Effectiveness of *Romanomermis culicivorax* (Nematoda Mermithidae) in *Anopheles pseudopunctipennis* and *Culex quinquefasciatus* (Diptera, Culicidae) in Mexico

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Effectiveness of Romanomermis culicivorax *(Nematoda, Mermithidae) in* Anopheles pseudopunctipennis *and* Culex quinquefasciatus *(Diptera, Culicidae) in Mexico.*— Field tests were conducted with *Romanomermis culicivorax* Ross & Smith, 1978 as control of mosquito larvae in three natural sites located in Oaxaca State, Mexico. Infective nematodes were disseminated at 1000/m². Both species of mosquito larvae were infected but populations of anopheline mosquitoes were more susceptible than culicine.

Key words: Biological control, *Romanomermis culicivorax*, *Anopheles pseudopunctipennis*, *Culex quinquefasciatus*.

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Introduction

Oaxaca State has a high rate of malaria. This area is situated between parallels 19° of the north longitude and meridians 93° and 99° of west longitude. Its climatic conditions such as temperature and rainfall are appropriate for the development of *Anopheles pseudopunctipennis* Theobald, 1901 and for the transmission by *Plasmodium vivax* Grassi & Feletti, 1890 and *Plasmodium falciparun* Welch, 1897. From 1988 to 1994 an intensive program against vector *A. pseudopunctipennis* was carried out in Oaxaca State, consisting of application of insecticides inside houses as antilarvarian treatments. Mass treatment were carried out in zones of major epidemiological risk.

R. culicivorax has been evaluated successfully against various *Anopheles* species in field conditions (Petersen & Willis, 1974; Petersen, 1976) and at least 52 species of mosquitoes in their larval stage are parasitized in natural conditions (Petersen, 1973).

The infective or pre-parasitic larvae of this nematode species have a short life and must find a larvae of the host mosquito between 36 and 48 h after emergence. The pre-parasitic enters the larvae by cuticular route by means of a stiletto. Seven days later, post-parasites emerge killing mosquitoe larvae (Platzer, 1982). This paper describes the results of introducing the mermithid *Romanomermis culcivorax* in *Anopheles albimanus* and *Culex quinquefasciatus* natural breeding places in Oaxaca State and evaluates its parasitic capacity as biolarvicide.

Material and Methods

Three natural sites of 200, 180 and 150 m^2 situated in Oaxaca coast were used. The sites were examined before the application of the nematode to establish the larval density of *A. pseudopunctipennis* at sites 1 and 2 and of *Culex quinquefasciatus* Say, 1823 at site 3.

The pre-parasites were obtained from six laboratory cultures of nematodes produced in the Institute of Tropical Medicine 'Pedro Kouri'. Cultures were flooded with distilled water. A total of 745,000 pre-parasites were determined which were

Table 1. Characteristics of the study and control sites of mosquito larvae treated with *Romanomermis culicivorax*.
Características de las zonas de control y estudio de las larvas de mosquito tratadas con *Romanomermis culicivorax*.

Sites	Area (m^2)	Depth (cm)	Main vegetation	Type of water	pH	°C
1	200	30	filamentous algae	fresh	7.1	29
2	180	26	filamentous algae	fresh	7.2	29
Control	120	29	filamentous algae	fresh	7.1	29
3	150	38	-	fresh	6.9	28
Control	90	34	-	fresh	6.9	28

Table 2. Means of infection and percentages of mortality in mosquito larvae by *Romanomermis culicivorax*. (Means followed by different letters are significantly different p = 0.05). C. Control.

Medias de infección y porcentaje de mortalidad en larvas de mosquito con Romanomermis culicivorax. *(Medias con superíndices distintos son significativamente diferentes p = 0,05). C. Control.*

Sites	Number of larvae	Larval density/m² Pre-treatment	Post-treatment	%	Infection 72h post-treatment Instars	$\bar{X}$	Mortality %
A. pseudopunctipennis							
1	66,000	330	5	85	I	3.0[a]	91
					II	2.7[a]	91
					III	1.5[b]	80
					IV	1.1[b]	77
2	41,500	231	4	84	I	2.7[a]	94
					II	2.6[a]	91
					III	1.5[b]	80
					IV	1.0[b]	72
C. 1	31,680	264	328	-		-	-
C. quinquefasciatus							
3	59,400	396	6	79	I	1.8[a]	89
					II	1.6[a]	86
					III	1.16[b]	77
					IV	0.8[b]	71
C. 2	17,820	198	215	-		-	-

calculated by volumetric dilution method (Petersen & Willis, 1972). Pre-parasites were transported to the field in a 3 l plastic container. The final dilution was made with water from the sites. The pre-parasites were applied at rates of 1000/m². Sites 1, 2 and 3 received 200,000, 180,000 and 150,000 pre-parasites respectively from a 5 l compressed air sprayer (Holder-planta 5, Gebr. Holder, Germany) at a pressure of 2 at.

Two control sites (120 and 190 m²) were established.

The temperature at time of application was 29 °C. *Anopheles* and *Culex* mosquito larvae (1st-4th) instars were sampled 72 h post-application and 140 larvae per site (35 larvae per instar) were returned to the laboratory and observed under a compound microscope for nematode infection. Mosquito larvae populations were determined again seven days later by collecting samples each 2 m, using a larval net 20 cm in diameter and 20 cm in depth with a handle of 2 m (Dubitskij, 1978). Data from field tests presented normal distribution and were analysed statistically using analysis of variance (ANOVA) ($p < 0.001$) and

Duncan's multiple range test ($p = 0.05$) to compare the mean of infection ($\bar{X}$) per larvae (1st-4th) instar. To determine the influence of mosquito species and larval instars on the mean value of infection an ANOVA of double classification ($p < 0.005$) was used.

Results

The ecological characteristics of control sites and study sites pre-treatment are shown on table 1.

Table 2 shows the total number of larvae and larvae density (m^2) prior to treatment.

The comparison of mean values of infection by 1-way ANOVA showed differences in all sites ($p < 0.001$): site 1 $F = 17,875$, site 2 $F = 18,452$; site 3 $F = 9,091$.

The percentage of larvae infected 72 h post-treatment in each site, and the mean infection for each instar are presented in table 2.

The comparison of infection means from all sites by 2-way ANOVA showed significant differences among instars from each specie ($F = 9,973$; $p < 0.001$) and between both species ($F = 22,921$; $p < 0.001$). Duncan's test showed that 3rd-4th instars *A. pseudopunctipennis* and *C. quinquefasciatus* mosquito larvae differed from 1st-2nd instars, indicating younger larvae (I, II) were more susceptible to infection than older ones (III, IV).

Estimation of larval densities seven days post-treatment are shown in table 2, as well as percentage of mortality.

Discussion

Younger larval stages of *A. pseudopunctipennis* and *C. quinquefasciatus* were more susceptible to parasitism and *A. pseudopunctipennis* mosquito larvae showed the highest index of infection. This is probably due to the fact that the latter adopts a horizontal position below the water surface, thereby facilitating the invasion by the pre-parasitic larvae of the nematode, which exhibits negative geotropism (Rojas et al., 1987). Recent observations (Montoya & Gallo, unpublished results) indicate that the mermithid *R. culicivorax* shows a high infective capacity in larvae of *Anopheles albimanus* Wiedemann, 1821 and *Anopheles nuñez-tovari* Gabaldon, 1940, killing the younger stages within 28 hours, and the parasite does not attain a post-parasitic form in these larvae.

Petersen et al., 1978 reported that *R. culicivorax* was mass produced for the treatment of 144,000 m^2 of *A. albimanus* breeding area in Lake Apastepeque in El Salvador. The edges of Lake Apastepeque were treated eleven times in seven weeks to control *A. pseudopunctipennis* and *A. albimanus* mosquito larvae. The *Anopheles* population was reduced and parasitism of mermithid averaged 94% post-application. Larvae of 1st and 2nd instars collected from treated sites 1, 2, and 3 were dead. The cause was considered to be multiparasitism.

Although *C. quinquefasciatus* mosquito larvae showed the lowest rate of parasitism, that of 1st and 2nd instars was the highest, due the slight chitin formation in the cuticle which facilitates the penetration by the pre-parasites stiletto.

The percentage of parasitism found in *A. pseudopunctipennis* larvae showed a high pathogenic effect. The increment of larval density at control sites 1 and 2 was attributed to the continual hatching of new larvae. The percentage of mortality observed in *A. pseudopunctipennis* and *C. quinquefasciatus* larvae and the reduction of larval density at sites 1, 2, and 3 showed the possible use of the parasite *R. culicivorax* to control these species of mosquito larvae as an alternative to chemical control in Oaxaca State, Mexico.

Resumen

Efectividad de Romanomermis culicivorax *(Nematoda, Mermithidae) en* Anopheles

pseudopunctipennis *y* Culex quinquefasciatus *(Diptera, Culicidae) en México*

Se realizaron pruebas de campo con *Romanomermis culicivorax* Ross & Smith, 1978 como control de larvas de mosquito en tres áreas naturales situadas en el estado de Oaxaca, México (tabla 1). Los nemátodos infectantes se diseminaron a 1000/m^2. Resultaron infectadas las larvas de mosquito de las dos especies, sin embargo, las larvas de *Anopheles* resultaron ser más susceptibles que las de *Culex* (tabla 2). Se observó además que los mayores niveles de parasitismo ocurrieron en los estadios más jóvenes I y II, los cuales resultaron más susceptibles a la invasión de los preparasíticos infectivos. Los valores de pH y temperatura calculados en el momento de las aplicaciones no influyeron negativamente en la capacidad infectiva de los preparasíticos.

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