Biological Control of Mosquitoes with Mermithids[1]

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Abstract: Mermithid nematodes parasitizing mosquitoes have substantial potential for vector control. Studies on the physiological ecology of *Romanomermis culicivorax* have defined some of the general requirements of mermithid nematodes and produced general guidelines for the experimental release of mermithids in biological control. Experimental field studies have established the biological control potential of *R. culicivorax,* but further development and utilization of this parasite will require a substantial commitment of scientific man-years and funds. *Key words:* Integrated Pest Management, physiological ecology, *Romanomermis culicivorax.*

The Phylum Nematoda has five orders with 14 families of obligate insect parasites, but only the Mermithidae have been found in natural populations of mosquitoes (29, 30). Mosquitoes are also utilized by filarioids as intermediate hosts, but these nematodes have no promise for biological control and will not be considered further. There are many reports of mermithids in mosquito populations but, because the nematode identifications were based on larval stages, they are often incomplete or inaccurate. However, there appear to be 15 acceptable species in eight genera (29,30). These mermithids can be divided into two groups: those that complete the parasitic phase of life primarily in the larval stages of the mosquito host and those that complete most of the parasitic growth phase in the adult mosquito (Table 1). In the first group there are four genera and ten species. Of the 133 natural and laboratory infected host species listed, 53% are *Aedes* sp., 20% are *Anopheles* sp., 19% are *Culex* sp., and the remainder of the hosts are species of *Armigeres, Culiseta, Deinocerites, Mansonia, Orthopodomyia, Psorophora, Toxorhynchites, Tripteroides, Uranotaenia,* or *Wyeomyia* (17,29,30). It may appear obvious from this list that mermithids have had their greatest success as parasites of culicine mosquitoes, particularly those in the genus *Aedes.* However, the list probably more accurately reflects the geographic distribution of entomologists interested in biological control organisms and, therefore, such an assumption is probably premature. A similar assumption, and subsequent explanation can be made for hosts of mermithids maturing in adult mosquitoes. There, of 14 host species 86% are *Aedes* sp.

HOST SPECIFICITY

In most cases, we have little information on host specificity other than that the hosts are generally mosquitoes. Some exceptions are *Hydromermis churchilliensis,* which naturally infects chaoborid midges as well as *Aedes* sp., and *Romanomermis culicivorax,* which also can infect blackfly larvae under abnormal conditions (30). These examples appear to be extraordinary exceptions to the specificity for mosquito larvae. Specificity within mosquitoes has received little attention. *Perutilimermis culicis* demonstrates species specificity and parasitized only *Aedes sollicitans; Strelkovimermis peterseni* demonstrates generic specificity and parasitized only *Anopheles* sp. (13).

The most thorough examination of host specificity has been carried out mainly by Dr. Petersen with *R. culicivorax* (17,30). More than 82 species of mosquitoes in 13 genera have been infected in the laboratory or found infected under natural conditions. *R. culicivorax* was unable to complete its development in five mosquito species: *Aedes triseriatus, Anopheles sinensis, Culex territans, Mansonia uniformis,* and *Psorophora ferox.*

ENVIRONMENTAL LIMITATIONS

Most investigations of environmental limitations of mermithids as biological control agents have used *R. culicivorax,* but the observations are probably valid for other temperate zone mermithids.

Temperature: Petersen (13) reported that *R. culicivorax* was infective when air temperatures were above 15 C. Subsequently, Brown and Platzer (1) and Galloway and Brust (8) found that some infec-

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Table 1. List of mermithids, their hosts, and sites of collection.*

Mermithid	Mosquito host	Locality
Mermithids maturing primarily in larval stages of the host:		
Hydromermis churchilliensis	*Aedes*—3 sp.	Manitoba
Octomyomermis muspratti	*Aedes*—8 sp.	Zambia
	Anopheles—2 sp.	
	Culex—5 sp.	
Octomyomermis troglodytis	*Aedes sierrensis*	California
Romanomermis culicivorax	*Aedes*—29 sp.	Florida,
	Anopheles—3 sp.	Louisiana
	Armigeres subalbatus	
	Culex—18 sp.	
	Culiseta—4 sp.	
	Deinocerites pseudes	
	Mansonia—2 sp.	
	Orthopodomyia signifera	
	Psorophora—7 sp.	
	Toxorhynchites rutilus	
	Tripteroides bambusa	
	Uranotaenia—3 sp.	
	Wyeomyia smithii	
Romanomermis communensis	*Aedes*—4 sp.	Manitoba
Romanomermis hermaphrodita	*Aedes*—2 sp.	Manitoba
Romanomermis iyengari	*Anopheles subpictus*	India
Romanomermis kiktoreak	*Aedes*—5 sp.	Northwest Territories
Romanomermis nielseni	*Aedes*—5 sp.	Wyoming
	Culex—2 sp.	
Strelkovimermis peterseni	*Anopheles*—9 sp.	Louisiana
Mermithids maturing primarily in adult stages of the host:		
Culicimermis schakhovii	*Aedes*—6 sp.	USSR
Culicimermis sp.	*Aedes*—4 sp.	Manitoba
Empidomermis cozii	*Anopheles funestus*	West Africa
Paramermis canadensis	*Aedes*—2 sp.	British Columbia
Perutilimermis culicis	*Aedes sollicitans*	Louisiana, New Jersey

*Derived from Petersen and Chapman (17) and Poinar (29,30)

tions occurred at water temperatures of 12 and 10 C, respectively. However, the optimum temperature was in the 21–33 C range. In addition, the optimal temperature for development of the parasitic stage of *R. culicivorax* was in the 20–32 C range. Petersen (14) found that *Romanomermis nielseni* postparasites developed and completed oviposition within 7 wk at 17 C. Increasing the temperature to 23 C suppressed oviposition but not postparasite development. In contrast, *R. culicivorax* required 23 wk to complete oviposition at 17 C, but only 3–7 wk at 23 C. This information provided a unique physiological comparison between the two species. Temperature is an important consideration in the use of *R. culicivorax* as a biological control agent; its use in colder temperature zones is precluded (8).

pH: Petersen (15) found that *R. culicivorax* was fully infective from pH 5.4 to 7.9 (4.8 to 8.5 were transitory pH exposures). Hence, pH of most natural waters should not be a limiting factor.

Salts: Petersen and Willis (20) reported that mild salinity (0.04M NaCl) inhibited the infectivity of *R. culicivorax*. This was confirmed by Brown and Platzer (2) who also reported the hierarchy of ion toxicity on a molar basis for *R. culicivorax* as follows: cations, sodium < potassium < calcium: and anions, chloride < carbonate = sulfate < nitrate < nitrite < phosphate. Therefore, it appeared that *R. culicivorax* would not be an effective biocontrol agent in feedlot runoffs, fertilizer plant wastewater, and brackish water situations. Recently, Petersen (16) has found that *Octomyomermis muspratti* is tolerant of diluted

seawater (3,000–4,000 μmhos/cm) and water from tree holes (10,000 μmhos/cm; high in organic matter). These findings suggest that *O. muspratti* has great potential for mosquito biocontrol in polluted waters.

Oxygen: In investigations to determine why polluted water compromises the infectivity of *R. culicivorax,* Brown and Platzer (3) reported the effects of lowered oxygen availability on the infective nematode. Transient exposure to low O_2 tension increased the survival, and thereby the infectivity, of the preparasites of *R. culicivorax.* This effect was explained as resulting from induced quiescence under low O_2 tensions and hence greater viability under the test conditions. More recently, I have found that the preparasites of *R. culicivorax* stop moving within 8 h in water high in organic content but low in O_2 (6). Therefore, lowered oxygen tensions in polluted waters may be responsible for the inability of *R. culicivorax* to infect mosquitoes under such conditions.

INTEGRATED PEST MANAGEMENT

Chemicals: Integrated Pest Management, the multipronged approach to pest control, encourages assessment of pesticide effects on mermithid infections. The first assessment was that of Mitchell et al. (11) in Taiwan, who found that the usual levels of Abate, Dieldrin, and Gama HCH did not adversely affect *R. culicivorax* infections. Finney et al. (7) reported that Altosid 5E, an insect growth regulator, at concentrations typically used for control of mosquito larvae did not interfere with any phase of the parasite's development. However, host mortality was considerably increased when Altosid 5E and *R. culicivorax* were used in combination on *Aedes aegypti* in laboratory experiments. This finding suggests a unique chemical-biological control approach in mosquito control: "If the chemical don't get 'em, the nemas will!" Platzer and Brown (25) reported that a variety of copper-based organic algicides and copper sulfate didn't compromise the infectivity of *R. culicivorax* as long as the concentrations were in the ranges usually used for algae and weed control. In summary, it appears that *R. culicivorax* is tolerant of a variety of chemical pest control measures used in aquatic ecosystems and combination treatments are possible.

Invertebrate predators: Most research on environmental limitations has been directed towards evaluation of abiotic factors. Mitchell et al. (11) were the first to recognize that ostracods preyed on mermithid preparasites. Similarly, copepods and young gammarids will attack preparasites (27), and the dynamics of copepod predation on mermithids has been reported in detail (28). Both satiated and starved adult copepods *(Cyclops vernalis)* attack preparasites rapidly in small volumes of water (less than 2 ml). This attack or predation rate is dependent on the volume of water, thus indicating that searching or hunting behavior controls predation rate. In laboratory studies with larger volumes of water (one liter), it was shown that mermithid preparasite populations were reduced significantly by copepod densities of 20–100/liter. Although field studies have not been carried out, I suspect that copepod density might play a significant role in the success or failure of field applications.

Invertebrate predators also may reduce the potential of establishment of aquatic mermithids (27). Diving beetles and gammarids, dragonfly and damselfly naiads, and small crayfish attack mermithid postparasites rapidly (27). Isopods, however, will attack postparasites only if other food is limited. Such predation may account for poor or no establishment of the mermithid after field application of *R. culicivorax.*

FIELD APPLICATIONS

Essentially all field releases of mermithid parasites of mosquitoes have been conducted with *R. culicivorax,* although in one study, Petersen and Willis (22) released *S. peterseni* and after 5 yr obtained more than 80% parasitization. In 1971 Petersen and Willis (21) treated 10 natural sites 20 times with preparasites of *R. culicivorax* and obtained 65, 58, and 33% parasitism of second, third, and fourth instars, respectively, of the *Anopheles* sp.; 94% of second-instar anophelines and 64% of all *Anopheles* were infected at rates of 1,000 preparasites/m^2 of surface area application. Application rates less than 1,000 preparasites/m^2 of surface area were less effective, but higher

application rates did not increase the number of infected mosquito larvae. Establishment of the nematode was observed in 7 of the 10 sites.

Petersen et al. (19) treated fallow rice fields in Louisiana infested with *Psorophora confinnis* and *Anopheles quadrimaculatus*. Increasing the application rate from 180 to 1,450 preparasites/yd^2 of surface area increased the parasitism of *P. confinnis* from 10 to 38%. They estimated that 3,900 preparasites/yd^2 would give 95% infection. Sixteen percent of *A. quadrimaculatus* were infected at the rate of 181 preparasites/yd^2 and 61% at 724/yd^2 of surface area. They estimated that 1,300 preparasites/yd^2 of surface area would give 95% infection. Caution should be used in making this type of prediction. Brown et al. (4), for example, found that increasing treatment rates from 1,000 to 25,000 preparasites/m^2 of surface area did not significantly increase the number of *Culex tarsalis* larvae infected.

Petersen and Willis (22) made a total of 30 releases in 21 sites (15 at rates of 1,000 and 15 at rates of 2,000 mermithid preparasites/yd^2 of surface area) to control *Anopheles* larvae. The primary species present was *Anopheles crucians*. At the lower rate, an average of 76% of the hosts were infected and parasitism averaged 60, 80, 86 and 77% in first through fourth instars, respectively. At 2,000 preparasites/yd^2, an average of 85% of the larvae were infected. Poor correlation was found between infection rates and vegetation and water depth.

Brown et al. (4) conducted field tests against four species of mosquitoes in three natural (rice fields and ponds) and two artificial (1- and 6-m^2 ponds) habitats at treatment levels ranging from 706 to 25,000 preparasites/m^2 of surface area. Anopheline larvae were more susceptible than culicine. Although both *C. tarsalis* and *Culiseta inornata* were reported in laboratory studies to be good hosts for *R. culicivorax*, field studies with these two species were disappointing.

Levy and Miller (10) applied *R. culicivorax* at the rate of 3,600 preparasites/m^2 of surface area to control mosquitoes breeding in a grassy field. In 10 potholes and ditches sampled in the field, they found 88–100% infection in *Culex nigripalpus, Aedes taeniorhynchus, Psorophora columbiae,* and *Psorophora ciliata*. In some cases all larvae were killed within 24 h due to multiple parasitism.

Levy and Miller (9) released preparasites into two unused sewage settling tanks at 64,000 and 110,000 preparasites/m^2 of surface area and obtained 37 and 54% infection of *C. pipiens quinquefasciatus,* respectively. Although these treatment levels seem high, large numbers of *C. p. quinquefasciatus* larvae were present and host: parasite treatment ratios were approximately 5:1 and 3:1. High concentrations of phosphates, chlorides, and carbonates probably lowered the infectivity of the preparasites, as much higher infection levels would be expected in this species at the dosages applied.

Petersen et al. (18) successfully controlled *Anopheles albimanus* and *Anopheles punctipennis* in Lake Apostepeque, El Salvador, with *R. culicivorax*. The mosquito breeding areas, a 2–5 meter band (total area = 10,700 m^2) around the lake was treated 11 times during 7 wk with 2,400–4,800 preparasites/m^2 at each application. The *Anopheles* population declined from 10 per sample dip prior to the first preparasite application to 0.6 per dip by the end of the release period; a 17-fold reduction in the number of mosquito larvae. This was the first successful large-scale attempt to control mosquitoes with a parasite.

Another technique for application of *R. culicivorax* was reported by Petersen and Willis (23) who distributed the eggs and postparasitic stages of *R. culicivorax* in 13 habitats known to breed pasture mosquitoes. They reported 52% of *Aedes atlanticus,* 59% of *Aedes tormentor,* 38% of *P. columbiae,* and 51% of *Psorophora howardii* were parasitized after the pastures were flooded. Brown-Westerdahl et al. (5) found that early season application of *R. culicivorax* postparasites was effective in providing continuous partial control (weekly mean infection = 60%) of *A. freeborni* and *C. tarsalis* throughout the rice growing season in Northern California. Infections were observed up to 12 m from point of application of 1,500 postparasites. The nematodes overwintered, and infected sentinel and native mosquitoes were found

the following season. Platzer and Eby (26) also showed that postparasites can produce sufficient eggs to persist for two seasons of mosquito control.

In field releases of mermithid nematodes, it is important that the application be made by trained personnel who thoroughly understand the environmental limitations of the organism. Under such conditions, the full potential for biological control will be achieved with mermithid nematodes.

ESTABLISHMENT

Petersen and Willis (26) have conducted the only long-term studies on the ability of *R. culicivorax* to become established after application. They showed that this nematode can become established in many semipermanent and permanent mosquito breeding sites, but that certain problems existed: lack of host populations, periodic lack of breeding water in the semipermanent sites, and inaccessibility due to changes in site access. Periods of little or no water tended to reduce the levels of infection in susceptible hosts, and flushing of the sites by heavy rains reduced nematode populations. It also appeared that sites which produced most mosquitoes also produced most *R. culicivorax* infections. Petersen and Willis (24) noted that recycling occurred in 10 sites 6–29 wk after treatment with preparasites. In 1974 three of the sites treated in 1971 were still producing infected hosts (7–25%). Five of six sites treated in 1971 and 1973 and 7 of 12 sites treated only in 1973 were producing parasitized hosts in 1974.

Petersen and Willis (23) released *S. peterseni* in Louisiana in 1971. Two years later 88% of the *Anopheles* sp. were parasitized. Nickle (12) reported that *R. culicivorax* was established and overwintered in Maryland where winter temperatures dropped as low as –17 C.

SUMMARY

Although substantial potential has been attributed to the effectiveness of mermithids for vector control, more time and research is required to realize this potential. Additional studies on the biology of *R. culicivorax* and other mermithids are needed if we are to fully utilize the biocontrol potential of these nematodes. A greater commitment of scientific and economic resources are required. Biocontrol with *R. culicivorax* has been under development for 10 yr; at the current level of interest and funding it will probably require 10 more years to obtain effective use of this or alternate organisms in biological control of vector populations. In addition, we need a strong commitment from agencies to train personnel in the handling and use of such biological control organisms.

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