EXPERIMENTAL RELEASE OF *ROMANOMERMIS CULICIVORAX* (MERMITHIDAE:NEMATODA) TO CONTROL MOSQUITOES BREEDING IN SOUTHWEST FLORIDA[1]

R. LEVY AND T. W. MILLER, JR.

Lee County Mosquito Control District,
P.O. Box 2237, Fort Myers, Florida 33902

ABSTRACT. The mermithid nematode *Romanomermis culicivorax* Ross and Smith was released at a dosage rate of ca. 3.6×10^3 preparasites/m^2 of water surface in an attempt to control mosquitoes breeding in a grassy field. Results from the release of ca. 9×10^6 preparasites indicated that $96.5 \pm 2.8\%$ of the 1st-4th instar *Psorphora columbiae* Dyar and Knab, *Ps. ciliata* F., *Culex nigripalpus* Theob. and *Aedes taeniorhynchus* (Wiedemann) larvae from 8 potholes and ditches in the field were parasitized by *R. culicivorax,* and marked reductions in host populations were observed in samples 24 hr post-treatment. No larvae were found in 2 additional sampling sites 24 hr post-treatment that contained high populations of 1st and 2nd instar *Ps. columbiae* and/or *Cx. nigripalpus* prior to preparasite release.

Experimental field releases of preparasites of the mermithid nematode *Romanomermis culicivorax* Ross and Smith (=*Reesimemris nielseni* Tsai and Grundmann, auct., partim.) against natural populations of *Anopheles freeborni* Aitken, *An. crucians* Wiedemann, *An. quadrimaculatus* Say (Petersen et al. 1972, 1973; Petersen and Willis 1972a, 1974), *Psorophora columbiae* Dyar and Knab [=*Ps. confinnis* (Lynch-Arribálzaga)] (Petersen et al. 1973), and *Culex pipiens quinquefasciatus* Say (Levy and Miller 1977) have indicated its potential as an effective biological control agent of mosquitoes that breed in diverse habitats. Furthermore, data concerning the non-target effects of *R. culicivorax* have indicated that these parasites are virtually host specific for mosquito larvae, and therefore pose no threat to other organisms which might accidentally be exposed to the introduced parasite (Ignoffo et al. 1973, 1974).

Since tests with this nematode have indicated its use as a safe and effective biological control agent of several species of mosquitoes which breed in semi-permanent and permanent aquatic environments, the Lee County Mosquito Control District, Fort Myers, Florida, has initiated a series of research projects aimed at evaluating the mosquito control potential of *R. culicivorax* under a variety of field conditions. The present study describes the results of an experimental field release of preparasitic (infective stage) nematodes to control mosquitoes breeding in a grassy field.

METHODS AND MATERIALS. A section of a grassy field (ca. 50m × 60m) located in a residential section of North Fort Myers, Lee County, Florida, was used to evaluate the mosquito control potential of *R. culicivorax.* This field has been used previously as a site for a tree nursery and subsequently contained numerous planting potholes, ditches and low areas that differentially flooded and drained in relation to the amount of rainfall.

Pre-treatment examination several hours prior preparasite application indicated that a high population of 1st-4th instar *Cx. nigripalpus* Theob., and *Ps. columbiae* larvae were present in a number of potholes and ditches throughout the field. In addition, lesser numbers of *Ae. taeniorhynchus* (Wiedemann) and *Ps. ciliata* F. larvae were also present. It should be

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noted that with the exception of *Ps. ciliata*, approximately 60% of all larvae collected were 1st-2nd instar (about 100 larvae examined). No natural parasite activity was indicated in the sampling.

Pre-treatment bioassays were conducted to determine the effects of the water on preparasite viability and infectivity to *Cx. nigripalpus* larvae under laboratory and field conditions, even though water standing in the field was presumed to be quite fresh due to several days of rain in the area. In the laboratory test, two 400 ml glass beakers were filled with 200 ml of water collected from the field and two were filled with equal amounts of dechlorinated reverse osmosis (RO) water. A 20:1 preparasite to mosquito larval ratio was used in this test (i.e. 500 preparasites/25 larvae/beaker). Ten larvae from each beaker were examined for parasitism 24 hr after exposure. The field bioassay consisted of applying ca. 50 g of sand containing nematode eggs to one pothole in the test site which contained 1st and 2nd instar larvae.

Seven sand and sphagnum moss cultures containing eggs of *R. culicivorax* were flooded with dechlorinated RO water for 18 hr to obtain preparasitic nematodes for the major field release (Levy and Miller 1977). Preparasite population determinations (3 replicates/culture) (Petersen and Willis 1972b) indicated that ca. 9×10^6 infective stage nematodes were available for the field release, thereby providing an estimated application dosage rate of ca. 3.6×10^3 preparasites/m^2 of water surface.

Preparasitic nematodes were applied by pouring directly from flasks or with a compressed air sprayer (Levy et al. 1976) to flooded areas where sampling indicated that mosquito larvae were concentrated. The water temperature at the time of field release was 28°C.

Mosquito larvae (1st-4th instar) were sampled 24 hr post-treatment from 10 locations (3 dips per location with a pint dipper) throughout the field and examined under a compound microscope for nematode parasitism. In addition, mosquito larvae determined to be infected were separated into beakers containing field water, fed finely ground rabbit chow, and maintained at 26°C (ambient) to observe parasite development and post-parasite emergence.

RESULTS AND DISCUSSION. The laboratory bioassay to determine immediate effects of the habitat water on preparasitic *R. culicivorax* resulted in 100% parasitism of the 1st and 2nd instar *Cx. nigripalpus* larvae examined in all containers. In addition, the pre-test application of sand containing nematode eggs to one pothole resulted in 68.2% parasitism of the 22 *Cx. nigripalpus* larvae (mainly 2nd instar) examined 24 hr post-treatment. These preliminary tests indicated that the water in the test sites had no detectable adverse effects on nematode egg hatch or on the viability and infectivity of the preparasitic nematode.

Results from sampling 10 potholes and ditches 24 hr after preparasite application indicated that 164 of the 170 mosquito larvae (96.5 ± 2.8%) were infected with *R. culicivorax* (Table 1). The mosquito population in 8 sampling sites had been substantially reduced [i.e. average decreases from 84 (50–125) to 17 (10–25) per dip], and in 2 sites no larvae could be found. For the most part, the reduction in host numbers 24 hr post-treatment was attributed to early death of many 1st and 2nd instar larvae due to multiple penetrations by the nematodes. Laboratory observations of 10–15 1st–2nd instar *Cx. nigripalpus, Ps. columbiae,* and *Ae. taeniorhynchus* larvae 5 days post-field infection by *R. culicivorax* resulted in 100% mortality of all larvae by the emerging postparasites, and therefore indicated the nematode kill potential for these species of mosquitoes as well as the potential for recycling in this field situation.

Microscopic examination of the mosquito larvae collected 24 hr post-treatment indicated that the majority of the 1st–3rd instar *Cx. nigripalpus* larvae were multiply parasitized. In general, the observations of nematodes (usually 1 mm long or less at the time of examination) within the hemocoel of this species was not difficult since the cuticle was quite transparent. Lit-

Table 1. Results of experimental release of preparasitic *R. culicivorax* to control several species of mosquitoes breeding in a grassy field.

Sample site designation	Mosquito species	Larval instars	Number larvae sampled	% Parasitism for all larvae
Pothole	*Cx. nigripalpus*	1–2	20	100
Pothole	*Cx. nigripalpus*	1–2	6	100
	Ps. columbiae	2–4	15	
	Ps. ciliata	3–4	2	
Pothole	*Ps. columbiae*	2–4	29	88
	Cx. nigripalpus	2–3	5	
Pothole	...	...	0	...[a]
Ditch	*Ps. columbiae*	2–4	20	100
Pothole	*Ps. columbiae*	2–4	20	100
Ditch	*Ae. taeniorhynchus*	2–4	15	100
Ditch	...	...	0	...[a]
Pothole	*Ps. columbiae*	2–4	20	100
Pothole	*Ps. columbiae*	2–4	7	89
	Cx. nigripalpus	2	11	

[a] 1st–2nd *Cx. nigripalpus* and/or *Ps. columbiae* larvae present prior field release, but no larvae present 24 hr post-treatment.

tle difficulty was encountered locating the nematode(s) in 2nd instar *Ps. columbiae* and *Ae. taeniorhynchus* larvae. However, some difficulty was encountered when examining 3rd and 4th instar larvae of these species as well as *Ps. ciliata,* due to their darkly pigmented cuticles. In these cases, parasites were mainly observed in the more transparent anal segments, anal gills, and air tubes of the mosquito larvae. Late instar larvae that were determined to be unparasitized were verified with a phase contrast microscope.

Previous experiments have shown that *Ps. columbiae, Ps. ciliata,* and *Ae. taeniorhynchus* are susceptible to parasitism by *R. culicivorax* under field and/or laboratory conditions (Petersen 1975, Petersen et al. 1968, Petersen et al. 1973); however, no data prior to this report have indicated that *Cx. nigripalpus,* a potential encephalitis vector of southwest Florida (Anonymous 1976), is a highly susceptible host for this nematode. In addition, no previous information concerning field parasitism of *Ae. taeniorhynchus* by *R. culicivorax* has been reported. This is probably because *Ae. taeniorhynchus* is a salt-marsh mosquito and typically breeds in water that is higher in salinity or chloride content than *R. culicivorax* can tolerate (Petersen and Willis 1970). However, fresh water breeding of *Ae. taeniorhynchus* in roadside ditches and grassy fields in water containing concentrations of salts sub-lethal to preparasites is not uncommon in the Lee County area and therefore indicates a potential for using *R. culicivorax* against this mosquito on a limited scale.

Our results indicated that *Cx. nigripalpus, Ps. columbiae, Ps. ciliata* and *Ae. taeniorhynchus* are highly susceptible to parasitism by preparasitic *R. culicivorax* under some natural field conditions and that natural populations of these larvae (mainly 1st and 2nd instar) can be substantially reduced or even eliminated within 24 hr when application rates are ca. 3.6×10^3 preparasites/m^2 of water surface. In addition, preliminary data from our field bioassay as well as data presented by Petersen and Willis (1976) have indicated that significant levels of parasitism were achieved in the field when cultures containing nematode eggs and adults were applied as a pre-hatch treatment.

The aforementioned data, as well as the strong propensity for natural establishment and recycling that *R. culicivorax* has exhibited (Petersen and Willis 1972a, 1974 and 1975) indicate that this nematode is a very promising biological control agent for use against several species of mosquitoes breeding in certain fresh water environments of southwest Florida.

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