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RELATIONSHIP OF DENSITY, LOCATION OF HOSTS, AND WATER VOLUME TO PARASITISM OF LARVAE OF THE SOUTHERN HOUSE MOSQUITO BY A MERMITHID NEMATODE[1]

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ABSTRACT. When the volume of water in six square outdoor ponds varied from 25 to 900, from 50 to 1800, and from 75 to 2700 liters, no significant differences in the mean incidence of parasitism by *Reesimermis nielseni* Tsai and Grundmann in *Culex pipiens quinquefasciatus* Say could be detected as a result of dilution. The incidence of parasitism was somewhat suppressed when most densities reached 0.4 host larvae per cc, but this reduction could be overcome by increasing the ratio of parasites to hosts at the time of exposure. Parasitism was higher in host larvae held in the corners of ponds of four or more square meters of surface area than when larvae were held at the edge or the middle of the ponds. Parasitism was lowest in larvae held in the middle of the ponds. Also, parasitism was higher when the hosts were held in screened containers that permitted a water surface-screen contact than when they were held in containers that prevented such contact.

Thus water volume was not a major factor in determining the incidence of parasitism by *R. nielseni,* and preparasitic *R. nielseni* exhibited thigmotactic and negatively geotactic behavior.

Reesimermis nielseni Tsai and Grundmann, a mermithid nematode parasite of mosquito larvae, is a promising biological control agent that has received considerable attention. Nevertheless, little is known about the way the infective stage (preparasitic nema) finds its host. An investigation was therefore conducted at Lake Charles, Louisiana, to delineate the importance of the densities of parasite and host on the incidence of parasitism by *R. nielseni* of *Culex pipiens quinquefasciatus* Say and to study the host seeking behavior of the preparasitic stage. In a previous laboratory study, parasitism by *R. nielseni* was observed to decrease by about 50 percent when water volumes were increased from 0.2 to 30 liters (Petersen and Willis, 1970). However, the factors of surface area, container shape, and depth of water were not considered. The present study was planned to compensate for these variables and was made in outdoor screened enclosures in ponds that permitted the use of greater volumes of water.

[1] In cooperation with McNeese State University, Lake Charles, Louisiana.

EFFECT OF DILUTION. Six ponds measuring 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 m^2 by 40 cm deep were made by lining redwood frames with two layers of 16-gauge polyethylene plastic. They were filled to the desired depth with tap water and treated with sodium thiosulfate to remove the chlorine. Then five hundred 6 to 8-hour-old first instar *C. p. quinquefasciatus*, the desired number of preparasitic nematodes, and rabbit pellets (a food source for the mosquitoes) were introduced into each pond. Both the mosquito larvae and the nematodes were obtained from laboratory colonies. Four to five days after the introduction, 200–300 larvae were recovered from each pond and held in the laboratory until the incidence of parasitism was determined. The ponds were pumped out, cleaned and refilled for the test the next week.

In the first test (replicated 5 times), the six ponds were filled to a depth of 10 cm, and 500 first instar *C. p. quinquefasciatus* and about 5,000 preparasitic *R. nielseni* were added to each. The water volume in the six ponds ranged from 25 to 900 liters, and the density of the parasites ranged from 0.2 to 0.005 per cc. Regression analysis showed that the difference in mean incidence of parasitism (Table 1) was non-significant. In a second test the ponds were filled to a depth of 20 cm and water volumes ranged from 50 to 1800 liters. Again, after 5 replications, no significant regression could be demonstrated in the incidence of parasitism when correlated with the increase in water volume. In a third test the ponds were filled to a depth of 30 cm (5 replications) and even though the volumes ranged from 75 to 2700 liters, still no significant differences in mean parasitism could be detected as a result of the dilution.

EFFECT OF HOST DENSITY. The effect of host density was studied in a fourth test by filling the ponds to a depth of 15 cm and introducing 15,000 host larvae into each pond. In the first two trials, about 150,000 preparasitic nemas were

TABLE 1.—Effect of water volume on the incidence of parasitism of *C. p. quinquefasciatus* by *R. nielseni*.[1]

Pond	Volume (liters)	No. hosts per cc	No. preparasitics per cc	Mean percentage parasitism [2, 3]
		10-cm depth		
V-1	25	0.02	0.2	77.2
V-2	100	0.005	0.05	89.2
V-3	225	0.002	0.02	62.0
V-4	400	0.001	0.01	65.2
V-5	625	0.0008	0.008	63.8
V-6	900	0.0005	0.005	61.9
		20-cm depth		
V-1	50	0.01	0.1	84.0
V-2	200	0.0025	0.025	71.0
V-3	450	0.001	0.01	39.5
V-4	800	0.0006	0.006	78.3
V-5	1250	0.0004	0.004	51.1
V-6	1800	0.0003	0.003	70.0
		30-cm depth		
V-1	75	0.007	0.07	54.8
V-2	300	0.002	0.02	51.2
V-3	675	0.0007	0.007	47.6
V-4	1200	0.0004	0.004	56.4
V-5	1875	0.0003	0.003	51.2
V-6	2700	0.0002	0.002	41.5

1 All exposures made at 10:1 parasite to host ratio.
2 All tests replicated 5 times.
3 No significant regression occurred.

TABLE 2.—Effect of high host densities on the incidence of parasitism of *C. p. quinquefasciatus* by *R. nielseni.*

Pond	No. hosts per cm² of surface area[1]	No. hosts per cc[1]	No. preparasitics per cm² of surface area[2]	No. preparasitics per cc[2]	Percent parasitism			
					Trial 1	Trial 2	Trial 3	Mean
V-1	6.0	0.4	60 (66)[3]	4.0 (4.4)[3]	54.6	64.8	93.5	71.0
V-2	1.5	0.1	15 (16.5)	1.0 (1.1)	58.4	73.5	92.5	74.8
V-3	0.67	0.044	6.7 (7.3)	0.44(0.49)	63.7	85.2	91.4	80.1
V-4	0.37	0.025	3.7 (4.1)	0.25(0.27)	94.0	84.4	89.6	89.3
V-5	0.24	0.016	2.4 (2.6)	0.16(0.18)	74.5	71.7	91.4	79.0
V-6	0.17	0.011	1.7 (1.8)	0.11(0.12)	79.5	89.0	83.3	82.3

[1] 15,000 first-instar *C. p. quinquefasciatus* per pond.
[2] 150,000 preparasitic *R. nielseni* introduced to each pond in trials 1 and 2; 165,000 preparasitics introduced in trial 3.
[3] Densities for trial 3 in brackets.

introduced; and in the third, about 165,000. In trials one and two there were 6.0 host larvae per sq. cm of surface area (0.4 larvae/cc) in the 0.5 m² pond (V-1), and there was a lower incidence of parasitism than in the ponds with high volumes of water (Table 2). Thus, at very high host densities, the incidence of parasitism seems to be somewhat suppressed, perhaps because of increased activity of the hosts at these densities or because the hosts take in the parasites as food. Nevertheless, as in the first three tests, host densities that were below one mosquito per sq. cm of surface area showed no significant difference in incidence of parasitism that might result from dilution. Still, the higher ratio of parasites to hosts in trial three resulted in higher incidences of parasitism at the lowest volumes of water. Perhaps at high host densities, higher ratios of parasites to hosts are needed to effect levels of parasitism similar to those obtained at lower host densities. Nevertheless, the results indicate that high levels of parasitism can be obtained even at very high host densities.

EFFECT OF HOST LOCATION. The previous extensive laboratory observations of *R. nielseni* infecting mosquitoes had not revealed any host orientation by the infective stage parasite and had led us to the conclusion that parasitism occurred by chance collision (Petersen *et al.* 1968). However, the data in these present tests indicated that random collision between the preparasitic nema and its host was not the only process involved in producing the levels of parasitism observed. For example, one could not expect similar levels of parasitism to occur by chance at densities of one host and 10 parasites in both 50 and 5400 ml of water. Then if contact does not occur through accidental collision, other behavioral responses are occurring that increase the chance of contact. Since mosquito larvae tend to concentrate at the water surface and along the edges of objects and bodies of water, a test was designed to determine whether these same responses occurred in the preparasitic nemas of *R. nielseni.*

In a series of tests the host larvae were restricted to specific locations within the ponds by placing them in cylindrical plastic containers (11.5 cm in diameter by 14 cm deep) similar to those described by Chapman *et al.* (1970). Six of the containers were prepared by cutting two 9 x 15-cm openings in the sides of each (1 cm from the bottom and 3.5 cm from the top) and covering the openings with 55-mesh stainless steel screen. To determine if preparasitic nemas concentrate at the surface, another set of 3 containers was prepared with 4.5 x 15-cm openings (1 cm from the bottom and 8.5 cm from the top) and were covered like the other containers. Three like containers were then placed in the 1-, 2-, and 3-m ^{2} ponds in such a way that the top 5 cm remained out of water. Thus in two ponds, the screened portion of the containers touched the water surface and in one pond, the top of the screened portion was about 3.5 cm below the water surface. One container was placed in a corner, one in the center, and one in the middle of a far edge of each pond, and during the trials, all four corners and sides were tested in an attempt to remove any bias resulting from outside influences and the different sets of containers were placed in different ponds in each trial.

Once the containers were in place, 250 24-hour-old first-instar larvae and a few rabbit pellets were added to each, and the desired number of preparasitic nematodes was placed in each pond at a point about equidistant from the three containers. Three days later, the mosquito larvae were returned to the laboratory and handled as previously described. The test was replicated four times (Table 3). In the 1 sq. m surface area pond (V-2), location had little effect on the mean incidence of parasitism. However, in the 4 sq. m surface area pond (V-4), the mean incidence of parasitism was almost 20 percent higher in the corner container than in the other two; furthermore, the corner container had the highest incidence of parasitism in all four trials. Also, in the 9 sq. m surface area pond (V-6), the corner container always had a much higher incidence of parasitism than the other two locations and parasitism was noticeably higher at the edges of this pond than in the center.

As noted, (Table 3), in each trial, the screened openings of the containers in one pond were about 3.5 cm below the surface of the water, and the openings in the containers in the other two ponds were at the surface of the water. Regardless of the size of the pond with the containers with openings below the surface of the water, the incidence of parasitism was significantly lower when the opening was below the surface. The results indicate that preparasitic *R. nielseni* are then apparently negatively geotactic and concentrate near the surface of the water. Nevertheless, the same general pattern of

TABLE 3.—Incidence of parasitism by *R. nielseni* of larvae of *C. p. quinquefasciatus* held in three different locations in square ponds.

Position	Replications				
	1	2	3	4	Mean
			Pond V-2[1]		
Corner	19.8[2]	79.5	89.1	23.5[2]	53.0
Center	7.0	86.2	95.3	14.9	50.8
Edge	32.1	83.4	100	26.3	60.4
			Pond V-4		
Corner	92.9	12.1[2]	82.6	48.1	58.9
Center	40.0	0.9	71.6	44.6	39.4
Edge	47.5	3.5	63.7	41.8	39.1
			Pond V-6		
Corner	71.7	30.5	15.1[2]	66.8	46.0
Center	13.6	13.9	5.3	24.9	14.4
Edge	44.8	16.4	10.4	35.3	26.7

[1] Pond V-2 had a surface area of 1 sq. m and a volume of 150 liters; Pond V-4 had a surface area of 4 sq. m and a volume of 600 liters; Pond V-6 had a surface area of 9 sq. m and a volume of 1350 liters.

[2] Pond containing larval containers with top of screens below surface of the water (see text).

parasitism with reference to location was there in all ponds with all containers.

These data indicate that water volume is not itself a major factor in determining the incidence of parasitism of mosquitoes by *R. nielseni*. They also indicate that preparasitic *R. nielseni* are negatively geotactic and thigmotactic in behavior. These responses greatly enhance the chances that this parasite will contact a suitable host since most mosquito species exhibit the same responses. Indeed, the greater susceptibility of anopheline mosquitoes to parasitism compared with culicine mosquitoes (unpublished data) may be partly the result of the behavior of anopheline larvae: they suspend themselves horizontally at the surface of the water where they would be more apt to be contacted by randomly swimming preparasitics. The swimming behavior of preparasitic *R. nielseni* also improves the potential of this parasite for mosquito control (see Petersen, 1973, for discussion of other factors). The nematode can now be applied to many mosquito breeding areas with the assurance that dilution or large open surfaces of water will not greatly reduce its effectiveness.

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OBITUARY

Boyd M. Lafferty

Boyd M. Lafferty, Executive Secretary of the Cape May County Mosquito Extermination Commission, Cape May, New Jersey died June 26, 1973. Boyd experienced complications following heart surgery. He was just 2 weeks from his 56th birthday. For 25 years he guided a greatly expanded mosquito control program in his county which is highly dependent on resort and recreational activity for its economic base. Boyd Lafferty met successfully and with great directness a multitude of mosquito problems associated with economic development in a county having an unusually high ratio of tidal wetlands to its total area. He was active in the affairs of the New Jersey Mosquito Extermination Association and the AMCA, and he will be long remembered by those who worked with him.—D. M. JOBBINS