EXPERIMENTAL RELEASE OF A MERMITHID NEMATODE TO CONTROL *ANOPHELES* MOSQUITOES IN LOUISIANA [1]

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ABSTRACT. The mermithid nematode *Reesimermis nielseni* Tsai and Grundmann was released 30 times (15 at rates of 1000 and 15 at rates of 2000 per square yard of surface area) in attempts to control *Anopheles* mosquito larvae (principally *Anopheles crucians* Wiedemann) in natural habitats. At the lower rate, 76 (52–100) percent of the hosts were infected, and parasitism averaged 60, 80, 86, and 71 percent in 1st- through 4th-instar hosts, respectively. At the higher rate, 85 (36–100) percent of the *Anopheles* larvae were infected, and 10 of the 15 treatments produced levels of parasitism in excess of 90 percent. *Reesimermis nielseni* can efficiently locate and parasitize *Anopheles* larvae. In general, vegetation and depth of water had little influence on ultimate levels of parasitism.

The preparasitic stage of the mermithid nematode *Reesimermis nielseni* Tsai and Grundmann was released 30 times at two rates to test the effect of the nematode on the larval stages of *Anopheles* mosquitoes (principally *Anopheles crucians* Wiedemann) in various types of habitat.

Previously, Petersen and Willis (1972a) showed that several species of mosquitoes including *Anopheles* were readily parasitized when *R. nielseni* was released into natural habitats and that the parasite became established in many of these sites. Furthermore, *R. nielseni* was found to infect 50% and 80–85% of the *A. freeborni* Aitken in California rice fields when the parasites were applied at rates of 500 and 1000 per square yard of surface area, respectively (Petersen *et al.* 1972). Also, Petersen *et al.* (1973) reported that 3900 and 1700 preparasitic *R. nielseni* per square yard of surface area would be required to achieve 94+% parasitism of early-instar *Psorophora confinnis* (Lynch Arribálzaga) and *A. quadrimaculatus* Say, respectively, in Louisiana rice fields.

The present study was made to determine: (1) the control of *Anopheles* that could be expected at two rates of application, (2) the effect of different habitats on levels of parasitism, and (3) the reliability of *R. nielseni* against *Anopheles* mosquitoes.

MATERIALS AND METHODS. The infective stage of *R. nielseni* (preparasitic juveniles) was obtained from laboratory cultures, and the numbers per unit volume of water were determined by volumetric dilution as described previously (Petersen and Willis 1972b). Then the nematodes were transported to the field in stock cultures where final dilutions were made by using water from the test site. The parasites were applied at rates of 1000 or 2000 preparasitic nematodes per square yard of surface area from a compressed air sprayer (2 gallon capacity) with a standard fan nozzle. Treated areas ranged from 225 to 550 square yards; at sites that exceeded 550 square yards only portions of the area were sprayed. Also, only vegetated edges of open ponds were treated.

Anopheles populations were sampled 24-hours post-treatment, and all larvae obtained were returned to the laboratory and examined by microscopic dissection for nematode parasitism.

Twenty-three semipermanent to permanent sites were selected for treatment that were low in salinity, devoid of *R. nielseni*, and good producers of *Anopheles* mosquitoes. The sites varied greatly in size, shape, depth, and amounts of floating

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and emergent vegetation present. However, most were exposed to direct sunlight, and many possessed high populations of top minnows and other mosquito predators. The sites included fallow rice fields (1, 2, and 3); open ponds (4, 5, 6, 7, 8, 9, 10, 11, and 12); habitats created by oil drilling operations (13, 14, and 15); densely vegetated ponds (16, 17, 18, 19, and 20); fresh water swamps (21 and 22); and an irrigation canal (23).

RESULTS. When 15 sites were treated with ca. 1000 preparasitic nematodes per square yard of surface area (Table 1), parasitism ranged from 52 to 100 percent and averaged 76 percent 24-hour post-treatment. The highest mean incidence of parasitism occurred in 3rd-instar larvae (86%) with 12 of the 15 sites producing parasitism in excess of 80 percent in this instar. Parasitism was lowest in 1st-instar larvae (60%). Also, 15 treatments were applied to 13 sites (2 treated twice) at a rate of ca. 2000 preparasitics per square yard of surface area (Table 2); 85 percent of all larvae collected contained one or more nematodes 24-hour post-treatment. Parasitism ranged from 36 to 100 percent, and ten of the treatments produced levels of parasitism in excess of 90 percent. Again, 3rd-instar hosts appeared most susceptible with 11 of 14 treatments producing 100 percent parasitism. Parasitism was lowest in 4th-instar larvae (70%).

Where the incidence of parasitism was compared with the type of habitat, little or no correlation was observed. For example, sites 16, 17, 19, and 20 (heavily vegetated) exposed to the same dose produced widely differing levels of parasitism (Table 2). Similar results were also obtained in the open water sites where only the edges were treated. Also, one site (13) consistently produced poor results at both doses though the host populations were very high at the time of treatment. Apparently other unknown factors reduced the effectiveness of the preparasitic *R. nielseni*.

Previously, older larvae of *Culex p. quinquefasciatus* exposed to *R. nielseni* in the laboratory proved to be less susceptible to parasitism than younger larvae (Petersen and Willis 1970), but in nature, 3rd-instar *Anopheles* larvae appeared to be the most susceptible and 1st- and 4th-instar larvae appeared to be the least

TABLE 1. Parasitism of *Anopheles* spp. larvae by *R. nielseni* at 24 hours post-treatment with doses of 1000 parasites per square yard of surface area.

Site no.	Percentage parasitism by instar (number of larvae sampled) [1]				
	First	Second	Third	Fourth	All
12	35 (20)	60 (25)	86 (7)	25 (4)	51.8 (56)
13	0 (19)	56 (34)	83 (24)	89 (9)	54.6 (86)
21	100 (3)	67 (30)	33 (12)	44 (9)	57.4 (54)
6	46 (43)	85 (13)	100 (9)	0 (3)	58.8 (68)
11	44 (9)	61 (18)	80 (15)	50 (10)	61.5 (52)
9	43 (21)	76 (25)	76 (25)	60 (20)	64.8 (91)
2	50 (2)	50 (2)	100 (9)	50 (6)	73.7 (19)
19	53 (30)	85 (27)	100 (13)	100 (1)	74.6 (71)
23	86 (7)	71 (7)	91 (11)	67 (3)	82.1 (28)
18	72 (18)	95 (44)	43 (7)	100 (2)	84.5 (71)
5	86 (7)	100 (9)	100 (14)	86 (22)	92.3 (52)
4	91 (11)	91 (11)	93 (14)	100 (14)	94.0 (50)
14	33 (3)	100 (17)	100 (24)	100 (15)	96.6 (59)
7	.. (0)	100 (5)	100 (19)	93 (14)	97.4 (38)
22	100 (1)	100 (22)	100 (36)	100 (9)	100 (68)
Means & totals	59.9 (194)	79.8 (289)	85.7 (239)	70.9 (141)	76.3 (863)

[1] Percentage parasitism was determined from number of hosts penetrated by *R. nielseni* even though host resistance may have been evident.

TABLE 2. Parasitism of *Anopheles* spp. larvae by *R. nielseni* at 24 hours post-treatment with doses of 2000 parasites per square yard of surface area.

Site no.	Percentage parasitism by instar (number of larvae sampled) [1]				
	First	Second	Third	Fourth	All
13	35 (20)	35 (20)	55 (20)	0 (9)	36.2 (69)
13	0 (5)	52 (40)	69 (29)	79 (39)	62.8 (113)
17	44 (9)	80 (5)	100 (3)	.. (0)	64.7 (17)
19	.. (0)	100 (6)	.. (0)	40 (5)	72.7 (11)
21	89 (9)	95 (22)	82 (17)	50 (6)	85.2 (54)
8	.. (0)	100 (13)	100 (21)	72 (18)	90.4 (52)
1	.. (0)	100 (5)	100 (17)	73 (11)	90.9 (33)
5	89 (9)	89 (9)	100 (15)	87 (16)	91.8 (49)
15	100 (9)	80 (10)	100 (6)	.. (0)	92.0 (25)
7	100 (3)	100 (9)	100 (11)	75 (8)	93.5 (31)
10	93 (13)	94 (35)	100 (11)	75 (4)	93.6 (63)
21	100 (6)	100 (24)	100 (23)	87 (8)	98.4 (61)
20	.. (0)	100 (1)	100 (12)	100 (7)	100 (20)
3	100 (3)	100 (1)	100 (13)	100 (5)	100 (22)
16	.. (0)	.. (0)	100 (4)	.. (0)	100 (4)
Means & totals	74.9 (86)	87.5 (200)	93.3 (202)	69.7 (136)	84.8 (624)

[1] Percentage parasitism was determined from number of hosts penetrated by *R. nielseni* even though host resistance may have been evident.

susceptible. Therefore, when we observed lower than expected incidence of parasitism in 1st-instar larvae in the present test, we attributed it in part to the continual hatching of new larvae after the numbers of parasites were reduced. As a result, we divided the 1st-instar larvae taken in six of the 30 collections into early or late stages (based on the presence of the so-called collar that results from the developing head capsule of the next instar) before dissection; parasitism averaged 13 and 45 percent, respectively. Similarly, when the 4th-instar larvae taken in 14 of the collections were divided into early or late stages based on size and prepupal development, the mean incidence of parasitism was 88 and 70 percent, respectively. However, the levels of parasitism may sometimes have been higher than we estimated. In some samples, a majority of the *Anopheles* spp. were multiple-infected, which often results in the early death of the host, especially in early instars. Thus, the more heavily infected hosts probably died and were not present during the sampling. For example, site 19, which was treated with 2000 preparasites per square yard produced no 1st- or 3rd-instar larvae and only 6 2nd-instar larvae (all heavily infected) 24-hour post-treatment. Since only 2 of 5 4th-instar larvae were infected, the mean incidence of parasitism was only 73 percent. Obviously it may have been much higher. Also, at the time the hosts were examined, the nematodes were very small and could have been occasionally overlooked, particularly in the larger hosts.

Resistance to the nematode was observed in some host larvae after all 30 treatments (previous observations indicated that resistant hosts developed normally). Resistance was not observed in 1st-instar hosts and it averaged less than 1 percent in 2nd-instar hosts from 29 of the treatments, 22 (0–67) percent in 3rd-instar larvae, and 51 (0–100) percent in 4th-instar larvae. Resistance was about 20 percent higher in late 4th-instar larvae than in early 4th-instar larvae. Finally, site 13, previously mentioned as having the lowest incidence of parasitism, also had the highest incidence of host resistance in the 2nd- (70%) and the 3rd- (67%) instar hosts.

Much of the resistance in *Anopheles* appeared to be related to the thickness of

the host cuticle since most of the dead nematodes were in the integument of the host rather than in the haemocoele. Also, when melanized nematodes were free in the haemoecoele, at least some seemed to have been weakened in the effort to enter the host to the point that they died or could not resist the defense mechanisms (many hosts with melanized nematodes also had one or more normal developing nematodes). In addition, *Anopheles quadrimaculatus* comprised about 5–20 percent of most of the samples, and this species is known to be moderately resistant to *R. nielseni*. Therefore, since the larvae were not identified by species, many of those exhibiting resistance were undoubtedly *A. quadrimaculatus*.

Generally 2 or 3 (range 1–5) sites were treated on a given day from a given stock of preparasitic *R. nielseni*. When the levels of parasitism from these sites were compared, the data indicated that at least one stock produced especially high levels of infection, one stock produced lower than normal levels, and the other nine stocks produced a variety of levels. These variations among the nine latter stocks were apparently the result of environmental factors or sampling error.

The study demonstrated that preparasitic *R. nielseni* efficiently locate and parasitize *Anopheles* larvae and that the amounts of vegetation or the amount of untreated open water have little influence on the ultimate levels of parasitism. Also, levels of parasitism can be increased by increasing the dose of nematodes. The data also suggest that doses in excess of 2000 preparasitic nematodes per square yard of surface area may result in many cases of multiple infection. Therefore, too large a dose would result in early death of both hosts and parasites and would lessen the chance that the parasites might become established.

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