Effects of Temperature and Photoperiod on the Infection of Two Mosquito Species by the Mermithid Romanomermis culicivorax[1]

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Abstract: Successful invasion by the mermithid *Romanomermis culicivorax* declined linearly from 93.6 to 1.5% in *Culex tarsalis* and from 73.1 to 1.6% in *Aedes dorsalis* larvae exposed in the laboratory at 18, 16, 14, 12, and 10 C. Larvae of *C. tarsalis* were more susceptible than those of *A. dorsalis* at 18 and 16 C, but this relationship was reversed at 12 C. Larval mortality during the 48-h exposure period was due primarily to nematode infection. Photoperiod had no effect on infection. *Key Words:* biological control, Culicidae, Nematoda, Mermithidae.

The development of mass-culture laboratory techniques by Petersen and Willis (10) for *Romanomermis culicivorax* Ross and Smith (=*Reesimermis nielseni* auct. partim.) has stimulated tremendous interest in the application of mermithids for mosquito control. Field trials have been conducted in Louisiana (11), Taiwan (5), California (8), and Manitoba (Galloway and Brust—unpublished observations) against larvae of *Anopheles, Culex, Psorophora,* and *Aedes* species.

If *R. culicivorax* is to be considered as a suitable control agent of mosquitoes common to Canada and the northern United States, information regarding its ability to infect mosquito larvae at low temperatures must be gained. Petersen and Willis (9) found that preparasitic juveniles of *R. culicivorax* were active in the field in Louisiana from April to November when the mean water temperatures exceeded 65 F (18.3 C) but were less active when temperatures dropped below 55 F (12.8 C). Mitchell et al. (5) determined that preparasites of *R. culicivorax* were unable to infect larvae of *Culex pipiens fatigans* Wiedemann in the field in Taiwan at low temperatures (minimum of 6-8 C). Kurihara (4) found that the rate of parasitism of *C. pipiens molestus* Forskal by *R. culicivorax* decreased from 30 C to 15 C. The present laboratory study was conducted to determine the ability of *R. culicivorax* to infect mosquito larvae at constant temperatures ranging from 10 C to 18 C.

MATERIALS AND METHODS

Host mosquitoes selected for this experiment were *Culex tarsalis* Coquillett and *Aedes dorsalis* (Meigen). Larvae of *C. tarsalis* were obtained from a laboratory colony which originated from a field population at Glenlea, Manitoba. *Aedes dorsalis* larvae were obtained from eggs laid by blood-fed females collected in the Winnipeg area. Eggs were hatched in a low oxygen environment to give a rapid and synchronous hatch. Larvae of both *A. dorsalis* and *C. tarsalis* were 20-24 h old when exposed to preparasitic juvenile nematodes.

Our colony of *R. culicivorax* was obtained from Dr. J. J. Petersen and originated in Louisiana. Colony maintenance was similar to that described by Petersen and Willis (10). Sand containing mermithid eggs and preparasites was flooded with dechlorinated tap water after the eggs of *C. tarsalis* hatched. *Aedes dorsalis* were hatched immediately afterwards. Only preparasites that could be recovered during the first 12 h after flooding were used in each experiment. Preparasite numbers were estimated from five 1-ml aliquot subsamples. Both mosquito larvae and preparasites were acclimated at 15 C for 12 h to reduce the temperature shock before being introduced to any experimental temperature. Preparasites and larvae were allowed further adjustment to each experimental temperature for an additional hour before treatment.

Rearing pans (15-cm diam) contained 30 larvae and approximately 300 preparasites in 100 ml of larval medium which consisted of 0.2 gm of finely ground liver powder/liter of dechlorinated tap water. Temperatures of 18, 16, 14, 12, and 10 C were selected and regulated by a water bath

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within ± 0.1 C (2) for the experiment. To ensure that all larvae remained in the first instar for the duration of the exposure period, no temperatures higher than 18 C were investigated. Five photoperiods (24L: 0D, 16L:8D, 12L:12D, 8L:16D, and 0L: 24D) were utilized at each temperature to measure their effects on infection. Three replicates of each treatment were conducted for both *A. dorsalis* and *C. tarsalis*.

Mosquito larvae were exposed to infection for 48 h, washed over a 250-μm (60-mesh) screen to separate any remaining preparasites, and dissected to determine the incidence of infection and the number of nematodes present in each larva. Numbers of surviving larvae were recorded in all treatments. A series of five pans of 30 larvae each at each temperature was used to determine larval survival in the absence of mermithid infection.

RESULTS AND DISCUSSION

Survival of *C. tarsalis* larvae in the control pans averaged 98.6% and that of *A. dorsalis*, 96.4%; there was no significant difference ($P<0.05$) between the survival of the two species at any temperature (Table 1).

Except for 10 C, survival of larvae of both species, when exposed to infection, was reduced at all temperatures and survival of *A. dorsalis* larvae was lower than that of *C. tarsalis* larvae. At 10 C, mean percent survival for both mosquito species did not differ from that in control pans. No difference occurred in the survival of *C. tarsalis* larvae at 18, 16, 14, or 12 C, whereas survival of *A. dorsalis* larvae varied significantly between temperatures. Larval survival under the described experimental conditions was not correlated with either percent infection of surviving larvae or number of nematodes per infected host.

The percent infection and the mean number of nematodes per infected host followed a linear reduction for both *C. tarsalis* and *A. dorsalis* as temperature was reduced. Paired t-tests yielded different infection levels between species at 18, 16, and 12 C (Table 1). At the highest temperatures (18 and 16 C), *C. tarsalis* larvae were more heavily infected than were *A. dorsalis* larvae. At 12 C, this relationship was reversed, and *A. dorsalis* larvae were more heavily infected. At 10 C, few larvae of either species were infected. Although Brown and Platzer (1) found *C. pipiens* larvae more susceptible to infection by *R. culicivorax* in total darkness, no significant effect of photoperiod was detected in our

TABLE 1. The response of *Culex tarsalis* and *Aedes dorsalis* larvae to infection by *Romanomermis culicivorax* under five different temperature regimens.

Temperature (C)	Survival of control larvae (%)[a]	Survival of larvae exposed to infection (%)[b]	% of surviving larvae infected[b]	No. nematodes/host in infected larvae[b]
		Culex tarsalis		
18	96.7 ± 0.0	90.5 ±2.3*	93.6 ± 2.0***	2.59 ± 0.08**
16	99.0 ± 1.3	85.8 ± 2.9***	86.0 ± 2.9***	1.72 ± 0.08NS
14	98.0 ± 0.8	93.8 ± 2.0***	46.2 ± 5.5NS	1.24 ± 0.04NS
12	99.3 ± 0.7	93.8 ± 1.4***	31.9 ± 5.9***	1.15 ± 0.03**
10	100.0 ± 0.0	99.3 ± 0.4*	1.5 ± 0.5NS	1.00 ± 0.00NS
		Aedes dorsalis		
18	98.7 ± 0.8	83.8 ± 1.9*	73.1 ± 3.4***	1.86 ± 0.07**
16	94.0 ± 2.2	63.1 ± 4.6***	71.2 ± 2.4***	1.79 ± 0.07NS
14	95.3 ± 2.3	70.7 ± 4.4***	49.6 ± 3.6NS	1.52 ± 0.07NS
12	95.3 ± 1.3	64.4 ± 4.7***	48.6 ± 5.3***	1.44 ± 0.09**
10	98.7 ± 0.8	96.7 ± 0.9*	1.6 ± 0.6NS	1.00 ± 0.00NS

[a]Mean ± standard error; 5 replicates of 30 larvae each.
[b]Mean ± standard error; pooled data; 15 replicates of 30 larvae each. Paired t-test; level of significant difference between larvae of the two species; asterisks (*, ** and ***) indicate significant difference at $P<0.05$, 0.01, and 0.001 respectively; NS = $P>0.05$.

study and no interaction between temperature and photoperiod was evident..

The pest species of mosquitoes in Manitoba and other parts of Canada differ from those encountered in Louisiana, Taiwan, or California. In the spring, snow-melt species such as *Aedes communis* (Degeer), *A. fitchii* (Felt and Young), *A. trichurus* (Dyar), *A. excrucians* (Walker), and *A. stimulans* (Walker) are major pests in woodland areas. In Manitoba, *A. dorsalis* and *A. spencerii* (Theobald) often occur in large numbers in ditches filled with water from melting snow. These early mosquito populations can cause considerable irritation to man from May to August.

The low temperature conditions under which many spring snow-melt mosquitoes develop may severely restrict the use of the Louisiana strain of *R. culicivorax* in their control. For example, larvae of *A. communis* and *A. diantaeus* Howard hatch and complete their development in woodland pools where mean daily temperatures may rarely exceed 10 C. Results of this experiment indicate difficulties in the application of *R. culicivorax* against certain mosquito species at low temperatures.

Tsai and Grundmann (14) found several early spring species of mosquitoes infected by *Romanomermis nielseni* (Tsai and Grundmann) under natural conditions in Wyoming. Much confusion surrounds the taxonomic definition of this particular mermithid complex. The Wyoming and Louisiana populations may in fact represent two distinct species (7, 13), or two subspecies. The nematode material used in our experiment was maintained under laboratory conditions at 26 $\pm$ 1 C for several generations. Proper environmental conditioning or accurate microclimatic simulation could affect the capabilities of *R. culicivorax* to infect larvae of certain species of mosquitoes at low temperatures. Trials against spring *Aedes* in Manitoba are presently in progress in an attempt to evaluate *R. culicivorax* as a potential control agent under field conditions.

Mosquito larvae often die before the mermithid has completed its parasitic development (3, 12). At temperatures of 12 to 18 C, almost all mortality in both species resulted from infection by *R. culicivorax*. Petersen (6) has shown that different mosquito species vary in their relative susceptibility to infection. From our results, it can also be concluded that different species vary in their ability to support successful development of one or more parasites, at least over the temperature range examined.

In our experiments, successful infection by preparasites of *R. culicivorax* decreased with temperature; yet larval mortality did not follow a similar trend, although this circumstance was largely a result of infection. Low temperatures impose a thermal stress on larval growth. Infection by one or more mermithids applies additional stress on growth to the point of premature death of some hosts. The results suggest that larvae of both *A. dorsalis* and *C. tarsalis* are more easily killed by the sustained infection as the stress from low temperature increases. At 10 C, almost no infection occurred and larval survival was high, an occurrence not significantly different from survival of larvae in control pans.

Larval behaviour may be an important factor in successful parasitism by preparasitic juveniles of *R. culicivorax* (7). In Manitoba, *A. dorsalis* larvae develop under early spring snow-melt conditions at lower temperatures than do *C. tarsalis* larvae which develop later in the season. Nevertheless, in the laboratory, *C. tarsalis* larvae were more active than *A. dorsalis* larvae at low temperatures. The greater susceptibility of *A. dorsalis* larvae at 12 C, relative to that of *C. tarsalis* larvae, may be explained by the reduced activity of the *A. dorsalis* larvae.

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