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Studies on the Biology of Three Mermithid Parasites (Nematoda:Mermithidae) of Mosquitoes

by

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STUDIES ON THE BIOLOGY OF THREE MERMITHID PARASITES (NEMATODA:MERMITHIDAE) OF MOSQUITOES

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ABSTRACT

Temperatures of 10°C and 15°C were shown to be suitable for long term storage of postparasitic juvenile Romanomermis culicivorax Ross and Smith, 1976. Age groups of 24, 48, 96, and 144 hour old juveniles were investigated. An age difference of up to 96 hours was found not to be a significant factor in successful storage of the material. Developmental response during the post-treatment of 26±1°C was slower for material stored at 10°C than that for material kept at 15°C.

Observations and collections of a mermithid nematode, Romanomermis communensis Galloway, 1977, were made during the springs of 1976 and 1977 at Goose Creek, Manitoba. Fifty pools were sampled and levels of infection determined. Soil samples collected from pools at Goose Creek in August, 1976 were found to contain embryonated eggs. The proposed life cycle of this mermithid is outlined.

The effects of low temperatures on the growth and development of R. communensis eggs was investigated. Nematode eggs embryonated at 10, 15, and 20°C. Eggs at 5°C failed to develop. Some hatching (<5%) was observed at 15 and 20°C but none at 10°C by the end of the 32 day study period.

Development at temperatures of 10, 15, and 20°C did not produce a synchronous hatch. There is some evidence that eggs enter a period of dormancy.

Preliminary observations and an evaluation of a Culicimermis sp. parasitic in adult Aedes vexans (Meigen) in Manitoba have been made. Material was collected from the field and levels of infection were determined. Four nematode developmental sites were found in May, 1977. Establishment of a viable laboratory colony of this nematode failed. Much of the limited juvenile material came under attack by a parasitic fungus, Catenaria anguillulae Sorokin.

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CHAPTER I

INTRODUCTION

Mermithid nematodes found parasitizing various species of mosquitoes and black flies within the past ten years have stimulated a great deal of interest among researchers in the field of biological control. The potential of many of these nematodes is just now beginning to be realized.

The development of parasitic nematodes to the stage where their usage is not only practical but economical involves a great many problems. Years of research are involved in learning about the biology of these nematodes. Life cycle, preferable hosts, possible ecological damage, and their potential as biological control agents in various habitats are areas that need to be studied.

The full potential of any parasitic nematode can only be realized if mass production and long-term storage techniques have been developed and are shown to be economical. As well, geographical and climatic limitations of mermithids may pose problems in the widespread use of any one parasitic nematode.

Biological studies included in the thesis were carried out on three species of mermithids, Romanomermis culicivorax Ross and Smith, 1976, Romanomermis communensis Galloway, 1977, and a Culicimermis sp. The objectives were: to investigate various temperatures (5, 10, 15, 20^{o}C)

with regard to their potential for the long-term storage of R. culicivorax postparasitic juveniles; to study the life cycle of R. communensis both in the field and laboratory, and determine its overwintering stage; to study the embryonation of R. communensis eggs at 5, 10, 15 and 20°C and see if a synchronous hatch could be stimulated; and to evaluate the potential of a Culicimermis sp., parasitic in Aedes vexans (Meigen) in Manitoba, as a biological control agent.

CHAPTER II

Review of Pertinent Literature

Nematodes of the family Mermithidae are known to be parasitic in many insects, spiders, crustaceans, and other invertebrates. Most of the nematodes parasitizing insects of medical and veterinary importance are mosquito and black fly mermithids (Welch 1960b). Culicidae, Simuliidae, Chironomidae, and Ceratopogonidae are some of the more common aquatic Diptera that have been found to be parasitized by mermithids.

A great deal of what is known about mermithids infecting mosquitoes has been reported by mosquito biologists with limited knowledge of nematode biology. Such reports have provided valuable information concerning the distribution and importance of mermithids, but little towards their biology or rearing (Galloway 1977).

Records of mermithid nematodes found parasitizing mosquitoes in North America date back as early as 1903 when J.B. Smith collected two preadult worms in New Jersey. These were subsequently described as Agamomermis culicis, Stiles, 1903. The first complete description based on adult material was that of Hydromermis churchillensis Welch, 1960 by Welch, (1960a), which he found infecting Aedes communis (Degeer), at Churchill, Manitoba.

To date, 11 species of mermithids have been recorded as infecting mosquitoes from 6 genera. Eight of the 11 have been described since 1970 (Galloway 1977).

Basic information concerning the life cycles of mermithids infecting mosquitoes has been provided by Iyengar (1929), Muspratt (1945, 1965) and Welch (1960a). More recently, our knowledge of mermithids as parasites of mosquitoes has expanded greatly.

Nickle (1972) noted that there are four types of life cycles found in mermithids. Two types can be described for those mermithids that parasitize mosquitoes: 1.) completion of development in the adult 2.) completion of development in the larval mosquito.

Perutilimermis culicis, Nickle, 1972 and A. canadensis (Steiner 1924) are two mermithids which develop to maturity within the adult mosquito. Much of the biological information pertaining to this type of life cycle has been presented by Petersen et al. (1967) and Galloway and Brust (1976).

Mermithid eggs hatch when flooded with water and the resulting preparasites penetrate the cuticle of early instar mosquito larvae, and enter the haemocoel. Development of the parasites in the haemocoel is very slow during the larval and pupal stages of the host. In the adult, development of the parasitic juveniles is rapid.

Petersen et al. (1967) noted that a blood meal by the female mosquito appeared to be an important factor in

the development of Perutilimermis culicis, Nickle, 1972. However, Galloway and Brust (1976) have found that a blood-meal by the host was not essential in the development of a Culicimermis sp. in Aedes vexans (Meigen), noting that it can develop in both male and female mosquitoes.

The parasitic juveniles emerge from the abdomen of the adult host and become free-living. The postparasites move into the soil substrate where they moult to adults and mate. Gravid females deposit their eggs in the soil. The eggs embryonate, and once fully developed, hatch when flooded with water.

The effect of parasitism on the host is usually fatal. Petersen et al. (1967) noted that some females can survive such an infection and subsequently take a blood meal and lay a reduced number of eggs. The overall effects of parasitism on the host include the reduction of the fat body, and the castration of the females (Steiner 1924; Petersen et al. 1967; Trpiŝ et al. 1968; Trpiŝ 1971). Effects upon male hosts are not entirely known but Rubtsov and Isaeva (1975) have indicated that infected male mosquitoes are capable of mating.

Our knowledge of mermithids that complete their life cycle in the larval host far exceeds information from those in adult hosts. The development of mass rearing techniques for Romanomermis culicivorax Ross and Smith, 1976 by Petersen and Willis (1972) facilitated extensive studies

with this mermithid nematode. As a result, a great deal has been learned about its biology and effectiveness as a biological control agent under various conditions (Chapman et al. 1968; Gordon et al. 1974; Levy and Miller 1977a, 1977b; Pao-Shu 1976; Petersen 1972, 1973a, 1973b, 1973c, 1975a, 1975b; Petersen et al. 1973).

In this type of life cycle, as well, the eggs hatch and the preparasites gain entry into the haemocoel of the larvae of the mosquito host. Development of the parasitic juveniles in the larval host is rapid. In the latter stages of development, the nematodes can be seen coiled in the thorax. The time required for development is related to temperature. Galloway (1977) has noted that, in the laboratory, R. culicivorax required as little as 5 days for development before emerging from the host, while in spring snow-melt pools an excess of 30 days was required.

The effects of this type of parasitism on the larval host includes depletion of the fat bodies, and inhibition of the development of wing pads, leg rudiments, and other preadult structures (Bailey and Gordon 1973). With the emergence of the nematode from the larva, death of the host soon follows.

Beckel and Copps (1955) and Welch (1960a) have observed mermithids, which usually complete their development in the larva, to pass into the pupal and adult stages of A. communis. Pao-Shu (1976) has reported similar

observations for R. culicivorax in Culex pipiens fatigans Wiedemann. The occurrence of this is thought to be rare, and may be related to low temperature or the stage of host infected (Galloway 1977). Its significance is that it could provide a vehicle of dispersal for the nematode parasite which is usually restricted to the larval habitat of its host.

The postparasitic juveniles move into the substrate where they will eventually moult to adults and mate. Poinar and Otieno (1974) have indicated that the postparasitic juveniles undergo a double moult to the adult stage. Females of R. culicivorax are very fecund, laying as many as 2,480 eggs in a period of 18 days (Petersen 1975b).

The embryonation of the nematode eggs proceeds in a fashion similar to that described by Poinar and Gyrisco (1962a) for Hexamermis arvalis Poinar and Gyrisco, 1962. Hatching of the fully embryonated eggs can usually be induced by flooding with water. However, in the case of R. communensis, Galloway, 1977, eggs could not be stimulated to hatch synchronously. This may be related in some way to the synchronization of the nematode's life cycle to that of its natural host, A. communis.

An interesting facet of mermithid biology is that sex is determined during parasitic development (Christie 1929). Similar observations have been made on R. culicivorax (Petersen 1972), Diximermis peterseni, Nickle, 1972

(Petersen and Chapman 1970), and Romanomermis spp. (Galloway and Brust 1976). Petersen (1972) has noted that the number of parasites per host, host species, host size, and the quantity and/or quality of food taken in during the period of parasitic development may influence the sex and number of postparasitic juveniles produced.

Those mermithids which complete their development in the adult host are more host-specific than those which develop in larvae (Petersen 1973b). Many of the mermithids which complete development in the larvae have been found parasitizing only certain species of mosquitoes, even though others may be present in the aquatic environment (Welch 1960a; Petersen et al. 1967; Petersen 1973a; Petersen and Chapman 1970; Iyengar 1929). This, in many instances, may be a result of host resistance, rather than host specificity, and may also involve such factors as larval activity in the aquatic environment, cuticle thickness of the host and synchrony of mermithid and mosquito hatch.

Host resistance may be evident in the form of melanization of the nematode by the mosquito. It has been reported by Welch (1960a) to occur in A. communis as a response to infection by H. churchillensis. Similar reports have been made about R. culicivorax being melanized in various mosquito hosts (Petersen et al. 1968; Petersen et al. 1969; Petersen 1975a, Mitchell et al. 1974).

The potential of mermithid nematodes as biological control agents of various aquatic Diptera, (e.g. Culicidae, Simuliidae) is very promising. They are found infecting natural field populations, and should produce no ecological damage, and no pollution of the environment. As well, they are relatively host specific as demonstrated by Ignoffo *et al.* (1973; 1974) for *R. culicivorax*.

By far the most extensively studied mermithid species, with regard to its potential as a biological control agent, is *R. culicivorax* (Mitchell *et al.* 1974; Petersen *et al.* 1973). *R. culicivorax* is one mermithid that can be mass reared, and at the present time is being commercially produced by Fairfax Biological Laboratories under the label of Skeeter Doom® (Nickle 1976). The *in vitro* culture of *R. culicivorax* is still in the preliminary stages of investigation (Roberts and van Leuken 1973; Finney 1976).

It is evident that before a mermithid nematode can be used as a biological control agent, mass production techniques are essential. This can only be accomplished through extensive studies of the life cycle, the preferred hosts and host-parasite interactions.

CHAPTER III

The Effects of Low Temperature Storage on Postparasitic Juveniles of *Romanomermis culicivorax*

INTRODUCTION

Romanomermis culicivorax Ross and Smith, 1976 is a biological control agent that has received considerable attention in recent years. This mermithid nematode has a wide host range: 16 species infected in nature, and 57 species in the laboratory (Petersen 1975b).

With the development of mass rearing techniques for *R. culicivorax* (Petersen and Willis 1972) considerable interest has been generated in the application of this nematode for mosquito control in various parts of the world. However, to be used effectively, large numbers of preparasites must be available on demand.

Present storage techniques for this nematode are inadequate in many respects; time material can be stored, cost of storing. The general practice at the present time is to store free-living stages in moist sand at a temperature of 27± 1°C. The sand is then flooded as needed to obtain preparasites. Cultures of the non-parasitic stages of nematodes maintained in this way reached their peak preparasite production between 11 and 16 weeks, though small numbers hatched as long as 34 weeks after the postparasites were placed in sand (Petersen 1975b).

Earlier studies by Dr. B.V. Helson, in our laboratory indicated that the postparasitic juvenile stage of R. culicivorax was the most suitable for long term storage at low temperatures. The present study was undertaken to investigate the effects of low temperature storage on four age groups of juvenile nematodes. From these results, recommendations have been made concerning the long term storage of juvenile R. culicivorax.

MATERIALS AND METHODS

The original culture of R. culicivorax was kindly supplied by Dr. J.J. Petersen, Gulf Coast Mosquito Research Laboratory, United States Department of Agriculture, Lake Charles, Louisiana.

Rearing methods used in this study were similar to those described by Petersen and Willis (1972). High levels of infection with a balanced sex ratio of postparasites were obtained by combining approximately 1800 preparasites with 300 day-1 old larvae of Culex pipiens quinquefasciatus Say. The larvae were reared in 33x23x6 cm. plastic pans containing 1 liter of chlorine-free tap water at 26± 1°C and a photoperiod of 16L: 8D. Infections were made on each of three successive days in order to stagger the emergence of postparasitic nematodes. Larvae were fed a liver powder paste every day. After six or seven days, the larvae were placed on nematode collecting trays, and at selected intervals, the trays were transferred to new pans.

In this manner, nematodes were assigned to specific age groups as explained below.

The postparasitic nematodes were separated by sex, based on morphological differences described by Tsai and Grundmann (1969). Four age groups of postparasitic nematodes were each placed at four storage temperatures: 5, 10, 15, and 20°C (Fig. 1). The age groups consisted of: 1) 12-24 hour old postparasitic juveniles hereafter referred to as 24 hour old juveniles; 2) 36-48 hour old juveniles designated as 48 hour old juveniles; 3) 84-96 hour old postparasitic juveniles designated as 96 hour old juveniles; 4) 132-144 hour old postparasitic juveniles, designated as 144 hour old juveniles.

Five replicates of each age group were placed at the four different temperatures (Fig. 1). Each replicate consisted of 25 male and 25 female postparasitic juveniles in 50 g. of boiled silica sand in a 115 cc (5 cm h, 5.5 cm d.) glass jar with a screw type lid. When the postparasitic nematodes reached the particular age designated for storage, they were placed into the glass jars with the moist sand and moved directly to their assigned storage temperatures. Replicates 1, 2, and 3 were examined after 4, 6, 9, 12, and 15 weeks of storage for all four age groups at all four temperatures. Replicates 4 and 5 were used as controls, to determine whether the results would be affected by the examining process.

The following were recorded: 1) mean per cent survival of males; 2) mean per cent survival of females; 3) mean per cent moulting of males; 4) mean per cent moulting of females; 5) mean per cent gravid females.

After 15 weeks of storage, it was noted that survival and development were best at 10 and 15°C. All 5 replicates of all age groups kept at these temperatures were placed at 26± 1°C for an additional 3 weeks. After this post-treatment period, the contents of each jar were examined to determine the mean per cent survival of males and females, the mean per cent surviving females gravid. Egg production and the presence of preparasites 24 hours after flooding was noted.

An arc sine transformation (Eisenhart 1947), was carried out on the per cent females moulting during 15 weeks at all four temperatures at all ages, the per cent females gravid after 15 weeks at all four temperatures and at all ages, and the per cent live gravid females after the three week post-treatment. The data was then analysed using a two-way analysis of variance.

RESULTS

The mean per cent survival of males of the four age groups at the four storage temperatures studied was followed over the 15 week storage period. These results are indicated in Figures 2-5. General trends are evident in these graphs.

Survival of males at 10, 15, and 20°C was high for all four ages. However, survival of all four ages at 5°C was poor.

A two-way analysis of variance was carried out on the data obtained after 15 weeks. This indicated significant variation in survival rates at the four storage temperatures. ($F= 316.6$ (d.f. 3,32) $p<0.001$). Partitioning of the sum of squares of the temperatures (Snedecor and Cochran 1967) indicated that much of the significant variation was due to the 5°C treatment ($p<0.001$) as is apparent from Figures 2-5. Variation between the remaining treatments was also significant ($p<0.05$) but this could not be further specified. No significant variation was found between the survival rates of the four age groups ($p>0.1$).

The mean per cent survival of females of all four age groups stored at the four temperatures for 15 weeks is shown in Figures 6-9. Material of the four age groups stored at 10, 15, and 20°C had high rates of survival, while material stored at 5°C showed poor survival. A two-way analysis of variance indicated a significant variation in survival between the four storage temperatures ($F= 316.5$ (d.f. 3,32) $p<0.001$). Partitioning of the sum of squares showed most of the variation was due to the 5°C temperature ($p<0.001$); the residual sum of squares was not significant ($p>0.1$). No significant variation was found between the age groups at each of the temperatures ($p>0.1$).

The mean per cent moulting is shown for both males (Table 1) and females (Figs. 10-13). At 10 and 15°C males began to moult to adults sooner than females. Males and females of all four ages stored at 20°C showed 100% moulting after four weeks. All males of age groups 48, 96, and 144 hours moulted to adults after four weeks at 15°C but the 24 hour old males were slower (Table 1). The age of the male material affected the rate of moulting throughout the duration of the 15 weeks at 10°C (Table 1). Males of all four ages stored at 5°C showed no moulting after 15 weeks.

The rate of moulting of surviving females (Figs. 10-13) was slower than that of the males, but similar trends were observed. At 10 and 15°C the rate of moulting increased with increasing age of the juvenile material, but at 5°C there was no moulting.

After an arc sine transformation was carried out on the per cent of surviving females which had moulted at 15 weeks, two-way analysis of variance was carried out. This analysis showed that there was significant variation in moulting between temperatures ($p<0.001$), and age groups ($p<0.05$) and a significant interaction between these treatments ($p<0.05$). Further analysis showed that there were only two significant effects. At 10°C fewer 24 and 48 hour old females had moulted than had those in other treatments (a partitioning of the sum of squares of that material

against all the rest ($F= 976.2$ (d.f. 1,24) $p<0.001$). Additionally at $10^{o}C$, fewer 24 hour old females than 48 hour old females had moulted. ($F= 405.4$ (d.f. 1,24) $p<0.001$). No other significant effects were found ($F= 0.66$). The proportion of surviving females which were gravid for all the treatments is shown in Figures 14-17. It is evident that the material stored at $20^{o}C$ became gravid much more rapidly than material at 5, 10, and $15^{o}C$. Two-way analysis of variance indicated a significant variation between temperatures. Partitioning the sum of squares of 10 and $15^{o}C$ versus $20^{o}C$ accounted for 91% of the variation ($F= 765.8$ (d.f. 1,24) $p<0.001$). The $5^{o}C$ treatment was not considered because of the low survival. The remaining variation was found between 10 and $15^{o}C$ ($F= 79.4$ (d.f. 1,24) $p<0.001$).

Decreased development of both juveniles and adults is evident at $15^{o}C$ (Fig. 14-17). This is manifested by the lower number of gravid females. Females of all ages stored at $10^{o}C$ for 15 weeks showed no signs of being gravid.

A significant variation due to age was found ($F= 3.89$ (d.f. 3,24) $p<0.05$). This is most evident at $20^{o}C$. Approximately 30% of the females in the 24 and 48 hour old age groups had become gravid after four weeks (Fig. 14-17). The two older age groups, 96 and 144 hours old, showed a more rapid rate of females becoming gravid at week four in storage. One would assume from Figures 14-17 that the two

younger age groups stored at 20°C had attained a higher per cent of gravid females by week 15, than did those of the two older age groups. This is not the case. Females of the two older age groups came into egg production sooner than did the 24 and 48 hour old females. By week nine some of these females had already spent their eggs and were not considered as being gravid. While females of the two younger age groups came into production slower, more remained gravid for a longer period of time, thus accounting for the higher per cent of females gravid at week 15. Therefore while the figures do not indicate it, the overall mean per cent females gravid for all four ages stored at 20°C were comparable.

Material from 10 and 15°C was subjected to a post-treatment of 26± 1°C for a three week period after which the material was again examined to determine mean per cent survival of males and females for all age groups. The mean per cent survival of males and females is shown in Table 2. One hundred per cent moulting of the surviving males and females was noted after the three week post-treatment. The per cent gravid females after three weeks post-treatment is shown in Figure 18 for material stored at 10°C, and Figure 19 for material stored at 15°C.

As mentioned above, none of the surviving females of all four age groups stored at 10°C showed any signs of being gravid. After a three week post-treatment at 26± 1°C, the

mean per cent gravid females was higher in the older age groups. (Fig. 18).

Some females of all four age groups stored at 15°C for 15 weeks showed signs of being gravid. Response to the three week post-treatment was almost uniform with 94% to 98% of the surviving females showing signs of being gravid (Fig. 19). Two-way analysis of this data indicated a significant variation between temperatures ($p<0.001$); age groups ($p<0.05$); and a significant variation between temperature and age groups ($p<0.05$). The significant variation due to age is most apparent in those groups stored at 10°C. There is a definite relationship between the age of the material and the mean per cent gravid. This accounts for a large amount of the variation between age groups ($F= 41.8$ (d.f. 1,16) $p<0.001$). The remaining residual effects were not significant ($F= 0.85$).

Observations made after the material had been flooded for 24 hours indicated that egg production was greater at 15°C than at 10°C. Preparasites were recovered from all four age groups stored at 15°C, while preparasites were recovered from only the two oldest age groups, 96 and 144 hours, stored at 10°C.

The controls were examined at the end of the 15 week storage period and were found to be comparable in number of males and females surviving, mean per cent males and females moulting to adults, and mean per cent surviving females

becoming gravid. I would conclude from this that handling, washing, counting, etc. of replicates 1, 2, and 3 every 2 or 3 weeks had no significant effect on the results obtained.

DISCUSSION

The objective of this study was to establish guidelines, and propose recommendations for the long-term storage of R. culicivorax. In determining a suitable storage temperature, I felt that certain criteria must be met. Of primary importance was the survival of juveniles, adults and eggs. Secondly, during storage, the rate of sexual maturation of the juvenile nematodes should be inhibited. Thirdly, after storage, the material must resume normal activity when returned to higher temperatures, e.g. 27°C. Moulting, mating survival, and the rate of females becoming gravid therefore were major factors to be considered here, while egg production and the viability and the infectiveness of preparasites were also important factors in the evaluation of storage treatments.

Nematodes of all ages stored at 5°C showed a rapid decline in survival during the 15 weeks, thus not meeting one of the essential criteria of a suitable storage temperature. In addition to this those that did survive showed no signs of moulting or becoming gravid. Based on these results, 5°C was ruled out as being a suitable storage temperature for R. culicivorax postparasitic juveniles.

Nematodes of all ages stored at 20°C showed high rates of survival during the 15 weeks of storage, thus meeting one of the major criteria to be considered a suitable storage temperature. Males and females of all four ages, however, showed a rapid rate of moulting, with some females becoming gravid after only four weeks. Although the 20°C treatment met the criteria in one respect, it fell short of being considered a suitable long-term storage temperature. The rate of sexual maturation was not retarded enough to be considered adequate.

From this study, two temperatures, 10 and 15°C, appeared the most suitable for storage of all four age groups of R. culicivorax juveniles. At these two temperatures, survival is good, and the rate of sexual maturation is retarded. This is evident in both the rate of moulting and in the number of females becoming gravid during the 15 weeks.

Since both 10 and 15°C are suitable for long-term storage of R. culicivorax juveniles, other factors should be considered when selecting either one or the other temperature. At 15°C all four age groups performed well during storage and post-treatment periods. Nematodes of all four ages showed a uniform response to the post-treatment period, with respect to numbers being gravid, egg production, and the viability and infectiveness of the preparasites produced. Nematodes of all four ages

stored at 10°C in one way performed better than those at 15°C. None of the females at 10°C became gravid during the 15 weeks. However, the response of these nematodes to the post-treatment was not as uniform as in the case of the material stored at 15°C. The age of the material stored at 10°C definitely affected the rate at which the females became gravid during the post-treatment period. The production of eggs was greater in the 96 and 144 hour old groups and the preparasites recovered from them were found to be viable and capable of infection. At the time the material was checked, eggs were still in the process of embryonating at the 24 and 48 hour old age groups, therefore preparasites were not available for examination. This is not to say that the preparasites from these groups would not be just as viable and infective as the others.

It is possible from these results to project what might occur when nematodes are stored at 10 and 15°C for 20-30 weeks. Material stored at 15°C would undoubtedly continue to mature sexually. The rate at which the females are becoming gravid would increase. Whether or not egg laying would occur is questionable. At this temperature, there may be some inhibition to egg laying. If eggs were laid, then the rate of embryonation would be retarded.

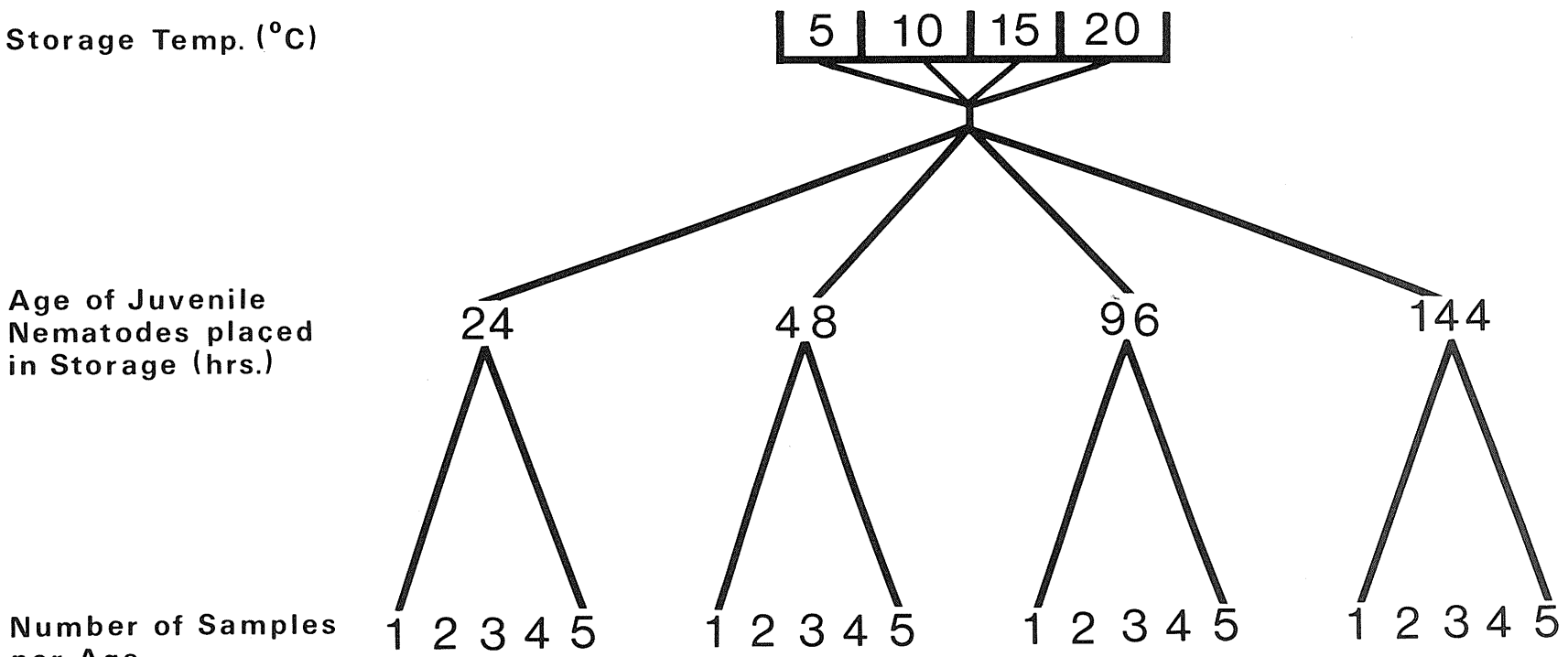
Material stored at 10°C for 15 weeks showed no signs of being gravid. However, if extended over a longer period, females might become gravid. There is however the possibility that mating is inhibited at this temperature and

therefore females would not become gravid. Survival at both these temperatures was good over 15 weeks of storage and there is no reason to suspect that it would not continue for up to 20-30 weeks.

The suitability of either 10°C or 15°C as storage temperatures is dictated by the requirements of the user. If a more gradual build up in the number of preparasites produced is desired, then storage of juvenile material at 10°C would be more suitable. If a quicker and more uniform response is desired, reaching full egg and preparasite production with 3-4 weeks following storage, then storage of juvenile nematodes at 15°C would be recommended.

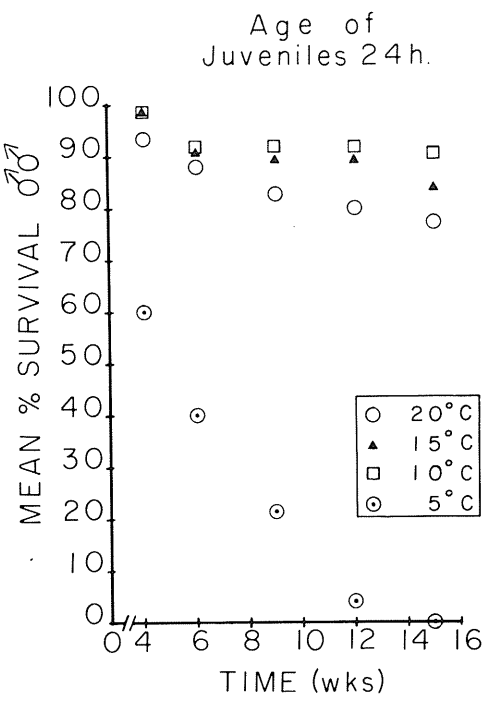
Figure 1

Diagram of the organization of the storage temperature study.

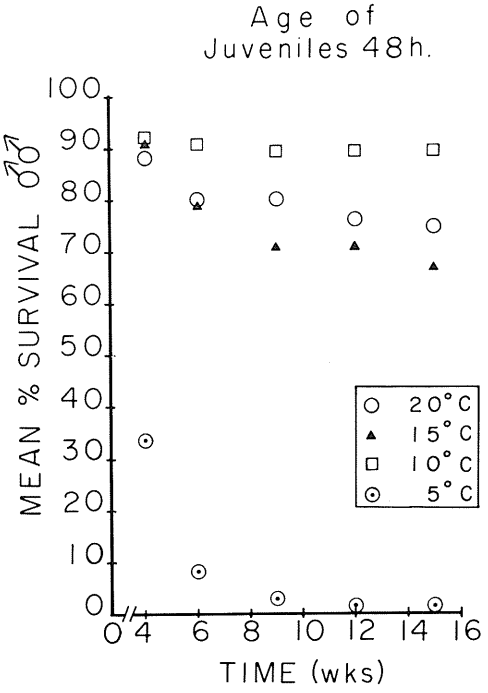

Storage Temp. (°C)
5
10
15
20
Age of Juvenile Nematodes placed in Storage (hrs.)
24
48
96
144
Number of Samples per Age
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5

Figures 2-5

Mean per cent survival of 24, 48, 96, and 144 hour old male R. culicivorax juveniles placed at four storage temperatures, 5, 10, 15, and 20°C, for 15 weeks.


Age of
Juveniles 24h.
MEAN % SURVIVAL ♂♂
100
90
80
70
60
50
40
30
20
10
0
0 4 6 8 10 12 14 16
TIME (wks)
20°C
15°C
10°C
5°C

2


Age of
Juveniles 48h.
MEAN % SURVIVAL ♂♂
100
90
80
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0 4 6 8 10 12 14 16
TIME (wks)
20°C
15°C
10°C
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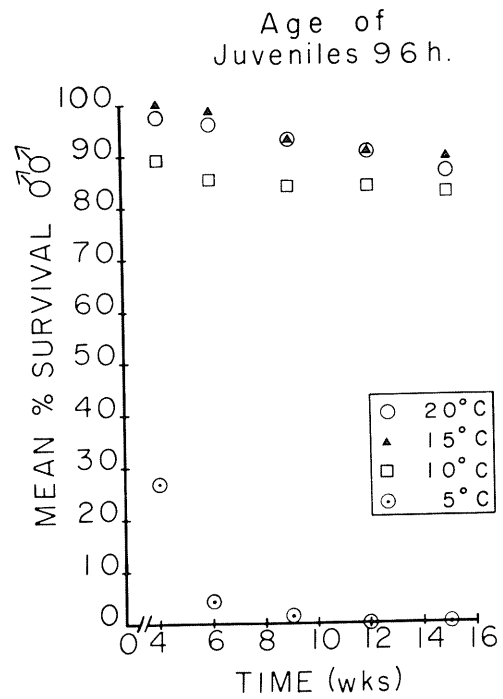
3


Age of
Juveniles 96h.
MEAN % SURVIVAL ♂♂
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TIME (wks)
20°C
15°C
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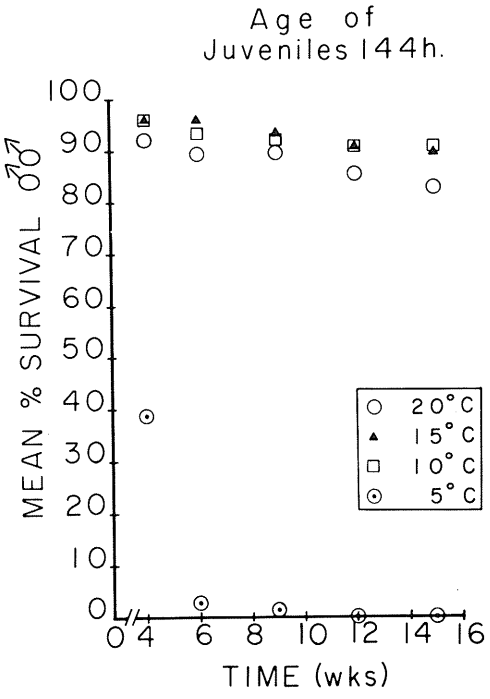
5°C

4


Age of
Juveniles 144h.
MEAN % SURVIVAL ♂♂
100
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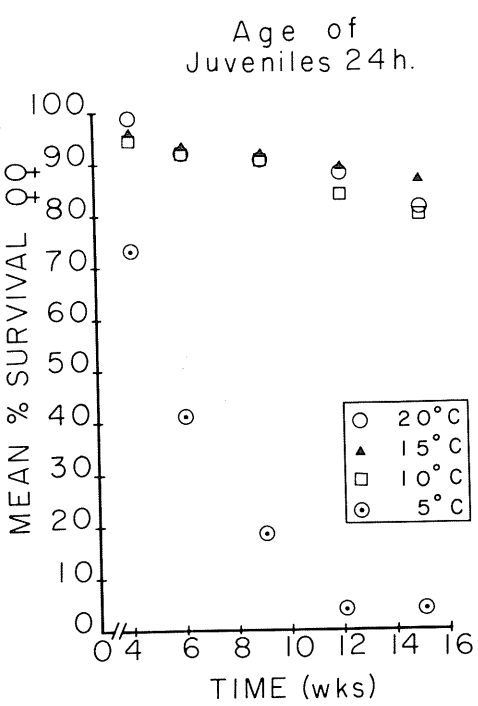
0 4 6 8 10 12 14 16
TIME (wks)
20°C
15°C
10°C
5°C

5

Figures 6-9

Mean per cent survival of 24, 48, 96, and 144 hour old female R. culicivorax juveniles placed at four storage temperatures, 5, 10, 15, and 20°C for 15 weeks.


Age of
Juveniles 24h.
MEAN % SURVIVAL ♀♀
100
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80
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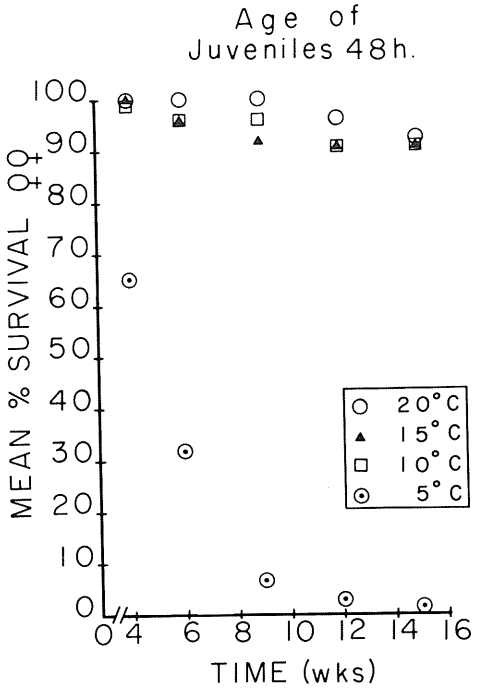
0 4 6 8 10 12 14 16
TIME (wks)
20°C
15°C
10°C
5°C

6


Age of
Juveniles 48h.
MEAN % SURVIVAL ♀♀
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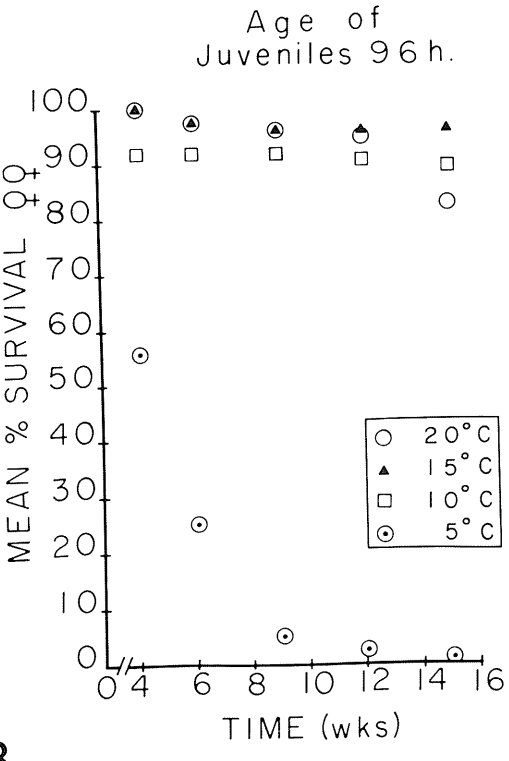
0
0 4 6 8 10 12 14 16
TIME (wks)
20°C
15°C
10°C
5°C

7


Age of
Juveniles 96h.
MEAN % SURVIVAL ♀♀
100
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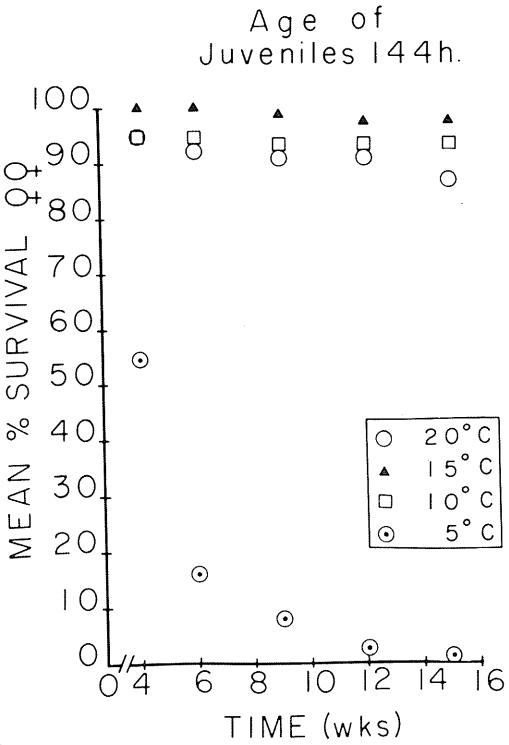
TIME (wks)
20°C
15°C
10°C
5°C

8


Age of
Juveniles 144h.
MEAN % SURVIVAL ♀♀
100
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80
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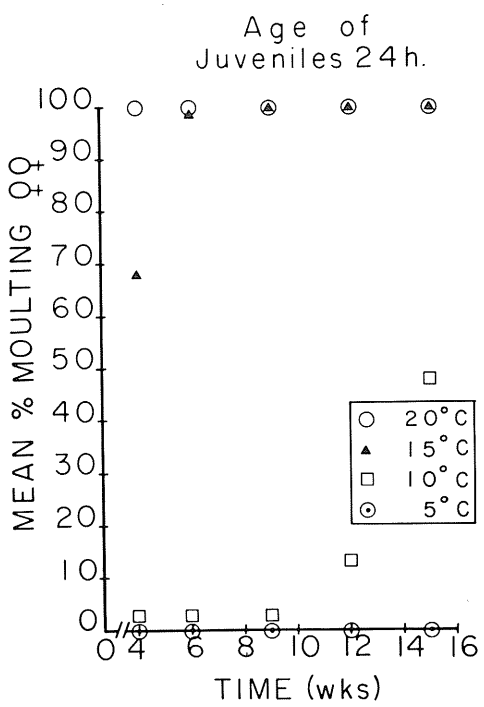
0 4 6 8 10 12 14 16
TIME (wks)
20°C
15°C
10°C
5°C

9

Figures 10-13

Mean per cent surviving R. culicivorax females which moulted within the four age groups (24, 48, 96, 144 hours) and the four temperatures (5, 10, 15, 20°C).


Age of
Juveniles 24h.
MEAN % MOULTING ♀♀
100
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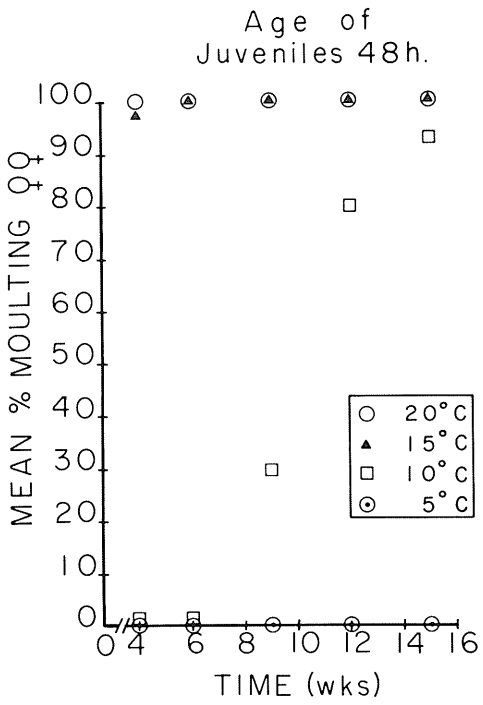
0 4 6 8 10 12 14 16
TIME (wks)
20°C
15°C
10°C
5°C

10


Age of
Juveniles 48h.
MEAN % MOULTING ♀♀
100
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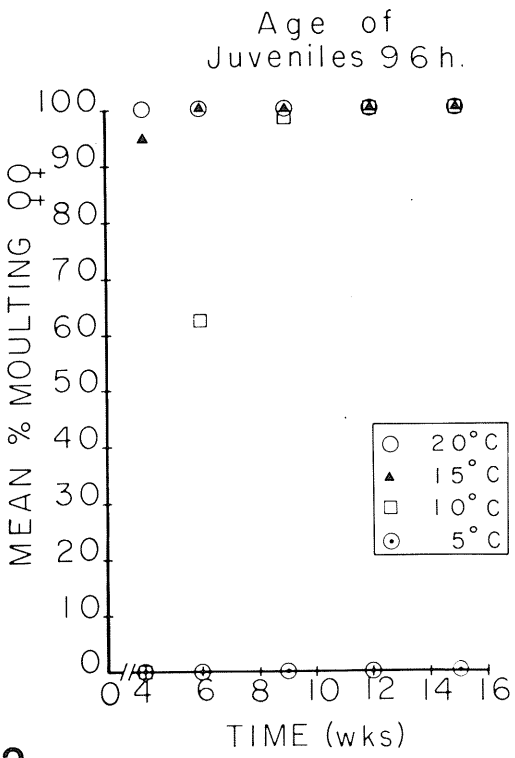
0 4 6 8 10 12 14 16
TIME (wks)
20°C
15°C
10°C
5°C

11


Age of
Juveniles 96h.
MEAN % MOULTING ♀♀
100
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80
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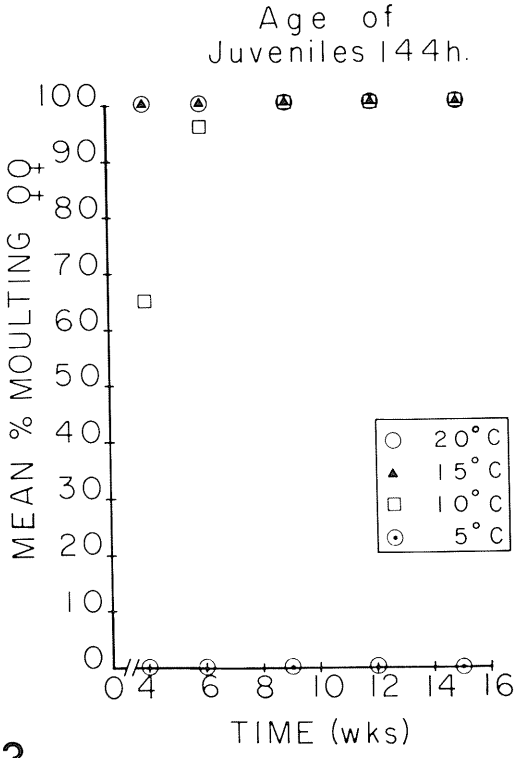
0 4 6 8 10 12 14 16
TIME (wks)
20°C
15°C
10°C
5°C

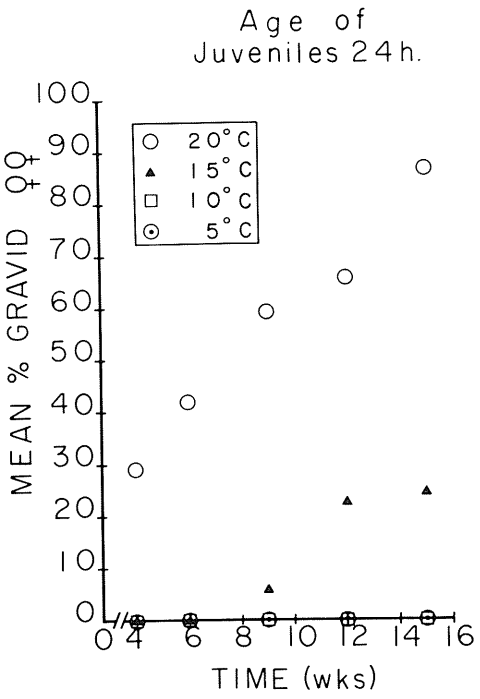
12


Age of
Juveniles 144h.
MEAN % MOULTING ♀♀
100
90
80
70
60
50
40
30
20
10
0
0 4 6 8 10 12 14 16
TIME (wks)
20°C
15°C
10°C
5°C

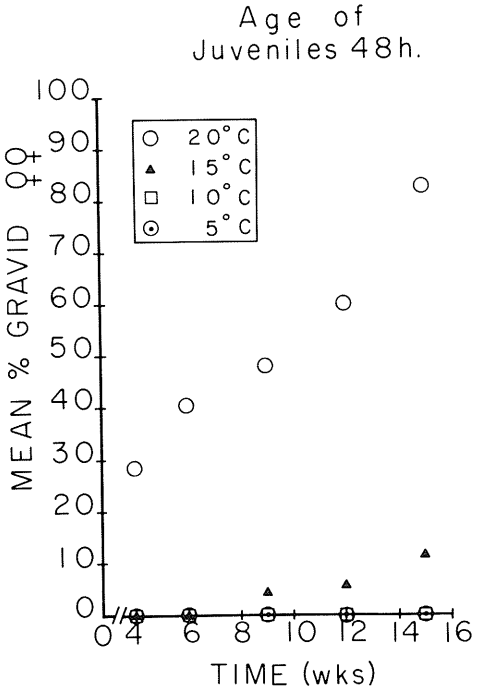
13

Figures 14-17

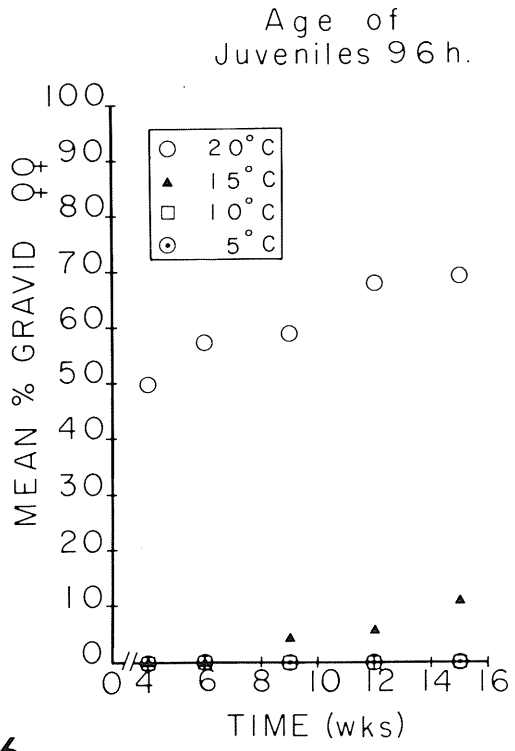
Mean per cent of surviving R. culicivorax females which are gravid within the four age groups (24, 48, 96, 144 hours) and the four temperatures (5, 10, 15, 20°C).


Age of
Juveniles 24h.
MEAN % GRAVID ♀♀
TIME (wks)
20°C
15°C
10°C
5°C

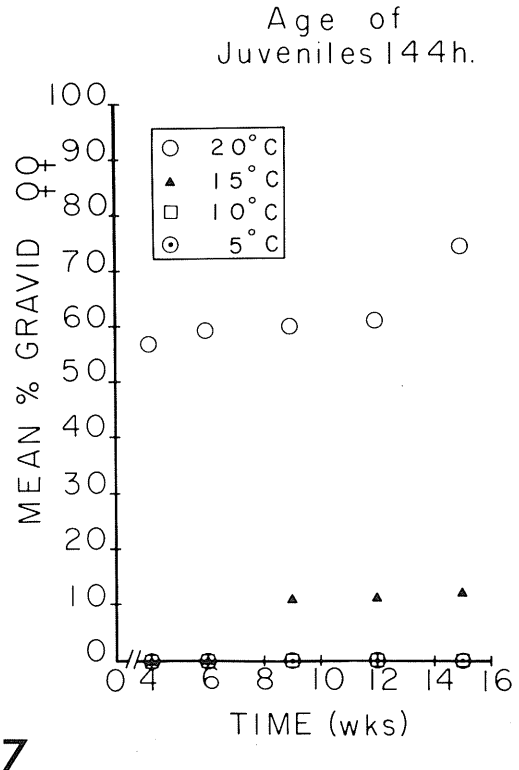
14


Age of
Juveniles 48h.
MEAN % GRAVID ♀♀
TIME (wks)
20°C
15°C
10°C
5°C

15


Age of
Juveniles 96h.
MEAN % GRAVID ♀♀
TIME (wks)
20°C
15°C
10°C
5°C

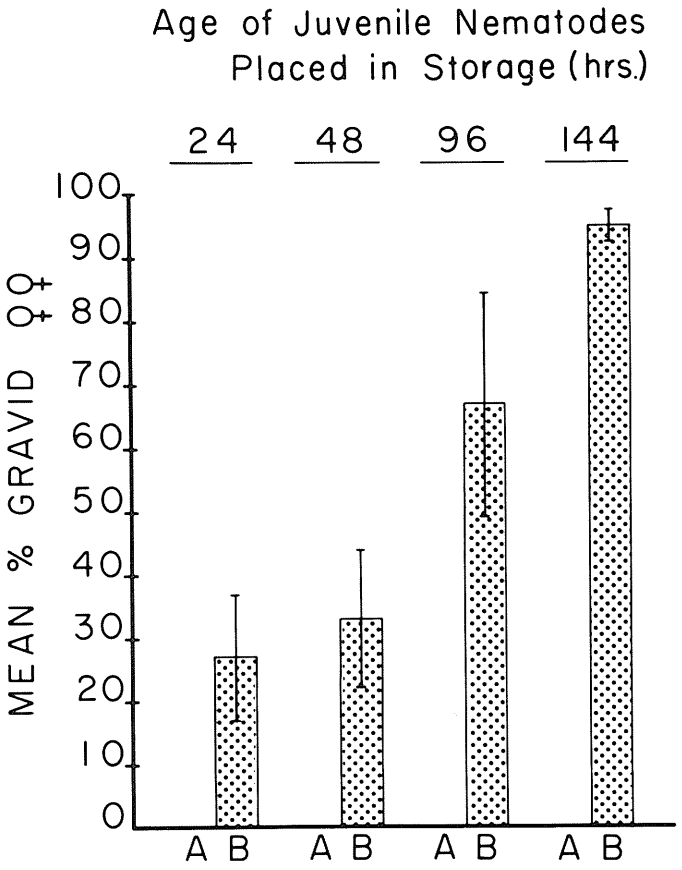
16


Age of
Juveniles 144h.
MEAN % GRAVID ♀♀
TIME (wks)
20°C
15°C
10°C
5°C

17

Figures 18 and 19

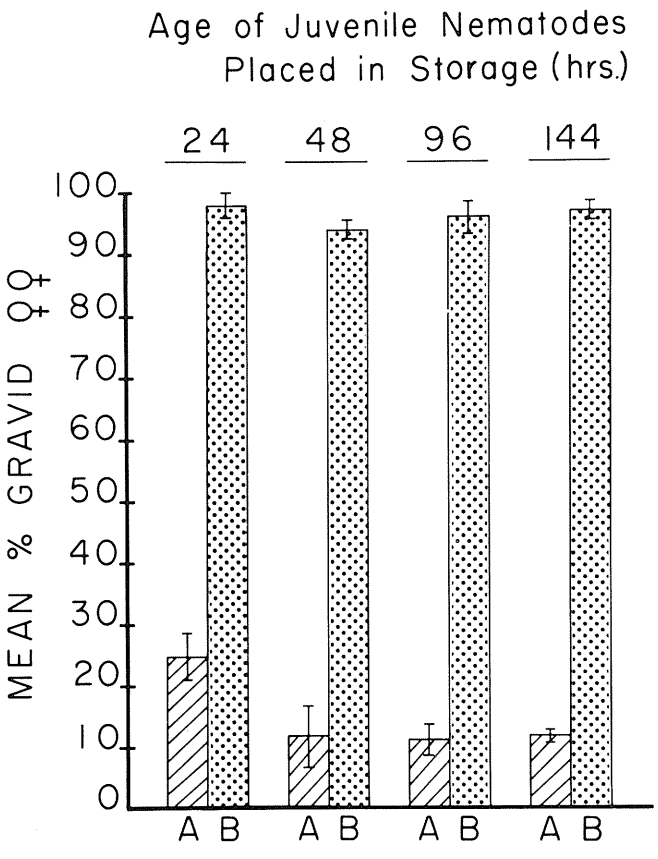
Mean per cent surviving R. culicivorax females which are gravid after 15 weeks storage at 10°C and 15°C, and three week post-treatment at 26± 1°C.



A= 15 wks. at 10°C
B= Same Material after 3 wks. at 26±1°C

Mean % Calculated from Surviving ♀♀

18



A= 15 wks. at 15°C
B= Same Material after 3 wks. at 26±1°C

Mean % Calculated from Surviving ♀♀

19

Table 1

Mean % male R. culicivorax juveniles moulting to adults during 15 weeks storage at 5, 10, 15, 20°C.

Wks. in Storage	4				6				9				12				15			
Temperature °C	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Age of Juveniles when placed in storage (Hrs.)	(%)				(%)				(%)				(%)				(%)			
24	0	0	97.3	100	0	4.8	100	100	0	5.1	100	100	0	49.6	100	100	0	81.9	100	100
48	0	1.7	100	100	0	17.2	100	100	0	88.9	100	100	0	94.3	100	100	0	100	100	100
96	0	13.7	100	100	0	79.2	100	100	0	100	100	100	0	100	100	100	0	100	100	100
144	0	84.7	100	100	0	100	100	100	0	100	100	100	0	100	100	100	0	100	100	100

Table 2

Mean % survival of male and female R. culicivorax of all four age groups (24, 48, 96, 144 hrs), stored at 15 and 10°C for 15 weeks (A), and after a 3 week post-treatment (B)

A = after 15 wks. in storage
B = after 3 wks. in post-treatment

Age of Juvenile Nematodes placed in Storage (hrs.)----		24				48				96				144			
Time (wks)--------		A		B		A		B		A		B		A		B	
		♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
15°C	no./25	23	21	23	20	18	24	18	22	24	23	22	23	24	24	24	22
		21	22	21	18	14	23	14	23	24	24	22	24	24	24	24	22
		19	22	19	18	18	21	17	21	19	25	19	25	19	25	19	23
	$\bar{X}$	21.0	21.7	21.0	18.7	16.7	22.7	16.7	22.0	22.3	24.0	21.0	24.0	22.3	24.3	22.3	22.3
	Mean % Survival	84.0	87.0	84.0	75.0	67.0	91.0	65.0	88.0	89.0	96.0	84.0	96.0	89.0	97.0	89.0	89.0
10°C	no./25	20	19	19	18	21	22	18	21	24	24	24	23	25	23	25	23
		24	16	24	15	24	21	23	21	21	21	21	20	23	25	22	25
		25	25	24	23	23	25	22	24	17	22	15	20	20	23	20	23
	$\bar{X}$	23.0	20.0	22.3	18.7	22.7	22.7	21.0	22.0	20.7	22.3	20.0	21.0	22.7	23.7	22.3	23.7
	Mean % Survival	92.0	80.0	89.0	75.0	89.0	91.0	84.0	88.0	83.0	89.0	80.0	84.0	91.0	93.0	90.0	93.0

CHAPTER IV

Field Observations on *Romanomermis communensis* Parasitizing *Aedes communis* (Diptera:Culicidae) at Goose Creek, Manitoba

INTRODUCTION

Jenkins and West (1954) noted that chemical control methods have met with limited success, in regions where mosquitoes are very abundant but where the larvae are widely dispersed and not concentrated such as occurs in the arctic and subarctic. In such regions natural control methods using parasites and predators would be valuable if practical.

Reports by Jenkins and West (1954), and Beckel and Copps (1955) of a mermithid nematode found parasitizing *Aedes communis* (DeGeer) larvae and adults stimulated further research to be conducted in the Churchill area of Manitoba. Welch (1960a), working at the Goose Creek area near Churchill during the summers of 1952, and 1953, described a new species of mermithid nematode, *Hydromermis churchillensis*. This was found parasitizing *A. communis* larvae near Goose Creek. More recent research by Dr. T.D. Galloway during the springs of 1974, 1975 and 1976 in the same area near Goose Creek revealed the presence of a mermithid nematode infecting *A. communis* larvae that was different from that described by Welch (1960a). This was a *Romanomermis* sp. and has been described as *Romanomermis*

communensis n. sp. by Galloway (1977). Various observations have been made on R. communensis since 1974. However, in this particular section, only the field observations made during the springs of 1976 and 1977 will be discussed.

MATERIALS AND METHODS

Based on temperature and precipitation records for the Churchill area, approximate hatching dates were determined for A. communis larvae during the springs of 1976 and 1977. The use of such records in determining the development of Aedes mosquitoes in this area was pioneered by Haufe and Burgess (1956) and Haufe (1957). It has been estimated that the hatching of A. communis in the Churchill area is two to three weeks later than the hatching of A. communis in southeastern Manitoba (Brust, pers. comm.). From these dates, the time most suitable for observations and collections of the mermithid nematode was determined.

A base camp was established at the north branch of Goose Creek, approximately 12 miles south of Churchill, Manitoba. During the period June 5-22, 1976 observations and sampling of pools were conducted in an 8 to 10 acre area just east of mile 498.2 on the Hudson Bay Railway (Fig. 20).

A large number of the pools sampled were in an area that had experienced forest fire damage during the early 1950's (Fig. 21). The vegetation present in the area was typical of that found growing in the transitional area

between boreal forest and tundra. Scrub spruce (Picea sp.) and various willows (Salix spp.) were predominant, while ground or dwarf birch (Betula glandulosa) were also abundant. Various grasses (Poa spp.) and sedges (Eriophorum spp.) dominated the low ground cover.

HABITAT. A. communis, a woodland species of mosquito, were found in typical breeding sites (Haufe 1957). Fifty snow-melt pools in boreal and transitional forest areas were staked, and tagged, and samples from each pool of approximately 30-40 mosquito larvae were collected by dipping. These were transferred to styrofoam cups for transport to the base camp. From the dissection of these preliminary samples, estimates of the per cent infection, the distribution of the nematode population, and the determination of the stage of development of nematodes were made. Mosquito larvae collected at the time were in second and third instars. The most abundant species found in the 50 pools sampled was A. communis. However, small populations of A. churchillensis Ellis and Brust, A. cinereus Meigen, A. diantaeus Howard, Dyar, and Knab, A. fitchii (Felt and Young), and A. pionips Dyar were also found to be present.

Based on preliminary dissections and levels of parasitism found in 1976, additional mosquito larvae were collected from those pools showing the highest levels of parasitism. Nematodes were allowed to develop normally and

as they emerged from the host, were sexed and placed in pans of moist silica sand for the return trip to Winnipeg. The total number of nematode juveniles obtained was 1,038: 660 males and 378 females.

Observations made during the spring of 1977 were not as extensive as those made in 1976. Conditions at the field site were much further advanced than had been estimated based on precipitation and temperature records for that area. Arriving on June 2, 1977 at the Goose Creek site, the area was considerably drier than previously. Adult mosquitoes were flying and seeking a blood meal. Some pupae and fourth instar larvae were present in the pools and parasitized larvae were obvious, as nematodes could be seen coiled in the thoracic region.

The nine pools which had shown the highest levels of infection in 1976 were selected for sampling. Approximately 40-50 larvae were collected from each pool and placed in styrofoam cups for the return trip to Winnipeg. Temperature readings of the pools were made at the time of sampling.

In the laboratory, nematodes were allowed to emerge from their hosts. These were sexed and placed in a pan of moist silica sand and placed at 20°C. A total of 215 nematode juveniles were obtained in 1977, 165 males and 50 females.

Soil samples were taken by Dr. R.A. Brust in August, 1976 from six pools previously shown to contain infected larvae. A total of 19 soil samples each approximately 85 cubic inches in size were taken: 10 from a depth of 0-2 inches, and 9 from a depth of 2-4 inches. These were obtained from the bottom and along the sides of these six pools. The samples were then returned to Winnipeg. Using a salt floatation method, sub-samples of each soil sample were examined for nematode eggs and preparasites.

RESULTS AND DISCUSSION

Incidence of Parasitism

Dissections made in 1976 showed that A. communis was the species with the greatest level of infection by R. communensis. Only 20 of the 50 pools sampled contained parasitized larvae. Infection rates in these 20 pools ranged from 3.3% to 95.5% (Table 3).

The percentage of parasitism in the nine pools sampled in 1977 ranged from 5% to 60%. However, these values are not a true indication of the actual levels of parasitism in the pools. Infected larvae are always slower in developing, and due to the precocious spring, many of the uninfected larvae had already pupated and the adults had emerged. The result was that there was a higher percentage of parasitism in the remaining larvae, and this did not serve as a suitable comparison with the 1976 records where sampling was done before pupation and emergence occurred.

Although the true percentage of infection could not be compared with 1976, the results do indicate the persistence of nematodes from one year to the next in the same pools (cf. Tables 3 and 4).

Many of the usual mosquito breeding habitats were dry at the Goose Creek site in 1977. Precipitation records for the Churchill area indicated above normal precipitation from November 1, 1976 to April 1, 1977. However, drier conditions prevailed from April 1, 1977 to June 1, 1977, with the Churchill area receiving only a total of 22.8 mm of precipitation, compared to a normal of 53.3 mm.

Based on climatic conditions at the site in 1977, development of the larvae was approximately three weeks ahead of normal. Pool temperatures recorded at the time of sampling averaged 19.1°C in the mid-afternoon, while air temperatures at this time were 9-10°C at the site. The importance of solar radiation in heating the pools of that area cannot be overstressed. In the future, when predicting larval development, the hours of sunshine per day may be an important factor to take into consideration.

Distribution of the Nematode

The nematode distribution in the 8 to 10 acre area near Goose Creek was rather irregular. Some pools of larvae exhibited high levels of parasitism while others adjacent to it showed no signs of parasitism or showed parasitism at much lower levels (Table 3). Similar observations have

been noted concerning other mermithid nematode parasites (Chapman et al. 1968; Petersen et al. 1968; Welch 1960a).

Development of the Nematode

Parasitic development of the nematode in the second and third instar host at the time of dissection was found to be variable, ranging from early, mid, and late crescent stages to more advanced parasitic stages of development.

Life Cycle

The life cycle of R. communensis is very similar to that described for H. churchillensis (Welch 1960a). Based on laboratory studies of this particular nematode, it is believed that the overwintering stage of the nematode is is the embryonated egg (see Ch. V). Eggs were found in soil samples taken from the bottom and along the sides of A. communis breeding sites in August, 1976. Embryonated nematode eggs were found to be present in soil samples taken from pools no. 34 and no. 48, at depths of 2-4 inches (Table 5). The presence of embryonated eggs in the pool soil in mid-August suggests this as the stage of over-wintering.

The exact stimulus for hatching is not known but it is thought to be associated with spring flooding conditions, e.g. snow-melt and rain forming pools in the area.

Hatching of the eggs releases the active free-living, preparasitic stage of the nematode into pools where newly

hatched first instar A. communis larvae are present. Contact between the parasite and the mosquito host may occur randomly, but once contact is made, it is hypothesized that the nematode preparasite becomes attached by a sticky substance which is exuded from the anterior end of the parasite (Galloway, pers. comm.).

Cuticular penetration by the parasite then takes place, with the nematode gaining entry into the haemocoel of the host. When entry has been gained, the once active preparasite becomes less active, and begins to absorb nutrients, e.g. body fluids, from the host. Multiple parasitism is quite common, and will influence the parasite's rate of development as well as the sex ratio. Petersen et al. (1968) and Petersen and Chapman (1970) noted the influence that multiple parasitism had on the sex ratios of Dixiermis peterseni Nickle, 1972 and in Romanomermis culicivorax Ross and Smith, 1976, in mosquitoes: as the number of nematodes per host increased, the ratio of males to females increased.

Parasitic nematodes are quite visible in the late fourth instar host and can be seen coiled in the thoracic region. Emergence of parasitic nematodes from the host usually occurs from late fourth instar, just prior to pupation. As a result, the host is killed due to loss of body fluids.

The postparasitic nematodes, are free-living, and

do not feed. These nematodes moult to adults, mate, and the females will deposit eggs in the soil at the bottom and along the sides of the larval pools. Welch (1960a) noted that the growth of the nematode is proportional to that of the host. It is therefore likely that factors influencing mosquito larval growth, e.g. food and temperature, will ultimately influence nematode size, fecundity, and sex ratio.

In 1976 it was estimated that parasitic development took approximately four to five weeks under field conditions. The entire life cycle can be completed only once a year in the Goose Creek area. A. communis is the only known host of the nematode, and this species hatches but once a year.

Effects of the Parasite on the Host

The presence of parasitic nematodes in the mosquito larva has the overall effect of delaying the larva's development. The nematode obtains its nourishment from the larva's body tissues. Depletion of the fat bodies is commonly observed. Development of leg rudiments, wing pads, and other pre-adult structures are prevented. Nematodes are usually visible in the fourth instar, coiled in the thoracic region. By this time the body of the host is nothing more than a cuticular sac of haemolymph with a gut system running the length of it.

The parasitized larvae are usually arrested at the fourth instar and fail to pupate. Beckel and Copps (1955)

and Welch (1960a) both reported finding low levels of parasitism occurring in A. communis adults. Dissections of pupae made in 1976 revealed the presence of melanized parasitic nematodes at low levels. The occurrence of nematodes passing into the adult mosquito is rare. However, when it does occur this might in part explain how the nematode parasites are moved from one breeding site to another. In addition, extreme flooding conditions which occur from time to time in the area may also account for movement of the nematode from one site to another.

Effects of the Host of the Parasite

Melanization of the parasite by the host in early larval instars has been recorded by Welch (1960a) and Petersen et al. (1968). Observations made in the spring of 1976 indicated a high incidence of melanization in larvae in some of the pools sampled, ranging from 8.3% to 33.3% (Table 3). Similar data was not obtained in 1977 as the nematodes had already begun to emerge from the larvae at the time of arrival at the field site, and no dissections were made.

Alternative Hosts

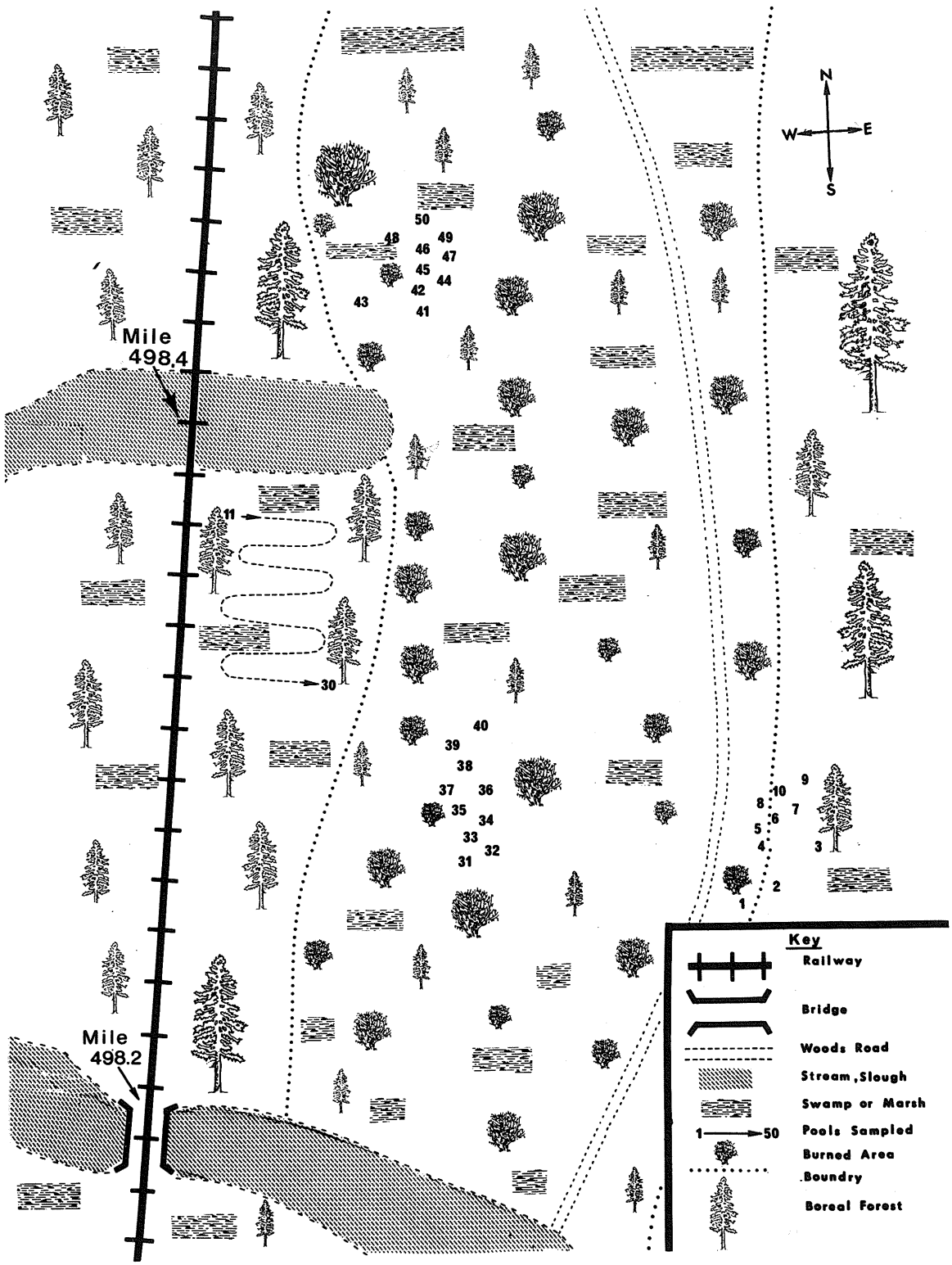
Welch (1960a) indicated that although larvae of A. punctor (Kirby), A. excrucians (Walk.) and A. pionips

were present in samples containing infected A. communis, none were found to be infected. Similar observations were made in 1976 and 1977 as small uninfected populations of A. cinereus, A. diantaeus, A. excrucians, A. fitchii, and A. pionips were also present in pools containing infected A. communis/churchillensis larvae. Host susceptibility and/or host resistance have often been cited as likely reasons for such behaviour (Petersen and Willis 1976; Petersen and Willis 1974; Petersen et al. 1969). However, in my study it may be due to the fact that the other mosquito species present in the pools hatched at a later date than did A. communis/churchillensis, when the nematode was no longer active in the pools.

This particular nematode does show promise as an effective biological control agent of some spring Aedes species of mosquitoes. However, further investigations are required into its life cycle, particularly into what stimuli are required to obtain a synchronous hatch of nematode eggs. Until such information can be obtained, its overall potential cannot be assessed.

Figure 20

Map of the Goose Creek area where observations and samples of nematodes were taken during 1976 and 1977.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45
46
47
48
49
50
Key
Railway
Bridge
Woods Road
Stream, Slough
Swamp or Marsh
1 → 50 Pools Sampled
Burned Area
Boundry
Boreal Forest

Figure 21

Photograph of sampling area at Goose Creek, taken in June, 1976. Area burned in 1952.

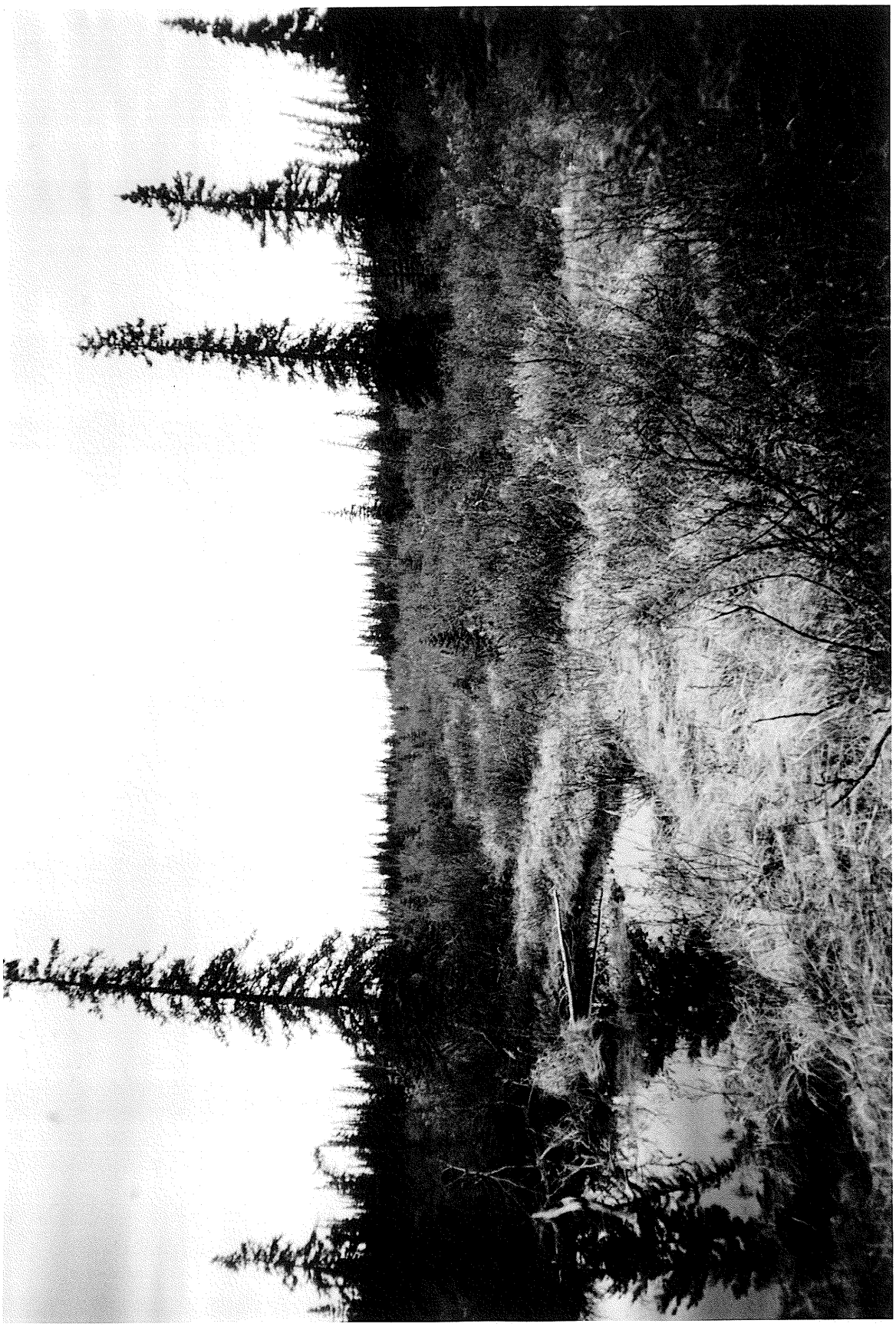


Table 3

Per cent parasitism and melanization of *R. communensis* in *A. communis* larvae in pools sampled at Goose Creek in June, 1976*

Pool #	% of larvae parasitized	% melanization in those larvae parasitized
G 76- 1	0	0
- 2	3.3	0
- 3	20.0	16.7
- 4	10.0	0
- 5	6.7	0
- 6	0	0
- 7	20.0	16.5
- 8	3.3	0
- 9	53.0	33.3
- 10	40.0	8.3
- 11	40.0	33.0
- 12	0	0
- 13	0	0
- 14	0	0
- 15	0	0
- 16	0	0
- 17	0	0
- 18	0	0
- 19	0	0
- 20	0	0
- 21	6.7	0
- 22	0	0
- 23	0	0
- 24	0	0
*- 25	-	-

*Pool not sampled.

Continued.....

Table 3 cont'd.

Pool #	% of larvae parasitized	% melanization in those larvae parasitized
- 26	0	0
- 27	0	0
- 28	0	0
- 29	0	0
- 30	0	0
- 31	0	0
- 32	0	0
- 33	52.4	0
- 34	74.0	10.5
- 35	0	0
- 36	0	0
- 37	0	0
- 38	0	0
- 39	3.3	0
- 40	0	0
- 41	93.3	0
- 42	95.5	0
- 43	33.3	0
- 44	63.3	0
- 45	0	0
- 46	63.3	0
- 47	26.3	0
- 48	80.0	0
- 49	0	0
- 50	0	0

Table 4

The presence or absence of parasitized *A. communis* larvae in pools sampled at Goose Creek in June, 1977

\+ present
\- absent

Pool #	Parasitism
G 76-9	+
-10	+
-11	+
-33	+
-34	+
-42	+
-44	+
-46	+
-48	+

Table 5

The presence or absence of *R. communensis* eggs in soil samples taken from pools in Goose Creek area in August, 1976

+ present
- absent

Pool #	# Samples	Depth	Nematode eggs
G-76-11	1	2-4''	-
G-76-34	4	2(0-2")	-
		2(2-4")	+
G-76-42	2	1(0-2")	-
		1(2-4")	-
G-76-44	4	2(0-2")	-
		2(2-4")	-
G-76-46	4	2(0-2")	-
		2(2-4")	-
G-76-48	4	2(0-2")	-
		2(2-4")	+

CHAPTER V

The Effect of Low Temperature on Embryonation of *Romanomermis communensis*

INTRODUCTION

Field observations and the potential of *R. communensis* have been discussed in the previous chapter. As indicated, much needs to be learned about its life cycle, and the stimuli involved in hatching the eggs.

This study was undertaken to determine the following: 1) if embryonation occurs at low temperatures, 2) in what stage of development the nematode overwinters, and 3) at what temperature a synchronous hatch of nematode eggs might be obtained. Temperatures of 5, 10, 15, and 20°C were investigated in this study.

MATERIALS AND METHODS

Nematode eggs obtained from nematodes collected at the Goose Creek site during June, 1976, were used in this study. As previously indicated, 1,038 nematodes were collected, 660 males and 378 females. These were placed in pans of moist sand and returned to Winnipeg by rail. The material in sand was placed at 20°C with a photoperiod of 16L: 8D in the laboratory 4-5 days after collection. The material was periodically sampled to determine the stage of development of the postparasitic nematodes. Gravid females were found to be present after approximately three weeks at 20°C. From this stock material, fifty gravid

females were selected and placed in a small petri dish containing 20 ml. of chlorine-free tap water and maintained at 20°C. Following a 4 hr. oviposition period, the females were removed from the petri dish. The number of eggs laid was then estimated and the volume of chlorine-free tap water was increased to give approximately 25-40 nematode eggs per milliliter. One milliliter of this water was pipetted into each of 8 wells, each 6 mm deep and 19 mm in diameter, on 9x12 cm plexiglass plates. The wells were covered with coverslips and sealed with vaseline. This was replicated four times for each of the temperatures studied. The four temperatures selected for study were 5, 10, 15, and 20°C, each with a L:D of 16:8.

Growth and development of the nematode eggs was recorded by drawings and photographs at each of the four temperatures during the 32-day study period.

RESULTS AND DISCUSSION

The eggs oviposited by R. communensis are spherical in shape and are 70-80 microns in diameter. They are whitish in color and are coated with a sticky substance, perhaps laid down at the time they are deposited. This substance allows the eggs to adhere to various surfaces and aids in the clumping of the eggs.

Embryonic Development

At the time the eggs are deposited, they are in the single cell stage. To aid in discussion, six stages of embryonic development of R. communensis eggs have been designated. They are: single-cell stage (Fig. 22a); two-cell stage (Fig. 22b); multicellular stage (Fig. 22c); early crescent, or the first indication of a worm shape (Fig. 22d); early coil stage (Fig. 22e); late coil stage (Fig. 22f). Similar stages were described by Poinar and Gyrisco (1962a), in their study of Hexamermis arvalis.

The results of the embryonation study at the four temperatures are shown in Figure 23. The six stages of embryonic development are indicated along the Y-axis, with the developmental time in hours shown along the X-axis. The points plotted on the graph represent those times at which 50% or more of the eggs had reached the stage indicated.

Material maintained at 10, 15, and 20°C developed to the late coil stage. This took somewhat longer at 10, and 15°C than at 20°C. Material at 5°C showed little development beyond the one-cell stage. It should be noted, however, approximately 10% of the eggs at 5°C did reach the two-cell stage. No further development of these eggs was noted at the end of the 32 day study period.

Eggs maintained at 20°C exhibited the most rapid development, reaching the two-cell stage after only 12 hours. The multicellular stage of development was reached

by 36 hours. An additional 47 hours was required for the eggs to reach the early crescent or early worm stage. The early coil stage was reached after 155 hours at 20°C. The eggs at 20°C took 395 hours or 16.5 days to reach the late coil stage. Development time from early coil to the late coil stage was 240 hours, considerably longer than it took the eggs to develop to the early coil stage.

Eggs maintained at 15°C required nearly twice as long as those at 20°C to reach the two-cell stage. To reach the multicellular stage it took approximately 75 hours and an additional 48 hours to reach the early worm stage. Three hundred and thirty-six hours were required for the eggs at 15°C to reach the early coil stage, and a total of 528 hours or 22 days to reach the late coil stage.

Development of eggs at 10°C took considerably longer, reaching the multicellular stage after 120 hours. To go from the multicellular stage to early worm shape it took an additional 240 hours. The early coil stage was reached after 600 hours, and the late coil stage was reached after 768 hours or 32 days.

Some hatching of nematode eggs (less than 5%) was noted after 443 hours at 20°C, and 672 hours at 15°C. Eggs at 10°C did not hatch before the end of the 32 day study period.

Poinar and Gyrisco (1962a) have indicated that the first moult in mermithids occurs in the egg. The shed

cuticle can be distinguished as a dark circular body in the hatched egg. This was not noted in my study. It may require embedding, sectioning and viewing under electron microscopy to determine the presence of a shed cuticle.

As was indicated in Chapter IV, soil samples collected from pools in the Goose Creek area in August, 1976 were found to contain nematode eggs in the late coil or fully embryonated stage of development. These findings, combined with the observations made during the embryonation study, indicate that the late coil or embryonated stage of development is the overwintering stage for R. communensis.

Tsai and Grundmann (1969) indicated in their description of the life history of R. nielseni (Ross and Smith, 1976) that the eggs appear to be resistant to freezing temperatures in soil. Eggs of R. communensis, which have experienced freezing conditions (e.g. -3°C) in the laboratory, have hatched after being returned to higher temperatures.

The indications are that this nematode completes one generation per year, synchronizing its life cycle to that of its natural host, A. communis/churchillensis.

Observations made during this study indicate that although some nematode eggs did hatch, the majority did not. This may be evidence that eggs become dormant for a certain period of time regardless of whether or not favorable conditions for hatching are present.

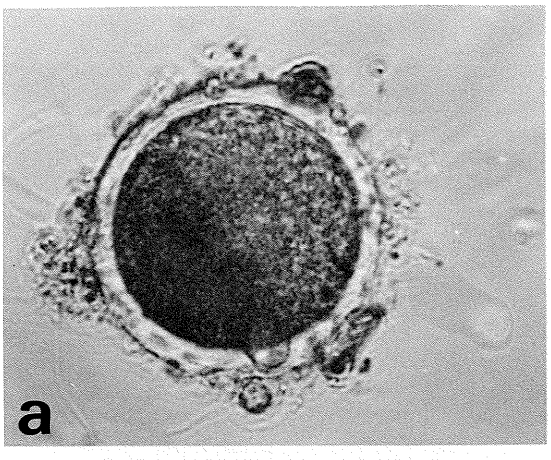
Recent work by Poinar (1977) on Empidomermis cozii

Poinar, 1977, a mermithid found infecting Anopheles funestus Giles, in West Africa, has shown that nematode eggs exhibiting a similar type of dormancy were induced to hatch synchronously by the addition of mosquito larvae into the container. He suggests that this indicates that the mermithids sensed the presence of the hosts.

Further investigations are necessary to establish what stimuli are necessary to obtain a synchronous hatch of R. communensis eggs. Research along the lines suggested by Poinar, 1977, may prove beneficial in the case of R. communensis. However, it is likely that some combination of cold temperature over time will trigger a more uniform hatching of the nematode eggs in the laboratory.

Figure 22 a-f

Stages of embryonic development of R. communensis eggs: (a) single-cell stage, ca. 500X; (b) two-cell stage, ca. 500X; (c) multicellular stage, ca. 500X; (d) early crescent or early worm shape, ca. 500X (e) early coil stage, ca. 250X; (f) late coil stage, ca. 500X.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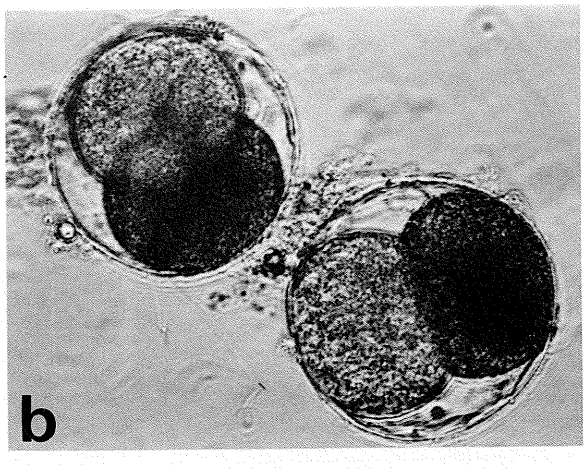
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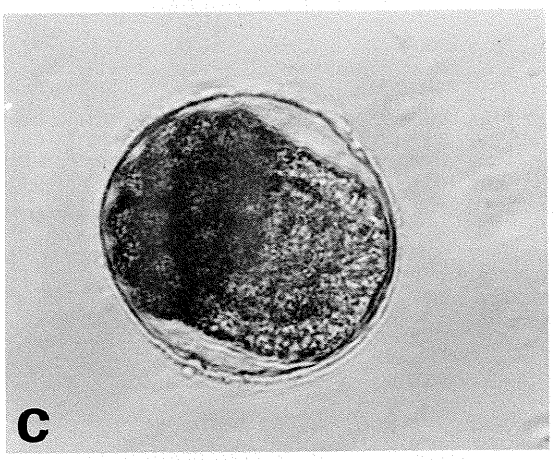
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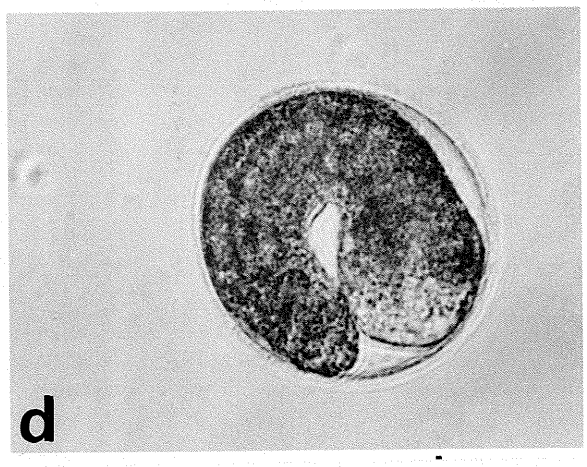
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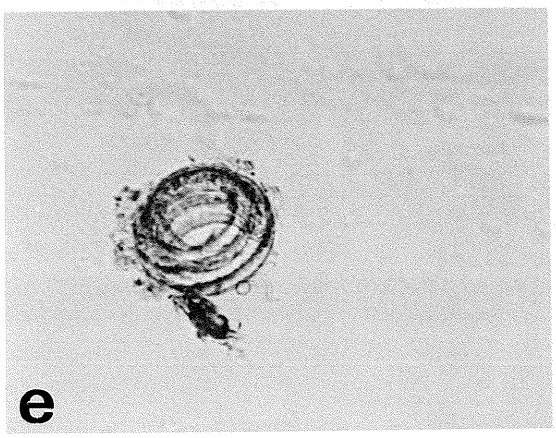
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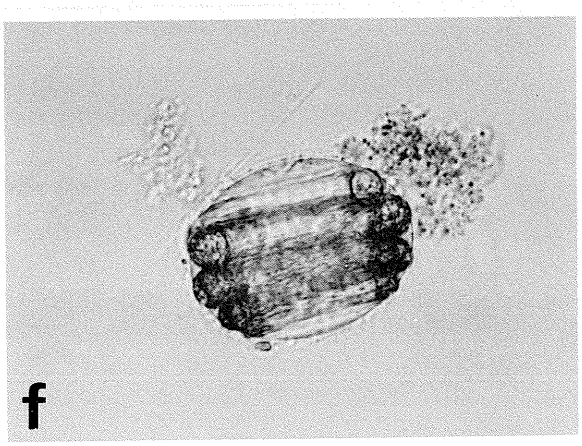
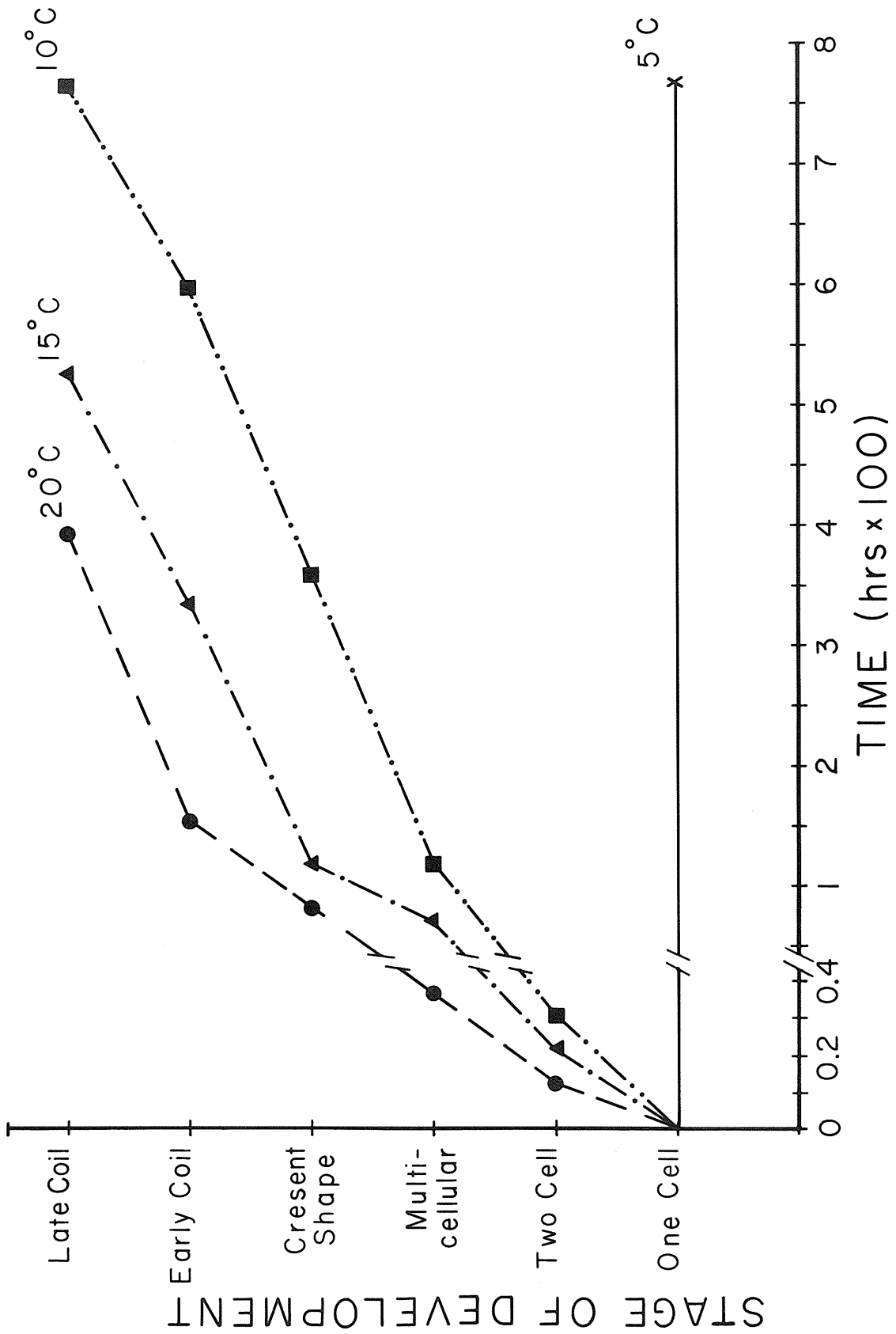

f

Figure 23

The rate of embryonation of R. communensis eggs at 5, 10, 15, 20°C.


STAGE OF DEVELOPMENT
Late Coil
Early Coil
Cresent Shape
Multi-cellular
Two Cell
One Cell
TIME (hrs x 100)
0
0.2
0.4
1
2
3
4
5
6
7
8
20°C
15°C
10°C
5°C

CHAPTER VI

Preliminary Observations and Evaluation of a *Culicimermis* sp. Parasitic in *Aedes vexans* (Meigen) in Manitoba

INTRODUCTION

Aedes vexans (Meigen) adults were found to be parasitized by a mermithid nematode as early as 1926 by E. Hearle in the Fraser Valley of British Columbia (Hearle 1926). More recent records by Trpiŝ, Haufe, and Shemanchuk (1968) near Haney in the Fraser Valley, B.C. showed a high incidence of infection of *A. vexans* females by a mermithid nematode.

Parasitized adult *A. vexans* were first recorded in the Winnipeg and Portage la Prairie areas of Manitoba during the summer of 1973 by Dr. T.D. Galloway, Department of Entomology, University of Manitoba. Various levels of infection had been noted in field collected *A. vexans* adults during the summers of 1974, 1975, 1976, and 1977. Based on observations made in the field and the laboratory, it is the purpose of this chapter to evaluate the potential of this nematode as a control agent of *A. vexans* in Manitoba.

Parasitized adult *A. vexans* females were collected from various localities in Manitoba during the period of 1973-1976. In addition to *A. vexans*, *A. sticticus* (Meigen), *A. dorsalis* (Meigen), *A. spencerii* (Theobald) have been found to be infected, but not to the same extent as *A. vexans* populations.

Unusually warm temperatures during late April and early May of 1977 combined with a 24 hour precipitation of 50.8 mm in the Winnipeg area on May 5, 1977, and an additional 14.6 mm on May 6, provided ideal conditions for the first A. vexans hatch of the year in the Winnipeg area.

Based on collections of A. vexans adults by Dr. T.D. Galloway and J. Harlos, Department of Entomology, between 1973-1976 in the vicinity of the University of Manitoba, and the levels of infection found at those times, it was suspected that the nematode breeding sites must be nearby.

MATERIALS AND METHODS

Several pools were sampled on May 9, 1977, on the University of Manitoba property. Preliminary dissections made by Dr. T.D. Galloway and myself, revealed parasitism by a mermithid nematode, a Culicimermis sp. Based on our early dissections of first and second instar larvae, the level of infection was estimated to be approximately 45-50%. A more extensive survey was undertaken of additional pools in the area and, as a result, three more locations were found to contain larvae infected with a nematode parasite within a 1.60 Km. radius (Fig. 24a-d). The nematode infections averaged 1.5 parasites per host.

The proximity of the nematode sites allowed us to make frequent field collections. Fourth instar larvae and pupae were kept in the laboratory and large numbers of

post-parasitic nematodes emerged from the adults.

Approximately 60,000 fourth instar larvae and pupae were collected from the breeding sites on May 14 and 15, 1977. Pupae were placed in styrofoam cups in adult emergence cages. Adults continued to emerge over a three to four day period. A high incidence of mortality of adult A. vexans was noted in some cages. It was later discovered by J. Harlos that most mortality was due to high levels of infection.

Parasitic development was followed throughout mosquito development in the field and the laboratory. Various parasitic stages dissected out of the larval hosts were prepared on slides. Photographs were taken and drawings and measurements were made.

RESULTS AND DISCUSSION

Development of the parasites in the hosts was not uniform in larvae exhibiting multiple infections. Dissections of adults emerging in the cages indicated higher levels of infection than those first indicated by preliminary dissections. Infection levels were 65% based on dissections of 376 A. vexans adults, 6-10 days old, with a range of 0-11 parasites per host, and an average infection level of 2.4 parasites per host (Dr. K.S. Kalpage, Department of Entomology, University of Manitoba, pers. comm.).

This data indicated that infections may have

occurred continously over the mosquito development period. If so, this would account for the lower levels of infection found previously based on dissections of first and second instar larvae from the field and the non-uniformity in the development of parasites in a host. However, it is also possible that early larval samples were too small (120) to accurately assess levels of parasitism.

Life Cycle

A general account of the mermithid nematode life cycle can be given based on field and laboratory observations. Nematode eggs, spherical in shape, are approximately 40-50 μ in diameter (Dr. T.D. Galloway, pers. comm.). These are located in the soil in A. vexans breeding sites where they are deposited by female adult nematodes during the previous year. The eggs likely spend the winter in an embryonated stage of development, however, further research is required to substantiate this. Hatching of the nematode eggs and of the first A. vexans mosquito eggs appear to be synchronous. The free-living preparasitic stage of the nematode is approximately 0.50-0.70 mm in length (Dr. T.D. Galloway, pers. comm.).

Infection of early larval instars is believed to take place soon after hatching. However, it may be possible that infection continues over the duration of the mosquito larval development. Once entry has been made into the haemocoel of the mosquito larva, the parasite becomes

less active. Development of the parasite in the larval host is apparently slow but some development does take place, as a noticeable thickening of the parasite occurs. The dissection of ten second instar larvae revealed that the mean length of a parasite was 0.75 ± 0.06 mm. The length and width of parasites dissected from ten A. vexans pupae showed a mean length of 1.28 ± 0.26 mm., and the mean width of 0.10 ± 0.01 mm. Parasites dissected from six 1 to 3 day old adult females had attained a mean length of 4.41 ± 2.4 mm. and a mean width of 0.10 ± 0.01 mm. Eight adult A. vexans mosquitoes, 2 to 6 days old, contained parasites having a mean length of 14.3 ± 2.6 mm., and a mean width of 0.15 ± 0.02 mm. Six adults, 5 to 10 days old, contained parasites having a mean length of 19.3 ± 3.03 mm. and a mean width of 0.16 ± 0.03 mm. These measurements are based on hosts that each contained an average of 2.4 parasites. The individual measurements from which these means were taken are quite variable depending on the numbers of parasites per host. This is the result of the influence that the number of parasites per host has on the nematode development, and possibly the result of continuous infection by the parasite throughout larval development should the latter occur.

Nematodes are usually found in the region of the malpighian tubules of the adults (Fig. 25). It is believed that movement to this region occurs during pupation.

Petersen et al. (1967) cited this as the likely time of migration to the abdomen for Perutilimermis culicis Nickle, 1972 found infecting A. sollicitans (Walk.) in Louisiana. Movement of the nematode out of the abdomen usually but not always results in the death of the host. The post-parasitic nematodes, escaping from their hosts, are free-living and do not need food.

It is speculated that the presence of nematodes in the abdomen of the female adult stimulates her to seek out favorable egg-laying sites, at which time the nematodes emerge. This could account for the re-introduction of the nematode into the mosquito breeding sites. Under these conditions, the nematodes then moult to adults and mate, and the females deposit their eggs in suitable substrate.

Effects of the Nematode on the Host

Early parasitic penetration by the active pre-parasite does not appear to affect the larval mosquito. As many as 23 parasites per host were recorded on several occasions. Petersen et al. (1967), working with A. sollicitans parasitized by the mermithid P. culicis noted that the larva can withstand higher levels of infection if penetrations occur over a long period of development.

As parasitic development is not extensive in the larval instars, there appears to be little effect on larval mosquito development. Pre-adult structures are formed normally and the parasitized larvae are able to pupate and

develop to adults.

Parasitized female adults readily seek a blood meal. Ovarian development appears to be variable in parasitized females. In some, complete atrophy results, while others are able to develop ovaries. A small percentage of females took a blood meal, developed eggs, and deposited a small number of eggs after the nematodes had emerged (Dr. T.D. Galloway, pers. comm.). Similar observations were noted by Petersen et al. (1967) in their work with P. culicis.

The effect upon a male host is not known. However, it is believed that the reproductive organs are injured as occurs in many females.

Alternate Hosts

Observations of this mermithid nematode indicate that it is less host specific than other nematode parasites which complete their development in the adult host (Petersen and Chapman 1970; Petersen 1973b). A vexans has been found to be the most extensively infected of the mosquito hosts, however, various levels of infection have been recorded in field collected A. spencerii (Theobald), A. sticticus, A. dorsalis, and possibly A. euedes Howard, Dyar, and Knab (Galloway and Brust, 1976). A. aegypti (Linnaeus), A. atropalpus (Coquillett), and Culex pipiens quinquefaciatus Say were also susceptible to infection in the laboratory. However, it was found that

the nematode could not reach maturity in the above, killing its host in the larval stage (J. Harlos, Department of Entomology, pers. comm.).

Evaluation of the Parasite Found Infecting *Aedes vexans* (Meigen)

This nematode appears to be very capable of infecting *A. vexans* and perhaps several other mosquito species. With an average incidence of infection of 2.4 parasites per host (noted during spring, 1977) there is some indication that *A. vexans* is a very susceptible host. Development of the mosquito does not appear to be affected drastically. Larval development proceeds normally, pupation occurs, and the adults emerge. Females will readily take a blood meal. However, female reproductive organs are adversely affected in many instances. A high incidence of host mortality was recorded as well, probably due to high levels of parasitism.

More male than female postparasitic nematodes were found. This is probably a result of the high levels of parasitism per host (Christie 1929; Petersen 1972).

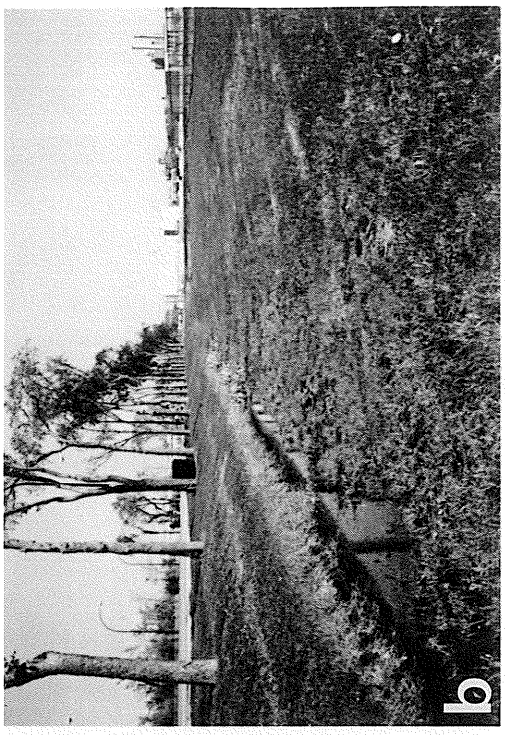
Establishment of a viable laboratory colony of this nematode in 1977 met with failure for several reasons: nematode material obtained had excessive numbers of males; the large numbers of host adults were difficult to handle effectively in the laboratory; material that was obtained was attacked by *Catenaria anguillulae* Sorokin, a fungus identified by Dr. G.L. Barron at the University of Guelph

as parasitic on many types of nematodes and other soil animals. J. Harlos of the Department of Entomology has cycled small numbers of the nematode through A. vexans during the past three years. However, the colony is too small to use for research purposes.

Although this parasitic nematode has been observed infecting native populations of A. vexans, its overall effect on control under natural conditions is no doubt limited. Mass production of the nematode would be necessary if it was to be used for effective mosquito control. Mass production is difficult because of the problems outlined previously and because A. vexans is a very difficult host to perpetuate in the laboratory. If a more readily colonized host could be found, mass production of the nematode might be possible.

Figure 24 a-d

Photographs of mermithid nematode developmental sites near the University of Manitoba. Nematode is a parasite of *A. vexans*.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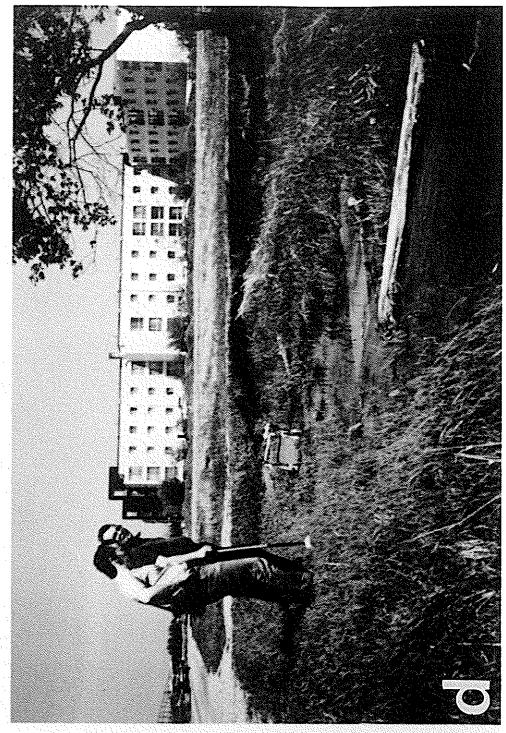
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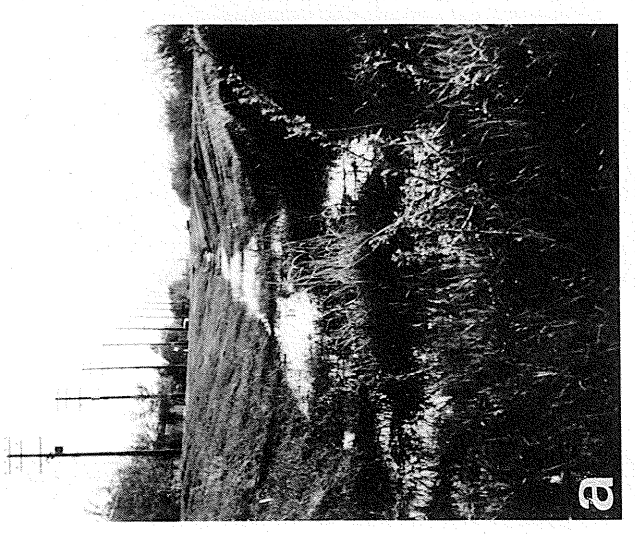
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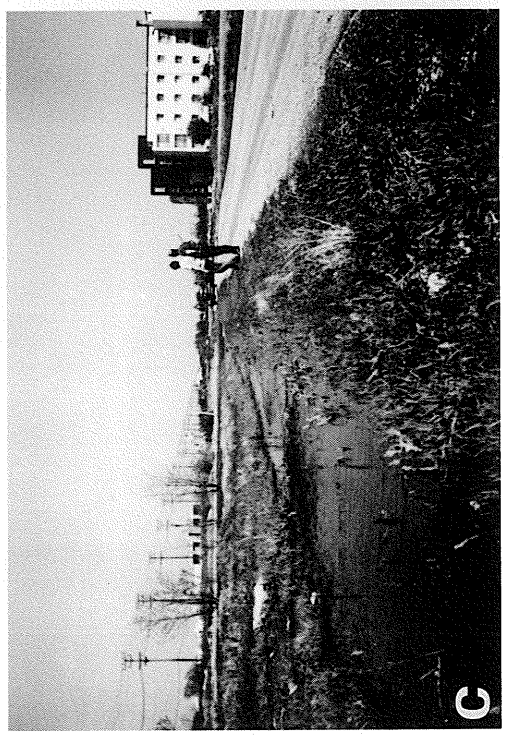
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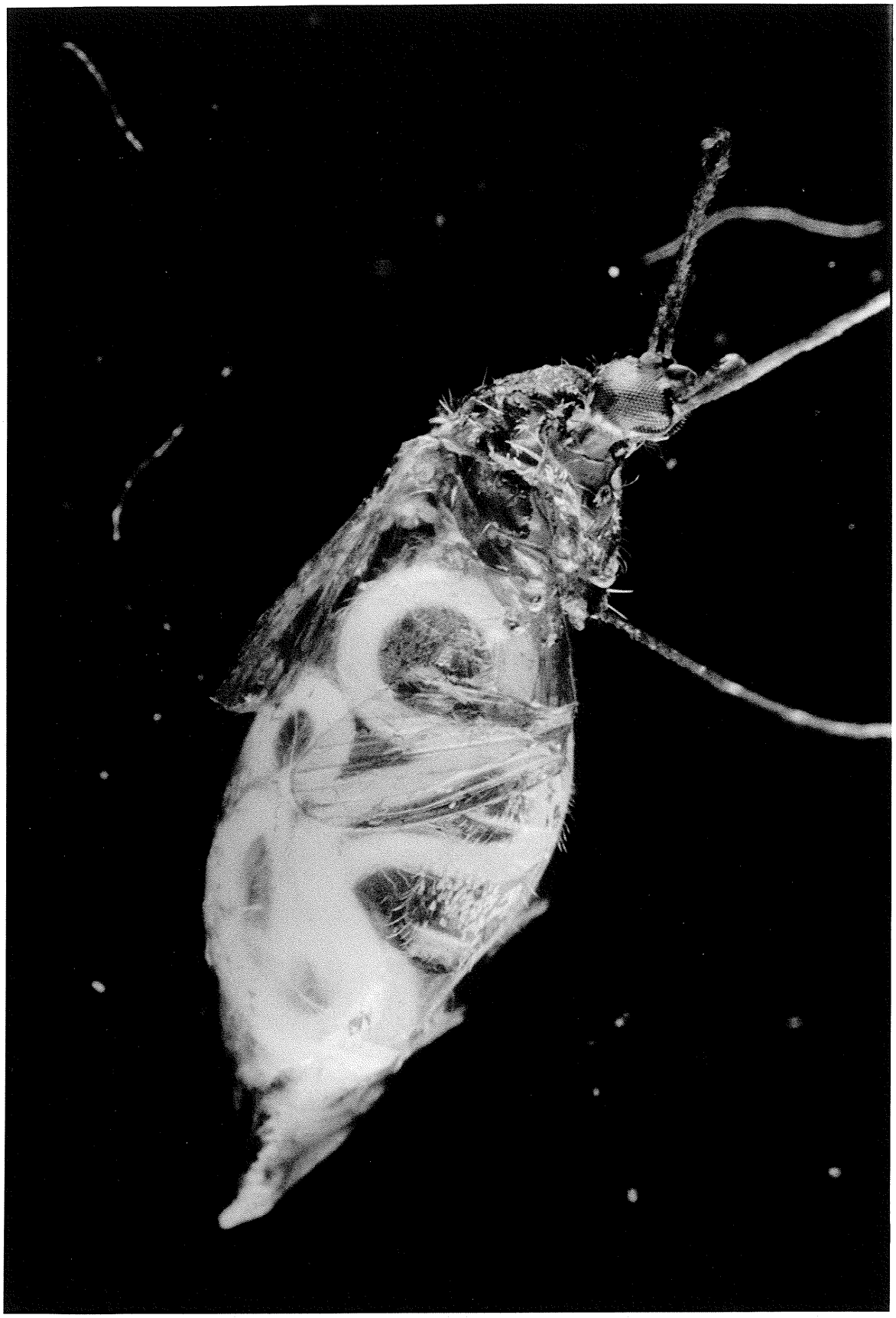
Figure 25

Mermithid nematode coiled in abdomen of a female *A. vexans* mosquito.



CHAPTER VII

CONCLUSIONS

From these studies, the following conclusions can be drawn:

1. Storage of R. culicivorax postparasitic juveniles at 10°C and 15°C will extend the expected shelf life and usefulness of the material for at least four months without adverse effects.
2. Age of the juveniles was found not to be a significant factor affecting the overall performance of R. culicivorax postparasitic juveniles during the storage and post-treatment stages.
3. Embryonated R. communensis eggs were found in soil samples from mosquito pools near Goose Creek, Manitoba in August, 1976. This indicates that the overwintering stage is the embryonated egg.
4. R. communensis shows some promise as an effective biological control agent against some spring Aedes species of mosquitoes. However, further investigations into its life cycle are required before its full potential can be assessed.
5. R. communensis eggs are capable of embryonating at 10, 15, and 20°C. Some hatching of eggs at 15, and 20°C was observed, but embryonation at these temperatures did not induce a synchronous hatch. Evidence indicates

that the nematode eggs enter a period of dormancy.

6. A mermithid nematode has been found infecting A. vexans and several other species of mosquitoes in areas of Manitoba. The overall effect of this nematode on control under natural conditions is no doubt limited. Further research is required before this particular nematode can be mass produced.

REFERENCES

1. Bailey, C.H. and R. Gordon. 1973. Histopathology of *Aedes aegypti* (Diptera:Culicidae) larvae parasitized by *Reesimermis nielseni* (Nematoda:Mermithidae). J. Invert. Pathol., 22:435-441.

2. Beckel, W.E. and T.P. Copps. 1955. Laboratory rearing of the adults of northern *Aedes* mosquitoes (Culicidae). Rep. Def. Res. Bd. Can. DRNL 7, 1955 1-9.

3. Chapman, H.C., T.B. Clark, J.J. Petersen, and D.B. Woodard. 1968. Louisiana lab takes intensive look at parasites for mosquito control. Pest Control, May, 1968, 1-6.

4. Christie, J.R. 1929. Some observations on sex in the Mermithidae. J. Exptl. Zool., 53:59-76.

5. Eisenhart, C. 1947. Inverse sine transformation of proportions, in Selected Techniques of Statistical Analysis, Eisenhart, Hastay, and Wallis (Eds.) McGraw-Hill, New York.

6. Finney, J.R. 1976. The *in vitro* culture of the mermithid parasites of mosquitoes and blackflies. Proc. 1st. Internat. Coll. Invert. Pathol., 225-226.

7. Galloway, T.D. 1977. 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. Ph.D. Thesis, University of Manitoba.

8. Galloway, T.D. and R.A. Brust. 1976. Observations on mermithid parasites of mosquitoes in Manitoba. Proc. 1st. Intern. Coll. Invert. Pathol., Kingston, Ontario. 227-231.

9. Gordon, R., C.H. Bailey, and J.M. Barber. 1974. Parasitic development of the mermithid nematode *Reesimermis nielseni* in the larval mosquito *Aedes aegypti*. Can. J. Zool., 52:1293-1302.

10. Haufe, W.O. 1957. Physical environment and behaviour of immature stages of *Aedes communis* (Deg.)(Diptera: Culicidae) in subarctic Canada. Can. Ent., 89:120-139.

11. Haufe, W.O. and L. Burgess. 1956. Development of Aedes (Diptera:Culicidae) at Fort Churchill, Manitoba, and prediction dates of emergence. Ecology, 37:500-519.

12. Hearle, E. 1926. The mosquitoes of the Lower Fraser Valley British Columbia and their control. Nat. Research Council (Can.) Rept., 17:1-94.

13. Ignoffo, C.M., K.D. Biever, W.W. Johnson, H.O. Sanders H.C. Chapman, J.J. Petersen, and D.B. Woodard. 1973. Susceptibility of aquatic vertebrates and invertabrates to the infective stage of the mosquito nematode Reesimermis nielseni. Mosquito News 33:599-602.

14. Ignoffo, C.M., J.J. Petersen, H.C. Chapman, and J.F. Novotny. 1974. Lack of susceptibility of mice and rats to the mosquito nematode Reesimermis nielseni Tsai and Grundmann. Mosquito News, 34: 425-428.

15. Iyengar, M.O.T. 1929. Parasitic nematodes of Anopheles in Bengal. Trans. 7th Congr. Far East. Assoc. Trop. Med. 1927, 3:128-135.

16. Jenkins, D.W. and A.S. West. 1954. Mermithid nematode parasites in mosquitoes. Mosquito News, 14:138-143.

17. Levy, R. and T.W. Miller, Jr. 1977a. Experimental release of a mermithid nematode to control mosquitoes breeding in sewage settling tanks. Mosquito News, 37:410-414.

18. Levy, R., and T.W. Miller, Jr. 1977b. Experimental release of Romanomermis culicivorax (Mermithidae: Nematoda) to control mosquitoes breeding in southwest Florida. Mosquito News, 37:483-486.

19. Mitchell, C.J., P.-S. Chen, and H.C. Chapman. 1974. Exploratory trials utilizing a mermithid nematode as a control agent for Culex mosquitoes in Taiwan. J. Formosan Med. Assoc., 73:241-254.

20. Muspratt, J. 1945. Observations on the larvae of tree-hole breeding Culicini (Diptera:Culicidae) and two of their parasites. J. Entomol. Soc. South Afr., 8:13-20.

21. Muspratt, J. 1965. Technique for infecting larvae of the Culex pipiens complex with a mermithid nematode and for culturing the latter in the laboratory. Bull. WHO, 33:140-144.

22. Nickle, W.R. 1972. A contribution to our knowledge of the Mermithidae (Nematoda) J. Nematol., 4:113-146.

23. Nickle, W.R. 1976. Toward the commercialization of a mosquito mermithid. Proc. 1st. Intern. Coll. Invert. Pathol. 241-244.

24. Pao-Shu, C. 1976. A study on Reesimermis nielseni for control of Culex pipiens fatigans in Taiwan. Bull. Inst. Zool. Acad. Sci., 15:21-28.

25. Petersen, J.J. 1972. Factors affecting sex ratios of a mermithid parasite of mosquitoes. J. Nematol., 4:83-87.

26. Petersen, J.J. 1973a. Relationship of density, location of hosts, and water volume to parasitism of larvae of the southern house mosquito by a mermithid nematode. Mosquito News, 33:516-520.

27. Petersen, J.J. 1973b. Role of mermithid nematodes in biological control of mosquitoes. Exper. Parasitol., 33:239-247.

28. Petersen, J.J. 1973c. Factors affecting mass production of Reesimermis nielseni, a nematode parasite of mosquitoes. J. Med. Ent., 10:75-79.

29. Petersen, J.J. 1975a. Penetration and development of the mermithid nematode Reesimermis nielseni in eighteen species of mosquitoes. J. Nematol., 7:207-210.

30. Petersen, J.J. 1975b. Development and fecundity of Reesimermis nielseni, a nematode parasite of mosquitoes. J. Nematol., 7:211-214.

31. Petersen, J.J. and H.C. Chapman. 1970. Parasitism of Anopheles mosquitoes by a Gastromermis sp. (Nematoda:Mermithidae) in southwestern Louisiana. Mosquito News, 30:420-424.

32. Petersen, J.J., H.C. Chapman, and O.R. Willis. 1969. Fifteen species of mosquitoes as potential hosts of a mermithid nematode Romanomermis sp. Mosquito News, 29:198-201.

33. Petersen, J.J., H.C. Chapman, and D.B. Woodard. 1967. Preliminary observations on the incidence and biology of a mermithid nematode of Aedes sollicitans (Walker) in Louisiana. Mosquito News, 27:493-498.

34. Petersen, J.J., H.C. Chapman, and D.B. Woodard. 1968. The bionomics of a mermithid nematode of larval mosquitoes in southwestern Louisiana. Mosquito News, 28:346-352.

35. Petersen, J.J., C.D. Steelman, and O.R. Willis. 1973. Field parasitism of two species of Louisiana rice field mosquitoes by a mermithid nematode. Mosquito News, 33:573-575.

36. Petersen, J.J., and O.R. Willis. 1972. Procedures for the mass rearing of a mermithid parasite of mosquitoes. Mosquito News, 32:226-230.

37. Petersen, J.J. and O.R. Willis. 1974. Experimental release of a mermithid nematode to control Anopheles mosquitoes in Louisiana. Mosquito News, 34:316-319.

38. Petersen, J.J., and O.R. Willis. 1976. Experimental release of a mermithid nematode to control floodwater mosquitoes in Louisiana. Mosquito News, 36:339-342.

39. Poinar, G.O., Jr. 1977. Empidomermis cozii n. gen., n. sp. (Mermithidae:Nematoda) a parasite of Anopheles funestus (Culicidae:Diptera) in West Africa. Can. J. Zool., 55:1475-1479.

40. Poinar, G.O., Jr., and G.G. Gyrisco. 1962a. Studies on the bionomics of Hexamermis arvalis Poinar and Gyrisco, a mermithid parasite of the alfalfa weevil, Hypera postica (Gyllenhal) J. Insect Pathol., 4:469-483.

41. Poinar, G.O., Jr., and G.G. Gyrisco. 1962b. A new mermithid parasite of the alfalfa weevil, Hypera postica (Gyllenhal). J. Insect Pathol., 4:201-206.

42. Poinar, G.O., Jr., and W.A. Otieno. 1974. Evidence of four molts in the Mermithidae. Nematologica, 20:370.

43. Roberts, D.W. and W. van Leuken. 1973. Limited growth of a mermithid nematode cultured in association with tissue-culture cells. Proc. 5th Intern. Coll. Insect Pathol. and Microbial Control.

44. Ross, J.R. and S.M. Smith. 1976. A review of mermithid parasites (Nematoda:Mermithidae) described from North American mosquitoes (Diptera:Culicidae) with descriptions of three new species. Can. J. Zool., 54:1084-1102.

45. Rubtsov, I.A. and N.M. Isaeva. 1975. Culicimermis schakhovii gen. et sp. n. (Mermithidae), a new parasite of mosquitoes. (In Russian). Vest. Zool., 1:39-40.

46. Smith, J.B. 1903. Report on mosquito investigations 23rd Ann. Rep. N. Jersey Agric. Exper. Station, pp 509-593.

47. Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. 6th edition. Iowa State University Press, Ames, Iowa.

48. Steiner, G. 1924. Remarks on a mermithid found parasitic in the adult mosquito Aedes vexans (Meigen) in B.C. Can. Ent., 56:161-164.

49. Stiles, C.W. 1903. A parasitic roundworm (Agamomermis culicis n. g., n. sp.) in American mosquitoes (Culex sollicitans) Bull. Hyg. Lab. U.S. Publ. Health and Mar. Hosp. Serv. 13:15-17.

50. Trpiŝ, M. 1971. Parasitical castration of mosquito females by mermithid nematodes. Helminthologia 1969, 10:79-81.

51. Trpiŝ, M.W., O. Haufe, and J.A. Shemanchuk. 1968. Mermithid parasites of the mosquito Aedes vexans Meigen in British Columbia. Can. J. Zool., 46:1077-1079.

52. Tsai, Y.-H. and A.W. Grundmann. 1969. Reesimermis nielseni gen. et sp. n. (Nematoda:Mermithidae) parasitizing mosquitoes in Wyoming. Proc. Helminthol. Soc. Wash., 36:61-67.

53. Welch, H.E. 1960a. Hydromermis churchillensis n. sp. (Nematoda:Mermithidae) a parasite of Aedes communis (DeG.) from Churchill, Manitoba, with observations on its incidence and bionomics. Can. J. Zool., 38:465-474.

54. Welch, H.E. 1960b. Potentials of nematodes in the biological control of insects of medical importance. Tech. Rept. Biol. Control Insects Med. Importance. Nov. 1960. pp 67-75.