EXPERIMENTAL RELEASE OF A MERMITHID NEMATODE TO CONTROL MOSQUITOES BREEDING IN SEWAGE SETTLING TANKS [1]

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ABSTRACT. Tests were conducted to determine if the mermithid nematode, *Romanomermis culicivorax* Ross and Smith (=*Reesimermis nielseni* Tsai and Grundmann, auct., partim.), would effectively parasitize and develop in *Culex quinquefasciatus* Say larvae breeding in an abandoned sewage settling tank.

Laboratory bioassays indicated that undiluted sewage water collected directly from the test sites on Sanibel Island, Lee County, Florida, had no measurable adverse affects on viability or infectivity of the preparasitic stage or on development of the parasitic stage.

Field releases of preparasites directly into 2 of the sewage settling compartments of the main system resulted in 37.3 and 53.7% parasitism of *Cx. quinquefasciatus* larvae at estimated parasite-to-host ratios of 3.4:1 and 4.6:1, respectively, and therefore indicated their potential use as biological control agents against mosquitoes which breed in some polluted water environments.

Tests aimed at evaluating the biological control potential of the mermithid nematode, *Romanomermis culicivorax* Ross and Smith (=*Reesimermis 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), and *Psorophora columbiae* Dyar and Knab [=*Ps. confinnis* (Lynch-Arribálzaga)] (Petersen et al. 1973) have indicated their effectiveness in suppressing the larval populations of these species in permanent and semi-permanent habitats that were low in salinity (Petersen and Willis 1970) and unpolluted (Anonymous 1972). Some researchers have indicated that polluted water will kill too many nematodes to provide adequate control of mosquito populations (Anonymous 1972), however, there are currently no laboratory or field data available to indicate the specific pollution parameters (phosphates, nitrates, etc.) that will limit the habitat range of *R. culicivorax*.

Therefore, experiments were initiated by the Lee County Mosquito Control District, Fort Myers, Florida, to determine if preparasitic (infective stage) *R. culicivorax* could have a potential use as a biological control agent in regulating the natural populations of the southern house mosquito, *Culex quinquefasciatus* Say breeding in certain polluted environments. Laboratory studies show larvae of *Cx. quinquefasciatus* to be highly susceptible to parasitism by this nematode (Petersen and Willis 1970, Levy, et. al. 1976).

METHODS AND MATERIALS

A sewage settling tank on Sanibel Island known to breed large populations of *Cx. quinquefasciatus* was used as the experimental release site (Fig. 1). Although this sewage treatment system was no longer in use, it contained a bottom layer of sewage sediments in various stages of decomposition that was presumed to be the cause of the pollution. Two sewage settling tank compartments of the main system containing 5.23 m^2 and 6.04 m^2 of water surface (test tank 1 and 2 respectively) were used as the test sites.

Pre-treatment estimates of the larval populations were determined by collecting several samples from each tank and extrapolating to the total water surface. Large concentrations of egg rafts, larvae (1st-4th), pupae were found.

[1] In cooperation with the Gulf Coast Mosquito Research Laboratory, ARS, USDA, Lake Charles, Louisiana 70601.

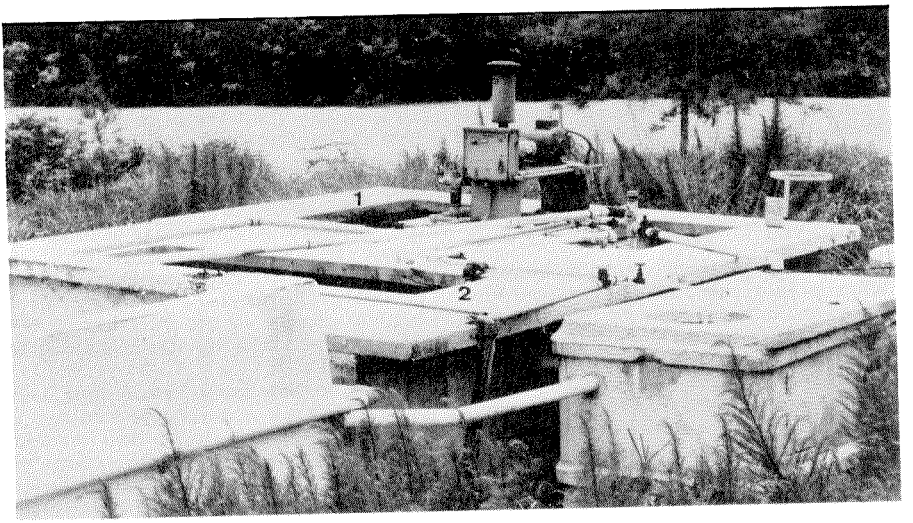

Fig. 1. Sewage settling tanks (test tank 1 and 2) where *R. culicivorax* was released against *Cx. quinquefasciatus* larvae.

LABORATORY EXPERIMENT. Bioassays to determine the effect of the sewage water in test tanks 1 and 2 on the viability and infectivity of preparasitic *R. culicivorax* were conducted several days prior to the field evaluation. Sewage water collected from the sewage settling tanks was diluted with dechlorinated reverse osmosis (RO) water to achieve the following test concentrations: 100,50,25, and 0% sewage water (100% RO water). Tests were conducted in 200 ml of the test water in 400 ml glass beakers. Three hundred 1–6 hr old preparasitic *R. culicivorax* (Petersen and Willis 1972b) and 25 1st-2nd instar *Cx. quinquefasciatus* larvae obtained from the Sanibel Island test site were added to each beaker to obtain a 12:1 preparasite to mosquito larva ratio.

Mosquitoes were fed ground rabbit chow, and the water in the beakers was swirled and aerated periodically with a pipette to prevent scumming. Tests were conducted at 26°C (ambient).

Seven days post-treatment, a sample of 15 *Cx. quinquefasciatus* larvae (4th instar) from each container were examined for nematode parasitism; a compound microscope was used to determine the average percent infection. Tests were replicated 3 times.

Chemical analyses of water samples from each sewage settling tank were performed to determine quantitatively the chemical nature of the sewage water.

FIELD EXPERIMENT. Two sand cultures containing nematode eggs were flooded with dechlorinated RO water 18 hr prior field dissemination providing about 1.2×10^6 infective stage parasites (Petersen and Willis 1972b).

Concentrations of 4×10^5 and 8×10^5 preparasitic nematodes were decanted directly from glass flasks into test tanks 1 and 2 respectively (approximately 6.4×10^4 and 1.1×10^5 preparasitic nematodes per m^2 of surface area). The water temperature at the time of preparasite release was 22°C. Samples of exposed mosquitoes from each tank were obtained 24, 48 and 72 hr post-treatment. Several hundred 1st-4th instar *Cx. quinquefasciatus* larvae

from each tank were examined using a compound microscope to determine the average percent parasitism by *R. culicivorax*. Twenty-five infected larvae from each test tank were placed in glass beakers to determine the effects of sewage water on postparasite emergence and viability.

RESULTS AND DISCUSSION

Data obtained from laboratory bioassays when preparasitic *R. culicivorax* were exposed to 1st-2nd instar *Cx. quinquefasciatus* larvae at a 12:1 ratio in undiluted sewage water, 50, 25 and 0% sewage water showed that nematode parasitism had occurred in all larvae examined in all test containers from both test sites. Postparasites obtained from mosquito larvae in undiluted sewage water appeared normal and exhibited no unusual behavioral characteristics when compared to postparasites recovered from other test concentrations, indicating the potential for natural recycling of the parasite in these environments.

Microscopic examination of 169 larvae from test tank 1 and 341 larvae from test tank 2 collected 24, 48 and 72 hr post-treatment indicated that an average of 37.3 (34.0–42.0)% and 53.7 (50.8–56.3)% of *Cx. quinquefasciatus* larvae (1st-4th instar) had been singly or multiply parasitized by *R. culicivorax* respectively. Postparasites were obtained from parasitized larvae in test tanks 1 and 2 and further indicated the mosquito kill and recycling potential of the nematode in this natural breeding situation.

Differences in percent parasitism between test tanks 1 and 2 were attributed primarily to density dependent factors (concentration of preparasites released in relation to the concentration of hosts present at the time of application) and not to water quality since water analyses from the 2 test sites did not appear to result in significant differences (Table 1). Approximation of larval populations resulted in an estimated 1.56×10^5 mosquito larvae present in test tank 2 at the time of application (average 125/dip). At this rate, a 5.1:1 parasite to host ratio existed. Petersen (1975) indicated that both the number of active preparasites as well as the infectivity of the preparasites decreased by about 15% for the first 24 hr after hatching and that infectivity decreased more rapidly after this time. Based on these data and the age of our preparasites at the time of application (22–24 hr old), it was assumed that at least a 10% decrease in infectivity could be expected. Therefore, the number of infective preparasites was probably reduced to 7.2×10^5 with a subsequent change in the infection ratio to 4.6:1. A ratio of 3.4:1 was calculated for test tank 1.

Based on these low infection rates, it was presumed that a higher percentage of parasitism, and subsequent host mortality could be achieved by simply increasing the number of preparasites released. Nevertheless, this level of infection is presumed to be favorable for observations on nematode recycling, since host populations were not drastically reduced.

The actual incidence of parasitism for *Cx. quinquefasciatus* larvae in the 2 test sites may have been somewhat higher than reported since some early mortality due to multiple penetrations probably occurred; also preparasites were small (about 1 mm long) and almost transparent at the time of microscopic examination and some were possibly overlooked, especially in the larger, more highly pigmented larvae. In addition, post-treatment hatch of eggs in each tank is expected to have contributed to a lower percent parasitism.

Quantitative analyses of water obtained from the test sites (Table 1) indicated that the concentration of certain pollutants (i.e. extremely high levels of orthophosphates, 7.63–10.05 ppm) were significantly higher than would be found in typical permanent (ponds) and semi-permanent (roadside ditches, potholes) fresh water situations which breed high concentrations of mosquitoes in Lee County. In addition, the high conductivity readings (1190–1300 micromhos/cm) and high chloride concentration (244–258 ppm) have indicated that *R. culicivorax* can survive in habitats with a

Table 1. Analyses of water obtained form sewage settling tanks used as experimental sites for release of preparasitic *R. culicivorax* against *Cx. quinquefasciatus* larvae.[a]

Parameter	Sewage settling tank 1 Release analysis[b]	Sewage settling tank 2 Release analysis[b]
Total solids (ppm)	990.000	970.000
PH	9.000	9.000
Conductivity (micromhos/cm)	1190.000	1300.000
Total Kjeldahl nitrogen (TKN as nitrogen, ppm)	1.197	1.577
Ammonia (NH_3 as nitrogen, ppm)	0.440	0.240
Nitrate (NO_3 as nitrogen, ppm)	0.100	0.120
Nitrate (NO_2 as nitrogen, ppm)	0.012	0.010
Chlorides (ppm)	244.000	258.000
Phenolphthalein alkalinity (calcium carbonate, ppm)	38.000	40.000
Total alkalinity (calcium carbonate, ppm)	312.000	324.000
Total hardness (calcium carbonate, ppm)	94.000	104.000
Calcium hardness (calcium carbonate, ppm)	39.000	70.000
Magnesium hardness (calcium carbonate, ppm)	55.000	34.000
Turbidity	7.000	16.000
Color	30.000	30.000
Orthophosphate (as phosphate, ppm)	10.050	7.630
Total organic carbon (as carbon, ppm)	32.000	29.000
Total inorganic carbon (as carbon, ppm)	76.000	75.000

[a] Analysis in cooperation with the Division of Environmental Protection, Lee County Environmental Laboratory, P.O. Box 398, Fort Myers, Florida 33902.

[b] Water obtained on the day of test.

salinity indices of this magnitude. Some previous experimental field releases of *R. culicivorax* have been conducted in semi-permanent and permanent habitats ranging from 22–400 micromhos/cm (Petersen and Willis 1972a).

It should also be noted that the pH readings (9.0) and total alkalinity levels (312–324 ppm) are rather high and not typically encountered in most mosquito breeding areas in Lee County (unpublished data). Levels of other indices of possible pollution (TKN, NO_3, NO_2, and NH_3 values) in water from the Sanibel test site were considerably lower than would be found in water from an active sewage settling system. However, the values are atypical for most fresh water mosquito breeding habitats of Lee County and are indicative of a certain degree of sewage pollution. The significance of the other parameters in Table 1 in relation to the habitat limitations of *R. culicivorax* is not known.

In general, even though our data have indicated that the water in the abandoned sewage settling system was polluted (mainly based on the high orthophosphate levels) and also contained a higher salinity than had been previously reported from fresh water mosquito breeding habitats used in field studies with *R. culicivorax*, it appeared to have little or no adverse effects on preparasite infectivity or on postparasite development. Furthermore, these tests have indicated that *R. culicivorax* may be an effective biological control agent against mosquitoes which breed in this type of specialized aquatic environment.

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THE BREEDING SITES AND SEASONAL OCCURRENCE OF *CULICOIDES FURENS* IN GRAND CAYMAN WITH NOTES ON THE BREEDING SITES OF *CULICOIDES INSIGNIS* (DIPTERA: CERATOPOGONIDAE)

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ABSTRACT. Of the seven species of *Culicoides* found in Grand Cayman, *C. furens* (Poey) was by far the dominant species. Only *C. furens* and *C. insignis* Lutz were taken in emergence traps. The density of emergence of the former was greatest in mangrove swamp and the latter in fresh water reed swamp. Within the mangrove, emergence of *C. furens* was not evenly distributed but favored bare mud over mud with pneumatophores and non-tidal rather than tidal areas. Most non-tidal bare mud occurred around mangrove pools in the lower, wetter parts of the swamp.

C. furens adults were present all year but with major peaks of abundance in late spring and early winter. Peaks were variable from year to year and variably demonstrated by different collecting methods. Light and emergence trap catches showed a very close relationship with swamp water levels and a lesser relationship with temperature.

INTRODUCTION

A 5-year program in Grand Cayman, using light, bait, suction and emergence trapping methods, showed the presence of 7 species of *Culicoides*: *C. furens* (Poey), *C. insignis* Lutz, *C. barbosai* Wirth and Blanton, *C. hoffmani* Fox, *C. pusillus* Lutz, *C. jamaicensis* Edwards and *C. panamensis* Barbosa. Of these, *C. furens* was by far the dominant species, constituting over 95% of the total catch (of over 400,000 biting sand midges or flies). *C. insignis* was widespread but its numbers were low while *C. barbosai* sometimes occurred in large

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