4	14.9	9.8	8.0	6.3	3.5	0.6	6.9

[1] When superparasitism occurred within a sampling date.
[2] When percent parasitism levels approach 100%, early mortality of the host often occurs.

If superparasitism becomes too stressful, on a per host basis, then the sex ratio of the resultant parasite progeny is affected. Petersen (1972) indicated that whenever ≧7 *R. culicivorax* parasites occurred within a single mosquito larval host, essentially 100% of the eventual offspring will be males. Data collected in this study indicate that superparasitism of ≧7 parasites/larvae was not a major factor and only occurred when the percentage of total parasitism exceeded 41%. When total percentages of ≧91% infection occurred, 17.3% of the mosquito larvae contained ≧7 nematodes. In addition, Kurihara (1979) demonstrated that mosquito larvae, particularly the early age classes (first and second instars), will suffer premature mortality when subjected to high levels of parasitism.

Postparasites of *R. culicivorax*, at dosage rates of 0.025 g and 0.050 g/m², were sufficient to establish and maintain recycling of nematode populations in riceland plots of southern Louisiana. The nematode population could survive the stressful conditions of overwintering, use of agrichemicals and the common farming practice of culturally rotating the land from a rice field to a fallow condition and back to a rice field again. Even though parasitism was at moderate levels in 1983 when the plots were fallow, the initial infection rates in the early summer of 1984 were very low. Factors such as seedbed preparation by grading of the land by tractor, may have contributed to the low parasitism.

Romanomermis culicivorax should not be used as the only means of riceland mosquito control. Infection rates of <55%, obtained in this study, are not acceptable in an abatement program. However, an integrated approach to riceland mosquito control should be implemented to include chemicals, biocontrol agents and water management strategies to achieve a more successful mosquito control effort.

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