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

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ABSTRACT. Cultures containing the mermithid nematode *Reesimermis nielseni* were introduced into habitats of floodwater mosquitoes in Louisiana as a pre-hatch treatment. Significant levels of parasitism were achieved when cultures were placed in damp habitats that possessed adequate amounts of vegetation or organic debris and were subjected to minimum flushing action when flooded.

Fifty-two percent of *Aedes atlanticus*, 59% of *A. tormentor*, 38% of *Psorophora columbiae*, and 51% of *P. howardii* were parasitized in 39 larval collections from 13 habitats. *Reesimermis nielseni* was observed to penetrate but failed to develop in larvae of *Psorophora ferox*.

Reesimermis nielseni Tsai and Grundmann, a nematode parasite of larval mosquitoes, has been experimentally released in semipermanent and permanent water breeding sites. High levels of parasitism were achieved, and the nematode exhibited a strong propensity for establishment and recycling (Petersen et al. 1972, Petersen and Willis 1972a, 1974, and 1975). Also, *Reesimermis nielseni* was found naturally parasitizing large numbers of *Psorophora columbiae* Dyar and Knab (=*confinnis*), *Aedes atlanticus* Dyar and Knab, and *Aedes tormentor* Dyar and Knab in semipermanent breeding sites when water levels fluctuated sufficiently to produce these species (Petersen et al. 1968, Petersen and Willis 1971). When *R. nielseni* was introduced into breeding sites of *Anopheles crucians* Wiedemann, 50–100% of the *A. atlanticus*, *A. vexans* (Meigen), *A. tormentor*, and *P. columbiae* in the site were parasitized (Petersen and Willis 1972a).

Since floodwater mosquitoes generally develop rapidly and are most susceptible to *R. nielseni* in the 1st and 2nd instars, *R. nielseni* must be applied to breeding sites within 24 hr after hatching of the host. Thus, the use of this parasite as a posthatch treatment is often impractical. A study was therefore made to determine the potential of *R. nielseni* in the control of floodwater mosquitoes when the parasite was introduced into temporary breeding sites as a pre-hatch treatment.

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MATERIALS AND METHODS. Two types of breeding sites were selected, (1) 6 pastures or open grassy areas that characteristically produced substantial broods of *P. columbiae* and *P. ciliata* (F.), and (2) 11 woodland breeding sites that characteristically produced adequate populations of *A. atlanticus, A. tormentor, P. ferox* (Humboldt), and *P. howardii* (Coquillett). The selected sites were generally well-defined and ranged in size from about 200 to 600 sq ft; most were known to produce mosquitoes after a substantial rain.

A 7–8 week old culture containing 15 gm of postparasites, adults, and eggs of *R. nielseni* in moist sand (Petersen and Willis 1972b) was portioned and placed throughout each breeding site when the site was free of water but still damp, and covered with leaf litter and other organic material. The moist substrate presumably permitted the nematodes to seek a suitable environment as the site dried. The actual number of preparasitic nematodes introduced was unknown because preparasite production from similar cultures in the laboratory varied 1–3 x 10^6; also, the nematode loss from introduction could not be determined.

Because of the localized nature of the summer rains, each site was monitored once a week, and all were sampled 24 or more hours after each flooding. All larvae collected were returned to the laboratory where the extent of parasitism was determined microscopically. Because larvae of *P. howardii* and *P. ciliata* are predacious, they were dissected as soon as possible. Also, when 24- to 36-hr-old larvae of *P. ferox* were collected, they were dissected to determine the presence of preparasites since this species is known to be resistant to the development of *R. nielseni*.

RESULTS AND DISCUSSION. Of the 17 sites treated with *R. nielseni*, 2 failed to produce larvae, and 1 failed to dry sufficiently so as to produce floodwater mosquitoes. The remaining 14 sites produced 1–6 broods of mosquitoes, and all but 1 possessed infected hosts.

In the 6 open pasture or grassy habitats, *P. columbiae* (95%) was the predominant floodwater species; *P. ciliata* (4%) and *A. atlanticus* (1%) made up the balance. In addition, on several occasions, these sites retained water long enough to produce broods of *Anopheles crucians*. One of the sites showed no parasitism after 5 broods of *P. columbiae;* it had very little vegetation or bottom litter, and this lack of protective cover may have been responsible for the absence of nematode activity. A second site produced limited parasitism after the 1st flooding and none after subsequent floodings. The extensive flushing of this site after heavy rains undoubtedly washed away most of the infective-stage parasites and infected hosts. The remaining 4 sites were flooded 2–6 times, and all were producing infected hosts (20–67%) after 15–18 weeks. The mean parasitism of *P. columbiae* collected 18 times from the 5 positive sites was 38%, and parasitism averaged 20 and 34% for *P. ciliata* and *A. atlanticus,* respectively (Table 1). The variations in levels of parasitism from flooding to flooding for 2 of the sites (G–1 and L–3) are shown in Table 2.

The 8 woodland sites produced 1 or more broods of floodwater mosquitoes, which included *A. atlanticus* (49%), *P. ferox* (19%), *A. tormentor* (15%), *P. howardii* (10%), *A. fulvus pallens* Ross (5%), and *A. vexans* (2%) (Table 1). One site produced a few parasitized larvae after the first flooding only. The limited parasite activity may have been caused by the introduction of a poor nematode culture or by some limiting factor in the habitat. One site that was flooded only once, 3 weeks after introduction of *R. nielseni,* produced 75 and 100% parasitism in *A. atlanticus* and *P. howardii,* respectively. Four sites, each flooded twice, produced a mean of 82% (34–100) parasitism after the initial floodings; the 2nd floodings produced 100, 100, 91, and 22% parasitism in *A. atlanticus* and *A. tormentor* after 3, 6, 8, and 17 weeks, respectively. Another site (C–7) produced a mean of 76% parasitism in *A. atlanticus* and 33% in *P. howardii* for 4 floodings,

Table 1. Summary of 13 releases of *Reesimermis nielseni* to control floodwater mosquitoes.

Species	No. sites	No. samples	No. larvae	Percent parasitism (Range)
		Open pasture sites		
Aedes				
atlanticus	1	2	4	33.5 (0–67)
Anopheles				
crucians	5	11	77	68.4 (0–100)
Psorophora				
ciliata	5	9	34	20.0 (0–100)
columbiae	5	18	723	38.2 (12–72)
		Woodland sites		
Aedes				
atlanticus	6	17	515	51.8 (0–76)
fulvus pallens	4	4	53	13.3 (0–50)
tormentor	5	9	161	59.3 (0–100)
vexans	3	4	28	5.0 (0–20)
Anopheles				
crucians	2	3	6	83.3 (50–100)
Psorophora				
ferox	6	12	202	0 –
howardii	8	15	102	50.6 (0–100)

and site E–6 produced 43% parasitism in *A. atlanticus* and 75% parasitism in *P. howardii* for 5 floodings (Table 2). The extent of parasitism varied with each flooding and did not appear to be correlated with either the number of floodings or the time intervals between floodings.

Psorophora ferox was the only species that was free of *R. nielseni* when permitted to mature to the 4th instar; how-

Table 2. Parasitism by *Reesimermis nielseni* of floodwater mosquitoes in 4 sites.

Site	Time (wk) after introduction of nematodes	Percent parasitism		
		A. atlanticus	*P. howardii*	*P. columbiae*
C–7	5	75	0	–
	6	100	20	–
	7	80	100	–
	14	48	13	–
E–6	1	1	–	–
	2	97	100	–
	3	8	–	–
	8	82	58	–
	9	29	67	–
G–1	2	–	–	0
	10	–	–	36
	14	–	–	14
	17	–	–	43
L–3	2	–	–	41
	5	0	–	47
	9	–	–	0
	10	–	–	83
	16	–	–	33
	18	67	–	67

ever, on 2 occasions, late 1st- and early 2nd-instar larvae of this species were dissected, and all contained infective stage parasites. These observations of high susceptibility to attack and high resistance to development of *R. nielseni* agree with observations made in earlier laboratory studies (Petersen 1975).

Though the numbers of *A. vexans* were low, the levels of parasitism (5%) indicated that this species is somewhat resistant to the attack of *R. nielseni*. Also, levels of parasitism in *A. fulvus pallens* (13%) were low, probably because the characteristic delayed hatch of this species allows it to miss the brunt of the infective-stage nematodes.

Reesimermis nielseni can thus produce significant levels of parasitism in floodwater mosquitoes when introduced in a manner to permit synchronous hatching of the eggs of the parasite and the host. The introductions of *R. nielseni* were most effective when they were made in damp habitats with adequate amounts of vegetation and organic litter that were subject to limited flushing action when flooded. The introductions produced significant parasite activity over 3–18 weeks and 1–6 floodings in 11 or 14 treated sites. Further observations are planned to measure the extent of long-term establishment and recycling of *R. nielseni* in such temporary breeding sites.

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