RESULTS OF PRELIMINARY FIELD APPLICATIONS OF *REESIMERMIS NIELSENI* (MERMITHIDAE:NEMATODA) TO CONTROL MOSQUITO LARVAE[1]

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ABSTRACT. A mean of 65, 58, and 33 percent of the second, third, and fourth instar larvae of the *Anopheles* spp., respectively, were parasitized by the nematode *Reesimermis nielseni* Tsai and Grundmann when 10 natural sites were treated 20 times with preparasitic juveniles of the nema. Dosage rates of 1000 preparasitic nemas per sq. meter of water surface resulted in 94 percent parasitism in second instar *Anopheles* larvae and 64 percent parasitism in all *Anopheles* sampled. Three sites treated with postparasitic nemas produced mean monthly parasitism of *Anopheles* larvae ranging from 20 to 24 percent with monthly highs of 100, 34, and 27 percent. All sites produced natural infections at least once after treatment, and *R. nielseni* now appear to have become established in at least seven of the sites.

Reesimermis nielseni Tsai and Grundmann has been studied extensively from 1966 to the present as a promising biological control agent against mosquito larvae (Petersen, 1972b; Petersen and Willis, 1970, 1971; Petersen *et al.*, 1968). Therefore, preliminary field releases of *R. nielseni* were made in an attempt to infect natural populations of mosquito larvae at the Gulf Coast Marsh and Rice Field Mosquito Research Laboratory, Lake Charles, Louisiana, during 1971. Except for a release of a *Romanomermis* sp. on Nauru Island in 1967 (Laird, 1971; results unknown), this release is the first reported attempt to infect natural populations of mosquitoes with mermithid nematodes.

MATERIALS AND METHODS. The preparasitic and postparasitic juveniles of *R. nielseni* used in the releases were obtained from laboratory cultures; the numbers released were determined by procedures described by Petersen and Willis (1972). However, at the time of this study, the techniques for the mass rearing of *R. nielseni* had not yet been developed fully, so treatments were attempted whenever sufficient infective-stage nemas were available. Dosages were therefore dictated by the availabilty of the nemas, and often only a portion of a test site could be treated at any given time.

Since laboratory tests showed that preparasitic nemas could be passed through a compressed-air sprayer with little or no loss of infectivity of the parasites, all preparasitic nemas were disseminated by using a 1 gallon-capacity compressed-air sprayer with a standard fan nozzle unless otherwise stated. Postparasitic nemas were released by placing small groups (about 0.15g wet weight) randomly over an area by hand.

Mosquito populations were sampled with standard pint dippers 1–3 days after the sites were treated with preparasitic nemas; all second to fourth instar larvae and pupae obtained were returned to the laboratory where the levels of parasitism were determined by microscopic dissection.

RESULTS. Eleven sites known to be free of *R. nielseni* from previous studies were selected for treatment; all were semipermanent to permanent and low in salinity (conductivities ranged from 22 to 400 micromhos/cm). However, the ponds varied both in size (from 209 to 3,000 square meters) and in the amounts of emergent and floating vegetation present. Most sites were exposed to direct sunlight and possessed high populations of top minnows and other mosquito predators. Also, the environmental conditions of most

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of the sites appeared similar to those at sites found to produce natural parasitism by *R. nielseni* (Petersen and Willis, 1971).

RELEASES OF PREPARASITIC NEMAS. Ten of the sites were treated one to three times (20 releases) with about 180 to 14,700 preparasitic nemas per square meter of surface area (Table 1).

The first release was made in site 2 in early April by casting about 1.1 x 10^6 preparasitics by hand over a 75-square-meter portion of the pond. Three days after treatment, 79 percent of the sampled *Anopheles* spp larvae were parasitized and three of four pupae contained nemas; most of the parasitized anopheline larvae were multiply infected (as many as 25–50 nemas/host). Dead larvae were observed at the site and upon examination appeared to have been killed by an overdose of nemas. Populations of *Anopheles* larvae appeared much lower in the release area after the treatment than in other areas of the pond, but the extent of the reduction could not be determined because no pretreatment counts were made. Since the number of nemas per infected host was so high, many of the uninfected larvae present (21 percent) had probably moved into the treated area after the infectivity of the nemas decreased.

Site 1 was similarly treated a short time later but with a lower dose of preparasitics (5,000/sq m). Again, though only 67 percent of the anopheline larvae were parasitized, many were multiple-infected, and 6 of 12 pupae were parasitized. These multiple attacks on the more susceptible early-instar larvae once more appeared responsible for the low populations 2 days after the treatment.

The preparasitic nemas in the 18 subsequent treatments at the other eight sites were all disseminated with the compressed-air hand sprayer. These results, too, are summarized in Table 1. Means of 65, 58, and 33 percent of the second, third, and fourth instar anopheline larvae, respectively, were parasitized; eight treatments produced 75 percent or higher parasitism

TABLE 1.—Parasitism in larvae of *Anopheles* spp. after preparasitic *Reesimermis nielseni* were disseminated from a pressurized spray can.

Site no.	No. preparasitics released per sq. m surface area	Sampling of larvae: No. days after treatment	Sampling of larvae: No. examined	Percentage parasitism by instar: Second	Third	Fourth	All
1	5000[a]	2	42	..	..	..	67
2	14,700[a]	3	67	..	..	..	79
3	2400	3	61	76	82	70	77
4	300	2	96	22	29	0	23
	1400	2	62	78	95	79	84
	670	1	58	0	63	25	26
5	1500	3	30	45	46	0	37
	3300	1	35	..	89	61	69
6	180	3	45	29	8	0	18
	1000	2	35	100	87	73	86
	1000	1	36	86	36	12	50
7	230	3	28	27	57	40	39
	460	2	7	50	80	..	71
	740	1	21	75	0	0	14
8	670	2	51	83	87	71	82
	1000	1	4	100	100	100	100
9	3000	3	49	71	50	25	47
	1300	1	6	50	..	100	67
10	670	3	45	37	100	0	42
	1000	1	73	89	22	5	20

[a] Preparasitics introduced by hand over a small portion of the habitat.

in second and third instar larvae, and seven produced 50 percent or higher parasitism in fourth instar larvae.

Our laboratory studies showed that host larvae become more resistant to penetration by nemas with age (Petersen and Willis, 1970); our data from the field releases generally were in agreement, but the differences were not as evident because of variations in the time between treatments and sampling and the increases in water temperature from April through July which permitted differential growth of infected larvae before examination.

The actual incidence of parasitism at the various sites may have been somewhat higher than reported since some early mortality due to multiple penetrations probably occurred; also, the nemas were so small (usually less than 1 mm long) at the time of dissection that some parasites were probably overlooked, especially in the larger hosts.

When the incidence of parasitism in anopheline larvae was compared against the numbers of preparasitic nemas applied per square meter of surface area, parasitism of second instar hosts was found to increase with an increase in the dose of parasites to 1,000 per square meter (94 percent parasitism in second instar larvae); it did not increase at higher doses (Table 2). In view of the uncontrolled variables between treatments and from site to site, it is difficult to explain exactly why parasitism was lower than expected at the higher doses. However, two of the six high doses were partial treatments of the most heavily vegetated site where unexposed larvae were apt to encroach and where loss of preparasitics left stranded on the floating mat of vegetation was expected. In four other treatments at the higher doses, one site produced no second instar hosts, and two others showed a lower incidence of parasitism in second than in third instar hosts, which could indicate early death of early-stage hosts.

The eight sites treated only with preparasitic nemas (1–3 times) were monitored weekly throughout the remainder of the year to determine the potential of the inoculative effect (natural recycling of this parasite) on host populations. Recycling of *R. nielseni* required from 6 to 29 weeks. All sites produced natural infections at least once during the remainder of the summer, and mean parasitism ranged from less than one percent to 40 percent in *Anopheles* spp. The highest monthly incidence, 64 percent, occurred at site 5. Once natural parasitism commenced, it remained fairly consistent throughout the remainder of the season in most sites.

RELEASES OF POSTPARASITIC NEMAS. In the 3 (of the 11) sites treated with postparasitic nemas, (2 of which were also treated with preparasitics), natural parasitism, once it began, continued in mosquitoes throughout the remainder of the season. Mean parasitism of *Anopheles* spp. at these sites ranged from 20 to 24 percent, with monthly highs of 100, 27, and 34 percent at sites 1, 2, and 11, respectively (Table 3). Site 11 was the only

TABLE 2.—Effect of doses of *Reesimermis nielseni* on the incidence of parasitism in larvae of *Anopheles* spp.

		Percentage parasitism of *Anopheles* spp. at the indicated larval instar			
Dose[a]	No. treatments	Second	Third	Fourth	All
180–250	2	28	32	20	28
251–500	2	36	54	0	47
501–750	4	49	62	24	41
751–1000	4	94	61	47	64
1001–1500	3	58	70	60	63
above 1500	3	73	74	52	64

[a] Number of preparasitics per square meter of surface area.

TABLE 3.—Parasitism (by month) in larvae of *Anopheles* spp. at 11 sites treated with preparasitic and/or postparasitic *Reesimermis nielseni.*

Site no.	No. weeks from first treatment to first natural parasitism	Percentage of *Anopheles* larvae parasitized (number of larvae sampled)								Highest percentage parasitized in a single collections
		June	July	August	September	October	November	December	All collections	
1[a, b]	7	......	20(64)	100 (7)	93 (84)	8 (12)	....	1(193)	22(364)	94
2[c]	..	33 (9)	37(19)	15 (27)	25 (65)	28(119)	17(181)	6(153)	24(466)	73
3	11	7(14)	0 (5)	....	0 (59)	0 (93)	....	0(101)	<1(272)	7
4	7	......	50 (4)	0 (27)	3(122)	2(169)	6(177)	9(101)	8(499)	23
5	6	8(38)	38(21)	64 (78)	57 (7)	64 (31)	55 (9)	0 (40)	49(184)	49
6	29	......	......	....	....	....	4 (27)	0 (14)	2 (41)	17
7	14	......	......	9(168)	28 (57)	1(191)	0 (82)	0 (9)	6(507)	38
8	6	......	18(56)	17 (81)	13 (45)	14 (77)	0 (13)	19 (16)	14(288)	40
9	16	......	......	....	11 (63)	8 (95)	17(171)	11(148)	13(477)	28
10	24	......	......	....	....	....	1(109)	0 (4)	<1(113)	2
11[d]	4	30(70)	34(59)	0 (2)	28 (78)	4(139)	0 (50)	....	20(355)	39

[a] Includes *Aedes vexans, A. alanticus, A. tormentor, Psorophora confinnis,* and *Anopheles* spp.
[b] *R. nielseni* introduced as preparasitic and postparastic juveniles.
[c] *R. nielseni* introduced at all stages of development.
R. nielseni introduced as postparasitic juveniles only.

site treated exclusively with postparasitic nemas; there, parasitism commenced 4 weeks after the release and averaged 30 percent from June through September though it decreased sharply thereafter.

Most of the mosquitoes produced in the 11 sites were permanent water breeding species, principally *Anopheles crucians* Wiedemann and *Anopheles quadrimaculatus* Say. *Uranotaenia sapphirina* (Osten Sacken), and *Culex erraticus* (Dyar and Knab) were also produced at most sites, but populations were small. Parasitism occurred in *U. sapphirina* and *C. erraticus* though it was generally lower than in the *Anopheles* spp.; however, site 5 produced 32 percent parasitism in *U. sapphirina* and 56 percent in *C. erraticus*. Site 1 produced several broods of floodwater species during the year, and parasitism ranged from 50 to 62 percent in *Aedes atlanticus* Dyar and Knab, *Aedes vexans* (Meigen) and *Psorophora confinnis* (Lynch-Arribálzaga) and was 100 percent in the only population of *Aedes tormentor* Dyar and Knab at this site. This was the first observation of *Aedes tormentor* serving as a host of *R. nielseni*. *Psorophora ferox* (Humboldt) occurred in site 1 but was free of parasites.

DISCUSSION. *Reesimermis nielseni* appeared to be established in at least 7 of the 11 sites where releases were made. The results demonstrate that this parasite can be an effective control agent against certain species of mosquitoes. Our attempts at inundative control (release of preparasitics) resulted in parasitism that ranged from 0 to 100 percent and averaged 55 percent in anopheline larvae for 20 treatments; but the exact doses that produced the most effective results could not be determined because of the many variables inherent in such diverse sites. The preparasitic *R. nielseni* were disseminated easily and simply by the same delivery systems used to spread insecticides. The potential for inoculative control was also demonstrated since *R. nielseni* completed its life cycle at least once at all 11 sites and appears to be established in at least 7 of these sites.

The recent development of a satisfactory method of mass culturing *R. nielseni* (Petersen 1972a, Petersen and Willis 1972) will permit more extensive and better controlled testing in the future.

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