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Susceptibility of *Psorophora columbiae* Larvae over Time to Parasitism by *Romanomermis culicivorax*[1]

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Abstract: The ability of *Romanomermis culicivorax* preparasites to penetrate and infect *Psorophora columbiae* decreased substantially after ca. 28 hours. Parasitism at temperatures typical of Louisiana rice fields (i.e., 26, 29, and 32 ± 0.5 C) showed a significant linear decrease ($P < 0.01$) as the percentage of older larval instars increased at the times of exposure. These data emphasize the need for a synchronous field application of preparasites to challenge the rapid development of early instars of *Ps. columbiae.* Applications of postparasites rather than insecticide treatments to potential mosquito breeding habitats may offer greater flexibility in larval mosquito control programs.

Key words: dark rice field mosquito, mosquito, nematode, parasitism, *Psorophora columbiae, Romanomermis culicivorax,* temperature.

The dark rice field mosquito, *Psorophora columbiae* (Dyar and Knab), is a major pestiferous species in the ricelands of the southern United States. Occasional natural populations of *Ps. columbiae* have been found to be heavily parasitized by a mermithid nematode, *Romanomermis culicivorax* Ross and Smith, at ambient summer temperatures (10).

In comparison to other mosquito species, *Ps. columbiae* has a very brief developmental period. Horsfall (3) reported that under field conditions with average water temperatures of 29–35 C, the larval development of *Ps. columbiae* can be completed in 4 days; McHugh and Olson (5) reported that at 34 C under laboratory conditions, development from egg eclosion to 50% adult emergence required only 4.5 days.

Because of the short developmental period of *Ps. columbiae,* larval susceptibility to infection by *R. culicivorax* may be of limited duration. Laboratory studies established that first and second instars of *Culex quinquefasciatus* Say were highly susceptible to infection by preparasites of *R. culicivorax,* whereas third and fourth instars were considerably less susceptible (8,11). However, exposure of mixed larval stages to preparasites of *R. culicivorax* resulted in significantly lower ($P < 0.05$) levels of parasitism in first instars than in second and third and about the same as fourth instars (8). Similar results were found in the field when mixed instars of natural populations of *Anopheles* were treated (9,12). Also, the preparasites are short lived, and their ability to infect *Cx. quinquefasciatus* decreases after 24 hours of age (7). Preparasites were found to be infective at 12–33 C, with optimum infectivity at 21–33 C (1).

Because of the rapid larval development of *Ps. columbiae* during the summer months when ambient temperatures are above 29 C, reduced susceptibility of the older larval instar, and the short time that *R. culicivorax* remains infective, it becomes extremely difficult to synchronize hatch of *Ps. columbiae* eggs with the introduction of *R. culicivorax* to achieve an optimum host–parasite interaction.

Our objective was to quantitatively define the period of greatest susceptibility to infection by *R. culicivorax* relative to the age of *Ps. columbiae* larvae.

Materials and Methods

Psorophora columbiae eggs were collected from wild females trapped in Jefferson Davis Parish, Louisiana. The eggs were hatched in a suspension of powdered Bacto nutrient broth (Difco Laboratories Inc.) and well water at a dilution of 1:1,000 by weight (6). During each hatching procedure the suspension was maintained at the same temperature as that used in the even-

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TABLE 1. Susceptibility of *Psorophora columbiae* larvae over time to parasitism by *Romanomermis culicivorax* at a constant water temperature of 26 C.*

Larval age (hours)	Age structure by instar at exposure				No. surviving larvae when parasitism determined		Parasitism (%) (± SE)
	I	II	III	IV	Control	Exposed†	
1	149	0	0	0	45	24	83.0 (6.8)
13.5	145	0	0	0	41	35	91.3 (0.5)
27	137	6	0	0	42	38	81.5 (3.8)
39	72	78	0	0	37	44	49.0 (3.5)
51	24	90	27	0	40	43	39.1 (6.4)
63	8	50	82	0	44	42	11.3 (4.6)
75	0	30	83	11	42	40	4.3 (1.8)
96	0	6	39	77	46	40	0 (0)

* Nematode to host inoculation ratio of 10:1.
† Mean for three replications at the termination of tests.

tual experiment. The temporal relationship between larval development and nematode infection was studied at constant temperatures of 26, 29, and 32 ± 0.5 C. To provide a uniform age structure, only the mosquitoes that hatched within the first 2 hours after flooding of eggs were used. Fifty mosquitoes were placed in each of thirty-two 600-ml beakers containing 400 ml well water. The mosquito larvae were fed as described by McHugh and Olson (5) and subjected to a 12 light : 12 dark photoperiod (14). *Psorophora columbiae* larvae were exposed to *R. culicivorax* at designated time periods that corresponded to larval developmental rates (5). Instar composition of the larval population was determined by head capsule width at each exposure period before inoculation. Preparasitic nematodes for each treatment were obtained by taking aliquots of sand containing *R. culicivorax* eggs from culture pans supplied from a mass rearing facility located at the USDA ARS Gulf Coast Mosquito Research Laboratory at Lake Charles, Louisiana. Twelve hours before treatment of the mosquito larvae, the aliquots of sand were flooded with well water at the test temperature to hatch the preparasites.

Four beakers were randomly selected at each of eight exposure periods. Three of the beakers were then inoculated with preparasites at a 10:1 parasite : host ratio. The fourth beaker was handled similarly but was not inoculated. Following treatment, the beakers were returned to the incubator for 24 hours. The beakers were then removed, and the mosquito larvae were concentrated and rinsed in a 150-μm-pore sieve to remove any free-swimming preparasites to prevent further parasitism. Mosquito larvae were then transferred to enamel pans (18 × 30 × 5 cm), reared to the fourth instar at ca. 27 C, and dissected to determine parasitism.

The resulting infectivity levels of larvae from the three treated beakers were statistically analyzed using a model based on three constant temperatures with eight replications over time for a total of 72 observations. Data were subjected to analysis of variance using SAS general linear models procedure for testing the hypothesis that parasitism percentages were equal (13).

RESULTS AND DISCUSSION

The ability of *R. culicivorax* preparasites to penetrate and infect *Ps. columbiae* larvae sharply decreased with host age after ca. 28 hours. At 26 C, parasitism of larvae declined from 81.5 to 49.0% between 27 and 39 hours (Table 1). At 29 C, parasitism decreased from 72.5 to 17.8% for larvae exposed when 24 and 34 hours old (Table 2); and at 32 C, parasitism decreased from 36.9 to 11.4% for larvae exposed when 28.5 and 35 hours old (Table 3).

Parasitism showed a significant linear ($P < 0.01$) decrease from newly hatched first instar to the oldest larval age class (Table 4). There was a significant ($P < 0.01$) temperature × time interaction which indicated that each temperature treatment needs to be considered separately. The first and second instars of *Ps. columbiae* were more susceptible to infection than were the

TABLE 2. Susceptibility of *Psorophora columbiae* larvae over time to parasitism by *Romanomermis culicivorax* at a constant water temperature of 29 C.*

Larval age (hours)	Age structure by instar at exposure				No. surviving larvae when parasitism determined		Parasitism (%) (± SE)
	I	II	III	IV	Control	Exposed†	
1	150	0	0	0	32	16	58.0 (13.3)
12	149	0	0	0	37	27	35.4 (2.1)
24	105	38	0	0	39	35	72.5 (3.2)
34	46	98	0	0	44	42	17.8 (2.5)
44	13	96	11	0	36	32	29.5 (13.0)
53.5	4	66	82	0	31	44	2.8 (1.8)
63	1	49	86	0	41	41	1.5 (1.5)
82.5	0	7	42	35	32	24	1.6 (1.6)

* Nematode to host inoculation ratio of 10:1.
† Mean for three replications at the termination of tests.

third and fourth instars. This was consistent with the findings of Petersen (8) and Petersen and Willis (11). The levels of parasitism were low at 32 C compared to the two lower temperatures, suggesting that 32 C may have been detrimental to the nematodes by shortening their period of infectivity. The upper infectivity threshold for this nematode has been reported as 33 C (2).

Some premature mortality appeared to have resulted from *R. culicivorax* infection of *Ps. columbiae,* as the control generally had a high survivorship to the fourth instar (Tables 1–3). Mortality was highest in hosts exposed at 1 hour of age in all three tests and was proportionately higher in host populations producing the higher levels of parasitism. These findings agree with those of Kurihara (4). Furthermore, some of the variability in parasitism rates in the younger age classes may have resulted from premature death of superparasitized larval hosts, thus resulting in the lower reported levels of parasitism.

The practical application of these data for operational mosquito control agencies is that inoculations of preparasitic *R. culicivorax* to mosquito breeding habitats may not be effective. Early research efforts emphasized the application of preparasites because this stage was easy to apply by conventional ground equipment and yielded immediate parasitism of currently available mosquito larvae. However, there are several biological constraints that must be considered when using preparasites in the initial application. The primary consideration is that preparasites must be applied to standing water containing first or second instars of mosquitoes to serve as hosts. The ephemeral preparasites and the brief period of maximum susceptibility of early instars, particularly *Ps. columbiae,* demand a

TABLE 3. Susceptibility of *Psorophora columbiae* larvae over time to parasitism by *Romanomermis culicivorax* at a constant water temperature of 32 C.*

Larval age (hours)	Age structure by instar at exposure				No. surviving larvae when parasitism determined		Parasitism (%) (± SE)
	I	II	III	IV	Control	Exposed†	
1	150	0	0	0	39	30	19.1 (5.9)
11	149	0	0	0	46	43	30.3 (4.7)
22	101	46	0	0	45	40	36.2 (7.8)
28.5	56	96	0	0	50	44	36.9 (1.8)
35	20	105	13	0	47	44	11.4 (2.1)
43.5	9	57	68	0	48	44	3.8 (2.0)
52	3	21	96	7	43	39	5.2 (2.7)
71	0	5	29	92	46	41	0.7 (0.7)

* Nematode to host inoculation ratio of 10:1.
† Mean for three replications at the termination of tests.

TABLE 4. Analyses of variance evaluating the susceptibility of *Psorophora columbiae* larvae over time to parasitism by *Romanomermis culicivorax*.

Source	df	MS	*F*
Time	7	0.5544	68.48**
Temperature	2	0.4506	55.66**
Temperature × time	14	0.0738	9.12**
Linear through time			
Within 26 C	1	2.7295	337.14**
Within 29 C	1	1.0292	127.13**
Within 32 C	1	0.2537	31.33**
Error	48	0.0081	

** $P < 0.01$.

rigid and synchronous application period in order to obtain optimum infection. These criteria severely inhibit mosquito control operations from using preparasites for the control of floodwater mosquitoes. In addition, it is difficult to detect freshly emerged larval populations and subsequently hatch the infective preparasite for treatment of breeding habitats and remain within the 28-hour period in which early instars are most susceptible. Andis et al. (1) have shown that the 475-ml dipper, the standard surveillance method used to detect mosquito larvae, is an inadequate tool for the detection of first-instar and second-instar *Ps. columbiae*.

The application of postparasites rather than preparasites offers more versatility in the treatment schedule of mosquito breeding habitats. Postparasites do not require immediate application, as do preparasites newly emerged from the egg. Also, the habitat need not be flooded at the time of postparasite application. Postparasites continue maturation both in the aquatic ecosystems and when applied to moist soil habitats. In moist soil, however, mature eggs of *R. culicivorax* will remain unhatched until the habitat is flooded, thus synchronizing egg hatch of both the parasite and the host.

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