portant arthropods for arbovirus isolation. Public Health Service, Center for Disease Control, Atlanta, Georgia.

Truman, J. W. and Craig, G. B., Jr. 1968. Hybridization between *Aedes hendersoni* and *Aedes triseriatus*. Ann. Ent. Soc. Amer. 61(4):1020–1025.

Venard, C. E. and Mead, F. W. 1953. An annotated list of Ohio mosquitoes. Ohio J. Sci. 53(6):327–331.

Zavortink, T. J. 1968. Mosquito Studies (Diptera: Culicidae) VIII. A prodrome of the genus *Orthopodomyia*. Contrib. Amer. Ent. Inst. 3(2):1–221.

PROCEDURES FOR THE MASS REARING OF A MERMITHID PARASITE OF MOSQUITOES [1]

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Abstract. Procedures were developed for the exposure of larvae of *Culex pipiens quinquefasciatus* Say to preparasitic *Reesimermis nielseni* Tsai and Grundmann to obtain high yields of nematodes. Also, methods of collecting large numbers of emerging postparasitic nematodes and of culturing them were evolved. Maximum nematode production was obtained when hosts were reared at densities of 0.35 square centimeter of surface area per host, were exposed to preparasitic nematodes at a 1:12 ratio and fed optimum amounts of food. These procedures can produce infective stage nematodes at a cost of about 7–10 cents per million.

The mermithid nematode *Reesimermis nielseni* Tsai and Grundmann was studied extensively from 1967–1971 at the Gulf Coast Marsh and Rice Field Mosquito Investigations Laboratory, Lake Charles, Louisiana. It was found to be an effective control agent against certain mosquito species and to be self-perpetuating once established (Petersen *et al.* 1968, Petersen *et al.* 1969, and Petersen and Willis 1970, 1971). However, an economical method of mass rearing *R. nielseni* had to be developed before extensive field testing could be accomplished. The method developed is reported here.

Since all known attempts to culture mermithid nematodes in vitro have failed, these parasites must be mass reared in a suitable arthropod host. *Culex pipiens quinquefasciatus* Say has proved to be such a host for *R. nielseni* because it is easily maintained in colony, is highly susceptible to attack by the nematode, and can be reared rapidly and in crowded conditions (Petersen and Chapman 1972). The procedures we evolved for the mass rearing of *C. p. quinquefasciatus* are modifications of those described by Gerberg (1970), Gerberg *et al.* (1969) and De Meillon and Thomas (1966).

Adult *C. p. quinquefasciatus* (a laboratory strain selected for diurnal feeding on guinea pigs) were maintained at high densities (densities not determined) in 91 x 61 x 61-cm cages (Fig. 1) in an insectary at 80±5% R.H. and 26–27° C. and provided with raisins as a source of carbohydrates. Then 3 to 5 days after the mosquitoes obtained a blood meal, egg rafts were collected by placing plastic oviposition containers (8 x 13 x 11 cm) half filled with water in the cages overnight. The egg rafts were then removed and counted, and the desired numbers were placed in separate containers for hatching. Rafts obtained at these conditions averaged about 125 eggs with 90+ percent hatch, and at 27° C., the holding temperature, essentially all the eggs hatched

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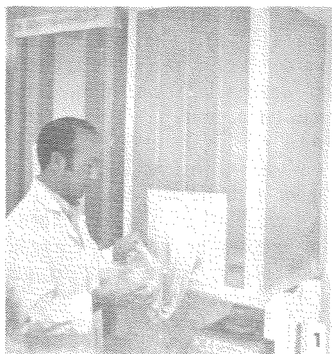

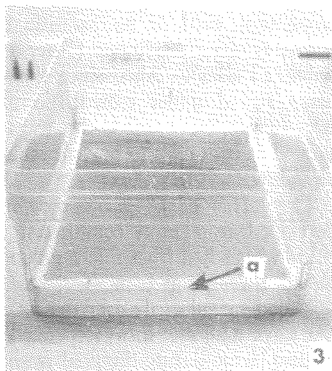

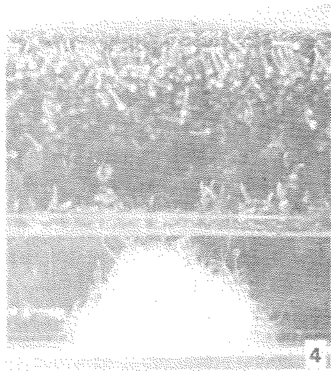

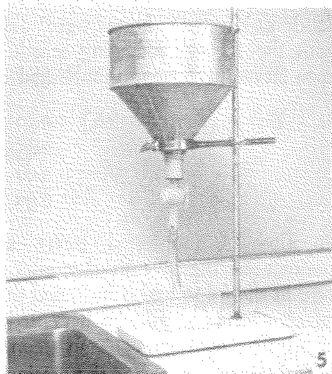

FIG. 1–6.—Equipment for the mass rearing of *Reesimermis nielseni*. (1) Oviposition container being placed in cage of adult *C. p. quinquefasciatus*. (2) Trays for exposing and rearing host mosquitoes. (3) Trays for separating emerging nematodes from hosts. (a) Screen divider. (4) Closeup of fig. 3 showing hosts above and nemas below screen divider. (5) Funnel (25 cm) equipped with shutoff valve for separating pupae from larvae by the ice water technique. (6) Containers used for maturating and ovipositing *R. nielseni*.

within 30 hours after they were removed from the adult cages.

Within a few hours after the egg rafts were counted and separated, a sufficient number of mature nema cultures were flooded with a liter of tap water (chlorine free) to provide the desired numbers of preparasitic nemas and then checked 2–3 hours after flooding for evidence that the hatch would be sufficient. (After a little practice, this estimate required only a few minutes so there was ample time for additional cultures to be flooded if needed.) Hatching of nemas was then allowed to continue 16–24 hours.

The inoculum of nemas desired was obtained as follows: The water from the flooded nema cultures was carefully collected in a common container, any water remaining in the sand was removed with absorbent paper towels, and the sand containing unhatched nemas was stored for future floodings. Then the volume of decanted water containing the preparasitics was determined, the water was mixed well, and a 10–ml aliquot was removed and used in the determination of the density of preparasitic nemas. First, the 10–ml sample was diluted to reduce the density of preparasitics to about 5–6 per 0.1 ml to permit easy counting (the dilution factor, usually 10:1 or greater, could only be determined by trial and error). Once the dilutions were made, ten 0.1 ml samples were pipetted into cells of a spot plate and the number of preparasites was counted under a dissecting scope to obtain the approximate number per milliliter of water. The numbers were then extrapolated for the volume of undiluted preparasites, and the extrapolation was used to determine the volume needed to provide the desired numbers of preparasitics. The number of times this procedure was repeated with a given sample depended on the desired accuracy. For an estimate of the inoculum for mass rearing, one replication was usually sufficient.

The mosquitoes were exposed to the nemas and reared in paraffin-coated 136 x 52 x 5-cm galvanized trays (Gerberg 1970) (Fig. 2). The trays were first filled to a depth of 3 cm (about 21 liters) with tap water which was allowed to reach room temperature; also, since preparasitic nemas are highly susceptible to chlorine, 1 ml of 5 percent sodium thiosulfate (about 1 drop/liter) was added as each tray was filled with water. Then larvae and preparasitic nemas were added at ratios of 1:7.5–1:12 hosts to parasites. If maximum production was desired, 20,000 larvae (about 3 larvae per sq. cm of surface area) were exposed to about 240,000 preparasitic nemas (1:12 ratio) since these numbers produced the most economical yield of postparasitic female nemas at these conditions (Petersen, unpublished data). Also, since the larvae being reared under crowded conditions were somewhat overfed, the trays were aerated at both ends to prevent scumming of the water surface.

The larvae of *C. p. quinquefasciatus* developed readily on commercial rabbit chow which had been finely ground and passed through a 30-mesh sieve. Also, this food spread easily over the surface of the trays and was easily separated from mature larvae during the final wash.

The largest yields of female nematodes and the most uniform growth of infected (and uninfected) hosts were obtained with the following feeding schedule for 1000 larvae (Petersen, unpublished data):

Day 0 (day of hatch)	0.30 g
1	0.45 g
2	0.60 g
3–7	0.90 g

Pupation of uninfected larvae usually commenced the seventh day after exposure in the trays, which indicated that the infected larvae should be harvested since emergence of nemas began soon after pupation started. Then the rearing trays were drained, and the mosquito larvae and pupae were collected on a 20-mesh screen, washed thoroughly, and concentrated in smaller holding trays (23 x 33 x 5 cm). They were then held 1–2 hours

to permit the expulsion of much of the food in the alimentary canals of the larvae, washed again, and placed into nema-collecting containers. These containers each consisted of two 36 x 25 x 10-cm plastic trays designed to fit into each other with a clearance of 2 cm between the bottoms of the trays (Fig. 3). The bottom of the upper container was cut away and replaced with 32-mesh nylon screen. As the postparasitic nemas emerged, they passed through the screen and dropped into the bottom tray. Dead and living hosts and pupae could thus be easily separated from the nemas by removing the upper tray (Fig. 4).

Enough mosquito pupae were produced during the mass rearing of the nemas to maintain the colony of mosquitoes at peak levels. Host resistance was not evident with this procedure but if it should develop colony mosquitoes would have to be raised separately. The ice-water technique (Ramakrishnan *et al.* 1964; Weathersby 1963; and Hazard 1967) was used to separate the pupae from the larvae in a 25-cm-diameter funnel (Fig. 5). The sudden chilling for a short period had no adverse effects on the mosquitoes or on nemas still infesting larvae.

After all postparasitic nematodes were harvested they were concentrated into a smaller container, washed by gentle agitation with a stream of water, and allowed to settle to the bottom; the suspended debris was decanted. This wash procedure was repeated one or two times as needed. Then 10 to 15 grams of nemas (wet weight) were added to paraffin-coated aluminum cake pans (22 x 33 x 5 cm) which contained clean coarse sterile sand (1.5 cm deep) covered to a depth of 1 cm with chlorine-free water (Fig. 6). (Fine grain sand was not used because it became tightly compacted when wet and thus inhibited movement of the nemas.) The cultures were covered with loose fitting plastic lids and stored. After about three weeks, any visible dead nemas were removed, the water was carefully decanted, and the excess water was absorbed with paper towels. The cultures were stored for an additional 4–15 weeks before use. The stored cultures could be flooded to induce hatch anytime after 7–8 weeks, but higher hatches occurred at the first flooding when the cultures were 11 to 16 weeks old (1–10 x 10^6 preparasitics). Also, additional hatches could be produced if the cultures were properly dried and stored for 3–4 additional weeks. We obtained substantial hatches from cultures sustained through three floodings if the cultures were not more than 18 to 20 weeks old. However, small hatches continued to occur in cultures up to 34 weeks and after six floodings.

Once the necessary equipment had been assembled and the simple techniques mastered, the only expense involved in mass rearing the nemas would be the salaries of the technicians, the cost of maintaining the bloodmeal source (guinea pigs) and food for the mosquito larvae. Thus, in essence, *R. nielseni* can be mass reared as cheaply as the host mosquito. If one technician can easily rear one million mosquito larvae per 40 hr week, if 80 percent parasitism yield 7.2–7.5 x 10^5 female nemas, and if female nemas produce a mean of 2500 eggs, preparasitic nemas can be produced at a cost of about 7–10 cents per million. However, the rates at which preparasitic *R. nielseni* must be added to the environment to be effective still remain to be determined. Preliminary data indicate that a rate of 1000 preparasitics per square meter is highly effective against susceptible species. Then production of the preparasitic nemas necessary for such an application would cost about $1.00 per 2.5 acres.

Literature Cited

De Meillon, B. and Thomas, V. 1966. *Culex pipiens fatigans* Wied. p. 101–114. In C. N. Smith, Insect Colonization and Mass Production. Academic Press, N.Y.

Gerberg, E. J. 1970. Manual for mosquito-rearing and experimental techniques. Amer. Mosq. Control Assn. Bull. 5. 109 pp.

Gerberg, E. J., Hopkins, T. M. and Gentry, J. W. 1969. Mass rearing of *Culex pipiens* L. Mosq. News 29:382–385.
Hazard, E. I. 1967. Modification of the ice water method for harvesting *Anopheles* and *Culex* pupae. Mosq. News 27:115–116.
Petersen, J. J. and Chapman, H. C. 1972. The development of a biological control agent of mosquitoes. Proc. Second Gulf Conf. Mosq. Sup. Fish Wild. Manag. In press.
Petersen, J. J. and Willis, O. R. 1970. Some factors affecting parasitism by mermithid nematodes in southern house mosquito larvae. J. Econ. Entomol. 63(1):175–178.
Petersen, J. J. and Willis, O. R. 1971. A two year survey to determine the incidence of a mermithid nematode in mosquitoes in Louisiana. Mosq. News 31(4):558–566.
Petersen, J. J., Chapman, H. C. and Woodard, D. B. 1968. Bionomics of a mermithid nematode of larval mosquitoes in southwestern Louisiana. Mosq. News 28(3):346–352.
Petersen, J. J., Chapman, H. C. and Willis, O. R. 1969. Fifteen species of mosquitoes as potential hosts of a mermithid nematode *Romanomermis* sp. Mosq. News 29(2):198–201.
Ramakrishnan, S. P., Krishnamurthy, B. S., and Singh, N. N. 1964. A simple technique for rapid separation of mosquito pupae by sudden chilling. World Health Organ./Vector Cont. 81. 3 pp.
Weathersby, A. B. 1963. Harvesting mosquito pupae with cold water. Mosq. News 23:249–251.

MATING COMPETITIVENESS OF CHEMOSTERILIZED MALE SOUTHERN HOUSE MOSQUITOES TREATED WITH TEPA[1]

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ABSTRACT. Male *Culex pipiens quinquefasciatus* Say sterilized as they crawled through polyvinyl tubes treated with tepa were competitive with normal males in outdoor cages; however, the data suggested that they might not survive as long as normal males. Also, the males were competitive whether, the ratio of sterile males to females was 9:1 or 1:1. Both laboratory-reared and outdoor-reared sterile males were competitive with normal males (eradication of caged population was achieved with both types of males when the ratio of sterile to normal males was 100:1). The caged populations had a rate of biotic increase greater than 29X but less than 99X.

Sterilization of mosquitoes with gamma irradiation was first demonstrated by Davis *et al.* (1959) with the common malaria mosquito, *Anopheles quadrimaculatus* Say, but these males were unable to compete sexually with normal males in the laboratory. Also, when Ramakrishnan *et al.* (1962) used irradiation to sterilize the southern house mosquito, *Culex pipiens quinquefasciatus* Say (=*C. p. fatigans* Wiedemann), they found that this method of sterilization reduced mating competitiveness. However, Murray and Bickley (1964) and Mulla (1964) found that male *C. p. quinquefasciatus* sterilized with chemicals in the larval stage were competitive with normal males in the laboratory. Also, Smittle *et al.* (1968) compared these two methods of sterilizing the southern house mosquito and confirmed that irradiation inhibited sexual activity of the males, whereas the chemosterilant apholate did not.

Except for the field release by Krishnamurthy *et al.* (1962), which was done in conjunction with the cited research by Ramakrishnan *et al.* (1962), these early studies with *Culex* were all made with controlled conditions in small cages in the laboratory. When Patterson *et al.* (1968)

[1] This paper reflects the results of research only. Mention of a pesticide or a commercial or proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the U.S. Department of Agriculture.

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