ESTABLISHMENT OF THE NEMATODE *DIXIMERMIS PETERSENI* IN THE FIELD IN SOUTHWEST LOUISIANA USING LABORATORY-REARED MATERIAL[1]

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ABSTRACT. A pond was treated 8 times (September, 1973—November, 1975) with material derived from a laboratory culture of the mermithid nematode, *Diximermis peterseni*. The inundative effect of these treatments against *Anopheles crucians* and *An. quadrimaculatus* was variable. The inoculative effect of the releases was followed for almost 3 years (April, 1974-June, 1976). Levels of parasitism increased each year from 15 to 37 to 42%. Infection levels averaged 20% during the warmer months (April through September) as opposed to 32% during the cooler months (October through March).

INTRODUCTION

The mermithid nematode *Diximermis peterseni*, a parasite of anopheline mosquitoes, has been reared and maintained at the Gulf Coast Mosquito Research Laboratory since March 1972. In southwestern Louisiana natural populations of *Anopheles quadrimaculatus, An. crucians,* and *An. punctipennis* have been collected that were infected with this nematode. Also, Petersen and Willis (1974) have reported the establishment of this nematode in an anopheline breeding site as a result of releases of naturally parasitized mosquito larvae into a previously uninfected area.

The present study was undertaken to determine whether material from laboratory-reared cultures could be introduced and established in a breeding site and the duration and levels of parasitism resulting from these releases.

MATERIALS AND METHODS

The test area was a small, permanent, rectangular pond of approximately 18 x 40m. During most of the year, the pond surface was almost covered with emergent vegetation (*Bacorpa caroliniana)* that provided excellent cover for larval populations of both *An. quadrimaculatus* and *An. crucians* (for the purpose of this study, the 2 species were not separated). Pretreatment larval samples showed no activity of *D. peterseni* in the pond.

The nematodes released in the study were obtained from a laboratory colony of *An. quadrimaculatus* that had been exposed to the nematode by using modifications of techniques reported by Petersen and Willis (1972) for the production of the nematode *Romanomermis culicivorax.* The parasites were introduced into the habitat either by using a sand culture that contained viable eggs or by pouring or spraying water containing preparasitic (infective) nematodes on the pond surface. The pond was usually sampled once or twice a week after the releases, and all anopheline larvae collected were returned to the laboratory to determine the extent of parasitism by microscopic examination.

RESULTS AND DISCUSSION

The first treatment (August 15, 1973) of the pond consisted of immersing 2 cultures containing nematode eggs in the pond where the preparasites (the infective stage) subsequently hatched. At 8 days post-treatment, 8% of the *Anopheles* larvae collected from the pond were parasitized. The culture was then removed.

In the 2nd treatment (September 18,

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1973), 7700 preparasites were poured into one end of the pond. The result was 25% infection in *Anopheles* larvae collected near the release site at 2 days post-treatment.

The 3rd treatment (September 25, 1973) like the first, consisted of immersing 2 cultures containing hatchable nematode eggs in the pond which produced infection levels of 21 and 42%, respectively, 2 and 6 days later. After the initial collections, samples were taken once or twice weekly for the next 70 days. The infection rate declined following the last release but then increased to a high of 47% on December 4, 1973.

In the 4th and 5th treatments (February 22 and March 1, 1974), a total of 122,800 preparasites were poured along one end of the pond. The pretreatment infection rate was 10%, and there was little change (13%) after the 4th treatment (88,200 preparasites). However, 7 days after the 5th treatment the rate was 48%. Thereafter, in the samples taken of the *Anopheles* larval populations from March 11 to 19, the infection rate ranged from 15 to 25%.

The 6th treatment (July 24, 1974) consisted of pouring 242,350 preparasites around the entire pond. At 24-hr post-treatment, infection rates were 22, 43, 58, and 38% for 1st to 4th-instar *Anopheles*, respectively. At 5 days post treatment, infection rates were 8, 36, 36, and 57%, respectively.

In the 7th treatment (August 29, 1974) 120,000 preparasites were introduced into the pond. The pretreatment collection yielded rates of infection of 28% in 1st instar larvae, 45% in 2nd instar larvae, 0% in 3rd instar larvae; and no 4th instar larvae were collected. At 5 days post treatment, infection rates were 36%, 38%, and 16%, again, no 4th instars were collected.

The 8th and final treatment (November 18, 1975) consisted of spraying 255,000 preparasites in a 4.5-m. swath across one end of the site with a 1-gal compressed air sprayer. The pretreatment collection showed infection rates of 4% in 1st instar larvae, 10% in 2nd instar larvae, and 0% in 3rd and 4th instar larvae. A collection at 1 day post-treatment yielded rates of 70, 100, 70, and 50%, respectively. Nine days after treatment, infection rates were 100% for 1st, 2nd, and 4th instar larvae and 69% for 3rd instar larvae.

The level of infection in the pond was monitored on a regular basis from April 1974 to June 1976 to determine the extent of parasitism due to recycling (inoculative effect) of the nematode (Table 1). However, the last 3 treatments (6, 7 and 8) occurred during this time frame, so no data were added to the monthly figure for 2 wk after each of the treatments. In this way, data from the immediate (inundative) treatment did not affect the integrity of the inoculative (recycling) data.

Table 1 also shows that infection levels generally increased from the 1st to the 4th instar, which was not unexpected since recycling exposes later instars to preparasites for a much longer period. Overall, the infection levels for the 1st to 4th instars averaged 15, 25, 37, and 45%, respectively. The mean infection level for all instars was 31% for the 3 years.

The infection levels for all instars in relation to the warmer months (April through September) and the cooler months (October through March) are shown in Table 2. Overall, rates were lower (20%) during the warmer period 24°C) and higher (32%) during the cooler period (15°C). The reasons for this inverse relationship are not clear, but it could be due to increased nematode activity and decreased activity of the predators that compete for mosquito larvae in the cooler months or from the longer exposure of the mosquito larvae due to the slower rate of growth in the cooler months.

Parasitism thus increased overall during the study period and seems quite impressive when one considers that the pond never dried and that there was competition from other kinds of parasites and predators for the anopheline hosts. Therefore, laboratory-produced *Diximermis peterseni* can be used successfully to infect mosquito populations, and a continuous introduction of the nematode is not required to maintain the infections. However, the apparent seasonal variation in

Table 1. Percentage of 1st to 4th instar anopheline larvae infected with *Diximermis peterseni* that were collected from a pond in southwest Louisiana (April 1974 through June 1976).

	Percentage infected—by instar and month												Average % infection of all instars		
	First			Second			Third			Fourth					
Month	1974	1975	1976	1974	1975	1976	1974	1975	1976	1974	1975	1976	1974	1975	1976
January	—	23	33	—	25	33	—	71	39	—	0	100	—	30	51
February	—	8	35	—	20	50	—	33	86	—	77	100	—	35	58
March	—	24	36	—	39	60	—	52	66	—	78	100	—	48	66
April	3	14	6	30	26	15	37	60	27	44	77	32	29	44	31
May	14	10	30	11	19	27	38	40	29	75	30	39	35	25	31
June	0	27	17	6	54	25	13	72	23	21	79	0	10	58	17
July	0	7	—	11	12	—	9	28	—	20	36	—	10	21	—
August	5	14	—	11	36	—	7	30	—	5	50	—	7	33	—
September	0	17	—	6	19	—	16	32	—	5	50	—	7	30	—
October	2	17	—	6	27	—	11	51	—	12	55	—	8	38	—
November	6	8	—	31	15	—	30	37	—	46	40	—	28	25	—
December	0	21	—	8	48	—	6	70	—	5	75	—	5	54	—
Average % infection	4	16	26	13	28	35	19	48	45	26	54	55	15	37	42

Table 2. Average percentage infection of anopheline larvae by *Diximermis peterseni* during the period April–September and the period October–March (April 1974 through June 1976).

Year	Period	Mean temperature (°C)	Mean % infection
1974	April–September	24.9	13
1974–1975	October–March	15.4	21
1975	April-September	24.8	26
1975–1976	October–March	15.1	42
1976	April–June	22.4	20

nematode activity would have to be carefully evaluated before a control program was initiated.

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