ESTABLISHMENT AND RECYCLING OF A MERMITHID NEMATODE FOR THE CONTROL OF MOSQUITO LARVAE [1]

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ABSTRACT. Three of five sites treated in 1971 by releasing *Reesimermis nielseni* Tsai and Grundmann produced *Anopheles crucians* Wiedemann with mean levels of parasitism ranging from 7 to 52% through 1974; five of six sites treated in both 1971 and 1973 produced *Anopheles crucians* with mean levels of parasitism ranging from 2 to 51% during 1974; and five of 12 sites treated only in 1973 produced *A. crucians* with levels of infection ranging from 11 to 85% in 1974. Parasitism in the remaining 7 sites ranged from 0 to 3%. One site treated in 1973 produced 100% parasitism for 14 weeks and 94% parasitism for the year in third- and fourth-instar hosts. *Reesimermis nielseni* often becomes established in many semipermanent and permanent water sites and will produce high levels of infection in *Anopheles* mosquitoes for an indefinite period.

Only a few releases of parasites and pathogens made in an effort to control limited populations of mosquito larvae have produced substantial levels of infection (Chapman 1974). Even fewer have resulted in successful establishment and recycling of the agents.

Perhaps the first successful recycling of this type occurred in 1958 when the fungus *Coelomomyces stegomyia* Keilin was introduced from Singapore into the Tokelau Islands to control *Aedes polynesiensis* Marks; by 1963, the fungus had spread from the original release sites and was found infecting larvae in 35% of 67 samples (Laird 1967). Later Reynolds (1972) reported the recycling of the protozoan *Pleistophora culicis* (Walker) in populations of *Culex pipiens fatigans* (= *quinquefasciatus* Say) on Nauru Island two years after release though the level of infection was very low; and Nolan et al. (1973) reported low levels of infection by the fungus *Coelomomyces macleayae* Laird in *Aedes triseriatus* (Say) in two treeholes one year after a number of treeholes were seeded with infected cadavers. Also, when the mermithid nematode *Diximermis peterseni* Nickle was introduced into a natural site in the spring of 1971, 88% of the *Anopheles* spp. were parasitized two years later (Petersen and Willis 1974a).

Likewise, on several occasions, the mermithid nematode *Reesimermis nielseni* Tsai and Grundmann, a parasite of larval mosquitoes, was released at sites in the field to control populations of mosquito larvae (Petersen et al. 1972, 1973, Petersen and Willis 1972, 1974b, Hoy and Petersen 1973, and Mitchell et al. 1974). The results showed *R. nielseni* to be a good control agent in certain environments. In one of these studies (Petersen and Willis 1972), all of the 11 sites treated with *R. nielseni* produced natural infections at least once after treatment in 1971, and the nematode appeared to be established in at least 7 of the sites by the end of the year. The present study was conducted as a follow-up to the 1971 tests. Establishment was investigated at 5 sites treated in 1971, at 5 treated in 1971 and 1973, and at 12 treated in 1973 (Petersen and Willis 1972, 1974b).

MATERIALS AND METHODS. As noted, after *R. nielseni* was released in the summer of 1971 at 11 sites where *Anopheles* mosquitoes were breeding, samples of larvae were collected once a week whenever possible. This sampling was continued through December 1974. Also, 6 of the 11 sites were treated again in 1973.

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Finally, 17 additional sites were treated for the first time in 1973; however, of these, 5 were not conducive to mosquito production in 1974 and were not included in the study.

Samples of 50 hosts were collected at random, taken to the laboratory, separated to species and instar, and dissected and examined for nematodes; when necessary, small larvae were held until positive identification could be made. The data from samples collected between early December and the end of March each year were not included in the results because low temperatures have an adverse effect on the activity of *R. nielseni* (Petersen and Willis 1971). Also, meaningful sampling at many sites was sometimes impossible because of a lack of host populations, lack of breeding water during some periods, and inaccessibility due to changes in access to sites.

RESULTS. Three of 5 sites treated only in 1971 continued to produce parasitized larvae of *Anopheles crucians* Wiedemann through 1974; mean percentage parasitism for the 3 years ranged from 7 to 52% (Table 1). At the M-2 site, parasitism remained low until November 1974; then a substantial increase was observed (48% in 3 samples). It will remain to be seen if this increased activity will be carried over into 1975. The M-3 site, though dry for more than a month on two occasions in 1974, showed a substantial increase in the incidence of parasitism over previous years, and *R. nielseni* now appears to be well established there since 100% of *A. crucians* sampled in 1 month (4 samples, between two dry periods) were parasitized. The third site, R-3, remained dry throughout most of the 1974 season, and only 3 samples were taken; however, all 3 produced infected larvae (34–100%). One of the 2 sites that did not produce infected hosts through 1974, M-1, was not sampled after 1972 because of no parasite activity during 1971 and 1972. This site is located at the edge of a stream and is subject to extensive flushing action during heavy rains. The other, C-5, showed gradually diminishing parasitism over the 4 years that apparently disappeared in 1974. This site was also generally unfavorable for mosquito production through most of 1973 and 1974; only 2 samples were obtained in 1974.

Five of 6 sites treated in both 1971 and 1973 produced infected *A. crucians* through 1974. The sixth site (R-1) produced only 1 host infected from recycling after the release in 1971, and none after

Table 1. Summary of the recycling of *Reesimermis nielseni* in *Anopheles crucians* in 11 sites after releases during the summers of 1971 and/or 1973.

Site	No. treatments	% samples containing nematodes (no. samples) 1971	1972	1973	1974	Mean percent parasitism due to recycling 1971	1972	1973	1974	1971–1974
Sites treated in 1971 only										
M-1	3	7(15)	0(13)	...	...	2	0	..	..	..
M-2	3	33(18)	25(12)	0(2)	40(15)	5	14	0	10	7
M-3	2	74(19)	43(7)	50(4)	100(14)	19	19	9	65	28
R-3	2	79(14)	58(12)	80(5)	100(3)	69	20	43	78	52
C-5	1	67(3)	57(7)	33(9)	0(2)	37	28	6	0	18
Sites treated in 1971 and 1973										
R-1	3	10(10)	0(7)	0(2)	...	<1	0	0	..	..
R-2	4	44(16)	38(13)	100(3)	87(24)	8	4	31	21	16
G-1	3	22(18)	9(11)	62(8)	50(4)	13	4	7	26	12
G-2	3	13(15)	0(3)	0(1)	100(1)	<1	0	..	33	11
A-1	2	71(14)	30(20)	33(3)	25(4)	20	6	1	2	7
C-1	2	90(31)	47(17)	30(20)	95(20)	21	26	5	51	27

the releases in 1973. There seemed to be some factor in this habitat that was deleterious to the infective stage of the nematode because low levels of infection and a very high degree of host resistance to infection was apparent when the releases were made (Petersen and Willis 1974b). Site G-2 showed less than 1% parasitism due to recycling after the 1971 release; however, in the only sample taken after the 1973 release, 33% of the *A. crucians* were parasitized. The R-2 site averaged 6% parasitism for 1971 and 1972 following the 1971 releases and 26% for 1973 and 1974 after the 1973 release; also, nematode activity increased markedly during the last part of 1974 and reached a level of 62% in early November. The trend at the G-1 site was similar to that at the R-2 site. At the A-1 site, parasitism by *R. nielseni* decreased after the 1971 introduction, and this trend continued after the 1973 release; *R. nielseni* was not observed during the latter part of 1974 and may have been lost from this site. At the C-1 site, nematode activity averaged 24% during 1971 and 1972 but dropped to about 10% before the 1973 release. After that release the decline continued during the remainder of the year, but in 1974, parasitism averaged 51% in 20 samples, and 19 of the 20 samples contained infected larvae. This site held water and produced substantial populations of *A. crucians* throughout most of the year.

Of 12 sites treated for the first time in 1973 (Table 2), 7 produced mean levels of infection of 3% or less in 1974. However, 5 of these 7 sites were sampled twice, and one three times. Also, the sites that failed to exhibit recycling had such high levels of infection (84–97%) immediatelly after the treatments that establishment may have failed because of the early mortality of multiply-infected hosts. In the remaining 5 sites, parasitism from recycling ranged from 11 to 85%.

Two of the five sites (H-1 and L-1) treated for the first time in 1973 produced such substantial populations of mosquito larvae throughout most of 1974 that we were able to make extensive observations. H-1 was a large freshwater swamp. A portion (1100 square yards) was treated on two occasions and samples were taken at weekly intervals during 1974 (Figure 1). Until mid-April (sampling began the last week of March), no parasitism was observed. This was expected because previous studies in southwestern Louisiana have shown that *R. nielseni* resumes activity about the first of April (Petersen and Willis 1971). Infections increased through April and May, reached 100% in *A. crucians* during the first week of June, and averaged 94% the next 4 weeks. Then the site dried up and failed to produce mosquitoes for the next 8 weeks. Once breeding resumed in the site, parasitism averaged only 31% until the latter part of October when it again built up to 100% during the first week in November. Thereafter, activity declined steadily, apparently because of the lateness of the season. Thus, 24 of the 25 samples of *A. crucians* taken between the first of April and the last of

Table 2. Summary of the recycling of *Reesimermis nielseni* in *Anopheles crucians* in 12 sites during 1974 resulting from a release during the summer of 1973.

Site	% samples containing nematodes (No. samples)	Mean percent parasitism	Site	% samples containing nematodes (No. samples)	Mean percent parasitism
G–3	100 (1)	2	I–1	0 (3)	0
G–4	0 (1)	0	I–2	0 (2)	0
G–5	100 (1)	11	I–3	100 (1)	3
G–6 [1]	67 (6)	23	T–1	0 (1)	0
E–1	0 (1)	0	H–1 [1]	96 (25)	47
E–2	100 (1)	81	L–1 [1]	100 (25)	85

[1] Two releases made during 1973.

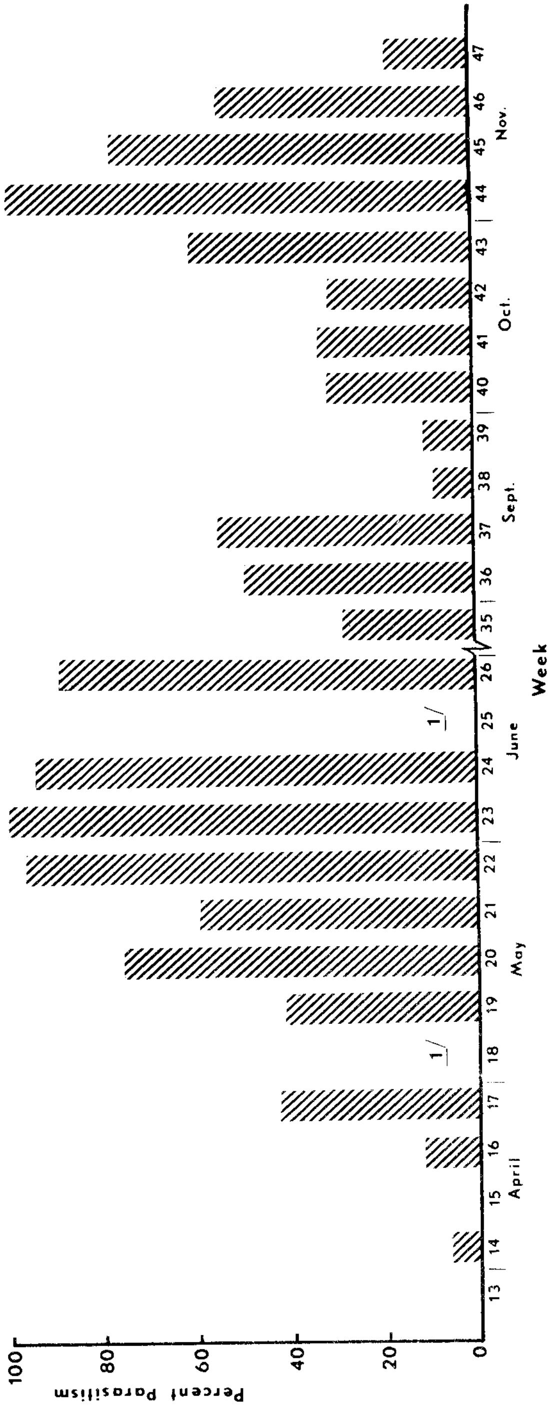

Fig. 1. Weekly incidence of parasitism of *Anopheles crucians* by *Reesimermis nielseni* during 1974 in a fresh water swamp (H–1).

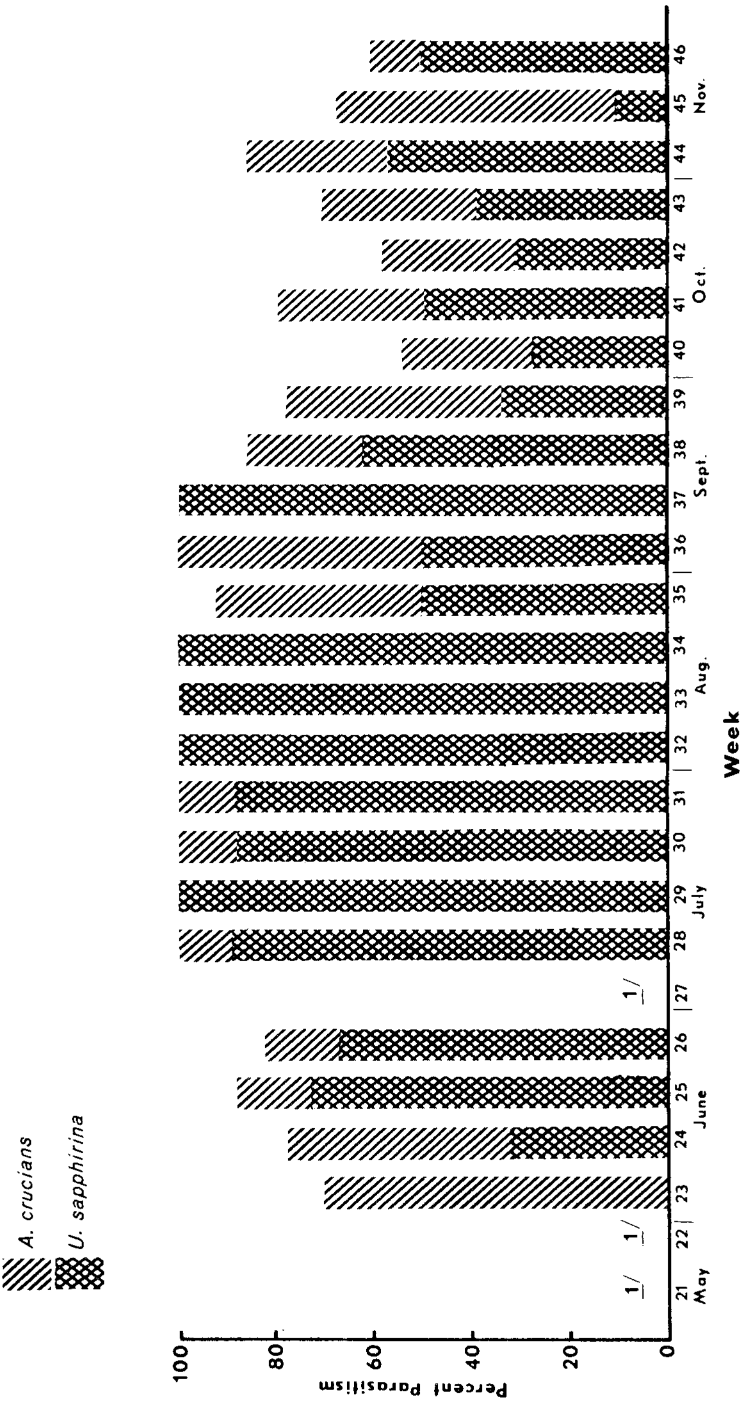

Fig. 2. Weekly incidence of parasitism of *Anopheles crucians* and *Uranotaenia sapphirina* by *Reesimermis nielseni* during 1974 in a permanently flooded drainage ditch (L-1).

November, at the H-1 site, produced infected larvae and mean parasitism in this species was 47%. Likewise, mean parasitism of *Anopheles quadrimaculatus* Say, collected 4 times, was 27%, of *Uranotaenia sapphirina* (Osten-Saken) collected 11 times, was 41%, and of *Culex erraticus* (Dyar and Knab), collected 2 times, was 66%.

The L-1 site is a heavily vegetated drainage ditch supplied continuously with water by a leaking water main. The site was treated on two occasions during August 1973, but levels of infection reached only 16 and 41%. However, when the site was sampled for the first time in early June 1974, infection due to recycling had reached 70% in *A. crucians*. Figure 2 shows the results of the weekly sampling at that site through the third week of November. Six samples were taken after that date, but only two *A. crucians* were collected, both infected. Parasitism reached 100% by the second week in July and remained at essentially this level for the next 10 weeks. Then it decreased to a mean of 72% and remained at this level the remainder of the year.

Levels of infection at the L-1 site seemed to be consistently lower in early instar larvae of *A. crucians*. When larvae from the samples were grouped by early (first and second), and late (third and fourth) instars, older larvae had substantially higher parasitism; 15 of 23 samples of older larvae showed 100% infection compared with 9 of 23 samples of younger larvae (Fig. 3). Parasitism averaged 70, 84, 93, and 94% for the first, second, third, and fourth instars, respectively, and 85% overall. This higher level of parasitism in older larvae was undoubtedly caused by the longer exposure to attack by the nematode. As a result, essentially no

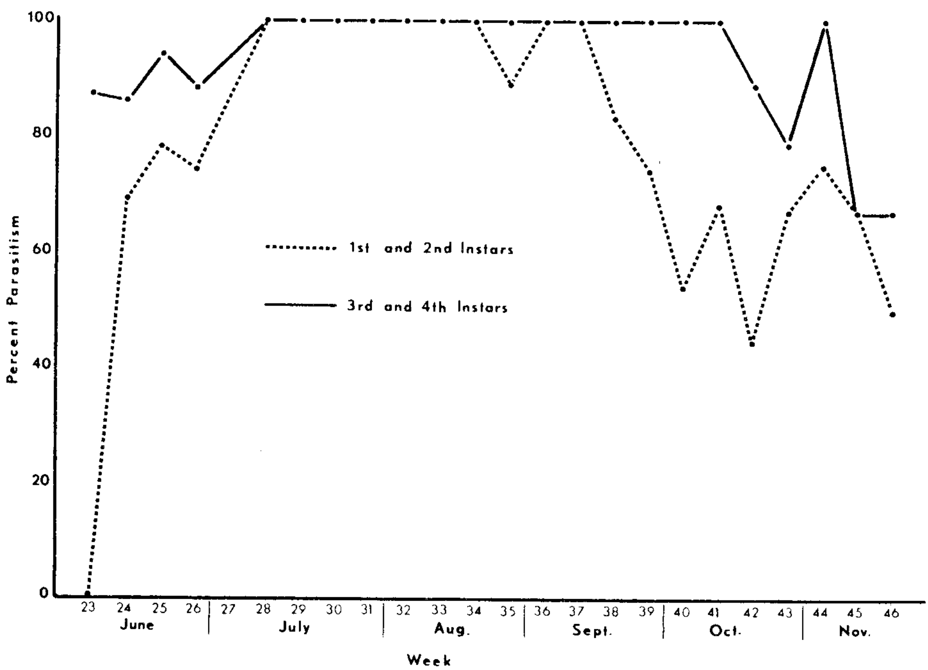

Fig. 3. Weekly incidence of parasitism of early and late instars of *Anopheles crucians* by *Reesimermis nielseni* during 1974 (L-1).

A. crucians adults emerged from the site from early July to early October.

U. sapphirina was collected in all but one of the samples (first week in June), and the levels of infection in this species were always equal to or less than the levels in *A. crucians*. Parasitism averaged 51, 58, 68, and 80% for first, second, third, and fourth instars, respectively, and averaged 57% overall. Eight samples contained *A. quadrimaculatus* (average 41% parasitism) and five contained *Culex salinarius* Coq. (average 3% parasitism).

DISCUSSION. Observations at the sites following the releases of *R. nielseni* have shown that this nematode has potential for establishment and recycling for an indefinite period. However, a number of environmental factors apparently affect its success as a control agent of *Anopheles* mosquitoes. For example, sites (M-3, C-1, H-1 and L-1) that produced greater numbers of *A. crucians* for longer periods also tended to have a higher percentage of larvae parasitized. However, two sites (R-3 and E-2) were exceptions. Also, periods of little or no water in a site tended to reduce the levels of infection in subsequent host populations (Table 1); still, one site, M-3, was dry 5 weeks but produced 100% parasitism in populations of *A. crucians* for the next 4 weeks. Finally, when rains occurred, they were often very heavy (up to 8 inches) and caused extensive flushing of some of the sites (i.e. M-1), which seemed to reduce nematode populations.

The actual levels of control achieved in most sites were somewhat higher than reported because we did not usually consider the age of the hosts. When we did (as shown in Figures 2 and 3, week 40) we found that the influence of parasitism was lower (60%) when the earlier instars made up the greater portion of the populations than when only the older larvae were considered (100%).

As a result of unfavorable conditions all sites were not sampled to the extent desired, and only 5 sites produced sufficient mosquitoes to provide significant information about recycling and the impact of *R. nielseni* on mosquito populations. However, the available data show that *R. nielseni* can become established in many semipermanent and permanent water sites and will produce high levels of parasitism in *A. crucians* mosquitoes for an indefinite period.

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