THE UNIVERSITY OF MANITOBA

Application of the Mermithid Nematode, *Romanomermis culicivorax* Ross and Smith, 1976, for Mosquito Control in Manitoba and Taxonomic Investigations in the Genus *Romanomermis* Coman, 1961

by
Terry Don Galloway

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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 for 48 hours. 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 hr exposure period was due primarily to nematode infection. Photoperiod had no apparent effect on infection.

Low temperature was the primary factor limiting infection by R. culicivorax in mosquito larvae which develop in snow-melt pools. About 20% of A. canadensis and A. pionips larvae were infected at 5-10° C in pools treated with 50,000 preparasites per m^2. Little or no infection occurred in A. dorsalis, A. spencerii or A. communis larvae at temperatures below 10° C. R. culicivorax survived the winter in a natural mosquito habitat in Manitoba. Mosquito larvae were prematurely killed by high levels of multiple infection. Eight to 20% of A. vexans larvae which hatched after an early spring rain were infected when a pool was treated with 50,000 preparasites per m^2. Melanized nematodes were discovered in A. communis, A. canadensis and A. vexans larvae. No infection of Mochlonyx velutinus and Dixa sp. larvae or Paraleptophlebia (?) sp. nymphs was observed. Romanomermis culicivorax cannot be recommended at this time for the control of spring Aedes mosquitoes in Manitoba because of the low levels of infection, the number of

applications and the high number of preparasites required to obtain infection.

Mermithid parasites were obtained from stock cultures at 27 ± 0.5° C 3 weeks after post parasitic juveniles of R. culicivorax were placed in moist sand. Peak preparasite production occurred between 8 and 14 weeks, and then declined rapidly. After 32 weeks, only a small number of preparasites could be obtained from samples. When a 10 wk old culture was kept at 15° C, the number of preparasites obtained from samples diminished progressively over a 7 wk period. Pre-treatment levels of preparasites were obtained 6 weeks after the culture was returned to 27° C.

Parasitism by R. culicivorax caused symptoms characteristic of starvation in A. vexans larvae both in the laboratory and in the field. Infected larvae were reduced in size throughout their development and moulting was delayed through successive instars. Food supply of the host affected development time of the nematodes in laboratory experiments. It was apparent that A. vexans larvae suffered significant premature mortality as a result of mermithid infection. The percentage of larvae infected in artificial pools varied considerably over time and between pools treated at the same level. Excluding samples taken after the onset of pupation of uninfected larvae, mean per cent infection ranged from 7.2-18.8, 12.4-24.8, 9.2-23.2 and 38.0-49.0 when preparasites were applied at 10,000, 20,000, 50,000 and 100,000 per m^2 respectively to artificial pools. Nematodes were occasionally

melanized by the host. It was concluded that control of floodwater mosquitoes using R. culicivorax on a large scale in Manitoba is not practical at this time.

A brief taxonomic review of the Romanomermis species parasitizing mosquitoes is presented, along with the description of Romanomermis communensis sp. n. from Goose Creek, Manitoba. The type locality for R. communensis was the same as for Hydromermis churchillensis Welch, 1960, however, the latter species was not found in either 1974, 1975 or 1976. Consideration is given to the present status of H. churchillensis. Although R. communensis and R. culicivorax Ross and Smith, 1976 were successfully cross-mated in the laboratory, they are considered here as separate species. The description of R. hermaphrodita Ross and Smith, 1976 is supplemented by specimens collected from the type locality and host.

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CHAPTER I

Review of pertinent literature

Mosquitoes have attracted the attention of medical and veterinary research in the past 100 years. The true potential of these biting flies as vectors of disease is only now being appreciated. As a result, broad surveys to locate parasites and/or predators suitable as biological control agents have been conducted. Through such surveys and intensive studies in mosquito biology, numerous encounters with nematodes of the family Mermithidae have been recorded.

Many reports have been presented by mosquito biologists with little nematological experience. Such observations have provided valuable information on the distribution and importance of mermithids, but have contributed little towards their biology or rearing. Until recently, the work of Iyengar(1929; 1938), Muspratt (1945; 1965) and Welch (1960) provided the basic information on the life cycles of mermithid parasites of mosquitoes. Since 1969, numerous important contributions have been made, especially in North America.

Our knowledge of the mermithids which parasitize mosquitoes is expanding rapidly. Table 1 is a chronological representation of significant contributions and reports of mermithids in mosquitoes. For the most part, references to Romanomermis culicivorax Ross and Smith, 1976 have been excluded. Bibliographic lists such as Speer (1927) and

Jenkins (1964) have been omitted. No attempts have been made in this table to resolve problems of synonymy. Species names in brackets represent the presently recognized taxonomic status of the nematode discussed in each paper.

At present, 11 species of mermithids parasitizing mosquitoes have been recognized from 6 genera, including adult descriptions for each species. Of these 11, 8 have been described since 1970. Only 3 species, Romanomermis iyengari Welch, 1964, Octomyomermis muspratti (Obiamiwe and Macdonald, 1973) and Culicimermis schakhovii Rubtsov and Isaeva, 1975 have been described from the Old World.

Mermithid parasites of mosquitoes have two basic life cycle types. They may normally complete their parasitic development in 1) the adult or 2) the larval mosquito.

1) Mermithids which emerge from adult mosquitoes. Numerous references to nematodes in this group have been made (Table 1). Petersen et al. (1967), Rubtsov and Isaeva (1975) and Galloway and Brust (1976) have presented most of the biological information for these mermithids.

Preparasitic juveniles hatch from the egg when flooded with water and penetrate the cuticle of early instar larvae. Petersen et al. (1967) suggested that preparasites may also be injested resulting in their observed concentration in the head capsule shortly after exposure. Development takes place in the haemocoele of the host. Growth is slow during the larval and pupal stages of the

host. At 23-26° C, Perutilimermis culicis Nickle, 1972 emerged 5-9 days after female mosquitoes took blood (Petersen et al. 1967). Culicimermis schakhovii Rubtsov and Isaeva, 1975 required 10-12 days for the completion of its parasitic development in the adult mosquito (Rubtsov and Isaeva 1975). A Culicimermis sp. in Aedes vexans (Meigen) required 6-21 days development after eclosion of the host (Galloway and Brust 1976). A blood-meal by the female mosquito appears to be an important element in the development of P. culicis (Petersen et al. 1967). Rubtsov and Isaeva (1975) and Galloway and Brust (1976) found that a blood-meal by the host was not essential to development of Culicimermis and that they developed in male mosquitoes as well as females.

Postparasitic juveniles emerge from the abdomen of the host and burrow into the moist substrate where they form tightly coiled masses. After the final moult and copulation, eggs are laid in the soil by mated females. The eggs embryonate immediately and after completion of their development, will hatch when flooded with water.

Fat body is reduced and female mosquitoes are apparently castrated as a result of parasitism (Steiner 1924; Coz 1966; Petersen et al. 1967; Trpiš et al. 1968; Trpiš 1971; Rubtsov and Isaeva 1975), though infected females may mate if the number of nematodes present is low (Rubtsov and Isaeva 1975). Similarly, infected male mosquitoes were capable of mating (Rubtsov and Isaeva 1975).

Emergence of the mermithids most often results in immediate death of the host. Petersen et al. (1967), however, noted that some females could survive infection and subsequently take blood and lay a reduced number of eggs.

2) Mermithids which emerge from larval mosquitoes. Our knowledge of this group of mermithids far exceeds that of the former group. For the most part, this has been a result of the development of laboratory mass culture techniques for Romanomermis culicivorax Ross and Smith, 1976 by Petersen and Willis (1972a).

Fully embryonated eggs hatch when flooded with water and the preparasitic juveniles penetrate the cuticle of early instar larvae. For R. culicivorax in A. aegypti (L.), growth during the first three days is slow compared to the accelerated rate in subsequent days (Gordon et al. 1974). The period required for parasitic development is related to temperature. In the laboratory, R. culicivorax may require as little as five days, while in spring snowmelt pools it may require in excess of 30 days.

As the nematode approaches completion of its parasitic development, it becomes visible through the cuticle of the host. In culicines, the nematodes coil laterally in the thorax, while in anophelines they coil longitudinally through the thorax and abdomen (Petersen et al. 1968).

Mermithid species which normally emerge from larval hosts have occasionally been observed to pass into the pupal or adult stages. Beckel and Copps (1955) and Welch

(1960) observed juveniles of H. churchillensis in pupae and adults of Aedes communis (Degeer) at Churchill. Brust and Smith (1972) observed juvenile nematodes in adult A. hexodontus (Dyar) and A. impiger (Walker). R. culicivorax has also been observed in adult mosquitoes (Pao-Shu 1976). The observations of larval parasites continuing their development into adults in the field may be related to low temperatures or stage of host infected. Welch (1960) suggested that this may be an important method of dispersal for a nematode normally restricted to the larval habitat of its host.

Juvenile mermithids enter the substrate where they form coiled masses. In these masses, they apparently undergo a double moult to adults (Poinar and Otieno 1974; Ross and Smith 1976) and mate. Females lay large numbers of eggs in the substrate. Petersen (1975b) observed female R. culicivorax to lay an average of 2,480 eggs over 18 days.

Christie (1929) first observed that sex in Mermithidae was determined during parasitic development. This is apparent for R. culicivorax (Petersen 1972), Diximermis peterseni Nickle,1972 (Petersen and Chapman 1970) and Romanomermis spp. (Galloway and Brust 1976). As the number of nematodes increased, the sex ratio shifted from predominantly female to predominantly male. Petersen (1972) demonstrated that for R. culicivorax in Culex pipiens quinquefasciatus Say, single infections resulted in 91% female nematodes, while nearly 100% males were produced

when greater than seven nematodes were present. Welch (1960) found no variation in sex ratio in the observed multiple infections of H. churchillensis. Petersen (1972) determined that the production of male R. culicivorax could be favored by the reduction in quantity and/or quality of the food source.

Investigations into host specificity of mermithids in this group have been limited because of a lack of suitable laboratory rearing techniques and taxonomic confusion. Generally, they seem to demonstrate a lesser degree of host specificity than have mermithids which normally develop in adult mosquitoes (Petersen 1973b). Muspratt (1945; 1965) observed Octomyomermis muspratti to infect 7 Aedes spp. and 1 Culex sp. in the field as well as 2 Culex spp. in the laboratory. R. culicivorax exhibits varying degrees of host specificity (Petersen 1975a) but developed in every mosquito species examined except Culex territans Walker and to a limited extent, A. triseriatis Say (Table 2). D. peterseni possesses clearly defined limits to host specificity by developing only in anopheline mosquito larvae (Petersen and Chapman 1970). A list of larval and adult hosts found infected by various mermithids is presented in Table 1.

Host resistance by larval mosquitoes to mermithids was first recorded by Welch (1960) who observed melanized juveniles present in the haemocoele of A. communis larvae. R. culicivorax has been observed to be melanized by larvae of A. triseriatus, Culex salinarius Coquillett, C. territans,

Culiseta melanura (Coquillett), Psorophora ferox (Humboldt) and P. discolor (Coquillett) (Petersen et al. 1968; Petersen et al. 1969; Petersen 1975a) as well as by larvae of Anopheles sinensis Wiedemann (Mitchell et al. 1974). R. culicivorax was not affected by the presence of melanized Neoaplectana carpocapsae Weiser, 1955 in A. aegypti larvae (Hall et al. 1975). N. carpocapsae is normally killed by the formation of a humoral capsule by the mosquito host larvae (Welch and Bronskill 1962; Dadd 1971). Investigations into the structure and suppression of the humoral capsule surrounding the nematodes have been conducted by Bronskill (1962), Poinar and Leutenegger (1971), Andreadis and Hall (1976) and Beresky and Hall (1977).

Records of mermithids of larval mosquitoes in non-culicid hosts are rare. Welch (1960) recovered H. churchillensis from a Mochlonyx sp. and a Chaoborus sp. Ignoffo et al. (1973) tested four species of freshwater fish and 16 species of invertebrates for their susceptibility to infections by R. culicivorax. Corethrella appendiculata Grabham was susceptible to R. culicivorax at high concentrations. Preparasites were able to penetrate into the haemocoele of Chironomus sp. larvae, a hydrophilid, a dytiscid and an adult haliplid, but were unable to develop and subsequently died. R. culicivorax was also able to enter larvae of Simulium vittatum Zetterstedt, S. venustum Say, and S. decorum Walker (Finney 1975) although information regarding the degree of development was not given.

Hansen and Hansen (1976) found that larvae of S. damnosum were attacked and killed by R. culicivorax preparasites but the nematode failed to develop. Obiamiwe and Macdonald (1973) attempted to infect three chironomid larvae with O. muspratti without success. Mice and rats were also immune to infection (Ignoffo et al. 1974).

Culture techniques for aquatic mermithids are inconsistent and with few exceptions, have met with little success. Iyengar (1929) first reared juvenile mermithids from several Anopheles species to adults in "moist sand slopes". Preparasites were eventually recovered and the methods of penetration observed. Welch (1960) reared H. churchillensis with limited success in moist sand. Muspratt (1965) succeeded in culturing O. muspratti in the Culex pipiens complex. Free-living stages were reared in wet sand which was allowed to dry to the desired moisture content. It was not until Petersen and Willis (1972a) cultured R. culicivorax that unlimited numbers of nematodes could be produced. The parasitic stages developed in C. pipiens quinquefasciatus larvae and free-living stages were kept in moist sterile sand in aluminum cake pans (22x35x 5 cm). At that time, their estimated cost of production was 7-10 cents per million preparasites. Petersen (1973c; 1975b) further expanded on factors affecting mass culture of R. culicivorax. Through the efforts of Dr. W.R. Nickle and Fairfax Biological Laboratories, R. culicivorax has become commercially available under the label Skeeter

Doom® (Nickle 1976). Both O. muspratti (Obiamiwe and Macdonald 1973) and D. peterseni (Petersen and Willis 1974b) have been reared in the laboratory with limited success.

In vitro culture of the parasitic stages of R. culicivorax is still very much in the experimental stage. Roberts and van Leuken (1973) observed growth from 750μ to 6-7 mm in six weeks when cultured in association with tissue-culture cells, though juveniles survived for eight weeks. Finney (1976) reported growth to the approximate size of mature juvenile mermithids but with degenerate trophosome and female genital primordia.

Ultrastructural studies have been conducted using the preparasitic juveniles of R. culicivorax (Poinar 1974a; Poinar and Hess 1974). Two brief reports on the ultrastructure of this species have revealed the uptake of ferritin particles through the cuticle of the parasitic stages (Poinar and Hess 1976) and the presence of crystal arrays of virus-like particles in the preparasite (Poinar and Hess 1977). Preparasites and adult males of R. culicivorax have been examined under SEM by Nickle and Högger (1974).

The potential of mermithids as biological control agents has often been stated (e.g. Welch 1965), but only two species attacking larval mosquitoes have been used in field trials.

R. culicivorax has been applied to field situations in Louisiana (Petersen and Willis 1972b; 1974a; 1975;

Petersen et al. 1973; Steelman et al. 1972), California (Petersen et al. 1972) and Taiwan (Mitchell et al. 1974). This nematode has been established and has achieved varying degrees of control of A. crucians larvae in suitable pools in Louisiana over a period of several years (Petersen and Willis 1975). Significant control has been achieved for certain floodwater species following placement of R. culicivorax cultures in the bottoms of suitable habitats (Petersen and Willis 1976). Field results have indicated the highly susceptible nature of Anopheles species to infection by R. culicivorax (Petersen et al. 1972; Petersen and Willis 1972b; 1974a). The widespread dissemination of preparasites over large breeding sites will be greatly enhanced if, as suggested by the preliminary results of Levy et al. (1976), preparasites can be applied using an aerial spray system.

Petersen (1973a) found that preparasites of R. culicivorax concentrated along the edges and surface of artificial pools. Volume of water in the pools had no effect on incidence of parasitism. Kurihara (1976) has suggested that preparasites of R. culicivorax may be actively attracted to larvae of C. pipiens molestus Forskål.

Field experiments in Louisiana (Petersen and Willis 1974b) have demonstrated that D. peterseni can become artificially established and produce substantial levels of infection in larvae of susceptible Anopheles species. The long-term potential of this mermithid species remains to be determined.

TABLE 1. Reports offering new information or observations on mermithid parasites of mosquitoes. Species names in brackets represent the tentative taxonomic status of the originally cited species name.

Mermithid species	Host species	Stage of host	Location	Author(s)
Agamomermis sp.	*Culex nemoralis* (*Aedes communis*)	larva pupa adult	Leipzig Saxony Germany	Stiles (1903) coll. 1889
Agamomermis culicis (*Perutilimermis culicis?*)	*Aedes sollicitans*	adult	New Jersey	Smith (1903)
Agamomermis culicis gen. et sp. nov. (*Perutilimermis culicis?*)	*Culex sollicitans* (*Aedes sollicitans*)	adult	Rutgers College	Stiles (1903)
Agamomermis culicis (*Perutilimermis culicis?*)	*Aedes sollicitans*	adult	New Jersey	Smith (1904)
Mermis sp. (*Agamomermis* sp.)	*Culex* - larver	larva	Aittosaari Saaksmaki	Levander (1904)
Agamomermis sp.	*Culex* sp.	larva	Ootacamund, India	Ross (1906)
Mermis sp. (*Agamomermis* sp.)	*Stegomyia fasciata* (*Aedes aegypti*)	larva	Labe, French Guinea	Gendre (1909)

Table 1 Cont'd.

Mermithid species	Host species	Stage of host	Location	Author(s)
Paramermis canadensis (Agamomermis canadensis)	Aedes vexans Aedes aldrichi (Aedes sticticus)	adult ♂ + ♀	British Columbia	Steiner (1924)
Agamomermis sp.	Anopheles sp.	-	USSR	Reingardta (1924) (in Rubtsov and Isaeva 1975)
Paramermis canadensis (Agamomermis canadensis)	Aedes vexans	adult	Fraser Valley, British Columbia	Hearle (1926)
Agamomermis sp. (Culicimermis schakhovii)	Aedes dorsalis Aedes cantans	adult ♂ + ♀	New Bavaria	Schakhov (1927)
Mermis sp. (Romanomermis iyengari)	Anopheles minimus var. varuna Anopheles pseudojamesi Anopheles sinensis Anopheles barbirostris Anopheles fulginosus Anopheles phillip-pinensis Anopheles tessellatus	larva	Lower Bengal Delta	Iyengar (1929)
Mermis sp. (Agamomermis sp.)	Anopheles fulginosus Anopheles subpictus	adult	Bengal	Iyengar (1929)

Table 1 Cont'd.

Mermithid species	Host species	Stage of host	Location	Author(s)
Eumermis sp. (probably Agamomermis sp.)	Anopheles sp. Aedes sp.	-	-	Steiner (1929)
Agamomermis sp.	Anopheles leucosphyrus	larva	Sumatra	Walandouw (1934)
Agamomermis sp. (Octomyomermis muspratti)	Aedes metallicus Aedes aegypti Aedes calceatus Aedes fulgens Aedes zethus Aedes marshalli Aedes hawthori Culex nebulosus	larva	Livingstone, Northern Rhodesia	Muspratt (1945)
Agamomermis sp.	Anopheles gambiae	larva	Livingstone & Barberton, Eastern Transvaal	Muspratt (1945)
	Anopheles rufipes	larva	Odzi, Southern Rhodesia	
Agamomermis sp.	-	larva	Frobisher Bay, Baffin Island	Freeman (1950) (in Jenkins & West, 1954)
Agamomermis sp.	Aedes vexans Culex salinarius Culex pipiens	larva	Delaware Co. Pa.	Stabler (1952)
Agamomermis sp.	Aedes cinereus Aedes impiger Aedes punctor Aedes communis	larva	Southeastern Alaska	Frohne (1953a; 1953b; 1954)

Table 1 Cont'd.

Mermithid species	Host species	Stage of host	Location	Author(s)
	Aedes excrucians Aedes pionips			
Agamomermis sp.	Aedes communis Aedes nigripes	larva	Churchill, Manitoba	Jenkins & West (1954)
Agamomermis sp.	Aedes nearcticus (Aedes impiger)	larva	Coral Harbour, Southampton Island	Jenkins & West (1954)
Agamomermis sp.	Aedes communis	adult	Churchill, Manitoba	Beckel & Copps (1955)
Agamomermis sp.	Anopheles annulipes	larva	Townsville, Queensland	Laird (1956)
Hydromermis churchillensis sp.n.	Aedes communis	larva pupa adult	Churchill, Manitoba	Welch (1960)
Hydromermis churchillensis	Mochlonyx sp. Chaoborus sp.	larva	Churchill, Manitoba	Welch (1960)
Hydromermis churchillensis (?)	Aedes pullatus Aedes communis	larva	Gothic, Colorado	Smith (1961)
Romanomermis iyengari sp. n.	Anopheles subpictus	larva	Bangalore, India	Welch (1964)
Agamomermis sp.	Aedes communis Aedes maculatus	adult ♀	Khoper River, USSR	Artyukhovski & Kolicheva (1965)
Agamomermis sp. (Octomyomermis muspratti)	Culex fatigans Culex pipiens Culex torrentium	larva	Livingstone, Zambia	Muspratt (1965)

Table 1 Cont'd.

Mermithid species	Host species	Stage of host	Location	Author(s)
Gastromermis sp.	Anopheles funestus	adult ♂ + ♀	Koumbia, Upper Volta	Coz (1966)
Agamomermis culicis (Perutilimermis culicis)	Aedes sollicitans	larva pupa adult	Louisiana	Petersen et al. (1967)
Agamomermis culicis (Perutilimermis culicis)	Aedes sollicitans	adult	Louisiana	Chapman et al. (1967)
Agamomermis spp.	Uranotaenia sapphirina Uranotaenia lowii Anopheles crucians Anopheles punctipennis Anopheles quadrimaculatus Culex erraticus Psorophora confinnis Corethrella brakeleyi	larva	Louisiana	Chapman et al. (1967)
Agamomermis sp.	Aedes vexans	adult	Haney, B. C.	Trpiš et al. (1968)
Reesimermis nielseni (Romanomermis nielseni)	Aedes cinereus Aedes communis Aedes fitchii Aedes increpitus Aedes pullatus Culiseta impatiens	larva	Lone Tree, Wyoming	Tsai et al. (1969)

Table 1 Cont'd.

Mermithid species	Host species	Stage of host	Location	Author(s)
Mesomermis sp.	*Orthopodomyia signifera*	larva pupa adult	Anthony Ferry, Louisiana	Petersen & Willis (1969b)
Agamomermis sp.	*Aedes vexans*	adult	Fraser Valley, British Columbia	Trpiš (1971)
Romanomermis sp. (*Octomyomermis muspratti*	*Culex pipiens molestus* (in laboratory)	larva	lab only	Obiamiwe (1969)
Agamomermis culicis (*Perutilimermis culicis*)	*Aedes sollicitans*	adult	Louisiana	Petersen & Willis (1969a)
Gastromermis sp. (*Diximermis peterseni*)	*Anopheles crucians*	larva	Louisiana	Petersen & Chapman (1970)
Agamomermis sp.	*Aedes impiger*	adult	Alaska	Gorham (1970)
Agamomermis sp.	*Aedes impiger* *Aedes pullatus*	larva	Alaska	Gorham (1970)
Agamomermis culicis (*Perutilimermis culicis*)	*Aedes sollicitans*	adult	Florida	Savage & Petersen (1971)
Romanomermis sp. (*Romanmermis culicivorax*)	*Anopheles crucians* *Uranotaenia* sp.	larva	Florida	Savage & Petersen (1971)

Table 1 Cont'd.

Mermithid species	Host species	Stage of host	Location	Author(s)
Gastromermis sp. (Diximermis peterseni)	Anopheles crucians	larva	Florida	Savage & Petersen (1971)
Corethrellonema grandispiculosum	Corethrella sp.	larva	Florida	Savage & Petersen (1971)
Agamomermis sp. (Romanomermis kiktoreak?)	Aedes impiger Aedes nigripes Aedes hexodontus	larva adult	Baker Lake, N.W.T.	Brust & Smith (1972)
Gastromermis sp. or Limnomermis sp.	Anopheles annulipes	larva	Mittagong, N.S.W.	Kalucy (1972)
Reesimermis muspratti (Octomyomermis muspratti)	Aedes, Culex, Anopheles spp.	larva	laboratory	Obiamiwe & Macdonald (1973)
Diximermis peterseni	Anopheles quadrimaculatus Anopheles crucians Anopheles punctipennis Anopheles albimanus Anopheles atropos Anopheles barberi Anopheles bradleyi Anopheles freeborni Anopheles stephensi	larva	Louisiana, Florida, New York	Petersen & Willis (1974b)
Octomyomermis troglodytis	Aedes sierrensis	larva	Northern California	Poinar & Sanders (1974)

Table 1 Cont'd.

Mermithid species	Host species	Stage of host	Location	Author(s)
Culicimermis schakhovii	Aedes cantans Aedes riparius Aedes excrucians Aedes cataphylla Aedes behningi Aedes communis	larva pupa adult	Leningrad, USSR	Rubtsov & Isaeva (1975)
Romanomermis hermaphrodita	Aedes impiger Aedes nigripes	larva	Camp Nanuk, Churchill, Manitoba	Ross & Smith (1976)
Romanomermis kiktoreak	Aedes impiger Aedes communis Aedes rempeli	larva pupa adult	Baker Lake, N.W.T.	Ross & Smith (1976)
Romanomermis sp. (Romanomermis hermaphrodita)	Aedes impiger	larva	Camp Nanuk, Churchill, Manitoba	Galloway & Brust (1976)
Romanomermis sp.	Aedes communis Aedes churchillensis	larva	Goose Creek, Churchill, Manitoba	Galloway & Brust (1976)
Culicimermis sp.	Aedes vexans Aedes dorsalis Aedes spencerii Aedes sticticus	larva pupa adult ♂ + ♀	Winnipeg, La Salle, Portage la Prairie, Manitoba	Galloway & Brust (1976)

TABLE 2. Species of mosquitoes infected by *Romanomermis culicivorax* naturally in the field, artificially in the field and artificially in the laboratory.

Natural infections in the field

Host Species	Location	Reference
Aedes atlanticus D.& K.	Louisiana	82, 88
mitchellae (Dyar)	Louisiana	82
vexans (Mg.)	Louisiana	82, 88
sollicitans (Walker)	Louisiana	88
Anopheles crucians (Wied.)	Louisiana Florida	82, 88, 112
punctipennis (Say)	Louisiana	82, 88
quadrimaculatus Say	Louisiana	82, 88
Culex erraticus (D.& K.)	Louisiana	82, 88
restuans Theo.	Louisiana	82, 88
Psorophora ciliata F.	Louisiana	82, 88
confinnis (Lynch-Arribalzaga)	Louisiana	82, 88
discolor (Coq.)	Louisiana	82, 88
Uranotaenia lowii Theo.	Louisiana	82, 88
sapphirina (Osten-Sacken)	Louisiana	82, 88
spp.	Florida	112
Culiseta inornata (Williston)	Louisiana	88

Table 2 Cont'd.

Artificial infections in the field

Host Species	Location	Reference
Aedes vexans (Mg.)	Louisiana	82
nigromaculis (Ludlow)	California	77
Anopheles bradleyi King	Louisiana	82
quadrimaculatus Say	Louisiana	82, 91, 93, 121
crucians Wied.	Louisiana	91, 93
freeborni Aitken	California	83
Culiseta inornata (Williston)	Louisiana	82
Psorophora cyanescens (Coq.)	Louisiana	82
confinnis (Lynch-Arribalzaga)	Louisiana	82, 121
Uranotaenia sapphirina (Osten-Sacken)	Louisiana	93
Culex erraticus (D.& K.)	Louisiana	93
tarsalis Coq.	California	83
pipiens fatigans Wied.	Taiwan	61
tritaeniorhynchus summorosus Dyar	Taiwan	61
fuscanus Wied.	Taiwan	61

Table 2 Cont'd.

Artificial infection in the laboratory

Host Species	Development	Melanization	Reference
Aedes aegypti (L.)	+	-	35, 38, 75, 80, 82
atlanticus D. & K.	-	-	82
canadensis (Theo.)	+	-	82
mitchellae (Dyar)	-	-	82
sierrensis (Ludlow)	+	-	82
sollicitans (Walker)	+	-	75, 80, 82
taeniorhynchus (Wied.)	+	-	75, 80, 82
thibaulti D. & K.	+	-	82
tormentor D. & K.	+	-	75, 80, 82
triseriatus (Say)	+	+	75, 80, 82
vexans (Mg.)	+	-	82
nigromaculis (Ludlow)	+	-	75
Anopheles barberi Coq.	+	-	82
bradleyi King	-	-	82
crucians (Wied.)	-	-	82
punctipennis (Say)	-	-	82
quadrimaculatus Say	+	-	82, 75
sinensis Wied.	-	+	61
albimanus Wied.	+	-	75
freeborni Aitken	+	-	75
Culex erraticus (D. & K.)	-	-	82
peccator D. & K.	+	-	82
pipiens pipiens L.	+	-	75
p. fatigans Wied.	+	-	61
p. quinquefasciatus Say	+	-	71, 75, 76, 80, 82, 87, 89
p. molestus Forskål	+	-	54

Table 2 Cont'd.

Host Species	Development	Melanization	Reference
restuans Theo.	+	-	80, 82
salinarius Coq.	+	+	75, 80, 82
territans Walker	-	+	75, 80, 82
annulus Theo.	+	-	61
t. summorosus Dyar	+	-	61
fuscocephalus Theo.	+	-	61
rubithoracis (Leicester)	+	-	61
neomimulus Lien	-	-	61
fuscanus Wied.	+	-	61
tarsalis Coq.	+	-	75
Culiseta inornata (Williston)	+	-	75, 80, 82
melanura (Coq.)	+	+	82
Orthopodomyia signifera (Coq.)	+	-	82
Psorophora ciliata Fab.	-	-	82
confinnis (Lynch-Arribalzaga)	+	-	75, 80, 82
cyanescens	-	-	82
discolor (Coq.)	-	+	82
ferox (Humboldt)	+	+	75, 80, 82
varipes (Coq.)	+	-	75, 80, 82
howardii Coq.	+	-	82
Uranotaenia lowii Theo.	-	-	82
sapphirina (Osten-Sacken)	+	-	82
Mansonia uniformis (Theo.)	+	-	53

CHAPTER II

Effects of temperature and photoperiod on the infection of two mosquito species by the mermithid *Romanomermis culicivorax*

INTRODUCTION

The development of laboratory, mass-culture techniques by Petersen and Willis (1972a) 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 (Petersen and Willis 1972b), Taiwan (Mitchell *et al.* 1974), California (Petersen *et al.* 1972), and Manitoba (Galloway and Brust - unpublished observations) against larvae of *Anopheles, Culex, Psorophora* and *Aedes* species.

If *R. culicivorax* is to be considered 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 (1971) 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.* (1974) 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 (1976) found that the rate of

parasitism of C. pipiens molestus Forskål 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 hr 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 (1972a). 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 hr after flooding were used in each experiment. Preparasite numbers were estimated from 1-ml aliquot subsamples. Both mosquito

larvae and preparasites were acclimated at 15°C for 12 hr 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 per litre of dechlorinated tap water. Temperatures of 18, 16, 14, 12 and 10°C were selected and regulated by a water bath within ± 0.1°C (Brust 1967) 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 hr, 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

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 3).

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

The per cent 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 3). At the highest temperatures (18° C 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 (1974)

found C. pipiens larvae more susceptible to infection by R. culicivorax in total darkness, no significant effect of photoperiod was detected in our study and no interaction between temperature and photoperiod was evident.

DISCUSSION

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 obtaining acceptable levels of infection by R. culicivorax against certain mosquito species at low temperatures.

Tsai and Grundmann (1969) found several early

spring species of mosquitoes infected by Romanomermis nielseni (Tsai and Grundmann) under natural conditions in Wyoming. A great deal of confusion surrounds the taxonomic definition of this particular mermithid complex. The Wyoming and Louisiana populations may in fact represent two distinct species (Petersen 1976; Ross and Smith 1976) or two subspecies. The nematode material used in our experiment was maintained under laboratory conditions at 26 ± 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 (Kerdpibule et al. 1974; Petersen and Willis 1974a). At temperatures of 12 to 18°C, almost all mortality in both species resulted from infection by R. culicivorax. Petersen (1975a) 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 pre-parasites of R. culicivorax decreased with temperature,

yet larval mortality did not follow a similar trend, though 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 in 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, 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 (Petersen 1976). 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 C. tarsalis larvae, may be explained by the reduced activity of the A. dorsalis larvae.

TABLE 3. 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 ± 0.08^{NS}
14	98.0 ± 0.8	93.8 ± 2.0***	46.2 ± 5.5^{NS}	1.24 ± 0.04^{NS}
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 ± 0.5^{NS}	1.00 ± 0.00^{NS}
		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 ± 0.07^{NS}
14	95.3 ± 2.3	70.7 ± 4.4***	49.6 ± 3.6^{NS}	1.52 ± 0.07^{NS}
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 ± 0.6^{NS}	1.00 ± 0.00^{NS}

[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 = $P > 0.05$.

CHAPTER III

Field application of the mermithid nematode, Romanomermis culicivorax to control spring Aedes mosquitoes in Manitoba

INTRODUCTION

Use of the mermithid, Romanomermis culicivorax Ross and Smith, 1976 for mosquito control has been investigated in Louisiana (Petersen and Willis 1972b; Petersen et al. 1972), California (Petersen et al. 1971), and Taiwan (Mitchell et al. 1974) and the results are promising. The suitability of this mermithid for widespread control of spring Aedes in North America is unknown. It has been shown that preparasitic nematodes suffer decreasing activity and infectivity at temperatures below 20° C (Kurihara 1976; Mitchell et al. 1974; Petersen and Willis 1971). Little information is available on either the winter survival of R. culicivorax in northern latitudes or the susceptibility of many spring Aedes to R. culicivorax. Field trials in snow-melt pools with this mermithid have not been attempted. Therefore, these studies were conducted to investigate whether or not R. culicivorax can be used to control spring Aedes in Manitoba.

MATERIALS AND METHODS

Site A

In 1974, this abandoned lot in Winnipeg (Fig. 1) contained numerous vehicle ruts, deep tire tracks and depressions that provided excellent breeding sites (Fig. 2-4) for A. dorsalis

(Meigen) and A. spencerii Theobald. Three pools were selected for treatment. Pools were measured and the surface area and mean depth for each determined (Table 4). All pools had grassy bottoms though Pool 3 lacked vegetation in several spots (Fig. 1-3).

In 1974, all three pools were filled with water from melting snow and early spring rainfall which induced a hatch of A. dorsalis and A. spencerii larvae. Larvae were sampled with a standard, circular (135 mm diam.), 850 ml. ladle. Numbers of larvae were estimated from a series of no less than 25 random samples. Pools 1 and 2 contained an average of 5-10 larvae/dip. A. dorsalis larvae outnumbered A. spencerii larvae 10:1 in all collections. Pool 3 contained 20-30 A. dorsalis larvae/dip. First and second instar larvae predominated in all samples though some ($< 5\%$) third instar larvae were present.

Preparasites were obtained from a laboratory culture maintained at $27^{o} \pm 0.5^{o}$ C. Sand containing embryonated eggs and preparasites was flooded with dechlorinated tap water 24 hr before application to field pools. Preparasite numbers were estimated 12 hr after flooding and allowed to acclimate slowly to field conditions before introduction into the pools. Preparasites were applied as uniformly as possible over the surface of each pool at a rate of $20{,}000/m^2$ on May 14, 1974.

Maximum and minimum temperatures were recorded for all pools each day immediately after treatment. Samples

of 50 larvae from Pools 1 and 3 and 100 larvae from Pool 2 were taken 12, 24, 48 and 72 hr after treatment. All larvae were returned to the laboratory, identified and dissected to determine the level of infection. No samples were taken after 72 hr when heavy rains flooded the entire site and all pools became confluent.

Site B

Site B was a woodland site and was selected to be representative of areas where spring snow-melt pools are commonly found (Fig. 5). The site was located 50 miles east of Winnipeg near the west boundary of the Sandilands Provincial Forest, and south of the Trans-Canada Highway No. 1. The pools in this site were studied previously by Dr. R.A. Brust and were known to be free of mermithid parasites of mosquitoes. Pool bottoms were covered by sphagnum and litter and various species of grasses and sedges. Most pools were small, steep-sided and relatively deep (Table 4, Fig. 6). A dense overhead canopy of conifers (cedar, Thuja occidentalis L.; balsam fir, Abies balsamea (L.); white spruce, Picea glauca (Moench)) provided shade which stabilized pool temperatures in the spring. Frost was present in the pool bottoms during the earliest trials and water temperatures remained near freezing.

The sequential appearance of numerous mosquito species in the pools coincided with increasing water temperatures. A. communis Degeer and A. churchillensis

Ellis and Brust[1] larvae appeared when mean daily temperatures were only 1° C. Hatching continued over a period of at least 7 days though temperatures were low enough to inhibit the first larval moult. Pools regularly froze over at night at this time. A. pionips Dyar, A. punctor Kirby, and A. diantaeus Howard, Dyar and Knab appeared as mean daily temperatures approached 4-5° C. Larvae of these species continued to hatch over a 7-10 day period and gradually increased in total numbers in the pools. A. canadensis Theobald and A. cinereus Meigen appeared as mean daily temperatures reached 6-7° C. Mochlonyx velutinus (Ruthe) and a Dixa sp. also began to hatch at this time. None of the pools at Site B contained all the species of mosquito larvae found.

Continuous temperature records were kept in treated pools with either a Weksler or Taylor temperature recorder from the time of each nematode application until the cessation of sampling. The temperature probe from the recorder was placed 5-8 cm from the pool bottom. All readings were rounded to the nearest half degree because of

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[1]Ellis and Brust (1973) defined A. communis and A. churchillensis as sibling species. As noted by these authors, both species were present at Site B in varying proportions among pools. No attempt to separate these species in samples was made with the understanding that all further references to A. communis include both A. communis and A. churchillensis.

the limits of accuracy of the temperature charts. Salinity (0/000) and conductivity (μmhos) levels were recorded at least once a week using a 4S1 Model 33 S-C-T metre.[1]

Preparasites were obtained from a laboratory culture maintained at $25^{o} \pm 0.5^{o}$ C. Sand containing embryonated eggs and preparasites was flooded at room temperature (20^{o} - 23^{o} C) 24 hr before introduction of preparasites into the pools. Preparasites were allowed to acclimate gradually to pool temperature before being introduced. Surface area and average depth were calculated before treatment. Preparasites were distributed as uniformly as possible into each pool at a rate of $50,000/m^{2}$ during both 1975 and 1976.

Random samples were taken at convenient time intervals after treatment using an 850 ml ladle. Where possible, a minimum of 75 larvae were collected per sample. Larvae were returned to the laboratory where they were kept at 3^{o} C to inhibit moulting. All dissections were performed immediately after samples arrived in the laboratory. All larvae were counted and classified by instar and by species where possible. Larvae of *M. velutinus* and *Dixa* sp. from Pools 18, 3, 38 and 51 and nymphs of a mayfly (*Paraleptophlebia* (?) sp.) from Pool 51 were examined for nematode infection.

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[1]Yellow Springs Instrument Co.

RESULTS AND DISCUSSION

Site A

Temperatures remained low though the pools were shallow and exposed to direct sunlight. Mean maxima were 15°, 14° and 12° C and mean minima were 3°, 1° and 4° C over the three day sampling period. Two hundred larvae were removed from each of Pools 1 and 3, and 400 larvae from Pool 2. No infected larvae were found, though both A. dorsalis (Chapt. II) and A. spencerii were susceptible to infection in the laboratory.

Site B

Salinity in the pools was very low (< 0.3 0/000) during the field experiments. Conductivity varied considerably between pools but we were able to detect an increase from a mean of 250 μmhos in April to 350 μmhos in May and June. Dissolved minerals undoubtedly increased in concentration through active dissolution from the pool substrate, but largely through evaporation. Table 5 gives temperature data for Pools 30, 38, 18 and 51. Only temperatures pertinent to the period of preparasite activity and infection (i.e. 7 days after treatment) are provided.

Pools 3, 30 and 50 contained only first and second instar A. communis larvae at the time of treatment (Table 4). No infected larvae were found in samples of 75 larvae per pool taken at two day intervals for 14 days. No records of 0° C appear (Table 5) although ice was frequently

observed on the pool surface.

Pool 18 contained infected larvae 48 hr after treatment. Only first and second instar larvae of A. canadensis and A. pionips were infected. A survey of the species in these two instars revealed that A. canadensis larvae outnumbered A. pionips 20:1. Most A. pionips larvae were in the third and fourth instars at this time. Small numbers of third and fourth instar larvae and pupae of A. communis and A. diantaeus were present at the time of treatment, but they, along with the third and fourth instar A. pionips larvae, were not infected. The sample size of third and fourth instar larvae was small (Table 6) and it is possible that infection did occur at a low level but was not detected.

Under laboratory conditions it was found (Chapt. II) that at low temperatures (10° C) the level of infection in A. dorsalis and Culex tarsalis Coquillett larvae was very low. In the field, recorded temperatures did not exceed 10° C during the first four days after treatment (Table 5), however substantial infection of susceptible larvae occurred (Table 6). Temperatures in the pool were recorded from a single probe placed 5-8 cm from the pool bottom. Haufe (1957) indicated that marked temperature variations occurred in pools of this nature, especially on sunny days during periods of maximum heating. Pools at Site B were partially or totally shaded most of the day, but similar temperature variations could be expected, and the temperature at the surface where mosquito larvae

congregate may have been higher for several hours each day. Preparasites of R. culicivorax concentrate at the surface and in the corners of artificial pools (Petersen 1973a). Infection of larvae in Pool 18 may have occurred at the edges and surface where temperatures would exceed those recorded during the day. Early instar larvae were observed to be more abundant near the edges of the pools at Site B. The behavioral characteristics of the parasite and the host, and the microclimatic anomalies in the pools may account for the observed infection.

The mean number of nematodes per host and percentage infected increased up to the May 16 sample (Table 6). It is unlikely that preparasites were active after this time. Two first instar larvae were found on May 20, but these were not infected. An increase in the total percentage of larvae infected was noticed on June 17 as a result of pupation of uninfected larvae.

A. canadensis and A. pionips larvae apparently cannot support the development of large numbers of nematodes under field conditions. Samples on May 16 and 20 contained several larvae which were heavily infected (i.e. ≥ 8 nematodes/host) (Table 7). The accumulation by larvae of these high numbers of parasites probably occurred subsequent to the May 14 sample. Few heavily infected larvae were found in samples taken after May 20. It was found (Chapt. II) that infected A. dorsalis and C. tarsalis larvae were most susceptible to premature death under

conditions of low temperature. The actual mortality of larvae in Pool 18 as a result of infection is difficult to assess. It can only be inferred from the disappearance of heavily infected larvae from the samples and the decreasing mean number of nematodes per infected host. The only indication of host resistance was observed in one partially melanized nematode in the abdomen of a third instar A. canadensis larva on May 22. Postparasitic juveniles were observed to emerge from their hosts on June 17, 36 days after treatment.

On April 28, 1976, sampling of A. communis larvae in Pool 18 was resumed for evidence of winter survival of R. culicivorax. On May 5, three second instar larvae from a sample of 82 first and second instar A. communis larvae were infected with one nematode each. All nematodes were completely melanized and apparently killed in the early crescent stage of development. Two were melanized in the head capsule and one in the thorax of their hosts. Samples taken May 7, 10 and 15 produced no further evidence of infection. Pool 18, after a dry spring, contained no standing water by May 17.

In 1975, Pool 3 contained relatively large numbers (50-75/dip) of first and second instar A. canadensis and A. cinereus Meigen larvae on May 23 when mermithid preparasites were introduced. Many third and fourth instar larvae of A. communis, A. pionips and A. diantaeus were also present. On May 25, a sample of 200 larvae contained 3

second and 4 third instar A. canadensis larvae that were infected. Each host was infected by a single nematode. Subsequent samples on May 27, 28, 29 and 31 produced no infected larvae. The reason for the disappearance of the infection is not known.

In 1975, Pool 38 contained low numbers (20-30/dip) of first and second instar A. canadensis and A. cinereus larvae. From samples of 100, 70, 130, 100 and 100 larvae on May 23, 25, 27, 28 and 29 respectively, only one infected second instar A. cinereus larva was found on May 27. The larva contained one nematode.

Samples of 75-100 A. communis larvae were taken from Pool 6 in 1976 on April 22, 24, 28 and May 5, 13 and 19. One second instar larva was found to contain a single nematode on April 28. No other infected larvae were found in any sample. Recorded temperatures had not exceeded 10^{o} C by April 28.

Heavy rainfall on May 23, 1975 resulted in a hatch of A. vexans Meigen larvae in Pool 51 located near the roadside at the edge of the bush. Temperature data are shown in Table 5. Levels of infection in A. vexans larvae for each sample are given in Table 8. A. vexans larvae are relatively resistant to infection by R. culicivorax (Petersen and Willis 1976). This is confirmed by the low incidence of multiple infections of A. vexans larvae in Pool 51. Third and fourth instar larvae and pupae of A. communis, A. cinereus, A. pionips, A. punctor, A. canadensis

and A. fitchii Felt and Young, though present in the pool when preparasites were introduced, were not infected.

Several hundred larvae of a Dixa sp. and M. velutinus from Pools 18, 3, 38 and 51 and nymphs of a Paraleptophlebia (?) sp. from Pool 51 were examined after treatment for evidence of infection but none was found. A Mochlonyx sp. was found infected by Hydromermis churchillensis in pools at Goose Creek, Manitoba (Welch 1960).

Temperature appears to be the major limiting factor in the application of R. culicivorax for control of spring Aedes in Manitoba. Nematodes reared and kept in the laboratory did not have sufficient capability to infect mosquito larvae in the field at temperatures below 8^{o} C. Infection that occurred in A. canadensis and A. pionips larvae at recorded temperatures of 5-10^{o} C was at levels unacceptable for control purposes. Under the prevailing low temperature conditions of snow-melt pools, application rates of preparasites were relatively high (50,000/m^2) to achieve minimal control. A large scale program utilizing such high numbers of nematodes is impractical.

Eggs of R. culicivorax apparently survived the Manitoba winter in a natural breeding site. Infection of A. communis larvae was possible at temperatures $<5^{o}$ C. Suitable environmental conditioning may have affected the hatch and infection potential of the nematode. The establishment of this mermithid in the field may be possible, provided parasitic development in its hosts under such conditions can be completed. None of the observed parasites

were alive at the time of dissection. Parasites either died as a result of unsuitable temperatures or developed so slowly that they were overcome by their hosts' defense mechanisms. Aedes communis may not be an entirely suitable host, since larvae melanized a high percentage of parasitic R. culicivorax when raised under laboratory conditions. It is not known whether laboratory conditioning of embryonated eggs could decrease the temperature threshold of activity of the preparasites so that they could be applied directly to the breeding sites. Recycling of R. culicivorax populations in the field would be an asset to its application in a biological control program but more observations are necessary.

Winter survival of R. culicivorax establishes a high degree of synchrony in the population as seen in H. churchillensis (Welch 1960) and Romanomermis spp. (Galloway and Brust 1976; Ross and Smith 1976) in subarctic Canada, and R. nielseni (Tsai and Grundmann 1969) in Wyoming. In the latter species, eggs are fully embryonated and hatch when flooded by the water from melting snow. Only those mosquito larvae which hatch simultaneously to those of the nematode are exposed to infection. Welch (1960) found that though A. punctor, A. pionips, A. communis and A. excrucians (Walker) were present in the same pools, only A. communis larvae were infected by H. churchillensis. It is apparent that a similar condition could exist in an overwintered population of R. culicivorax. Where the synchronizing effects of low temperature are absent, preparasites of R. culicivorax are present throughout most of the year and

contribute to the control of overlapping generations of many mosquito genera and species (Petersen and Willis 1971).

Preparasites are not infective for a sufficient time in the snow-melt pools. The sequential hatch of numerous Aedes spp. corresponding with increasing temperatures demands that preparasites be present for the duration of hatch to achieve infection. Mosquito larvae are most susceptible to infection in the first and second instars (Petersen and Willis 1970). Most snow-melt species of mosquitoes are univoltine in Manitoba with significant overlap in larval development among species. A single application of preparasites could only be expected to infect the susceptible larvae over a 3-4 day period. Mosquito larvae may continue to hatch for up to 8 weeks in the same pool, as observed at Site B. Infective preparasites from a single introduction would not be present for sufficient duration to contribute significantly to their control. However, the recent application technique of R. culicivorax against floodwater mosquitoes in Louisiana (Petersen and Willis 1976) may prove useful in the control of spring Aedes.

R. culicivorax cannot be recommended at this time for control of spring Aedes. The superior effectiveness, low cost and ease of storage and handling of chemical insecticides makes them the preferred control method. The high degree of host specificity (Ignoffo et al. 1973; Ignoffo et al. 1974) and the possibilities of environmental adaptation by R. culicivorax make it an attractive alternative to which continued research will be profitable.

Fig. 1. Site A, near Shore and Bison Drive, where the first field trial using *R. culicivorax* against *A. dorsalis* and *A. spencerii* larvae was conducted in 1974.

Fig. 2. Site A, Pool 1.

Fig. 1

Fig. 2

Fig. 3. Site A, Pool 2.

Fig. 4. Site A, Pool 3.

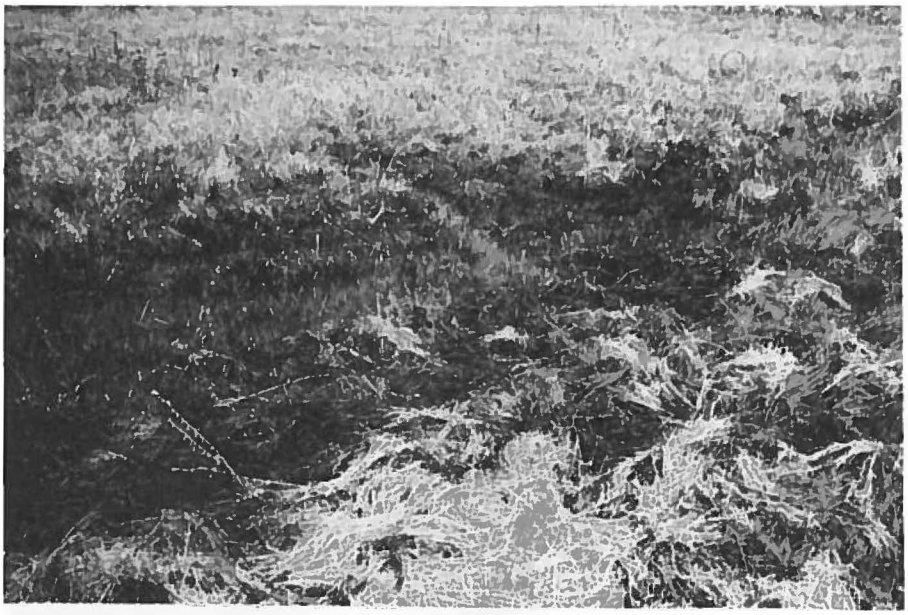

Fig. 3

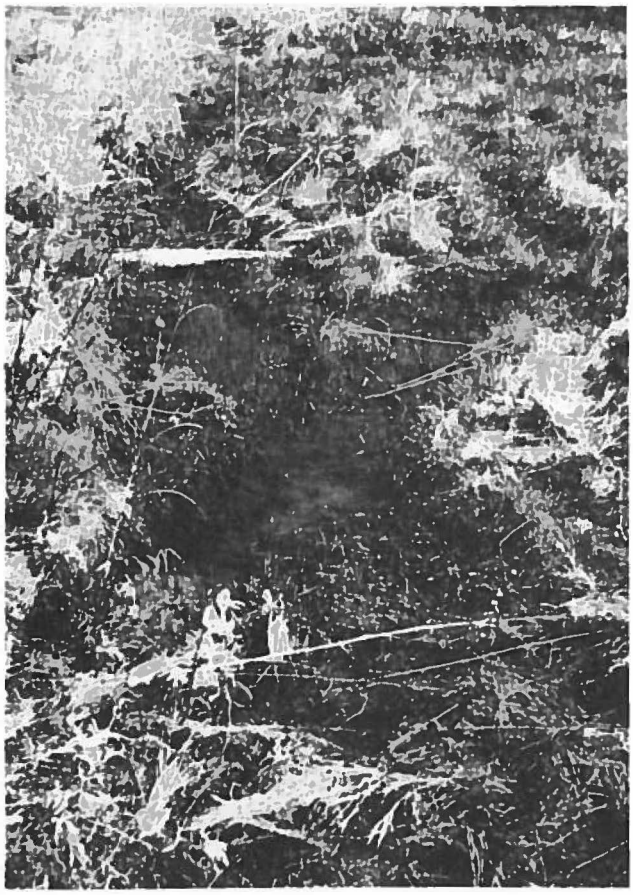

Fig. 4

Fig. 5. Site B, a typical woodland site in the Sandilands Provincial Forest where field trials using R. culicivorax against spring Aedes larvae were conducted in 1975 and 1976.

Fig. 6. Site B, Pool 3.

Fig. 5

Fig. 6

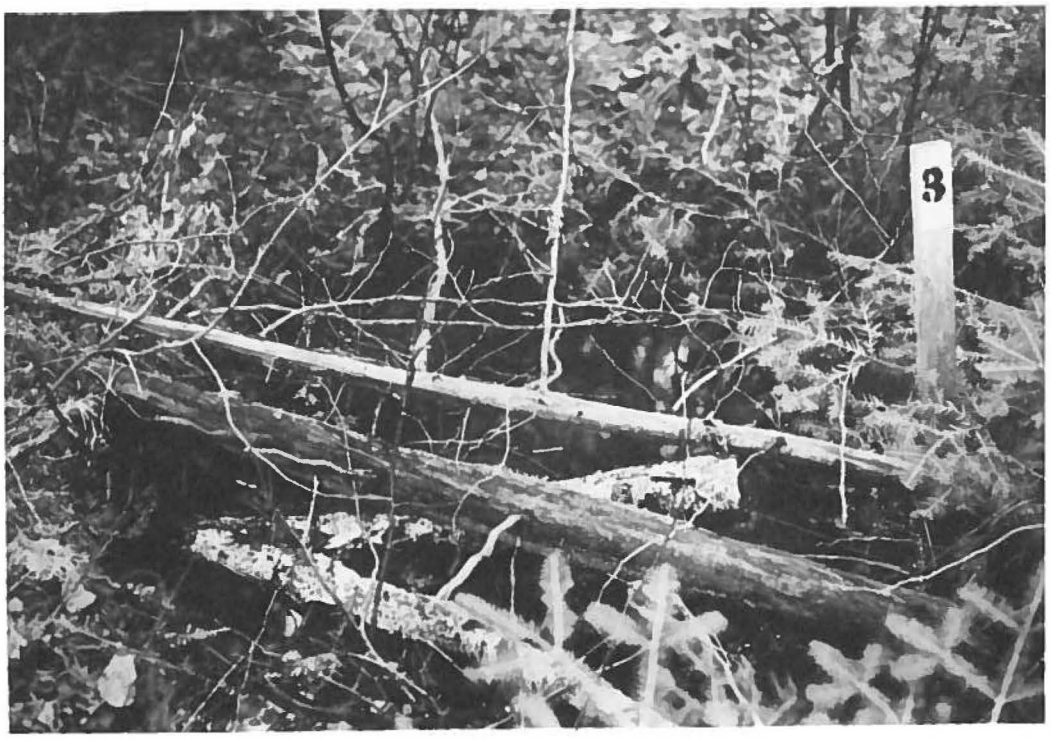

TABLE 4. Characteristics and date of treatment of Sites A and B for pools to which preparasites of *R. culicivorax* were applied at 20,000 and 50,000/m^2 respectively.

Pool No.	Surface area (m^2)	Mean depth (cm)	Date of treatment
		Site A	
1	4.5	10	May 14, 1974
2	1700	7	May 14, 1974
3	3.6	12	May 14, 1974
		Site B	
3	0.7	23	April 30, 1975
30	2.8	20	April 30, 1975
50	1.8	12	April 30, 1975
18	3.3	21	May 12, 1975
3	0.7	19	May 23, 1975
38	0.6	9	May 23, 1975
51	2.7	20	May 26, 1975
6	1.5	12	April 20, 1976

TABLE 5. Maximum, minimum and mean recorded temperatures in pools treated in 1975 with *Romanomermis culicivorax* in the Sandilands Provincial Forest, Site B.

Date	Maximum temperature (C°)	Minimum temperature (C°)	Mean temperature (C°)	Date	Maximum temperature (C°)	Minimum temperature (C°)	Mean temperature (C°)
Pool 30				Pool 38			
April 30	3.0	1.0	2.0	May 23	9.0	5.0	7.0
May 1	3.0	1.0	2.0	24	9.0	5.0	7.0
2	3.0	1.0	2.0	25	7.5	5.0	6.5
3	5.0	4.0	4.5	26	7.0	6.0	6.5
4	5.0	3.0	4.0	27	7.0	6.0	6.5
5	8.5	1.5	5.0	28	7.0	5.0	6.0
6	7.0	3.0	5.0	29	8.5	6.5	7.5
Pool 18				Pool 51			
May 12	7.0	6.0	6.5	May 26	15.0	13.0	14.0
13	8.0	7.0	7.5	27	15.0	10.5	12.5
14	8.0	7.0	7.5	28	15.0	10.0	12.5
15	8.0	5.0	6.5	29	14.0	11.0	12.5
16	10.0	7.0	8.5	30	13.0	9.5	11.5
17	9.5	8.5	9.0	31	13.0	8.0	10.5
18	12.0	9.0	10.5	June 1	15.0	9.0	12.0

Table 6. Distribution of infection of various mosquito larval instars by R. culicivorax after application of preparasites to Pool 18 on May 12, 1975.

Days after trmt.	Total No. of larvae	First Instar			Second Instar			Third Instar			Fourth Instar			% of total infected (Total no. infected)	Mean no./host (Mean no./Infected host)
		No.	% infected (No. infected)	Mean no./host (Mean no./Infected host)	No.	% infected (No. infected)	Mean no./host (Mean no./Infected host)	No.	% infected (No. infected)	Mean no./host (Mean no./Infected host)	No.	% infected (No. infected)	Mean no./host (Mean no./Infected host)		
2	200	15	20.0 (3)	0.30 (1.30)	172	25.6 (44)	0.50 (1.75)	9	0	0	4	0	0	23.5 (47)	0.45 (1.70)
4	200	12	25.0 (3)	0.25 (1.00)	184	33.2 (61)	1.01 (3.03)	4	0	0	0	-	-	32.0 (64)	0.93 (2.92)
8	165	2	0	0	35	40.0 (14)	0.60 (1.50)	105	30.5 (32)	0.91 (2.97)	23	0	0	27.9 (46)	0.79 (2.52)
10	167	3	0	0	22	22.7 (5)	0.23 (1.00)	128	39.1 (50)	0.61 (1.54)	7	0	0	33.5 (56)	0.50 (1.43)
21	48	0	-	-	4	0	0	5	0	0	39	18.0 (7)	0.36 (2.00)	14.6 (7)	0.29 (2.00)
28	11	2	0	0	2	0	0	5	20.0 (1)	0.20 (1.00)	2	50.0 (1)	0.50 (1.00)	9.1 (1)	0.09 (1.00)
36	23	0	-	-	0	-	-	16	18.8 (3)	0.19 (1.00)	7	71.4 (5)	1.71 (2.40)	34.8 (8)	0.65 (1.88)

TABLE 7. Frequency distributions of infection by R. culicivorax in larval samples from Pool 18, Sandilands Provincial Forest, in 1975.*

No. of nematodes/host	May 14	May 16	May 20	May 22	June 2	June 9	June 17
0	153	136	119	113	55	21	15
1	33	35	28	42	6	2	6
2	7	15	7	8	0	0	0
3	1	1	3	1	0		0
4	3	2	2	0	0		1
5	2	1	2	1	0		1
6	0	3	0	1	0		
7	1	0	1	0	0		
8		3	0	1	0		
9		2	0		0		
10		0	1		1		
11		0	1				
12		0	0				
13		0	0				
14		1	0				
19		0	1				
30		1	0				

*The frequency distribution of no samples follow a Poisson Distribution, as would be expected from the nonuniform degrees of susceptibility of larvae of different age and instar and the differential mortality levels.

TABLE 8. Levels of infection by *R. culicivorax* in *A. vexans* larvae in Pool 51, Sandilands Provincial Forest, after treatment at 50,000 preparasites/m^2 on May 26, 1975.

Sample date	Total sample size	No. of *A. vexans*	% of *A. vexans* infected	No. melanized	Mean no. nematodes/ infected host
May 28	125	109	8.3	2	1.00
May 30	100	93	18.3	1	1.12
June 6	100	90	12.2	0	1.09
June 9	69	49	20.4	1	1.10

CHAPTER IV

Preparasite production in *Romanomermis culicivorax* cultures at 27° C and 15° C

INTRODUCTION

Romanomermis culicivorax Ross and Smith (= *Reesimermis nielseni* auct. partim.) has shown considerable promise for mosquito control in various locations (Mitchell *et al.* 1974; Petersen *et al.* 1972; Petersen and Willis 1974a). The five month spring and summer mosquito seasons and the unpredictable summer generations of *Aedes* complicate the application of this mermithid for use in Canada. Reliable methods for long term storage must be available, especially where large quantities of material are involved.

Petersen (1975b) first recovered preparasites from his cultures after 7-8 weeks and substantial numbers were produced for up to 20 weeks. Preparasite numbers were greatly reduced by 34 weeks. Our studies were conducted to provide more detailed information on production in cultures at 27 ° C and to provide preliminary information on the possible use of low temperatures for long term storage.

MATERIALS AND METHODS

Our original culture of *R. culicivorax* was received from Dr. J.J. Petersen in 1973 and has been maintained according to methods of Petersen and Willis (1972a). Nematodes were kept in 5 kg of moist silica sand in 33x23x6 cm vinyl cake pans with sealing vinyl lids. Each pan received 5-10 gm of juvenile nematodes. Cultures were stored at 27

± 0.5°C and a photoperiod of 16L:8D.

Ten gram samples of sand containing nematodes and eggs were taken weekly from 10 culture pans. Since nematodes and eggs were not randomly distributed in the sand, the sand was gently stirred before any samples were taken to achieve greater uniformity between samples. Sampling bias was further avoided by acquiring each 10 gm sample as a composite of 15-20 subsamples taken at random from various locations in the pan. Samples were flooded with 30 ml of dechlorinated tap water and the number of live preparasites that were present after 24 hr was estimated from a series of 3-5 1-ml aliquot samples. Sampling was continued in all 10 pans from 1-33 weeks.

To investigate the effects of reduced temperature on storage, one pan of sand containing approximately 5 gm of juvenile nematodes was stored for 10 weeks at 27°C. The sand was then gently stirred and divided into two equal parts. One portion was subjected to 15°C (16L:8D) and the other to 27°C (16L:8D). Three random 2 gm samples were taken weekly from each portion and the number of preparasites that could be obtained after 24 hr estimated. After 7 weeks at 15°C, the portion was returned to 27°C. Sampling of both portions was continued as before for 6 weeks.

RESULTS

The cultures displayed a distinct pattern of preparasite production (Fig. 7). Preparasites could be recovered as early as the third week after juveniles were

placed in the sand. Individual cultures attained peak production (150-650 preparasites/gm of sand) between weeks 8 and 14, though one culture peaked as early as week 5. Three of the 10 pans reached peak production on week 11. Only one culture peaked in week 14, and none peaked later. Preparasite production between culture pans varied considerably though similar quantities of nematodes had been introduced. After week 14, the number of recovered preparasites declined. All cultures were essentially senescent by week 29. Few embryonated eggs were observed in the sand at that time.

The number of preparasites that were recovered from the 10-week-old culture placed at 15° C decreased much more rapidly than in that which was left at 27°C (Fig. 8). Estimates for week 1 were not available, as samples were accidentally flooded with distilled water instead of tap water and many of the preparasites died. After 7 weeks at 15°C, fewer than 4 preparasites/gm of sand could be recovered 24 hr after flooding. After 3 weeks at 27°C, the culture treated at 15°C showed signs of recovery. Production returned to pre-treatment levels by week 13. The culture which had remained at 27° C senesced with fewer than 20 preparasites/gm of sand that could be recovered by experimental week 13 (23 weeks of age).

DISCUSSION

Storage methods for R. culicivorax, as described by Petersen and Willis (1972a), are suitable for a relatively

short time at 27°C. After 20 weeks, production was so low that it would be impractical to obtain nematodes for control purposes (Fig. 7). If only half or less of the maximum number of preparasites can be obtained after 20 weeks, efficient low-cost production is difficult. Since mosquitoes occur in large numbers only during 4 to 5 months of the year in Canada, the remaining 7 to 8 months could be used to produce the large numbers of preparasites necessary for biological control during the mosquito breeding season.

Longevity and production of cultures in this experiment may have been influenced by weekly stirring, moisture content and coarseness of the sand. Condensation in the sealed pans may have been responsible for large numbers of hatched eggs. Although various bacteria and algae were abundant in some cultures, they had no obvious effects on preparasite production or longevity.

Crowding of R. culicivorax in the pans lengthens the time required for moulting to adults and the onset of egg laying (Galloway - unpublished data). Petersen (1975b) reported that some preparasites were recovered from his culture after 7-8 weeks. We first observed eggs to hatch in the third week. Petersen (1975b) placed 15 gm of juvenile nematodes into approximately the same quantity of sand that we used for 5-10 gm of juveniles. Although Petersen's (1975b) cultures began to produce much later than those described in this study, the ultimate life of

his culture resembled that of ours.

Romanomermis nielseni (Tsai and Grundmann 1969) and Hydromermis churchillensis Welch (Welch 1960) both overwinter in the egg stage. The embryonated eggs of R. culicivorax may be an excellent stage for long-term laboratory storage. The recommended storage temperature for Skeeter Doom® is 5° C. Reductions in refrigeration costs would result if 15° C, as suggested by our study, is a suitable temperature for storage.

The 2 week lag period (Fig. 8) which occurred before an increase in preparasite hatch was observed when cultures were returned to 27° C from 15° C may be a problem in storing cultures at 15° C. Since summer generations of Aedes vexans (Meigen), the major mosquito pest in Western Canada, occur following flooding from heavy rains, preparasites must be available immediately and dispersed quickly, especially when temperatures are high. They must be applied within 48 hr for peak infectivity (Petersen 1975b) and larval susceptibility.

Further investigations in long term storage of various stages of the nematode at a wide range of temperatures should be conducted before any definite recommendations are made.

Fig. 7. Weekly preparasite production (mean ± SE) in cultures of Romanomermis culicivorax stored at 27 ± 0.5° C.

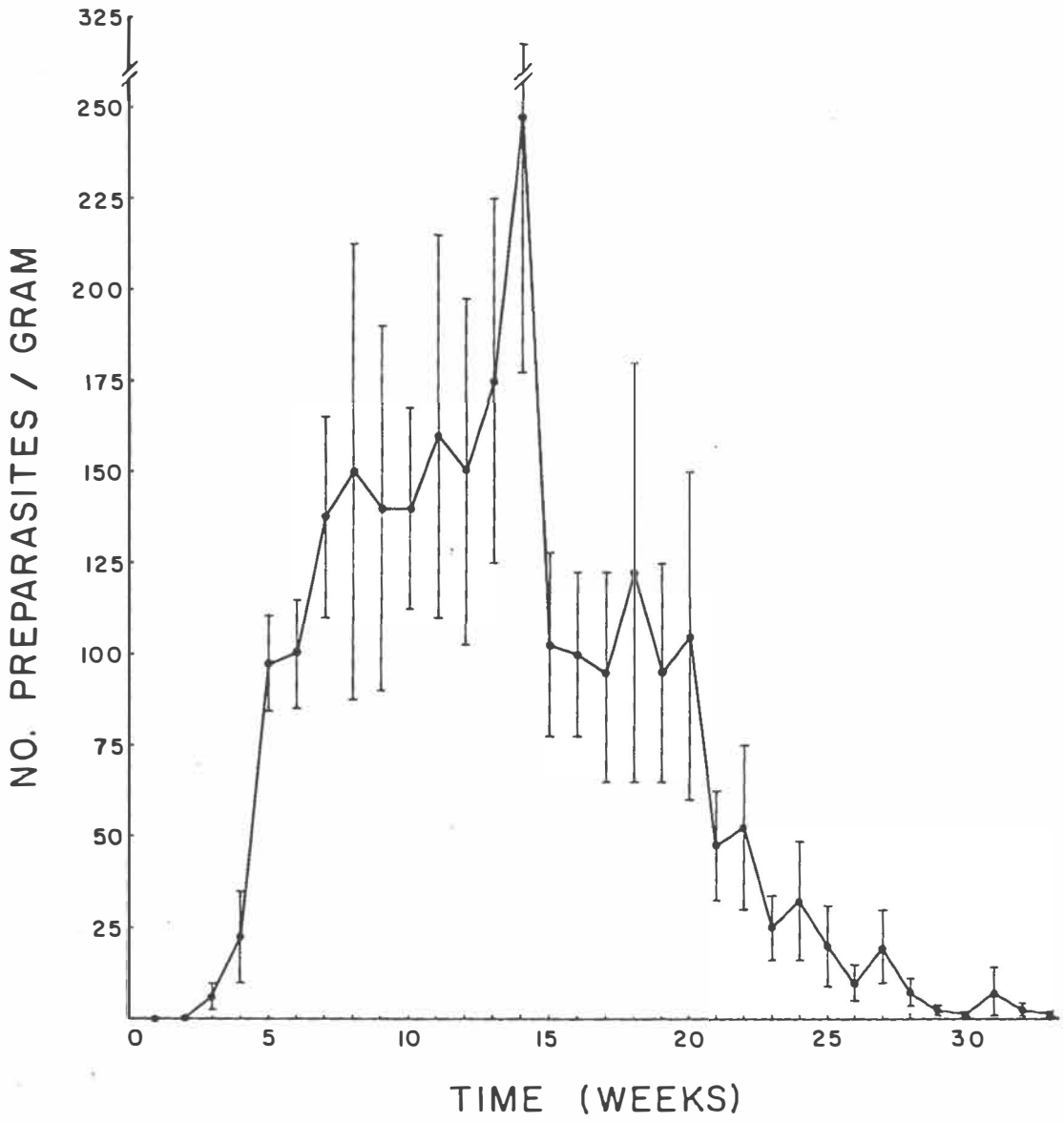
NO. PREPARASITES / GRAM
325
250
225
200
175
150
125
100
75
50
25
0
5
10
15
20
25
30
TIME (WEEKS)

Fig. 7

Fig. 8. Comparison of preparasite production when a 10-week-old culture was divided and one-half was maintained at 27° C and one-half was incubated at 15° C. The arrow indicates the time (7 weeks) when the culture incubated at 15° C was returned to 27° C.

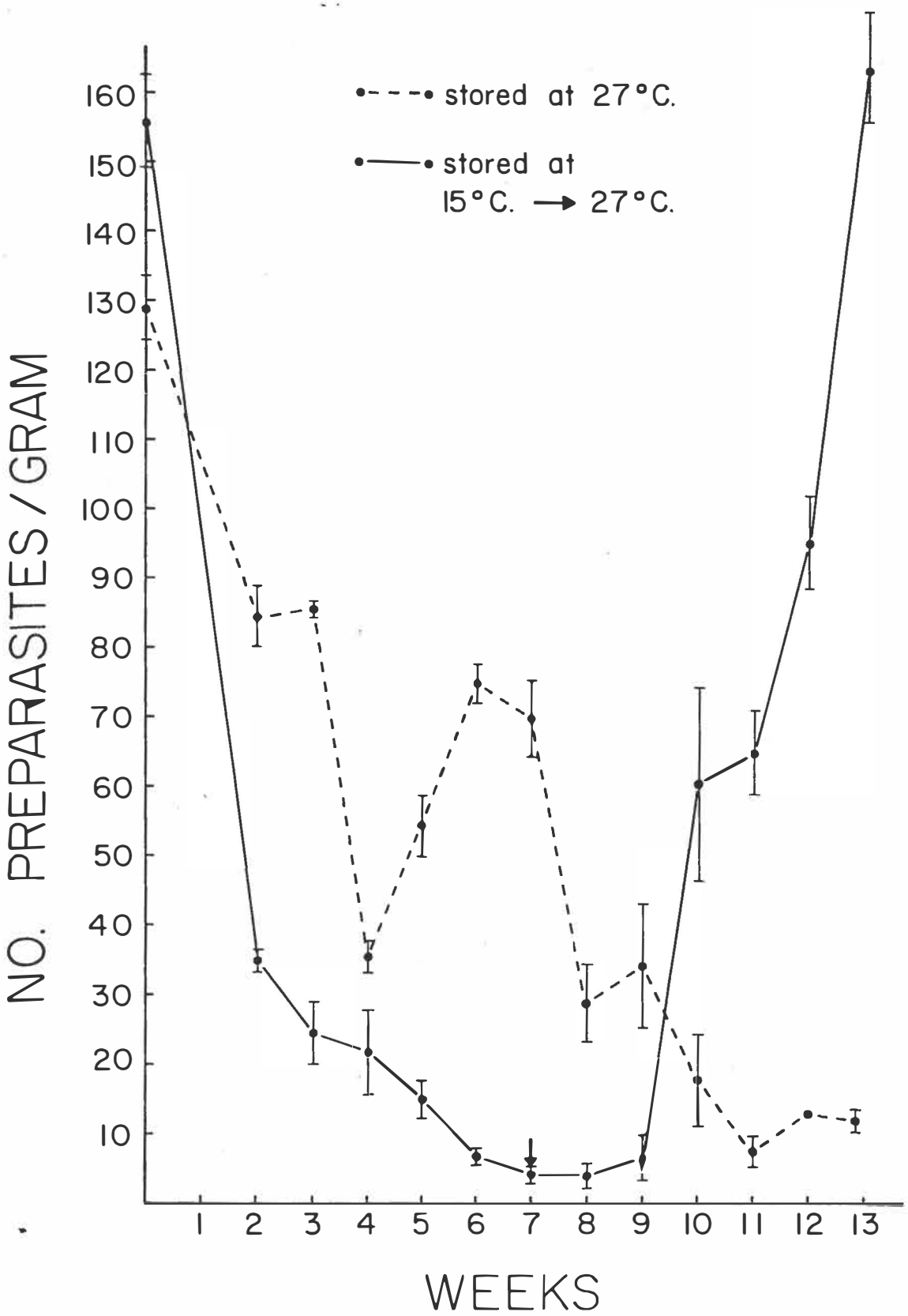
stored at 27°C.
stored at 15°C. → 27°C.
NO. PREPARASITES / GRAM
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
WEEKS

Fig. 8

CHAPTER V

The effects of parasitism by the mermithid nematode, Romanomermis culicivorax, on the growth and development of Aedes vexans larvae and preliminary results of field trials conducted in artificial pools

INTRODUCTION

The mermithid, Romanomermis culicivorax Ross and Smith, 1976 has repeatedly been evaluated for the control of mosquitoes breeding in permanent or semi-permanent pools (Mitchell et al. 1974; Petersen et al. 1972; Petersen et al. 1973; Petersen and Willis 1972b). Little attention has been given to floodwater mosquito control using this nematode. Petersen and Willis (1972b) observed 50-62% infection of Aedes atlanticus Dyar and Knab, A. vexans (Meigen) and Psorophora columbiae Dyar and Knab larvae when preparasites were applied directly to the pool surface. Petersen and Willis (1976) also placed laboratory cultures of R. culicivorax into suitable habitats of floodwater mosquitoes in Louisiana. A high percentage of A. atlanticus, A. tormentor Dyar and Knab, P. columbiae and P. howardii (Coquillett) larvae were infected. Aedes vexans larvae were moderately resistant to infection and low levels of parasitism (0-20%) were reported. Nickle (1976) achieved 90% infection of Anopheles punctipennis Say, A. crucians Wiedemann, Culex salinarius Coquillett, C. pipiens L., C. restuans Theobold and A. vexans larvae at undetermined application rates in Maryland. Neither Petersen and Willis

(1972b) nor Nickle (1976) stated the levels of infection in A. vexans larvae alone.

A. vexans is a ubiquitous species (Horsfall et al. 1973) that may locally attain serious pest status throughout its range. It is most abundant in flood-plain areas, or areas subject to periodic inundation after heavy rainfall. A. vexans comprises the greatest proportion of total summer mosquito populations in most years in the Winnipeg area (McLintock 1944; Brust and Ellis 1976). One-quarter to one-half of trapped adults from July to September were A. vexans in a small urban community surrounded by woodland (Brust 1974). It has been implicated as a possible vector of Western Equine Encephalitis in Saskatchewan (McLintock et al. 1970) and may be a suitable vector for the dog heartworm, Dirofilaria immitis Leidy, 1856 (Bemrick and Sandholm 1966). Horsfall et al. (1973) provide a further review of the economic status of A. vexans around the world.

Because of its wide distribution and high populations, A. vexans is a prime target for control in many areas. Little information is available on the effectiveness of R. culicivorax against this species. It was the purpose of this study a) to evaluate the control potential of R. culicivorax against A. vexans in Manitoba and b) to observe certain aspects of the host-parasite interrelationship.

MATERIALS AND METHODS

All A. vexans in this study were obtained from 8 cm diameter sod discs containing eggs. The source was a known breeding site at Portage la Prairie, Manitoba. Sods were kept at 30° C for 2-4 weeks, then inverted in 33 cm diameter porcelain basins and completely covered with chlorine-free tap water. Larvae that hatched up to 24 hr after flooding were used in the experiments. Except for occasional A. nigromaculis (Ludlow) and A. dorsalis (Meigen) larvae (less than 0.5%), all were A. vexans.

Preparasites were obtained from a laboratory culture maintained at 25° ± 0.5° C. Sand containing preparasites and eggs was flooded with chlorine-free tap water approximately 12 hr before the beginning of each trial. Flooded cultures were allowed to settle and the egg-free water was carefully decanted to minimize the number of unhatched eggs in the samples. Preparasite numbers were estimated from a series of 6 or more 1-ml aliquot subsamples.

Laboratory experiments:

Aedes vexans larvae were placed singly into 6-dram shell vials (23 x 85 mm) which contained 10 ml of larval medium (0.2 gm of finely ground liver powder (< 250 μm)/l of chlorine-free tap water). Ten preparasites were inoculated into each of 30 vials containing one A. vexans larva. Thirty larvae were maintained individually in vials to serve as controls. This experiment was repeated with 20 preparasites per vial.

Experiments at both infection levels and controls for each were replicated three times. Larvae were exposed to infection for 24 hr, at which time all larvae were transferred to fresh vials. Larval medium was changed every 48 hr thereafter to ensure sufficient food and to avoid pellicle formation on the water surface. In the second experiment, the quantity of liver powder in the medium was doubled when larvae reached third instar. Temperature was maintained at $23^{o} \pm 0.5^{o}$ C throughout the experiments.

Larvae were examined at 12 hr intervals until pupation was complete or postparasitic juvenile nematodes emerged. The period of each moult for all larvae was recorded and head capsule widths of cast exuviae were measured where possible. Records of larval mortality were kept and dead larvae dissected for the presence of nematodes. Emerging postparasites from each infected host were counted and sexed.

Field experiments:

Trials were conducted in artificial pools constructed from asbestos-cement boards (1 m x 1 m x 0.3 m) fastened at the corners with angle iron (Fig. 9). Pools were secured in place by driving the extensions of the angle iron corners into the ground. These 1 m^2 pools were lined with 20 mil polyvinyl plastic and covered with freshly cut grass sod known to be free of mosquito eggs (Fig.10). The sod lining more closely simulated the natural larval habitat and eliminated the necessity of feeding the larvae an

artificial diet. Pools were filled to a depth of 10 cm (total volume 0.1 m^3) with tap water and allowed to stand 12-24 hr before the introduction of mosquito larvae and nematodes. Fitted screen covers eliminated accidental introduction of predators and other mosquito species. Gravid females of Culiseta inornata (Williston) and Culex restuans Theobold were very persistent in seeking an oviposition site, and occasionally gained access to the pools through small openings. Egg rafts were removed when observed. Also, when these introductions occurred, preparasite activity had ceased and A. vexans larvae were in later instars and could be easily distinguished from unwanted species.

Since the number of A. vexans larvae that could be recovered from the sod discs was highly variable, the number of pools that were treated was determined by the number of larvae obtained from a single flooding. Two thousand 1-24 hr old larvae were counted and placed into each pool and allowed 30 minutes to disperse before the introduction of R. culicivorax preparasites. Preparasites were distributed as uniformly as possible over the pool surface at 10,000, 20,000, 50,000 and 100,000/m^2. Four pools at 10,000 and 20,000/m^2 and five pools at 50,000/m^2 were infected in 1974, while 1 pool each at 10,000 and 20,000/m^2 and six pools at 100,000/m^2 were infected in 1975. All treatments in each year were assigned to pools at random. Temperatures were continuously monitored using a

Weksler recorder.

Random samples of 50 larvae were returned to the laboratory on the following regime: 12, 24, 48, 96, 144 and 192 hr after treatment. Head capsule widths of larvae in all but the first trial and body lengths in all but the first two trials were measured before the larvae were dissected to determine the number of live and melanized nematodes in each host. Samples returned to the laboratory were kept at 3^{o} C to minimize moulting and growth until they were measured and dissected (maximum delay was 6 hr). A single control pool was examined to assess differences in growth parameters between larvae not exposed to infection and uninfected larvae in treated pools.

RESULTS

Laboratory experiments:

The results of infection of A. vexans larvae by R. culicivorax in single vials at 10:1 and 20:1 are shown in Table 9. Although only a slightly greater proportion of the total number of larvae were infected at 20:1, there were significantly greater numbers of nematodes per infected host ($P<0.05$). The sex ratios of nematodes in the 20:1 group more heavily favoured male production, coinciding with the higher infection rate. Survival of successfully invading nematodes was higher at 20:1 (Table 10). However, the improved diet in this trial increased larval survival in both infected and uninfected groups and was probably responsible for the observed in-

crease in nematode parasite survival. Survival of infected larvae after the third instar was lower than that observed in control larvae in both groups. No infected larvae survived through the fourth instar when postparasitic nematodes emerged. Two larvae infected at 10:1 failed to reach the fourth instar before postparasites were observed. One second and one third instar larva contained one partially melanized nematode each. In both cases, the nematodes were still alive and moving while their hosts were apparently dead at the time of dissection.

Infection by R. culicivorax had a significant effect on head capsule width of A. vexans larvae. Infected second, third and fourth instar larvae had significantly smaller head capsule widths ($P < 0.05$) than control larvae (Table11). There was a significant log linear relationship between head capsule width and larval instar (Fig.11). The difference between the slopes of the lines for infected (0.14) and uninfected (0.20) larvae was significantly different (ANCOVA; Snedecor and Cochran 1973), but the assumption of nonindependence was not strictly upheld.

The mean development time for each instar of infected A. vexans larvae was greater than that observed for control larvae. Infected larvae progressed to succeeding instars at different times and different rates (Fig. 12,13). In larvae infected at 10:1, the duration of the third instar was prolonged for some larvae. Control larvae in this

group spent more time in the fourth instar compared to the controls for the 20:1 group. Pupation in the former group did not commence until 192-204 hr, while the latter group had completed pupation by 192 hr. Postparasitic juveniles from the 10:1 group emerged sooner and over a shorter time period than in the 20:1 group. Nematodes emerging from third instar larvae emerged within the same period observed for those from fourth instar larvae.

Field experiments:

Mean daily temperatures for trials in 1974 and 1975 were above the minimum threshold of activity for R. culicivorax preparasites (Fig.14). However, daily minimum temperatures often dropped to where reduced activity and reduced levels of infection would be expected. Although temperatures between trials differed, we were unable to determine any correlation in observed infection and temperature between the same application rates at different times.

The proportion of larvae infected in each sample for all sample times is shown in Table 12. There was an increase in mean per cent infection at all treatment levels at least for the first 24 hr. The number of infected larvae continued to increase up to 96 hr in pools treated at 10,000 and 20,000 preparasites/m^2. This was followed by a significant drop in mean per cent infection at 144 hr for both treatments before a dramatic increase in infection as pupation in uninfected larvae began. A similar trend was

observed in pools treated at 50,000 and 100,000 preparasites/m^2 except that the recorded drop in infection occurred at 96 and 48 hr respectively, i.e. sooner by 48 and 96 hr. The characteristic increase in infected larvae was still evident in both pools at the time of pupation.

The mean numbers of nematodes per infected host for all samples are shown in Table 13. There was no apparent trend of increasing numbers of parasites per host with increased time as observed for the per cent infected. A. vexans larvae generally escaped infection by large numbers of nematodes at all treatment levels except in certain isolated cases. For example, the larvae in the first pool treated at 100,000 preparasites/m^2 on July 1, 1975 were more heavily infected (Table 14,15) and one larva in the 48 hr sample in trial D at 50,000 preparasites/m^2 contained 26 nematodes. A few larvae were observed with 8-16 nematodes per host, but only in their first two instars.

Samples could not be taken at 192 hr in trials C and D at 50,000 preparasites/m^2 and at 144 and 192 hr in trials E and F at 100,000 preparasites/m^2. Almost no larvae remained in these pools, though other pools at lower application rates still contained large numbers of larvae.

Melanized nematodes were commonly found at all treatment levels, in all samples taken during 1974 and 1975. Table 14 describes the percentage of parasitic nematodes which were either partially or totally melanized. There was no correlation between the number of nematodes

present in a host and the incidence of melanization of nematodes in that host. The incidence of melanization increased with time until 144 hr. A slight decrease was noticed before the proportion affected again increased significantly at 192 hr.

No significant differences in size or development rate occurred between larvae in the control pool and uninfected larvae in treated pools. Therefore, uninfected larvae were assumed to be representative of controls for comparisons of growth and development with infected larvae during the remainder of the experiment. No significant differences were observed between infected or uninfected larvae among pools in any one trial. Therefore, measurements were pooled for infected, or uninfected larvae of each instar, regardless of treatment level, and differences in mean head capsule width and body length tested (t-test).

Infection by R. culicivorax had a significant effect on growth and development of A. vexans larvae in the field, as observed in the laboratory. Mean time spent in each instar was greater for infected larvae (Figs. 15-19) and the moult to successive instars inhibited in heavily infected larvae. Head capsule widths of infected larvae were significantly smaller than those of uninfected larvae in the second to fourth instars in almost all trials (Table 15). Infected first instar larvae in trials E and F in 1975 were also significantly smaller. Body length, a highly variable character over time, was often signi-

ficantly reduced in all instars in infected larvae (Table 16).

An unidentified microsporidian infection was also present in the A. vexans larvae (< 0.5%) and appeared to retard development and growth. Larvae in which spores were observed were omitted from the sample. None of the sampled larvae contained both nematodes and significant numbers of microsporidial spores.

DISCUSSION

There is little information describing the effects of mermithid parasitism on growth rates of mosquito larvae. Welch (1960) observed that A. communis larvae infected by Hydromermis churchillensis Welch, 1960 failed to pupate and were present in the pools after uninfected larvae had completed their development. Mosquito larvae infected at an early stage by Octomyomermis muspratti (Obiamiwe and Macdonald, 1973) were retarded in their development (Obiamiwe and Macdonald 1973). Bailey and Gordon (1973) noted little difference in development of A. aegypti (L.) larvae infected by R. culicivorax. They also found that infected hosts did not differ significantly in weight gain until 4-6 days after infection, when infected larvae actually decreased in weight.

Insect parasites often have a considerable effect on growth and development rates of their hosts. Reduced weight or weight gain (Vinson and Barras 1970; Jones and Lewis 1971; Syme and Green 1972; Iwantsch and Smilowitz

1975), head capsule width (Muldrew 1967; Führer 1975a; Rechav and Orion 1975; Surgeoner and Wallner 1975) and development rate (Dowden 1938; Führer 1975a, b; Shapiro 1976) have often been observed. Many of these reports are not strictly analagous to mermithid parasitism, since proteins from the poison apparatus of ovipositing female Hymenoptera may contribute significantly to the observed pathologies (Vinson 1972; Vinson and Barras 1970). However, the parasite larva was largely responsible for pathological changes in the hosts of some species (Syme and Green 1972; Führer 1975a, b; Führer and Keja 1976).

The causes of the delayed larval moults and eventual inhibition of the pupal moult and the reduced size of infected A. vexans larvae are a matter of speculation at this time. Mermis nigrescens Dujardin apparently inhibits the moult of Schistocerca gregaria Forskål by reducing the rate of fat body protein synthesis and levels of fat body protein in the haemolymph necessary for moulting (Craig and Webster 1974). Apanteles tedellae Nixon larvae are capable of inhibiting the deposition of endocuticle during the intermoult periods of Epiblema tedella Cl. and Lissonota dubia Hgn. larvae (Führer 1975b). It has been suggested that the pupal moult of mosquito larvae infected by mermithids may be inhibited by an imbalance of host endocrine secretions or the presence of parasite neurosecretory materials (Welch 1965; Petersen and Willis 1970; Bailey and Gordon 1973).

Parasitic stages of R. culicivorax are found in the haemocoele of its hosts, bathed by the haemolymph. Mermithids conceivably cannot feed on solid materials and nutrient uptake must be, for the most part, transcuticular (Rutherford and Webster 1974; Poinar and Hess 1976). The mechanism of uptake for R. culicivorax may be similar to that suggested by Gordon et al. (1973) for M. nigrescens, as pointed out by Bailey and Gordon (1973).

The results of our experiments indicate that the observed reduction in head capsule and body length measurements and development time are probably related to depletion of nutrients available for host growth. The reduced size and extended period of development in parasitized larvae are reminiscent of the effects of starvation on A. vexans larvae (Brust 1968). In addition, many of the pathologies noted by Lower (1961) for starved Persectania ewingii (Westwood) larvae have been observed following parasitism of mosquito larvae by R. culicivorax. Bailey and Gordon (1973) observed the degeneration of the mid-gut epithelium in A. aegypti larvae infected by six R. culicivorax juveniles. The reduction of stored fat body was evident in all infected fourth instar A. vexans larvae in our studies. Also, the cuticle of infected third and fourth instar A. vexans larvae collected from artificial pools contained secondary pigments which produced a conspicuous gold-brown body colouration.

Host nutrition can have a significant effect on

the development of mermithid parasites of mosquitoes. Petersen (1972) found that host diet significantly affected the sex ratio of R. culicivorax. Starved individuals produced greater proportions of male nematodes. When hosts of O. muspratti were fed a vitamin-rich diet, nematodes were able to extend their development into the pupal and adult stages, while development was restricted to the larval stages in lesser fed hosts (Muspratt 1965). Although the primary aim of our laboratory experiments was not to investigate the effects of diet on the development of R. culicivorax, some observations were made. Postparasitic juveniles emerged earlier from hosts fed the lesser liver powder ration. Three infected A. vexans larvae in this group failed to reach the fourth instar and preparasites emerged from third instar larvae.

The retardation in host development following infection may have a considerable influence on the hostparasite relationship. Petersen and Willis (1970) and Galloway (1975) have shown that mosquito larvae are more susceptible to infection by R. culicivorax in the first two instars. Since the period of greater susceptibility is increased by the invasion of a single nematode, the chances of multiple parasitism are increased. This effect may be partially responsible for the non-random parasite distribution observed by Welch (1960) and in Chapt. III. Analysis of parasite distribution in A. vexans larvae in the present study

demonstrated significant agreement with a Poisson distribution only in the 12 hr samples. This may be a reflection of random infection initially, or the relatively low numbers of nematodes present in most samples at 12 hr. However, the presence of infrequent extremely heavily infected larvae suggests that other factors must be involved as well.

The increased larval duration as a result of infection may be particularly important in the control of multivoltine floodwater species. Larvae hatching in July and August in response to heavy rainfall may fail to complete their development before dessication of the habitat. Thus increased larval life could be a deterrent to establishment of the nematode in the habitat in the event the pools dry up quickly.

The extended period of development and reduced size of infected A. vexans larvae may be responsible for selective predation pressure, particularly after most uninfected larvae have pupated or emerged as adults. Smaller larvae may be available to a wider range of predators present in a pool.

Humoral melanization is a common feature of culicid and chironomid defence mechanisms (Salt 1970; Poinar 1974b) and may be of great importance to the widespread use of R. culicivorax for control of A. vexans. Invasion of A. triseriatus (Say) and Anopheles sinensis Wiedemann by R. culicivorax commonly results in melanization (Petersen et al. 1969; Petersen 1975a; Mitchell et al. 1974).

Neoaplectana carpocapsae Weiser is usually melanized by mosquito hosts (Welch and Bronskill 1962; Andreadis and Hall 1976) though the presence of encapsulated nematodes in A. aegypti does not appear to sensitize the host to invasion by R. culicivorax (Hall et al. 1975). Several observations of R. culicivorax infecting A. vexans larvae have been made (Petersen and Willis 1972b, 1976; Nickle 1976) but there has been no reference to melanization in this species. The incidence of melanized nematodes in A. vexans larvae sampled from our artificial pools increased but not as a result of increased melanotic activity. Since most melanized nematodes were in an early stage of development, it is probable that differential mortality of infected larvae containing no melanized nematodes occurred. It is conceivable that A. vexans larvae could increase their resistance to infection by R. culicivorax under heavy selection pressure.

Infection by R. culicivorax is responsible for significant mortality in mosquito larvae (Petersen and Willis 1974a; Kerdpibule et al. 1974; Chapt. II). Mortality in infected A. vexans larvae exceeded that in normal larvae kept under similar conditions in the laboratory. Evidence supports the occurrence of heavy mortality of infected A. vexans larvae in the field as well. The decrease in the per cent of infected larvae in the samples was undoubtedly in response to selective mortality. There was a dosage response in the per cent in-

fected where the observed decrease occurred earlier at higher preparasite application rates. However, we were unable to detect whether or not the most heavily infected larvae were selectively removed. Death may occur rapidly after the accumulation of large numbers of nematodes in some hosts and would rarely be detected in our samples.

Mortality caused by infection is an important factor in nematode release programs, particularly where establishment is desirable. Mortality in A. vexans larvae in some pools treated with 100,000 preparasites/m^2 was responsible for the premature elimination of nearly all infected larvae by the time the majority of uninfected larvae had completed pupation. Examination of a single representative sample from a treated pool may lead to misinterpretation of the actual level of control achieved. Particular attention to preparasite application rates under field conditions must be given with respect to larval densities, pool surface area and species present.

The results of applications in artificial pools and certain factors concerning the bionomics of A. vexans make practical control of this species using R. culicivorax rather difficult. A mean infection rate of less than 50% of surviving larvae was achieved when 100,000 preparasites/m^2 were applied. This estimate was inflated by disproportionately high levels of infection in the first of the six pools treated at this level. Applications of such high numbers of preparasites to the extensive breeding

habitats of A. vexans is not practical at this time. The high degree of variability in infection between pools treated at the same level established an element of unpredictability for control. Infection levels must be monitored following treatment. This involves extensive sampling and dissection of larvae from all treated pools, accompanied by a considerable time investment. A small percentage of A. vexans larvae demonstrated the ability to melanize parasitic R. culicivorax juveniles. Although unlikely, the selection of highly resistant strains of A. vexans exists. Aedes vexans larvae develop rapidly, especially if temperatures are high, and remain susceptible to infection for only a short time. If R. culicivorax can be effectively applied through the use of aircraft, as suggested by Levy et al. (1976), large areas can be treated in a short time. However, it is unlikely that R. culicivorax will be used alone in an A. vexans control program. It could be efficiently managed in conjunction with other control methods for treatment of larvae in later stages.

Widespread use of R. culicivorax for control of floodwater mosquitoes is impractical at the present time. However, this mermithid could become of singular importance in locations where the use of chemicals may be undesirable. Romanomermis culicivorax could also play a dominant role in mosquito abatement in the event of severe larvicidal chemical restrictions. Further research is necessary, especially in the area of field application and the response of established R. culicivorax populations in A. vexans habitats.

Fig. 9. An artificial pool used for treatment of Aedes vexans larvae with Romanomermis culicivorax. The screen cover eliminated unwanted mosquito predators.

Fig. 10. Pool bottoms were covered with freshly cut grass sod to provide food for growing Aedes vexans larvae and to more closely simulate field conditions.

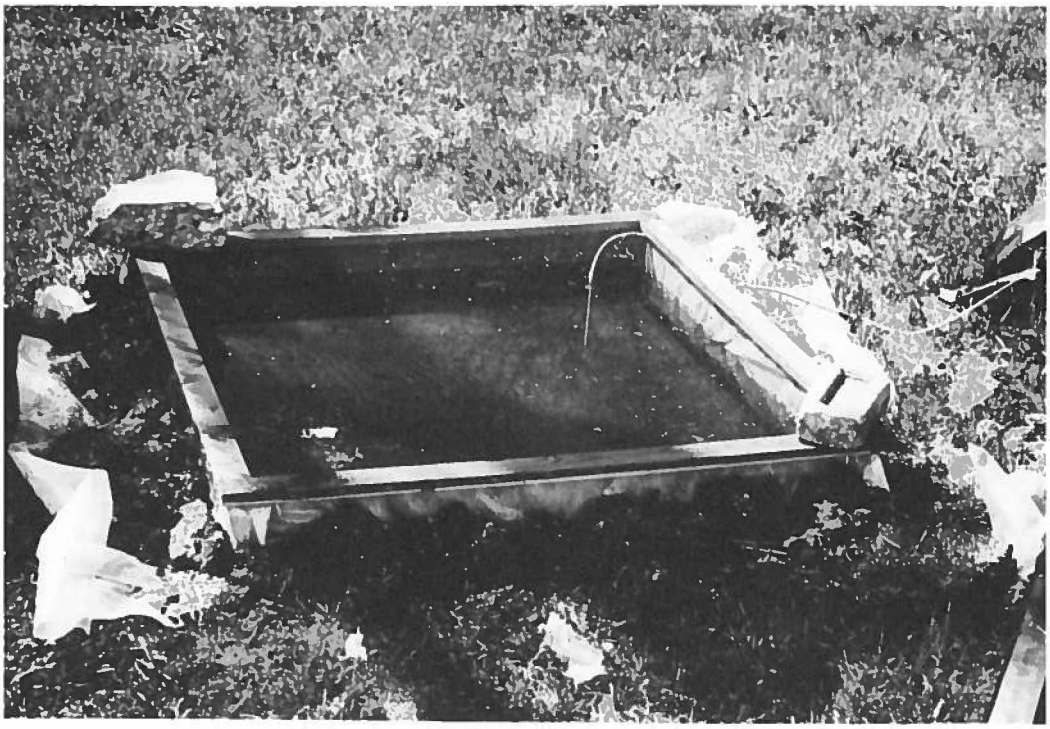

Fig. 9

Fig. 10

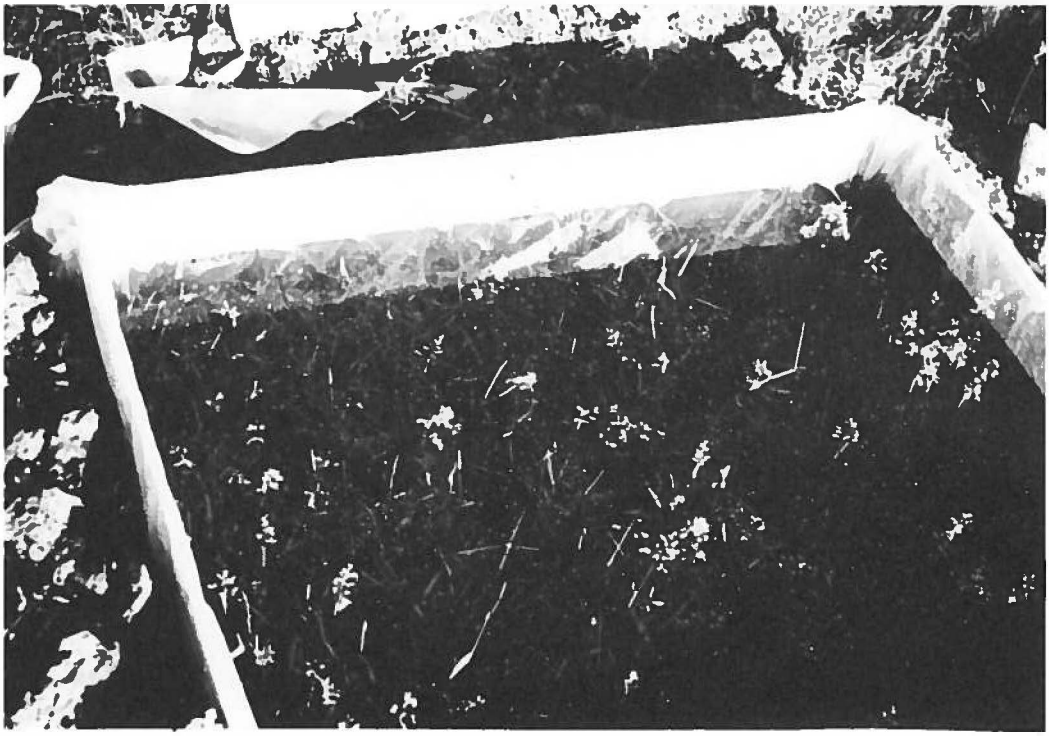

Fig. 11. Comparison of the linear relationships of head capsule width and larval instar between infected and uninfected Aedes vexans larvae reared singly in vials at 23° ± 0.5° C in the laboratory. Each larva was initially exposed to ten preparasites for 24 hr.

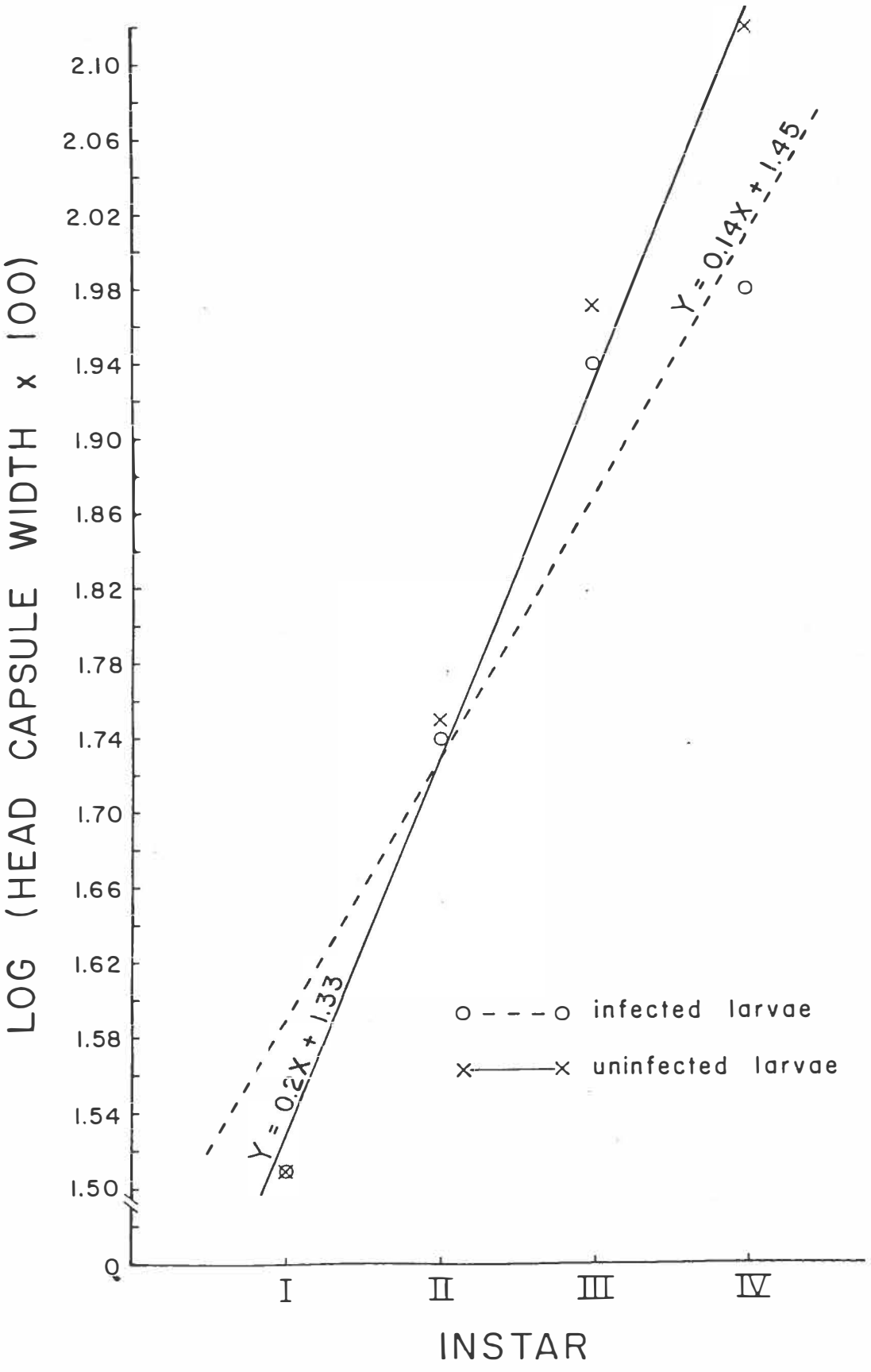
LOG (HEAD CAPSULE WIDTH x 100)
2.10
2.06
2.02
1.98
1.94
1.90
1.86
1.82
1.78
1.74
1.70
1.66
1.62
1.58
1.54
1.50
0
I
II
III
IV
INSTAR
Y = 0.14X + 1.45
Y = 0.2X + 1.33
o - - - o infected larvae
x——x uninfected larvae

Fig. 11

Fig. 12,13. The result of infection by Romanomermis culicivorax and its effect on development time of Aedes vexans larvae reared singly in vials in the laboratory. Fig. 12. Larvae exposed to 10 preparasites. Fig. 13. Larvae exposed to 20 preparasites. (I = infected; U = uninfected; subscripts refer to instar and P = pupation of uninfected larvae and NE = nematode emergence). Dotted portion refers to mortality in larvae resulting from nematode emergence.

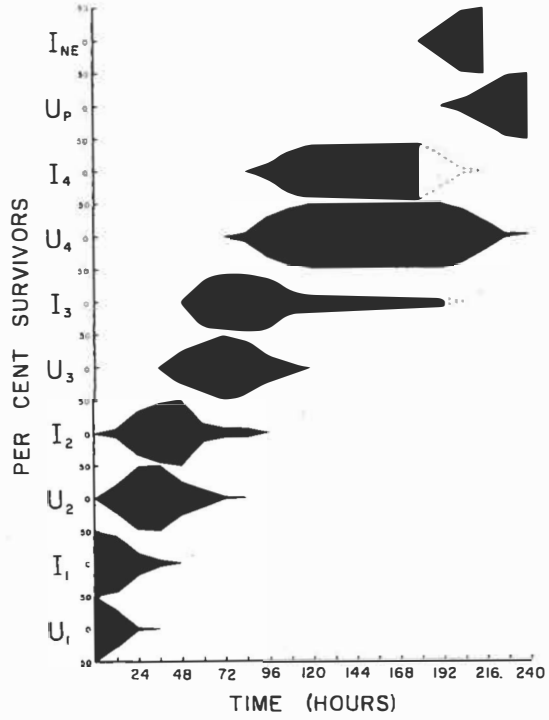
PER CENT SURVIVORS
I_{NE}
U_P
I_4
U_4
I_3
U_3
I_2
U_2
I_1
U_1
24 48 72 96 120 144 168 192 216. 240
TIME (HOURS)

Fig. 12

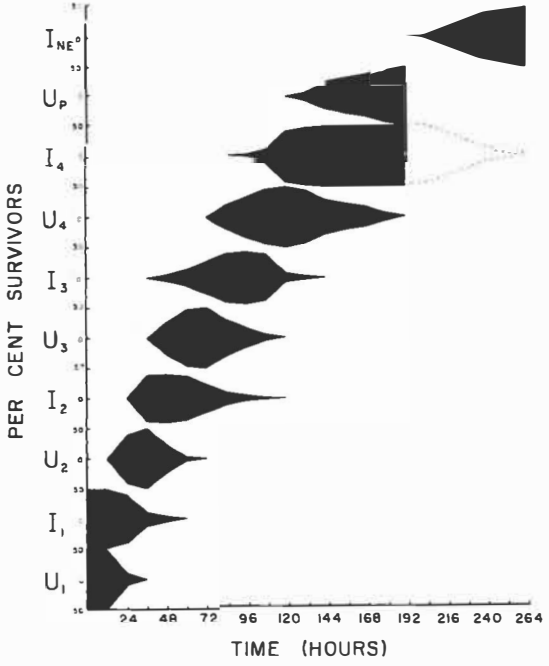
PER CENT SURVIVORS
I_{NE}
U_P
I_4
U_4
I_3
U_3
I_2
U_2
I_1
U_1
24 48 72 96 120 144 168 192 216 240 264
TIME (HOURS)

Fig. 13

Fig. 14. Mean ± maximum and minimum temperatures recorded in artificial pools in which Aedes vexans larvae were infected with Romanomermis culicivorax. Pools were treated A - June 26, B - July 7, C - August 8 (1974) and D - July 1, E - July 8, F - July 17 (1975).

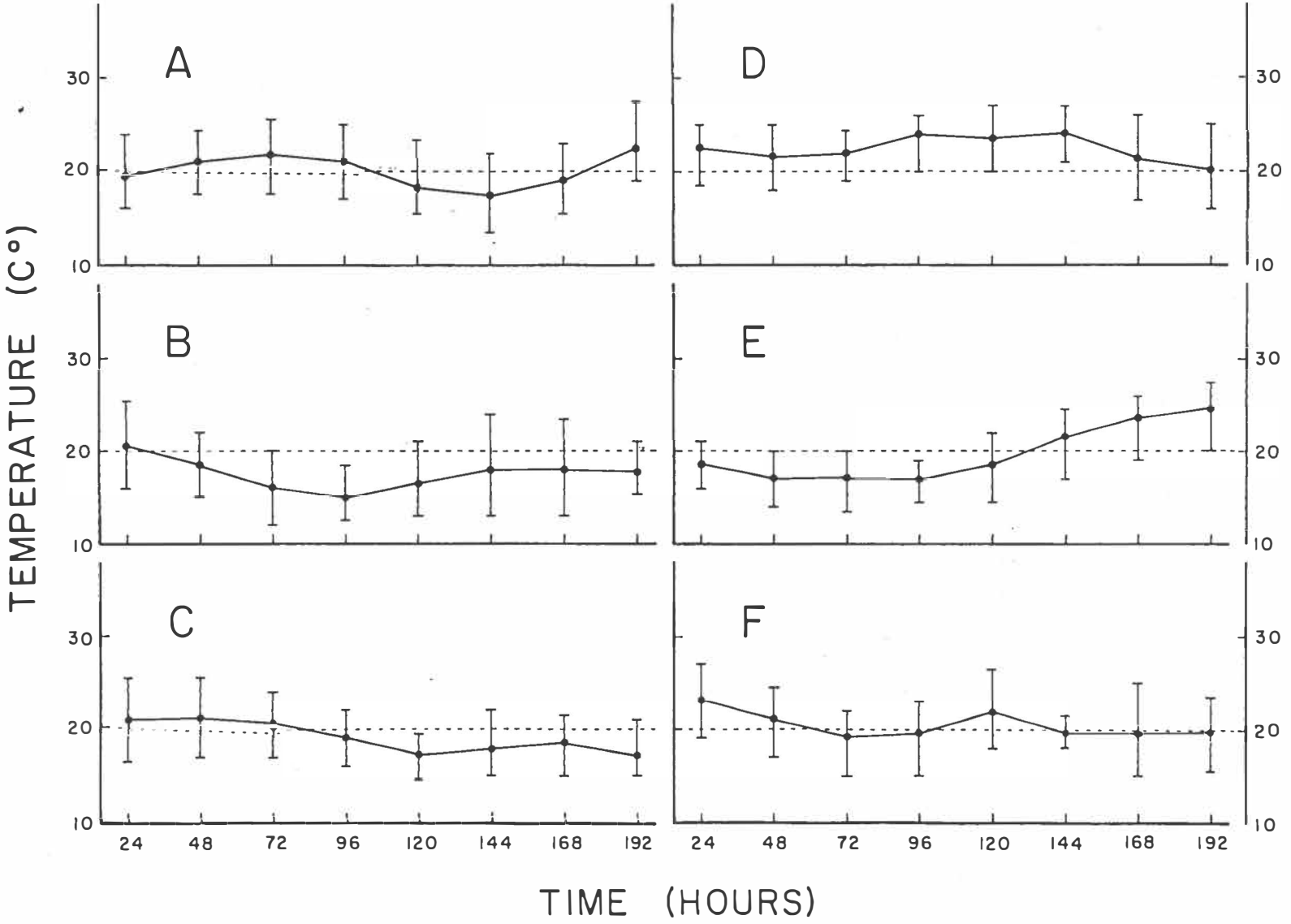
A
B
C
D
E
F
30
20
10
24
48
72
96
120
144
168
192
TEMPERATURE (C°)
TIME (HOURS)

Fig. 14

Fig. 15,16. The effects of infection by Romanomermis culicivorax on development time for each larval instar of Aedes vexans reared in artificial field pools. (Fig. 15, July 7; Fig. 16, August 8, 1974). I = infected, U = uninfected.

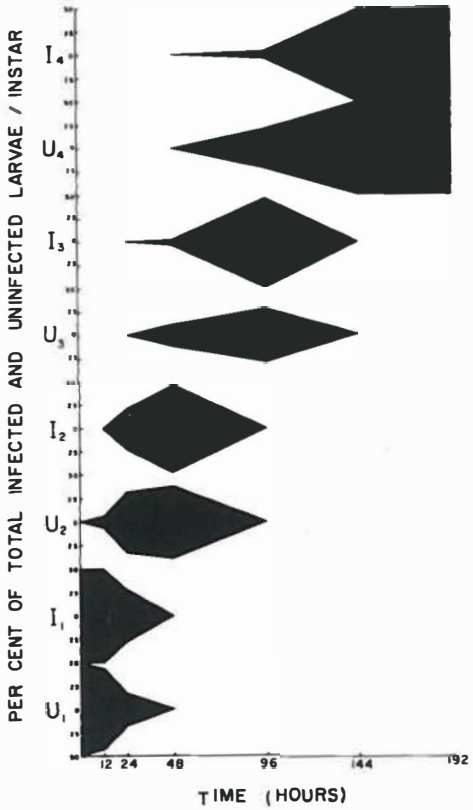
PER CENT OF TOTAL INFECTED AND UNINFECTED LARVAE / INSTAR
I4
U4
I3
U3
I2
U2
I1
U1
12 24 48 96 144 192
TIME (HOURS)

Fig. 15

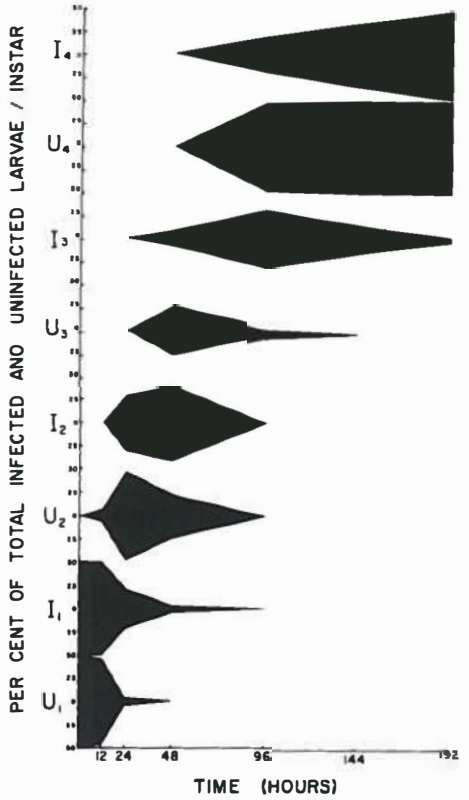
PER CENT OF TOTAL INFECTED ANO UNINFECTED LARVAE / INSTAR
I4
U4
I3
U3
I2
U2
I1
U1
12 24 48 96 144 192
TIME (HOURS)

Fig. 16

Fig. 17-19. The effects of infection by Romanomermis culicivorax on development time for each larval instar of Aedes vexans reared in artificial field pools. (Fig. 17, July 1; Fig. 18, July 8; Fig. 19, July 17, 1975). I = infected, U = uninfected.

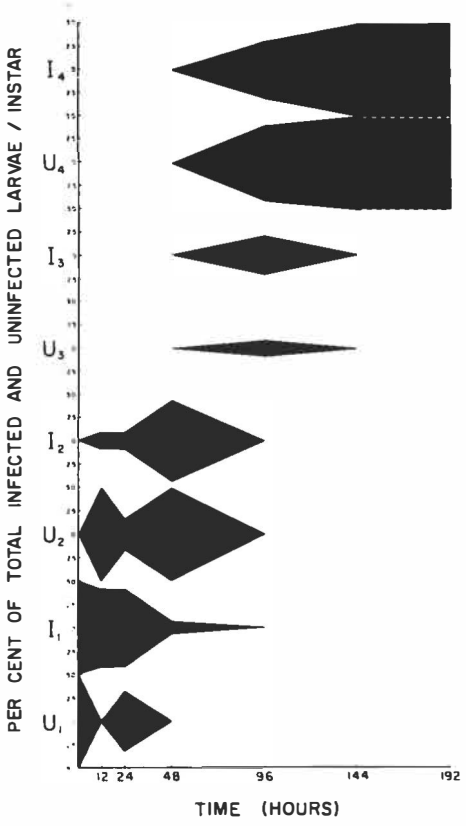

Fig. 17

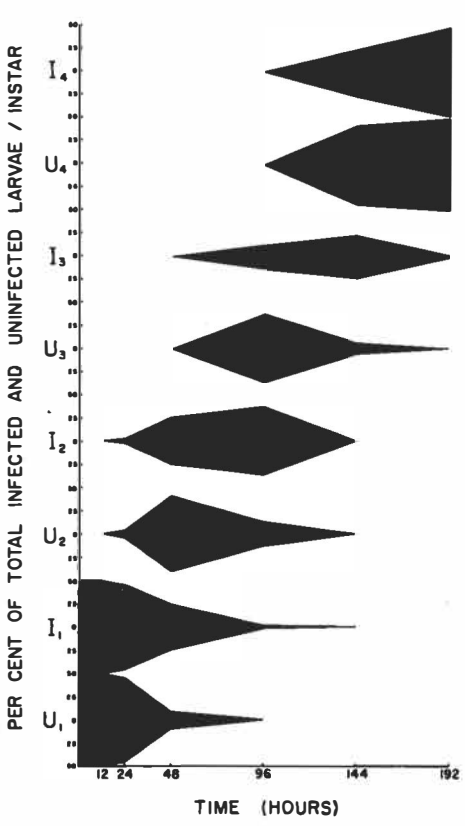

Fig. 18

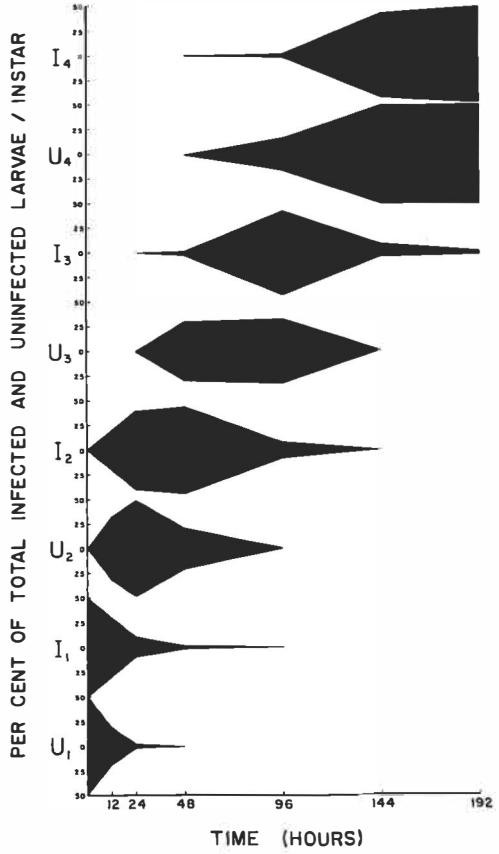

Fig. 19

TABLE 9. Results of infection of *A. vexans* larvaeby *R. culicivorax* in single vials at two infection rates at 23°C.

Trial	% infected	No. nematodes/ infected host	Total no. of nematodes	No. nematodes to complete development	No. developed nematodes/infected host	No. ♂ nematodes	No. ♀ nematodes
			10:1				
A	43.3	1.08	14	7	1.14	6	1
B	50.0	1.87	28	19	1.73	12	7
C	30.0	1.00	9	5	1.00	0	5
$\bar{x}\pm SE$	41.1±5.88	1.32±0.278	17.0±5.68	10.3±4.37	1.29±0.224	6.0±3.46	4.3±1.76
			20:1				
A	36.7	2.55	28	17	5.00	16	1
B	50.0	2.27	34	25	2.08	18	7
C	53.3	1.94	31	27	2.08	21	6
$\bar{x}\pm SE$	46.7±5.07	2.25±0.176	31.0±1.73	23.0±3.05	3.05±0.973	18.3±1.45	4.7±1.86

TABLE 10. Per cent survival in each instar of uninfected *A. vexans* larvae vs those infected by *R. culicivorax*. Larvae were reared singly in vials at 23° C. Numbers of surviving larvae are shown in parentheses.

	No. of larvae	No. infected	Instar I	Instar II	Instar III	Instar IV
10:1						
A	30	13	100.0(13)	76.9(10)	53.8(7)	0.0
B	30	15	93.3(14)	86.7(13)	73.3(11)	0.0
C	30	9	100.0(9)	100.0(9)	66.7(6)	0.0
$\bar{x}\pm SE$		12.3±1.76	97.8±2.23	90.1±6.69	64.6±5.73	
Control						
A	30	-	100.0(30)	80.0(24)	66.7(20)	33.3(10)
B	30	-	93.3(28)	70.0(21)	56.7(17)	6.7(2)
C	30	-	100.0(30)	100.0(30)	100.0(30)	83.3(25)
$\bar{x}\pm SE$			97.8±2.23	83.3±8.82	74.5±13.09	41.1±22.45
20:1						
A	30	11	90.9(10)	63.6(7)	45.5(5)	0.0
B	30	15	100.0(15)	93.3(14)	86.7(13)	0.0
C	30	16	100.0(16)	87.5(14)	87.5(14)	0.0
$\bar{x}\pm SE$		14.0±1.53	97.0±3.03	81.5±9.09	73.2±13.87	
Control						
A	30	-	100.0(30)	93.3(28)	80.0(24)	60.0(18)
B	30	-	100.0(30)	100.0(30)	73.3(22)	46.7(14)
C	30	-	100.0(30)	100.0(30)	90.0(27)	60.0(18)
$\bar{x}\pm SE$			100.0±0.00	97.8±2.23	81.1±4.85	55.6±1.33

TABLE 11. The result of infection by R. culicivorax and its effect on head capsule width (mm) of A. vexans larvae reared singly in vials at 23° C. Numbers of larvae examined are shown in parentheses.

	Instar I				Instar II			
	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$
10:1	0.32(13)	0.32(15)	0.32(9)	0.32(37)^{NS}	0.57(13)	0.55(14)	0.54(9)	0.55(36)*
Control	0.32(30)	0.32(30)	0.32(30)	0.32(90)^{NS}	0.58(30)	0.57(28)	0.55(30)	0.57(88)*
20:1	0.32(11)	0.32(15)	0.32(16)	0.32(42)^{NS}	0.56(7)	0.58(14)	0.60(16)	0.58(37)*
Control	0.32(30)	0.32(30)	0.32(30)	0.32(90)^{NS}	0.62(15)	0.63(15)	0.63(30)	0.63(60)*

	Instar III				Instar IV			
	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$
10:1	0.85(10)	0.86(13)	0.92(9)	0.88(32)*	1.07(7)	1.12(11)	1.18(6)	1.12(23)*
Control	0.90(24)	0.84(21)	1.00(30)	0.91(75)*	1.21(20)	1.13(17)	1.30(30)	1.21(67)*
20:1	-	-	0.87(14)	0.87(14)*	-	-	1.11(14)	1.11(14)*
Control	-	-	0.93(27)	0.93(27)*	-	-	1.17(18)	1.17(18)*

* $P<0.05$; NS $P>0.05$

TABLE 12. Per cent of *A. vexans* larvae infected by *R. culicivorax* in samples of larvae taken from artificial pools treated at four application rates and at various times after treatment.

Application rate (pre-parasites/ m^2)	Sample time (hr)	% Infected						$\bar{x}\pm SE$
		A	B	C	D	E	F	
10,000	12	0[a]	8[a]	8[b]	2[c]	18[f]	-	7.2±3.14
	24	0	2	8	12	30	-	10.4±5.34
	48	12	8	16	12	32	-	16.0±4.20
	96	12	36	16	12	18	-	18.8±4.45
	144	8	18	10	10	26	-	14.4±3.37
	192	18	30	56	84	34	-	44.4±11.65
20,000	12	10[a]	8[a]	8[b]	8[c]	28[e]	-	12.4±3.92
	24	18	10	12	14	28	-	16.4±3.19
	48	26	24	10	19	34	-	22.6±3.97
	96	46	26	10	12	30	-	24.8±6.56
	144	24	4	10	19	32	-	17.8±4.96
	192	46	36	22	86	44	-	50.8±11.32
50,000	12	10[a]	0[a]	10[b]	18[b]	8[c]	-	9.2±2.87
	24	12	4	16	38	22	-	18.4±5.71
	48	20	14	18	48	16	-	23.2±6.28
	96	16	20	20	28	12	-	19.2±2.65
	144	18	24	12	24	19	-	19.4±2.22
	192	76	81	-	-	96	-	84.3±6.01
100,000	12	94[d]	32[e]	24[e]	36[e]	34[f]	42[f]	43.6±10.35
	24	94	22	42	42	66	28	49.0±10.93
	48	92	38	32	14	48	22	41.0±11.30
	96	88	40	34	22	24	20	38.0±10.48
	144	92	44	46	28	20	0	-
	192	100	76	82	88	-	-	-

[a,b,c] Pools treated on June 26, July 7 and August 8, 1974 respectively.

[d,e,f] Pools treated July 1, July 8 and July 17, 1975 respectively.

TABLE 13. Mean number of parasites (R. culicivorax) per infected A. vexans larva in samples taken at various times from artificial pools treated at four different application rates.

Application rate (preparasites/m^2)	Sample time (hr)	Mean no. parasites/infected host						$\bar{x}\pm SE$
		A	B	C	D	E	F	
10,000	12	0.0[a]	1.0[a]	2.3[b]	2.0[c]	1.8[f]	-	1.4±0.42
	24	0.0	1.0	1.5	1.2	1.3	-	1.0±0.26
	48	1.0	1.0	1.0	1.1	1.3	-	1.1±0.06
	96	1.0	1.3	1.9	1.0	1.1	-	1.3±0.17
	144	1.0	1.0	1.3	1.0	1.2	-	1.1±0.06
	192	1.0	1.2	1.3	1.2	1.7	-	1.3±0.12
20,000	12	1.2[a]	1.0[a]	1.3[b]	1.0[c]	1.2[e]	-	1.1±0.06
	24	1.1	1.6	1.5	1.6	1.4	-	1.4±0.09
	48	1.0	1.7	1.0	1.0	1.9	-	1.3±0.20
	96	1.1	1.2	3.0	1.0	1.4	-	1.5±0.37
	144	1.2	1.0	1.3	1.6	1.2	-	1.3±0.10
	192	1.1	1.4	1.1	1.3	1.2	-	1.2±0.06
50,000	12	1.0[a]	0.0[a]	3.6[b]	1.0[b]	1.0[c]	-	1.3±0.60
	24	1.0	1.0	2.5	1.1	1.1	-	1.3±0.29
	48	1.0	1.0	1.0	2.2*	1.3	-	1.3±0.23
	96	1.0	1.0	1.7	1.1	1.0	-	1.2±0.14
	144	1.1	1.1	1.0	1.2	1.0	-	1.1±0.04
	192	1.1	1.1	-	-	1.3	-	1.1±0.04
100,000	12	3.8[d]	1.2[e]	1.1[e]	1.2[e]	1.6[f]	1.2[f]	1.7±0.43
	24	2.7	1.6	2.1	1.1	2.2	1.4	1.9±0.24
	48	2.2	1.6	1.9	1.1	1.1	1.3	1.5±0.18
	96	3.3	1.1	1.2	1.2	1.3	1.3	1.6±0.35
	144	3.4	1.3	1.6	1.7	4.0	0.0	-
	192	3.2	1.7	1.3	1.4	-	-	-

a,b,c Pools treated on June 26, July 7 and August 8, 1974 respectively.

d,e,f Pools treated on July 1, July 8 and July 17, 1975 respectively.

* 1 larva in the sample contained 26 nematodes.

TABLE 14. Incidence of melanization of parasitic R. culicivorax in A. vexans larvae for all pools at each sample time.

Sample time (hr)	Total no. of parasitic nematodes	Total no. melanized	Per cent melanized
12	397	2	0.50
24	459	4	0.87
48	453	4	0.88
96	432	11	2.55
144	390	6	1.54
192	655	29	4.43

TABLE15. Mean head capsule width ± standard error (mm) of each instar of infected vs uninfected *A. vexans* larvae from artificial field pools. Numbers of larvae measured are presented in parentheses.

	Trial	Infected larvae	Uninfected larvae
Instar I			
	B	0.32 ± 0.004^{NS} (21)	0.32 ± 0.003^{NS} (60)
	C	0.32 ± 0.003^{NS} (19)	0.32 ± 0.003^{NS} (148)
	E	0.32 ± 0.004^{a} (159)	0.32 ± 0.002^{a} (287)
	F	0.31 ± 0.002^{a} (93)	0.32 ± 0.002^{a} (112)
Instar II			
	B	0.50 ± 0.006^{c} (87)	0.53 ± 0.003^{c} (142)
	C	0.52 ± 0.005^{c} (34)	0.54 ± 0.003^{c} (183)
	E	0.52 ± 0.007^{NS} (81)	0.52 ± 0.005^{NS} (162)
	F	0.51 ± 0.003^{a} (77)	0.52 ± 0.003^{a} (145)
Instar III			
	B	0.83 ± 0.011^{c} (38)	0.86 ± 0.013^{c} (196)
	C	0.83 ± 0.009^{b} (21)	0.85 ± 0.012^{b} (71)
	E	0.80 ± 0.010^{c} (58)	0.85 ± 0.025^{c} (118)
	F	0.77 ± 0.012^{c} (32)	0.81 ± 0.019^{c} (89)
Instar IV			
	B	1.17 ± 0.024^{c} (64)	1.23 ± 0.031^{c} (292)
	C	1.15 ± 0.029^{c} (153)	1.22 ± 0.016^{c} (265)
	E	1.12 ± 0.016^{c} (180)	1.20 ± 0.007^{c} (160)
	F	1.16 ± 0.011^{c} (32)	1.21 ± 0.009^{c} (121)

Significance levels determined from Student's t-distribution: a = $P<0.05$; b = $P<0.01$; c = $P<0.001$; NS = $P>0.05$.

TABLE 16. Mean body length (mm) of each instar of infected vs uninfected A. vexans larvae from artificial field pools for each sample period. Numbers of larvae measured are presented in parantheses.

Trial	Time of sample (hr)					
	12		24		48	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
			Instar I			
C	1.30^{a} (7)	1.41^{a} (133)	1.49^{NS} (9)	1.46^{NS} (15)	-	-
E	1.16^{c} (76)	1.26^{c} (140)	1.33^{NS} (62)	1.38^{NS} (121)	1.48^{a} (29)	1.57^{a} (26)
F	1.32^{NS} (57)	1.28^{NS} (82)	1.42^{c} (34)	1.58^{c} (30)	-	-
	24		48		96	
			Instar II			
C	1.64^{a} (14)	1.76^{a} (111)	2.28^{b} (19)	2.41^{b} (61)	-	-
E	1.54^{NS} (5)	1.53^{NS} (12)	1.63^{c} (29)	1.86^{c} (114)	2.41^{c} (45)	2.65^{c} (36)
F	1.63^{b} (28)	1.79^{b} (58)	2.20^{NS} (49)	2.33^{NS} (74)	-	-

Continued.....

TABLE 16 (Continued)

Trial	Time of sample (hr)					
	48		96		144	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
			Instar III			
C	2.80^{NS} (4)	2.87^{NS} (65)	3.87^{NS} (11)	4.54^{NS} (5)	-	-
E	-	-	2.84^{c} (17)	3.21^{c} (99)	3.78^{NS} (40)	4.02^{NS} (16)
F	-	-	3.37^{c} (30)	3.77^{c} (62)	-	-
	96		144		192	
			Instar IV			
C	4.61^{c} (7)	5.58^{c} (120)	5.75^{c} (16)	7.01^{c} (127)	5.70^{c} (130)	6.81^{c} (18)
E	-	-	4.39^{c} (35)	5.11^{c} (109)	6.03^{NS} (145)	6.16^{NS} (51)
F	-	-	5.42^{c} (14)	6.10^{c} (43)	6.17^{NS} (17)	6.53^{NS} (33)

Significance levels determined from Student's t-distribution:
a = $\underline{P}$ 0.05; b = $\underline{P}$ 0.01; c = $\underline{P}$ 0.001; NS = $\underline{P}$ 0.05.

CHAPTER VI

Taxonomic review of the genus Romanomermis with a description of R. communensis sp. n. from Goose Creek, Manitoba.

INTRODUCTION

All but one species known in the genus Romanomermis Coman, 1961 are parasites of mosquito larvae. The potential for these mermithids as biological control agents of mosquitoes has been realized since the development of laboratory culture techniques for R. culicivorax Ross and Smith, 1976 (Petersen and Willis 1969a). The widespread use of R. culicivorax under the label Skeeter Doom® makes an immediate taxonomic and biological assessment of the North American Romanomermis species imperative.

Two mosquito mermithid populations native to Manitoba were investigated during this study. The first, 12 miles southeast of Churchill at Camp Nanuk, was at the designated type locality for R. hermaphrodita Ross and Smith, 1976. The second, 15 miles south of Churchill at Goose Creek, was at the designated type locality for Hydromermis churchillensis Welch, 1960.

MATERIALS AND METHODS

Juvenile mermithids were collected from parasitized hosts which had been placed singly in vials containing chlorine-free tap water and a small amount of liver powder. Nematodes were obtained from Aedes nigripes (Zetterstedt) and A. impiger (Walker) at Camp Nanuk and from A. communis

(Degeer) and probably A. churchillensis Ellis and Brust at Goose Creek. Juveniles were reared to adults in damp sand and stored at 20° C.

Nematodes to be used for taxonomic purposes were relaxed in warm water (55° - 65° C) and fixed in TAF for 24-48 hr. Specimens were transferred to hot lactophenol and maintained at 60° C for at least 24 hr. They were then processed following the rapid glycerine method of Goody (1957) and stored in anhydrous glycerine. Nematodes were allowed 1/2 - 1 1/2 hr in each solution before being transferred to the next to reduce distortion in the cuticle. Some specimens were stained with lactophenol-cotton blue and observed in lactophenol. Sections (10μ) were cut using a microtome and stained with Mallory's triple stain or haematoxylin-eosin from specimens embedded in Paraplast® to examine the hypodermal chords.

Body length and distance to the vagina from the anterior end were measured on the projected image of slide-mounted specimens. A Bausch and Lomb projector was used to project the image and measurements were calculated. All other body parameters were measured on a Wild compound microscope using either an ocular micrometer or a drawing tube and map-measurer. Illustrations were prepared using a Wild compound microscope and drawing tube. All measurements (mean range $\pm$ standard error) are presented in microns unless otherwise indicated. For terminology, refer to Goody (1957) and Ross and Smith (1976).

Taxonomy of Romanomermis Coman, 1961.

The genus Romanomermis was first established by Coman (1961) with his reassignment of Pseudomermis cazanica Băcesco, 1948 as the type species. Its generic diagnosis was expanded by Ross and Smith (1976) as follows:

"Eight hypodermal chords, six cephalic papillae in one plane, amphids of small-to-medium size (diameter much less than one-quarter of the body width at the level of the cephalic papillae), without obvious cuticular fibres; adult body bluntly rounded posteriorly; postparasitic juveniles with an elongate, pointed caudal appendage; male with two long (length greater than twice the body width at the level of the anus) spicules narrowing to a point distally; vagina of female pear-shaped, short (in length less than the width of the body); eggs in situ without obvious byssi."

The genus Romanomermis currently contains six described species, five of which parasitize culicids - R. cazanica (Băcesco, 1948) Coman, 1961; R. iyengari Welch, 1964; R. nielseni (Tsai and Grundmann, 1969) Ross and Smith, 1976; R. culicivorax Ross and Smith, 1976; R. hermaphrodita Ross and Smith, 1976; and R. kiktoreak Ross and Smith, 1976. Another Romanomermis sp., similar to R. culicivorax was reported by Nickle (1976) but its taxonomic status has yet to be defined.

Considerable confusion surrounding the taxonomy of this genus and its associated species began with the de-

scription of Reesimermis nielseni by Tsai and Grundmann (1969) and continued until the substantial improvements of Ross and Smith (1976). A brief synopsis of the events leading up to and including the revision of Ross and Smith (1976) appears below and in Fig. 20.

Tsai and Grundmann (1969) established the new genus, Reesimermis, separated from Romanomermis on the basis of its distally fused spicules in the male. The type species, R. nielseni was recovered from a number of larval spring Aedes spp. at Lone Tree, Wyoming. Coincidentally, Petersen et al. (1968) reported a Romanomermis sp. parasitizing larval mosquitoes in Louisiana. The Louisiana mermithid was subsequently reidentified as Reesimermis nielseni (Nickle 1972). Nickle (1972) also transferred R. iyengari to the genus Reesimermis and changed the diagnosis of the genus to two completely separate, though closely applied, spicules. The presence of separate spicules was confirmed by Nickle and Högger (1974), but they failed to point out the congeneric diagnoses of Reesimermis and Romanomermis. Rubtsov (1972) established the priority of Eurymermis and synonomized Romanomermis with it. This revision included the transfer of R. iyengari to Eurymermis. Rubtsov (1972; 1974) did not acknowledge Reesimermis as a valid genus. Obiamiwe and Macdonald (1973) described Reesimermis muspratti from tree-hole mosquitoes in Africa but they too failed to note the ambiguous status of the genus Reesimermis. Ross and Smith (1976), in the most comprehensive treatment

of this mermithid complex to date, identified the problem and synonomized Reesimermis with the preexisting genus Romanomermis and discarded the synonomy of the latter with Eurymermis. Romanomermis iyengari was reestablished and three new species were described, including R. culicivorax (previously identified as Reesimermis nielseni from Louisiana), R. hermaphrodita and R. kiktoreak. Ross and Smith (1976) also reallocated Reesimermis muspratti to the genus Octomyomermis.

Romanomermis communensis sp. n.

Fig. 21, A-E

Mermithidae Braun, 1883

Romanomermis Coman, 1961;

as expanded by Ross and Smith, 1976

ADULTS: Cross fibres in the cuticle visible only under high magnification; eight hypodermal chords at the mid body region; six cephalic papillae lying in a single plane; mouth opening terminal; amphids small ($\simeq$ 5x9), without a commissure, slightly smaller in the female; body elongate; tail bluntly rounded, no caudal filament; trophosome white; oesophageal tube ending within the region of the posterior 1/3 of the trophosome.

FEMALE: (n = 5-8) Body length 16.2 mm (13.5-20.7; SE = 1.0); body width at the cephalic papillae 55 (48-68; SE = 2.6), at the nerve ring 100 (88-104; SE = 2.9), near the vagina 165 (142-186; SE = 6.2), and at the posterior end of the trophosome 130 (110-144; SE = 4.8), giving ratios of 0.55:1.0:1.65:1.30; distance from the middle of the nerve

ring to anterior end 213 (180-280; SE = 16.0); distance from nerve ring to anterior/body length = 0.013; vagina somewhat pear-shaped with the canal ranging from straight and perpendicular to body wall to moderately bent at the mid region; V = 52 (50-55; SE = 0.01); cuticle thickness 4-7, usually thinnest medially, thicker at either extremity; distance of posterior end of trophosome to tail 131 (100-160; SE = 8.4).

The occasional female (less than 5%) possessed rudimentary copulatory muscles of the male (Fig.21, E). Several specimens collected in 1974 possessed fully developed male copulatory structures as well as the female reproductive compliment (Galloway and Brust 1976).

MALE: (n = 20-24) Body length 11.5 mm (8.5-16.7; SE = 0.43); body width at the cephalic papillae 41 (36-50; SE = 0.74), at the nerve ring 84 (76-98; SE = 0.97), at the mid body region 132 (114-156; SE = 2.0) and near the cloaca 119 (106-134; SE = 1.5), giving ratios of 0.48:1.0: 1.57:1.42; distance from middle of nerve ring to anterior end 208 (190-232; SE = 2.1); distance from middle of nerve ring to anterior end/body length = 0.018; two separate curved spicules, gently tapered to a point; thickened cone-shaped spicule guide present near cloaca (most evident in stained specimens); proximal base of spicules not normally flared; length 290 (244-344; SE = 4.9); width at base $\simeq 7$ (5-8; SE = 0.21), at middle $\simeq 4$ (3-5; SE = 0.07); ratio of body length to spicule length 39 (30-55; SE = 1.3); caudal

papillae in three rows with the middle row bifurcating to either side of the cloaca; middle row 23 (13-28; SE = 1.5) anterior to the bifurcation, 19 (15-26; SE = 0.41) in either row posterior to the bifurcation; lateral rows 33 (28-42; SE = 1.2); cuticle thickness 4-8, usually thinnest at the posterior end of the body.

POSTPARASITIC JUVENILE: (n = 20) Body similar in length and measurement to that of adult; trophosome white, caudal filament present, 75 (62-88; SE = 3.7); terminal gap greater in males and intersexes than in females.

TYPE HOST AND LOCALITY: Aedes communis (Degeer) collected in pools north of Goose Creek, Manitoba and east of the CN railway tracks, Hudson Bay Railroad, Mile 498.2-498.4. Romanomermis communensis evidently completed its development in Aedes churchillensis and also a Mochlonyx sp.

DEPOSITION OF TYPES: Holotype (♂) and allotype (♀) and three paratypes (2 ♂♂ , 1 ♀) deposited in Canadian National Nematode Collection, Ottawa; three paratypes (2 ♂♂ , 1 ♀) deposited in both University of California Nematode Collection, Davis, and United States National Nematode Collection, Beltsville, Maryland.

DIAGNOSIS; Romanomermis communensis males possess significantly shorter spicules (ratio of body length to spicule length 39.1) than other species of Romanomermis with the exception of R. nielseni (ratio 40.8) as described by Tsai and

Grundmann (1969). It can be separated from R. nielseni by its greater number of caudal papillae and the relatively closer position of the nerve ring to the anterior end. The nerve ring of both sexes in R. communensis is the most anteriorly located except for R. iyengari. Although the number of caudal papillae in R. communensis, R. kiktoreak and R. culicivorax are somewhat similar, R. communensis can be reliably separated from the other two species on the basis of the characters already described. The % V of R. communensis is significantly greater than those expressed by other Romanomermis species.

Romanomermis hermaphrodita Ross and Smith, 1976 and R. culicivorax Ross and Smith, 1976

Collections of material from the type locality of R. hermaphrodita and from a laboratory culture of R. culicivorax reared from A. aegypti (L.) larvae permitted morphological comparisons of these two species with the published descriptions of Ross and Smith (1976). Little difference was found to exist in the descriptions of R. culicivorax, therefore a complete description of the specimens from our cultures was considered unnecessary. Only selected parameters (Table 17) and figures (Fig.22, A-D) are presented here. It should be pointed out that although Ross and Smith (1976) described the spicules of R. culicivorax as being similar to those of R. iyengari, they failed to emphasize the presence of knobbed or flared bases in both species. Spicules of all other Romanomermis species

examined possessed no such swellings at their base. The present description for R. hermaphrodita differs considerably for some body parameters and may lend some insight into morphological variation in field populations of this species. Therefore, a complete species description for our examined material is included. A summary of selected morphometric relationships based on published and directly recorded information is presented in Table 17 for all Romanomermis species with the exception of R. cazanica.

Romanomermis hermaphrodita

Ross and Smith, 1976

Fig.23 , A-D

ADULTS: Cross fibres in the cuticle visible only under high magnification; eight hypodermal chords at the mid body region; six cephalic papillae lying in the same plane; mouth terminal; amphids small ($\simeq$7x10), commissure absent, not significantly different between sexes; body elongate (a = 75 for males; a = 90 for females); tail bluntly rounded, no caudal filament; trophosome white; oesophageal tube ending within the region of the posterior 1/3 of the trophosome.

FEMALE: (n = 20-33) Body length 15.0 mm (11.2-22.5; SE = 0.49); body width at the cephalic papillae 45 (36-60; SE = 1.1), at the nerve ring 115 (88-144; SE = 2.7), at the vagina 166 (132-228; SE = 4.7) and at the posterior end of the trophosome 129 (104-156; SE = 2.5), giving ratios of 0.39:1.0:1.44:1.12; distance from the middle of

the nerve ring to the anterior end 256 (188-316; SE = 5.6); ratio of distance from nerve ring to anterior / body length = 0.017; vagina pear-shaped, canal may be slightly bent particularly in young adults when trophosome still large; V = 49 (41-56; SE = 0.01); cuticle thickness 3-12, usually thinnest in the region of the vagina and thickening towards either end; distance of posterior end of trophosome to the tail 149 (96-212; SE = 6.4).

The derivation of the species name is based on the high incidence of intersexuality observed in the Camp Nanuk population (Ross and Smith 1976). Intersexes possessing slight to completely formed male copulatory structures and functional female reproductive organs were present in our collections in varying abundance (Galloway and Brust 1976).

MALE: (n = 7-13) Body length 10.4 mm (8.3-12.6; SE = 0.38); body width at the cephalic papillae 44 (40-48; SE = 1.1), at the nerve ring 96 (84-114; SE = 3.2), at the mid body region 139 (110-162; SE = 5.9) and at the cloaca 123 (100-152; SE = 6.9), giving ratios of 0.46:1.0:1.45: 1.28; distance from the middle of the nerve ring to the anterior end 257 (188-316; SE = 5.6); ratio of the distance from the middle of the nerve ring to the anterior end/ body length = 0.024; two separate, curved spicules, tapering to a point, flexible; proximal base of the spicules not obviously flared; length 361 (289-421; SE = 10.9); width at base $\simeq$6 (6-7; SE = 0.14), at middle $\simeq$5 (3-6; SE = 0.27);

ratio of body length to spicule length 29 (23-37; SE = 1.0); caudal papillae in three distinct rows with the middle row bifurcating to either side of the cloaca; middle row 15 (13-18; SE = 1.2) anterior to the bifurcation, 11 (10-12; SE = 0.4) in either row posterior to the bifurcation; lateral rows 16 (15-19; SE = 0.8); cuticle thickness 3-8, usually thinnest at the mid body region.

POSTPARASITIC JUVENILE: (n = 20) Body length and measurements similar to that of adults; trophosome white, no evidence of coloured granules present; caudal filament present, 91 (83-126; SE = 8.2); terminal gap greater in males and intersexes than in females.

DIAGNOSIS: Romanomermis hermaphrodita can be distinguished from R. iyengari and R. culicivorax by its lack of flared spicule bases and its relatively straight vaginal canal. The number and arrangement of caudal papillae separate it from R. kiktoreak and R. communensis. Its spicules are relatively longer than those of R. communensis and R. nielseni. The vulva is positioned more anteriorly in R. hermaphrodita than in R. communensis while the opposite is true for R. nielseni.

Care should be exercised when using the various diagnostic features for making comparisons between species and populations. Spicule length exhibited poor linear correlation with body length for males of R. communensis, R. hermaphrodita and R. culicivorax and may be of superficial value only. As shown by Hominik (1969) for three

species of mermithids parasitizing chironomids, V may also be a valuable character for separating Romanomermis species.

The status of Hydromermis churchillensis Welch, 1960

Mermithids described as R. communensis, were collected from the type locality, Goose Creek (Warkworth Creek of Welch 1960), and type host, Aedes communis, for Hydromermis churchillensis, following directions obtained from Dr. H. E. Welch. All specimens examined were R. communensis. No H. churchillensis were found.

The published description for H. churchillensis (Welch 1960) bears little resemblance to that for R. communensis. However, certain valuable characters for the former are lacking, including the number and distribution of caudal papillae in the male and the position of the nerve ring. Holotype and paratypes of H. churchillensis were examined from the Canadian National Collection, Ottawa and the University of Manitoba, Department of Zoology. No redescription or confirmation of this species could be attempted because of the poor condition of the mounted specimens. As a result, no valid comparison between R. communensis and H. churchillensis can be made at this time. Considering the absence of certain important morphological information, the condition of type material and its apparent disappearance from the type locality, H. churchillensis must be considered species inquaerenda until its validity can be established.

Cross-mating experiments

Cross-mating experiments between R. communensis and R. culicivorax were first attempted in 1975. Juvenile mermithids were placed in 250 gm of moist silica sand in 6 2-oz glass jars in the following scheme: 30 Louisiana males x 30 Louisiana females, 30 Goose Creek males x 30 Goose Creek females, 30 Louisiana males x 30 Goose Creek females, 30 Goose Creek males x 30 Louisiana females, 30 Louisiana females and 30 Goose Creek females. The nematodes had been sexed and kept separate until they were put into their respective containers. Juvenile R. culicivorax were held for no more than 4 days at 25° C while R. communensis juveniles were held for no longer than 7 days at 20° C. All nematodes were buried in the sand at the time of introduction. Screw-top lids were placed loosely on each jar to reduce evaporation. Nematodes were stored for eight weeks at 20° C before examination.

Survival in both populations was high after eight weeks: R. communensis ♂ - 90%; R. communensis ♀ - 87%; R. culicivorax ♂ - 93%; R. culicivorax ♀ - 90%. All survivors had moulted to adults. All females in the presence of males from either population contained eggs. Romanomermis communensis females appeared further developed in that some had laid nearly all their eggs. No evidence of parthenogenesis was observed in isolated females from either population.

Nematodes were returned to the sand in their re-

spective containers and placed at 20° C. Sand containing eggs was flooded with chlorine-free tap water 16 weeks later. Large numbers of preparasites were recovered after 24 hr from the Louisiana x Louisiana jar only, though eggs which were apparently fully embryonated were present in all jars which had contained mated females. Forty-three and 60 preparasites from the Louisiana male x Goose Creek female and Goose Creek male x Louisiana female crosses, respectively were placed in plastic pans (15 cm diameter) containing 100 ml of dechlorinated tap water with liver powder and 250 newly hatched Aedes aegypti larvae. Larvae were kept at 25° C. A total of 7 juveniles were recovered 8-11 days after preparasites had been introduced. Unfortunately, all were males, though apparently normal.

A second cross-mating experiment was conducted in 1976. Three replicates each of 20 males and 20 females were established as before for all combinations of the Goose Creek and Louisiana populations. Two jars each with 20 females from either population were also prepared. Romanomermis communensis juveniles were collected on site at Goose Creek where they were placed in damp sand and transported back to Winnipeg. Therefore, they ranged from 3-8 days old at the onset of the experiment. Romanomermis culicivorax juveniles were obtained from our laboratory colony and were only 1-2 days old. As before, jars containing nematodes were kept at 20° C.

Nematodes were examined 3, 8 and 16 weeks after

being placed in the sand. Results are presented in Table 18. No live R. communensis females were found at 8 and 16 weeks. Presumably, they had completed egg production and died. There was a marked delay in egg development in R. culicivorax females though all had moulted to adults. Few females contained eggs even after 16 weeks. The reason for this delay is unclear since R. culicivorax males successfully inseminated R. communensis females within three weeks. Live R. communensis males were present in the sand nine months after emergence from their host.

Sand containing eggs from R. communensis females was flooded after six months. So few preparasites were recovered that no infection trials were attempted.

The only results from cross-mating studies involving Romanomermis species reported to date are those of Petersen (1976). He was unable to obtain eggs from crosses between R. nielseni from Wyoming and R. culicivorax. Partially on these results, he concluded that the two populations indeed represented separate species, or at least separate subspecies. However, differences in development rates at the same temperature or differences in age may have rendered the two populations physiologically incompatable.

The successful crossing of R. communensis with R. culicivorax is confusing and raises some question on the status of these two allopatric species. Romanomermis communensis differs significantly from R. culicivorax in

morphology, its tolerance of low temperatures, hosts attacked and phenology. It remains to be determined to what degree these factors may be related to environmental adaptation. However, successful cross-mating does not prescribe conspecificity, especially following laboratory manipulation of the populations involved. In lieu of morphological and biological differences, the lack of F_2 and back crosses, insufficient F_2 biological information and the possibility of pseudogamy, R. communensis and R. culicivorax must be treated as valid separate species. Information on the effects of host and habitat on mermithid morphology may change the trend of describing new species from each new population and warrant reallocation of some Romanomermis species to subspecific status.

DISCUSSION

As long as populations of Romanomermis species remain distinct, taxonomic and ecological investigations are relatively straight forward. However, Nickle (1976) has reported a Romanomermis sp. found near Beltsville, Maryland which closely resembles R. culicivorax. This new population differs from R. culicivorax in its ability to successfully parasitize Culex territans. A more critical synthesis of the genus may be possible as additional populations of Romanomermis spp. are discovered.

Widespread use of R. culicivorax for biological control purposes may threaten this heretofore simplistic

approach. Its unreported application, establishment and hybridization with endemic populations may create an insurmountable taxonomic scramble. It is therefore imperative that broad surveys be conducted wherever possible to assess the local mermithid - mosquito fauna.

Fig. 20. Schematic representation of the taxonomic history of the genus Romanomermis.

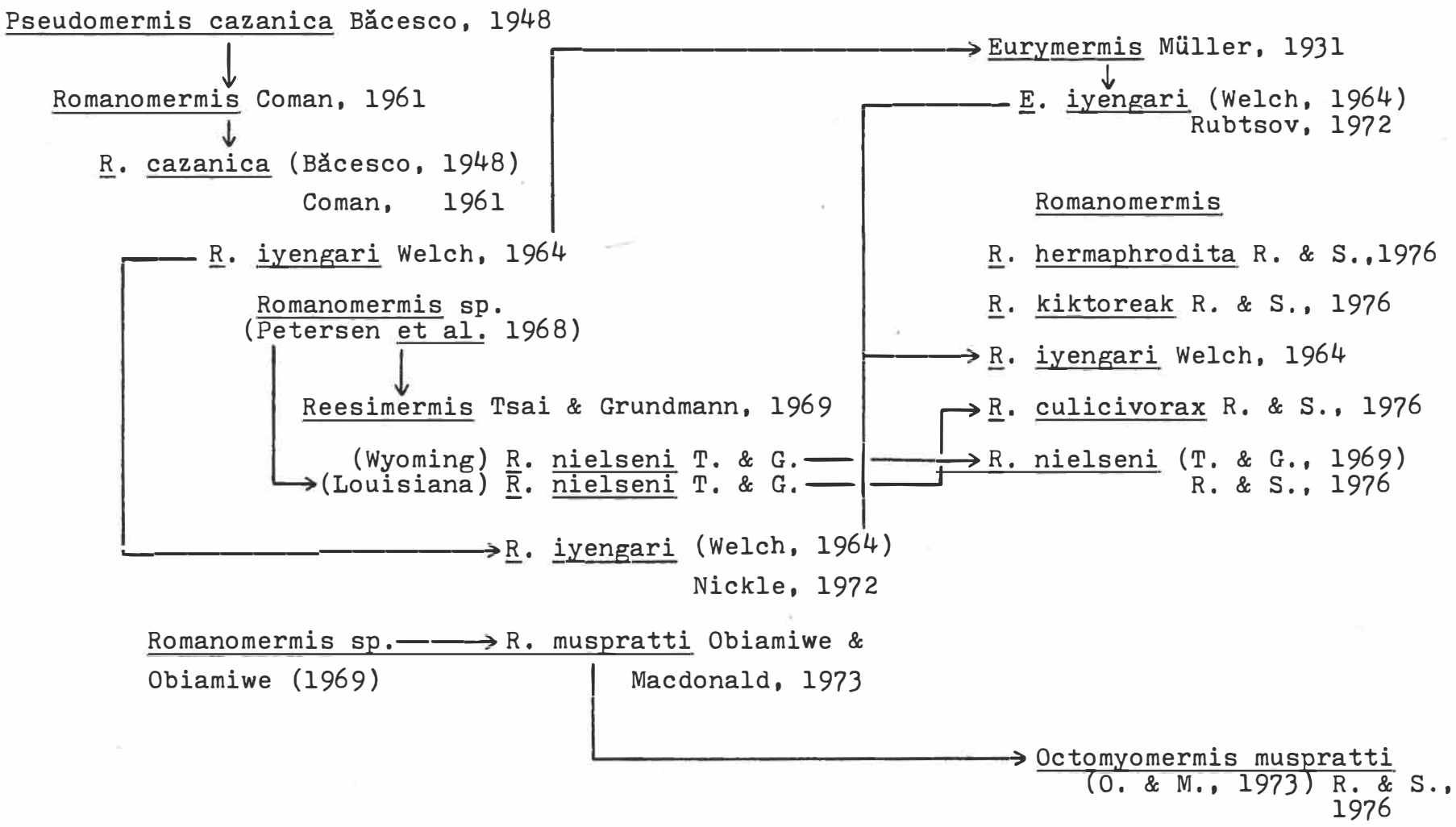
Pseudomermis cazanica Bǎcesco, 1948
Romanomermis Coman, 1961
R. cazanica (Bǎcesco, 1948) Coman, 1961
R. iyengari Welch, 1964
Romanomermis sp. (Petersen et al. 1968)
Reesimermis Tsai & Grundmann, 1969
(Wyoming) R. nielseni T. & G.
(Louisiana) R. nielseni T. & G.
R. iyengari (Welch, 1964) Nickle, 1972
Romanomermis sp. Obiamiwe (1969)
R. muspratti Obiamiwe & Macdonald, 1973
Octomyomermis muspratti (O. & M., 1973) R. & S., 1976
Eurymermis Müller, 1931
E. iyengari (Welch, 1964) Rubtsov, 1972
Romanomermis
R. hermaphrodita R. & S.,1976
R. kiktoreak R. & S., 1976
R. iyengari Welch, 1964
R. culicivorax R. & S., 1976
R. nielseni (T. & G., 1969) R. & S., 1976

Fig. 21,A-E. Romanomermis communensis sp. n. A-adult male, anterior end. B-adult female, anterior end. C-lateral view of adult male tail. D-lateral view of vaginal region of adult female. E-lateral view of adult intersex tail.

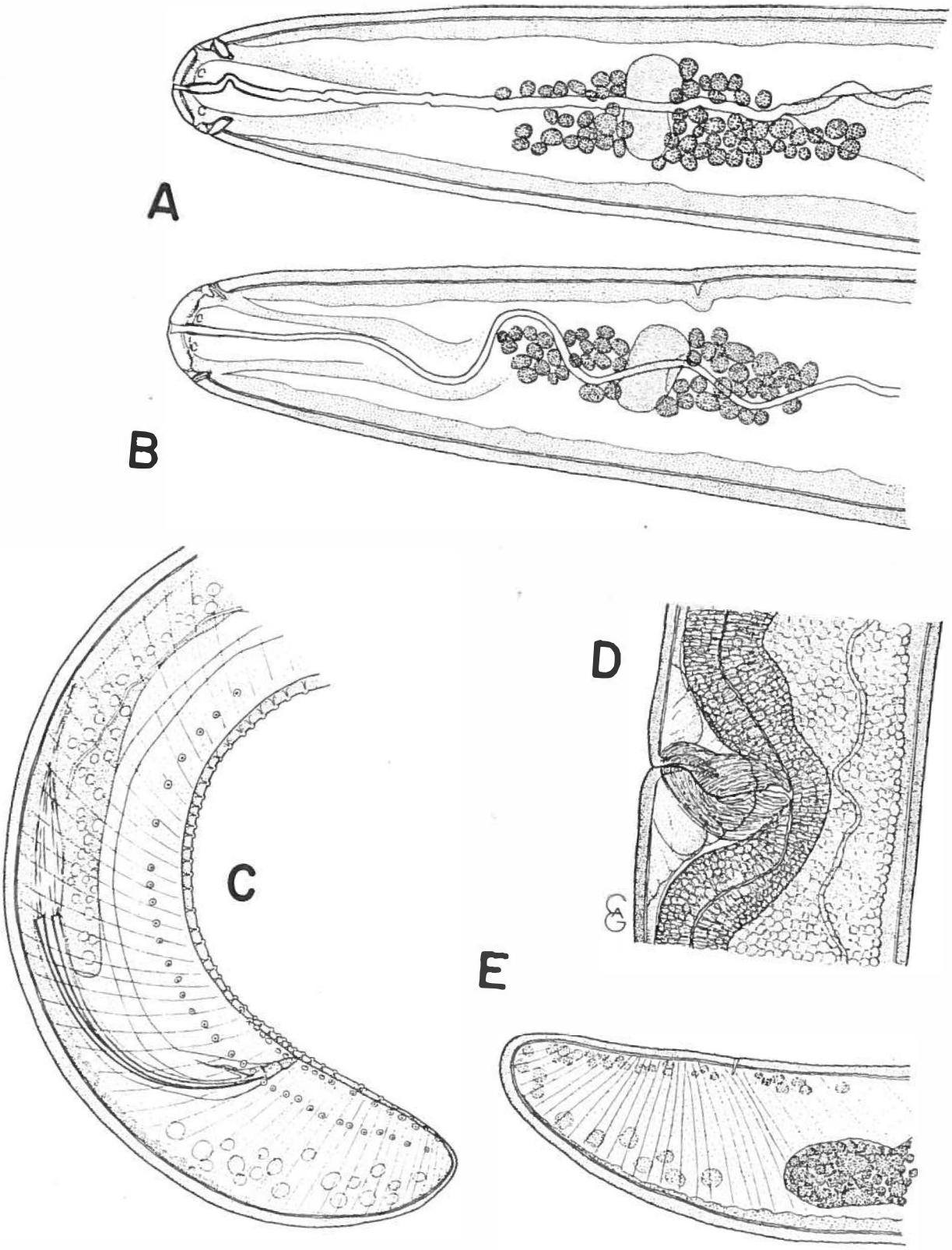

Fig. 21

Fig. 22, A-D. Romanomermis culicivorax Ross and Smith, 1976. A-adult male, anterior end. B-adult female, anterior end. C-lateral view of adult male tail. D-lateral view of vaginal region of adult female.

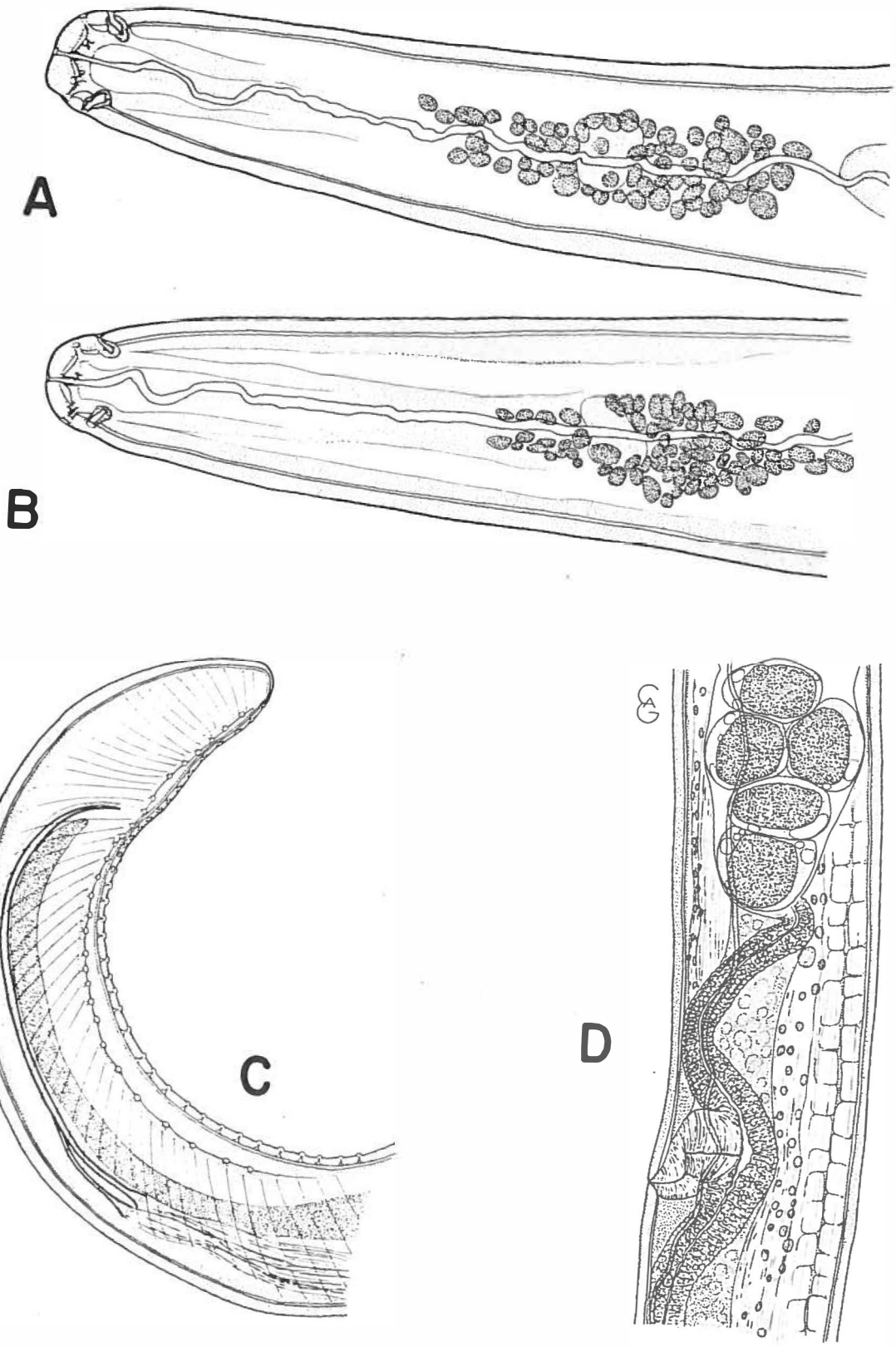

Fig. 22

Fig. 23, A-D. Romanomermis hermaphrodita Ross and Smith, 1976. A-adult male, anterior end. B-adult female, anterior end. C-lateral view of adult male tail. D-lateral view of vaginal region of adult female.

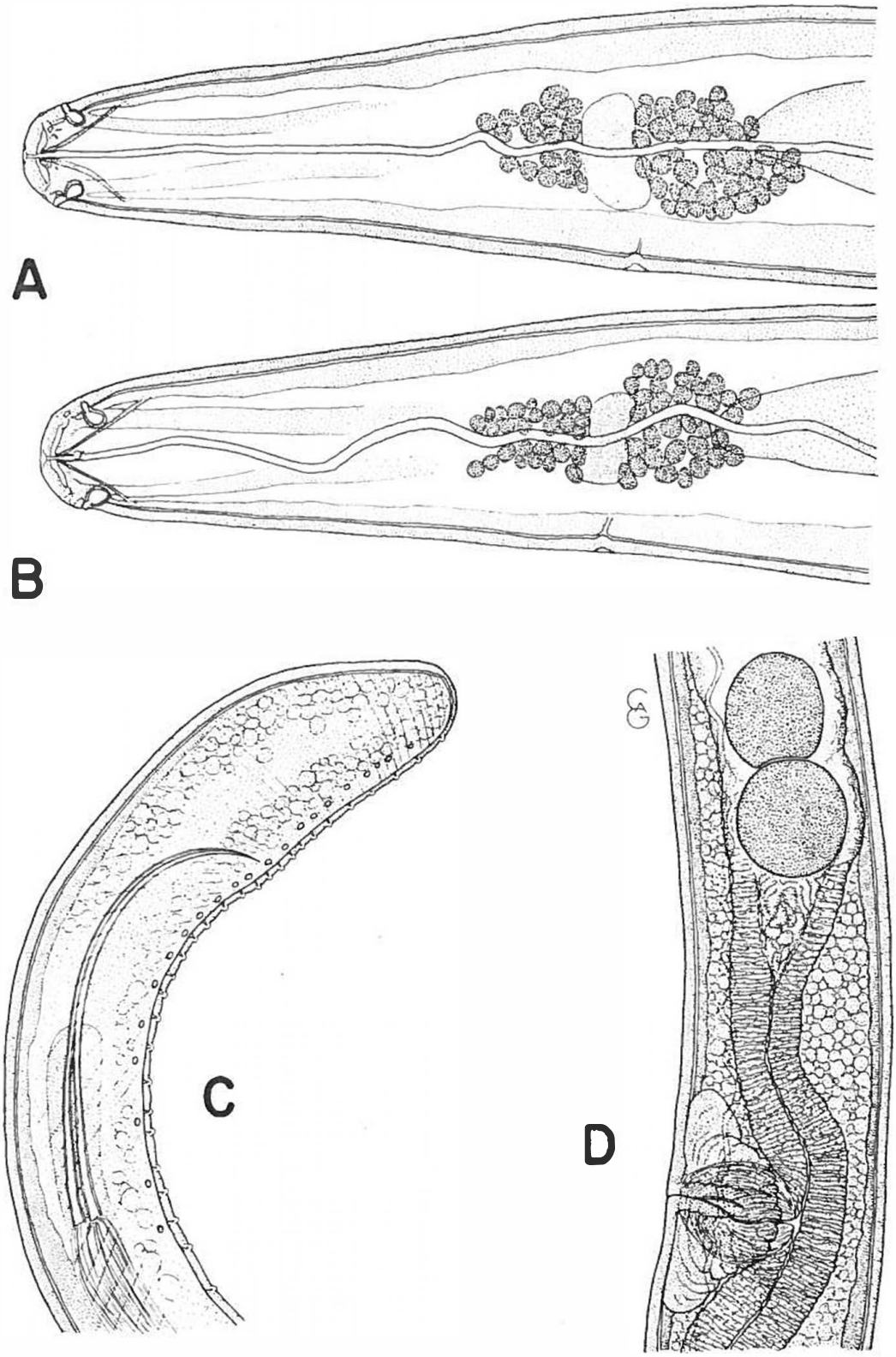

Fig. 23

TABLE 17. A comparison of selected body parameters for Romanomermis spp. which infect mosquitoes. The range for each parameter is presented in parentheses.

Body Parameter	Romanomermis sp.							
	iyengari[1]	nielseni[2]	culicivorax[3]	culicivorax[4]	hermaphrodita[3]	hermaphrodita[4]	kiktoreak[3]	communensis[4]
body length (mm)								
male	12.5 (7.0-14.9)	10.2 (6.2-14.1)	10.0 (7.2-12.9)	10.7 (8.7-12.6)	7.4 (5.7-9.0)	10.4 (8.3-12.6)	9.8 (5.6-12.6)	11.5 (8.5-16.7)
female	17.0 (11.7-20.6)	15.1 (11.0-21.2)	16.8 (11.3-21.0)	18.6 (14.6-22.7)	12.1 (7.1-17.5)	15.0 (11.2-22.5)	14.3 (12.0-18.0)	16.2 (13.5-20.7)
V (%)	49 (46-52)	45.7 (41.1-53.1)	48 (42-53)	48 (46-50)	50 (46-53)	49 (41-56)	48 (44-54)	52 (50-55)
Spicule length (μ)	478 (370-500)	250 (205-310)	397 (318-476)	393 (300-450)	318 (238-384)	361 (289-421)	350 (288-392)	290 (244-344)
body length/spicule length	26.2	40.8	25.2 (19-32)	27.4 (20.1-30.6)	23.5 (19.9-31.5)	29.2 (22.8-36.?)	28.2 (15.7-36.0)	39.1 (29.9-54.6)
number of caudal papillae								
middle row - total	29 (28-31)	- (19-22)	31 (26-39)	29 (23-36)	22 (20-26)	27 (24-31)	34 (28-38)	42 (28-56)
- ant. to cloaca	22 (21-23)	- -	23 (21-28)	20 (16-26)	15 (14-17)	19 (17-22)	24 (19-26)	29 (18-39)
- post. to cloaca	8 (7-8)	- -	8 (5-11)	9 (7-10)	7 (6-9)	8 (7-9)	10 (9-12)	13 (10-17)
lateral row	20 (17-23)	- (16-19)	21 (14-25)	29 (24-34)	16 (11-25)	16 (15-19)	28 (18-35)	33 (24-42)
nerve ring to ant. (μ)								
male	245 (232-256)	- -	214 (167-251)	229 (180-256)	215 (175-272)	250 (220-272)	239 (207-286)	208 (190-232)
female	209 (184-240)	- -	245 (203-282)	278 (228-316)	223 (183-264)	257 (188-316)	225 (210-237)	213 (130-280)
nerve ring/body length male	0.019	-	0.021	0.021	0.029	0.024	0.024	0.018
female	0.013	-	0.015	0.015	0.019	0.017	0.016	0.013

[1]Welch (1964); [2]Tsai and Grundmann (1969); [3]Ross and Smith (1976); [4]Data from present study.

TABLE 18. Survival of *Romanomermis communensis* (GC) and *R. culicivorax* (L) after 3, 8 and 16 weeks in cross-mating studies conducted at 20° C in 1976. Number of gravid females are shown in parentheses.

	males			females		
Cross	3 wk	8 wk	16 wk	3 wk	8 wk	16 wk
20 L ♂♂ x 20 L ♀♀						
A	13	13	11	20 (0)	18 (0)	15 (0)
B	17	16	14	17 (0)	17 (2)	13 (4)
C	14	13	9	18 (0)	18 (0)	16 (2)
20 L ♂♂ x 20 GC ♀♀						
A	16	16	12	17 (17)	0	0
B	18	18	17	18 (17)	0	0
C	18	18	16	20 (20)	0	0
20 GC ♂♂ x 20 GC ♀♀						
A	20	19	19	20 (20)	0	0
B	20	19	16	20 (20)	0	0
C	18	14	13	20 (20)	0	0
20 GC ♂♂ x 20 L ♀♀						
A	17	10	6	18 (0)	18 (0)	18 (0)
B	18	16	14	19 (0)	18 (0)	18 (2)
C	19	16	15	19 (0)	18 (0)	16 (0)

VII CONCLUSIONS

A number of factors contribute to the unsuitability of Romanomermis culicivorax as a biological insecticide for mosquito control in Manitoba at the present time.

1) Aedes spp. predominate as pest species in most parts of the province. Most Aedes spp. examined during this study demonstrated moderate to high degrees of resistance to infection by R. culicivorax.

2) Breeding sites for Aedes spp. may be numerous and widely dispersed (e.g. in woodland areas) or large and extensive (e.g. roadside ditches). Efficient methods for dissemination of R. culicivorax over such large areas have not yet been perfected.

3) Larval densities vary considerably and may be extremely high in certain habitats. An estimate of larval populations from each pool would be necessary to determine the application rate of preparasites to achieve an acceptable level of control.

4) Pools treated with preparasites of R. culicivorax must be monitored to determine the levels of control achieved. This can be done only through tedious dissections of larval samples from each pool.

5) Snow-melt Aedes spp. may hatch over an extended period of time and develop at low temperatures. The efficiency of R. culicivorax preparasites decreases proportionally with temperature and their infective life

expectancy is short. Therefore, repeated applications of large numbers of preparasites may be required in some habitats where a sequential hatch of numerous Aedes spp. exists. Low temperatures provide immunity to the earliest hatching species.

6) Summer Aedes spp. hatch synchronously after heavy rainfall and may develop rapidly if temperatures are high. Romanomermis culicivorax can be effectively applied only against early instar mosquito larvae. Alternative control methods would have to be used once larvae reached the third instar.

7) The number and frequency of summer generations of Aedes vexans is unpredictable. The present shelf-life of R. culicivorax cultures is only 3-5 months and methods of long-term storage are poorly developed. Therefore, reliable handling and efficient use of this nematode in a control program would be difficult.

8) Cost of production of R. culicivorax is prohibitive to extensive use in mosquito control. Considering the above factors and the high cost of production, R. culicivorax has extremely limited use as a biological insecticide.

Romanomermis culicivorax does have potential as a biological control agent of Culex and Culiseta spp. in Manitoba. These mosquitoes breed in semi-permanent or permanent habitats and are highly susceptible to infection by preparasites of R. culicivorax. They are not normally

serious pest species, but some (e.g. C. tarsalis) are responsible for the transmission of Western Equine Encephalitis. Therefore, populations of these mosquitoes only need to be maintained below epidemic levels. Established populations of R. culicivorax could contribute significantly to the control of these species, though more field experimentation is necessary before any definite recommendations can be made.

Romanomermis culicivorax appears to have its greatest potential in tropical climates, where highly susceptible Anopheles spp. are a considerable threat to human health as vectors of malaria. The added factor of resistance in mosquito populations to chemical insecticides contributes to the urgency of the development of alternative control methods.

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APPENDIX I

Mosquito species infected by *Romanomermis culicivorax*, either in the laboratory or in the field, during the present study (1973-1977) and their relative susceptibility as hosts

Species	Melanization	Susceptibility	Laboratory infection	Field infection
Wyeomyia smithii (Coquillett)	-	++	+	-
Culiseta inornata (Williston)	-	++++	+	+
Aedes (Ochlerotatus) canadensis (Theobald)	+	++	-	+
Aedes (O.) communis (Degeer)	+	++	+	+
Aedes (O.) dorsalis (Meigen)	-	++	+	-
Aedes (O.) flavescens (Muller)**	-	+	+	-
Aedes (O.) pionips Dyar	-	++	-	+
Aedes (O.) spencerii (Theobald)	-	+	+	-
Aedes (O.) sticticus (Meigen)	-	++	+	+
Aedes (O.) trichurus (Dyar)**	-	++	+	-
*Aedes (Finlaya) atropalpus** (Coquillett)	-	+++	+	-
Aedes (F.) epactius Dyar and Knab	-	++	+	-
Aedes (Stegomyia) aegypti Linnaeus	-	++	+	-
Aedes (Aedimorphus) vexans (Meigen)	+	++	+	+
Aedes (Aedes) cinereus Meigen	-	++	+	+
Culex pipiens quinquefasciatus Say	-	++++	+	-
Culex tarsalis Coquillett	-	++++	+	+

* *A. atropalpus* larvae, although quite susceptible to infection, suffered heavy mortality as a result of penetration by *R. culicivorax* preparasites.

** Results based on limited observations.