Sudia, W. D. and R. W. Chamberlain. 1967. Collection and processing of medically important arthropods for arbovirus isolations. U. S. Dept. Hlth., Edu., and Wlfr., Publ. Hlth. Srvc., Atlanta, Georgia.

Tempelis, C. H. 1970. Host preferences of mosquitoes. Proc. & Papers. 38th Annual Conf. Calif. Mosq. Control Assoc. 25–28.

Work, T. H., R. Vanis and H. G. Wallace. 1969. Arboviruses in southern California. Div. Inf. & Trop. Dis., Sch. Publ. Hlth., Univ. Calif., Los Angeles. (Unpublished manuscript) 8 p.

SUSCEPTIBILITY OF AQUATIC VERTEBRATES AND INVERTEBRATES TO THE INFECTIVE STAGE OF THE MOSQUITO NEMATODE *REESIMERMIS NIELSENI*

C. M. IGNOFFO,[1] K. D. BIEVER,[1] W. W. JOHNSON,[2] H. O. SANDERS,[2] H. C. CHAPMAN,[3] J. J. PETERSEN[3] AND D. B. WOODARD[3]

ABSTRACT. The nematode parasite of mosquito larvae, *Reesimermis nielseni*, is presently under consideration as a possible biological control agent of mosquitoes. This nematode has effectively suppressed field populations of mosquitoes in several areas of the United States. Safety tests evaluating possible effects of this nematode on nontarget aquatic organisms which might be exposed to the introduced nematode are reported herein.

The nematode *R. nielseni* is apparently quite host-specific. Neither trout, top minnows, nor any of 16 invertebrates tested supported development of the nematode. Little or no significant damage to aquatic organisms, other than mosquitoes, is anticipated after field introduction of *R. nielseni*.

A nematode parasite of mosquito larvae, *Reesimermis nielseni* Tsai and Grundmann, is receiving considerable attention as a possible biological control agent of culicine and anopheline mosquitoes (Anonymous, 1972). This nematode can be mass produced in labortory cultures of the southern house mosquito, *Culex pipiens quinquefasciatus* Say, (Petersen and Willis, 1972a). Moreover, preparasitic larvae of the infective stage have effectively suppressed developing field populations of *Anopheles* mosquitoes in California and Louisiana (Petersen, *et al.*, 1972; Petersen and Willis, 1972b).

Safety to organisms other than the target pest, in addition to production feasibility and field efficacy, must also be determined to insure the successful development of a biological control agent (Ignoffo, 1967; 1973). An evaluation of possible effects of *R. nielseni* in mammals is currently underway and should be completed within 6 months (Ignoffo, *et al.*, 1973). The evaluation of the possible effects of *R. nielseni* in representative aquatic organisms which might be exposed to artifically introduced preparasitic larvae is reported here. The studies were the combined efforts of the Fish Pesticide Research Laboratory, U. S. Department of Interior, BSFW, Columbia, Missouri; the Gulf Coast Mosquito Research Laboratory, Agr. Res. Serv., U. S. Department of Agriculture, Lake Charles, Louisiana; and Biological Control of Insects Research Laboratory, Agr. Res. Serv., USDA, Columbia, Missouri.

[1] Biological Control of Insects Research Laboratory, ARS, USDA, Columbia, Missouri 65201.

[2] Fish-Pesticide Research Laboratory, U. S. Dept. of Interior, Columbia, Missouri 65201.

[3] Gulf Coast Mosquito Research Laboratory, ARS, USDA, Lake Charles, Louisiana 70601.

SUSCEPTIBILITY OF VERTEBRATES

Eggs, yolk-sac fry, and swim-up fry of

four species of freshwater fish, i.e. rainbow trout (*Salmo gairdneri* Richardson), largemouth bass (*Micropterus salmoides* [Lacepede]), channel catfish (*Ictalurus punctatus* [Rafinesque]), and fathead minnows (*Pimephales promelas* Rafinesque) were exposed to preparasitic larvae of *R. nielseni*. Experiments were conducted in 500 ml glass beakers holding 300 ml of water for all species except rainbow trout swim-up fry where 4 liter aquaria were used. Tests were done using 20 fish per container. Exposure levels were 6.25 preparasitic larvae per ml of water, which is a ratio of 1250:1 for nematode larvae to trout swim-up fry and 94:1 for all other tests. This concentration of larvae is 1562 times the anticipated field rate of 5×10^6 preparasitic larvae per acre-ft[3] of water. Tests were conducted at 17± 1° C with rainbow trout and 21±1° C with the other species. Bass, catfish, and minnow tests began with the eyed egg and continued through the yolk-sac fry stage into the swim-up fry stage. Exposure times were 96 hours for bass and minnows and 10 days for catfish. Rainbow trout were exposed continuously from the eyed egg into the yolk-sac fry stage. Separate tests were conducted with the swim-up fry stage. Exposure times for the yolk-sac fry and swim-up fry were 10 days and 14 days respectively. Daily observations were made throughout the exposure period, and all fish were sacrificed and microscopically examined at the end of the tests to detect possible nematode invasion or tissue damage. All tests were triplicated.

Living preparasitic larvae of *R. nielseni* were observed throughout the short-duration tests and up to 8 days in tests of longer duration. Whole body examination of bass and minnows and examination of the buccal cavity, gills, gut, skin surface, and eyes of trout and catfish revealed no evidence of nematode invasion. In addition, nematodes could not be found in any body tissues.

SUSCEPTIBILITY OF INVERTEBRATES

TESTS ON A DAPHNID, AMPHIPOD, AND OLIGOCHAETE. Three aquatic invertebrates, the daphnid, *Daphnia magna* Straus; the amphipod, *Gammarus pseudolimnaeus* Bousfield; and an oligochaete, *Lumbriculus* sp., were exposed to heavy populations of preparasitic larvae of *R. nielseni*. Glass preparation dishes, 300 ml capacity, were used as test chambers. Each dish contained the test species (20–30 individuals) and 1000 preparasitic larvae in a total volume of 200 ml of water (1250 x the anticipated field rate). The numbers of daphnids, amphipods, and oligochaetes added to each dish were 20, 25, and 30, respectively; thus, the ratio of *R. nielseni* to the three species was 50:1, 40:1, or 33:1, respectively. Four dishes (3 containing nematodes, 1 without nematodes) were used for each of the species to be tested, and temperature was maintained at 21–23° C for 14 days. All animals were sacrificed after 14 days and examined microscopically for penetration or damage induced by *R. nielseni*. Also, as with the trout, living nematodes were observed during the first 8 days of exposure.

All test invertebrates were still living after 14 days of exposure. No evidence of penetration or damage by preparasitic *R. nielseni* was detected in any of the invertebrates despite a thorough examination of the body wall, coxal gills and digestive tract.

TESTS ON CHIRONOMID LARVAE. A series of tests was made with larvae of *Chironomus* sp. In the first test, 1st-instar larvae (40/container) were exposed to 0, 10, 100, 500, or 1000 preparasitic *R. nielseni* larvae/chamber. Five-hundred ml, plastic, wide-mouth jars containing 250 ml of water were used as test chambers. Two chambers were used for each rate of exposure. Tests were conducted at 23±2° C, air was bubbled through each container, and dog food was provided as food (Biever, 1965) for

the developing larvae. Subsamples of 2–3 chironomids were taken from each container at intervals during the test and examined for nematode entry or damage. The tests were terminated at 14 days when the larvae were nearly mature (4th instar). All surviving larvae were microscopically examined for evidence of nematode damage. No evidence of penetration or damage by *R. nielseni* was observed.

In a second test, ten 2nd-, 3rd-, and 4th-instar chironomid larvae were exposed to 100 or 500 preparasitic *R. nielseni* in chambers containing 250 ml of water. The tests were duplicated. Food was limited to prevent the larvae from developing to maturity. Half of the chironomid larvae were sacrificed after 1 day and the remainder after 8 days. Only 1 of 60 chironomid larvae was penetrated by the nematode. This occurred 1 day after treatment in a 4th-instar larva, and the nematode subsequently died without damage to the host.

Since one case of positive recovery was obtained in the second test, an additional test was conducted. This time groups of about 50 3rd- and 4th-instar chironomid larvae were each placed in 250 ml of water containing 75,000 preparasitic *R. nielseni*. After 1 day of exposure, 10 live chironomid larvae from each chamber were examined: 3 contained *R. nielseni* larvae, 2 dead and 1 alive. At 2 days posttreatment, 10 more chironomids were examined: 5 contained nematodes, 4 dead and 1 alive. At 8 days post treatment, none of the remaining chironomid larvae contained nematodes, and no mortalities were attributed to parasitism.

Apparently *R. nielseni* preparasitic larvae can penetrate larvae of *Chironomus* sp. when the population of *R. nielseni* is large but are unable to develop and survive.

TESTS ON PSYCHODID LARVAE. Fifteen 2nd- and 4th-instar larvae of *Psychoda* sp. were exposed to 500 or 1000 preparasitic *R. nielseni* larvae. This test was set up as previously described for *Chironomus* sp. but with three chambers/treatment. Half of the larvae were removed and examined 1 day after treatment and the remainder 5 days after treatment. This test was shorter because this species usually does not survive well for an extended period in a fully aquatic situation. No evidence of penetration or damage by *R. nielseni* was observed. In a second test, *Psychoda* sp. larvae were again exposed to greater concentrations of preparasitic *R. nielseni* larvae. About 25 2nd- and 3rd-instar *Psychoda* sp. larvae were placed in a petri dish (Biever and Mulla, 1966) containing 20 ml of water and 6000 preparasitic larvae of *R. nielseni*. Each test was replicated four times. At 1, 5, and 10 days posttreatment, 5 larvae from each unit were examined. As in the first test preparasitic *R. nielseni* failed to damage or penetrate *Psychoda* sp. larvae.

TESTS ON OTHER AQUATIC ARTHROPODS. Preparasitic *R. nielseni* were tested also against other nontarget organisms that commonly occur in aquatic habitats in Louisiana as follows: Immature and/or adult stages of Diptera (Chaoboridae), Coleoptera (Hydrophilidae, Dytiscidae, Haliplidae), Hemiptera (Notonectidae, Corixidae, and Belostomatidae), Odonata (Zygoptera, family not determined), and Ephemeroptera (Baetidae) were collected and exposed to high concentrations of preparasitic larvae (100–10,000 preparasitic larvae/host). Crayfish (*Cambarus* sp.) also were tested for susceptibility to *R. nielseni*. The test animals listed above were placed in 30 ml beakers containing 15–20 ml of water and exposed to preparasitic larvae for 1–7 days. Early instar mosquito larvae were exposed simultaneously to serve as treated controls. A total of 183 immature and 18 adults were tested. Only larvae of *Corethrella appendiculata* Grabham, a treehole breeding chaoborid, were susceptible to *R. nielseni* and only when they were exposed to high numbers of preparasitics (1300/host). No penetration occurred at a density of 60 preparasitic larvae/insect host. However, large numbers of preparasitic larvae (1300/host) either killed larvae of *Corethrella brakeleyi*

(Coquillett) because of multiple penetrations, or the nematodes failed to develop once they had successfully penetrated. Nematodes also penetrated 2 of 25 and 3 of 77 immature hydrophilid and dytiscid larvae, respectively, and one adult haliplid when they were exposed to 1200 to 10,000 nematodes/host. Nematodes, however, failed to develop and died soon after penetration. All mosquito larvae were heavily parasitized by preparasitic-stage *R. nielseni* larvae, but there was no evidence of penetration by the preparasitic nematodes in other exposed nontarget organisms.

DISCUSSION

The infective-stage larvae of *R. nielseni* are apparently quite host-specific for mosquitoes (Petersen, *et al.*, 1968). Of 61 species of mosquitoes exposed to *R. nielseni*, 4 were essentially resistant and 5 exhibited a moderate to high degree of resistance (Petersen, 1973). Neither 4 species of aquatic fish eggs, yolk sac fry, and swim-up fry nor any of the 16 invertebrates supported development of *R. nielseni*. Mice and rats also were not susceptible to intranasal, intraperitoneal, *per os*, or dermally administered preparasitic larvae of *R. nielseni* (Ignoffo, *et al.*, 1973 in press). Some penetration and development by *R. nielseni* was observed in *Corethrella* and *Chironomus* larvae, but the incidence was generally low even when high densities of *R. nielseni* were used with severely confined hosts. To date, the susceptibility of some 60 species of mosquitoes has been tested against *R. nielseni*. Only two species, *Anopheles sinensis* and *Culex territans*, were completely resistant; additionally, six species have shown varying degrees of resistance to the development of *R. nielseni*.

It is, of course, impossible to state that all aquatic organisms will respond like those tested. However, our studies support the belief that little or no significant damage to aquatic organisms, other than to mosquitoes, need be anticipated subsequent to artificial introduction of *R. nielseni* preparasitic larvae into aquatic habitats.

References Cited

Anonymous. 1972. Mass-produced nematodes down mosquitoes. Agric. Res. 20(11):3–4.

Biever, K. D. 1965. A rearing technique for the colonialization of chironomid midges. Ann. Entomol. Soc. Am. 58(2):135–136.

Biever, K. D. and Mulla, M. S. 1966. Effects of temperature on the developmental stages of *Psychoda alternata* (Diptera: Psychodidae). Mosq. News 26(3):416–419.

Ignoffo, C. M. 1967. Possibilities of mass-producing insect pathogens. *In* Proceed. International Colloq. Insect Pathol. and Microbial Control, Wageninger, The Netherlands, Sept. 5–10, 1966. pp. 91–117. North Holland Publ. Co., Amsterdam, 1967. 360 pp.

———. 1973. Effects of entomopathogens on vertebrates. Proc. New York Acad. Sci. 217: 141–172.

Ignoffo, C. M., Petersen, J. J., Chapman, H. C. and Novotny, J. F. 1973. Lack of susceptibility of mice and rats to the mosquito nematode *Reesimermis nielseni*. Environ. Entomol. (In press).

Petersen, J. J. 1973. Role of mermithid nematodes in biological control of mosquitoes. Experimental Parasitol. 33(2):230–247.

Petersen, J. J., Chapman, H. C. and Woodard, D. B. 1968. The bionomics of a mermithid nematode of larval mosquitoes in southwestern Louisiana. Mosq. News 28(3):347–352.

Petersen, J. J., Hoy, J. B. and O'Berg, A. G. 1972. Preliminary field tests with *Reesimermis nielseni* (Mermithidae: Nematoda) against mosquito larvae in California rice fields. Vector Views 19(7):47–50.

Petersen, J. J. and Willis, O. R. 1972a. Procedures for the mass rearing of a mermithid parasite of mosquites. Mosq. News 32(2):226–230.

———. 1972b. Results of preliminary field applications of *Reesimermis nielseni* (Mermithidae: Nematoda) to control mosquito larvae. Mosq. News 32(3):313–316.