# Penetration and Development of the Mermithid Nematode *Reesimermis nielseni* in Eighteen Species of Mosquitoes[1]

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*Abstract*: The susceptibility of 18 species of mosquitoes to the infective stage of the mermithid nematode *Reesimermis nielseni* was compared to that of *Culex pipiens quinquefasciatus*. Thirteen species were more susceptible, with three *Anopheles* species and *Culiseta inornata* the most susceptible. *Aedes triseriatus*, *Culex territans*, and *Psorophora ferox* were highly resistant. Resistance to *R. nielseni* appeared to be behavioral, physical, or physiological, and some host species exhibited one or more types of defense mechanisms. No noticeable differences were apparent in the degree of susceptibility of a native- and a laboratory strain of *C. p. quinquefasciatus* to *R. nielseni*. *Key Words*: parasitism, biological control, host resistance.

The mermithid nematode, *Reesimermis nielseni* Tsai and Grundmann, a promising biological control agent of larval mosquitoes, is known to parasitize 16 species of mosquitoes in nature and 57 species in the laboratory (3); however, it is not known to parasitize other invertebrates or vertebrates (1, 2). Earlier, when 15 species of mosquitoes were compared to determine their potential as laboratory hosts of *R. nielseni* (=*Romanomermis* sp.) (4), the nematode was found to develop normally in 12 species, but three species were highly resistant. However, the study was preliminary, and, although the 15 species were ranked according to susceptibility, no attempts were made to correlate host susceptibility to various levels of infection or to determine the types of resistance exhibited.

As procedures for mass production and field release of *R. nielseni* are improved, a better understanding of the susceptibility of various species of mosquitoes is needed before this nematode can be effectively used in the field. A study, therefore, was conducted at the Gulf Coast Mosquito Research Laboratory, Lake Charles, Louisiana, during 1972 and 1973 to supplement and expand the work of Petersen et al (4), in determining more exactly the susceptibility of different mosquitoes, and to determine the type of resistance exhibited.

## MATERIALS AND METHODS

Sixteen of the 18 species of mosquitoes tested (from five genera) were obtained from laboratory colonies. Larvae of native *Culex pipiens quinquefasciatus* Say were obtained from egg rafts collected from field sites, and larvae of *Psorophora confinnis* (Lynch Arribálzaga) were obtained from eggs of field-caught females which oviposited in the laboratory.

Tests were conducted by exposing first-instar larvae of 1-3 host species and *C. p. quinquefasciatus* ("control species") to 1- to 6-hour-old preparasitic larvae of *R. nielseni* from laboratory cultures as follows. The control host species was included to provide a basis for comparing susceptibility of species in different tests because variability in counting accuracy and infectivity of *R. nielseni* from different cultures made direct comparison between tests impossible. Fifty larvae of each host species were placed in each of nine 100-ml beakers, and then 50 preparasitic nematodes were added to three beakers; 250 to three beakers and 500 to three beakers. The mosquitoes were fed lightly, and after 24 h of exposure to the preparasites, were transferred to rearing containers. At the time of transfer, 10 larvae were removed from each container, dissected and examined for nematode penetration. Remaining larvae were maintained an additional 6-7 days at which time incidence of successful parasitic development was determined. A comparison of the level of penetration with the final level of parasitism provides a measure of physiological resistance. Tests were replicated three or more times when host numbers

Received for publication 23 September 1974.

[1]In cooperation with McNeese State University, Lake Charles, Louisiana 70601.

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permitted, and occasional tests were discarded because of high mortality.

The values reported for each species were derived by dividing the percentage of parasitism for a given dose of preparasitic nematodes by the corresponding value for the control species to obtain a comparative index of susceptibility. However, the variation in levels of parasitism from test to test at a given parasite-to-host ratio was so great that the results for the three ratios (1:1, 5:1, and 10:1) were not used in the final grouping of the susceptibility indices. Rather, the indices for the test species were related to ranges of incidence of parasitism in the control species. Mosquito species with indices greater than 1.00 were, therefore, more susceptible to *R. nielseni* than *Culex p. quinquefasciatus*.

## RESULTS

*Anopheles species*: *Anopheles albimanus* Wiedemann, *A. quadrimaculatus* Say and *A. freeborni* Aitken were all highly susceptible to *R. nielseni* (Table 1). These species were 6-9 times more susceptible to penetration and development than were *C. p. quinquefasciatus* when the incidence of parasitism was below 10% in the latter. No difference was noted between penetration and development at the end of the tests in any of the *Anopheles* spp., indicating little or no physiological resistance (Table 2).

*Aedes species*: *Aedes sollicitans* (Walker) was the most susceptible of the six *Aedes* spp. tested, and was about three times more susceptible than *C. p. quinquefasciatus* when parasitism was low. *Aedes nigromaculis* (Ludlow), *A. taeniorhynchus* (Wiedemann), and *A. tormentor* Dyar and Knab were about two times more susceptible than the control, and *A. aegypti* (L.) was slightly less susceptible to successful development. *Aedes aegypti* was somewhat resistant to penetration. The percentage of penetration was nearly always lower than the percentage of parasitism at 6-7 days for *Aedes* spp. except for *A. triseriatus*, indicating sampling error or that some parasitism was still taking place after dissection of the 24-hour-old hosts and that little or no physiological resistance could be measured in the five species. The incidence of penetration in *A. triseriatus* was noticeably lower than that of the control species, indicating a high physical or behavioral resistance in *A. triseriatus*. Physiological resistance was high in this species (parasitic development never exceeded 9%) and was attested to by the presence of melanized parasites in older larvae.

*Culex species*: At the lower levels of parasitism *Culex tarsalis* Coquillett and *C. salinarius* Coquillett were three times more susceptible than *C. p. quinquefasciatus* to both penetration and development by *R. nielseni*. *Culex pipiens pipiens* L. was more than twice as susceptible as the control. However, no distinct differences could be found in the susceptibility of native and laboratory populations of *C. p. quinquefasciatus*. Therefore, no detectable resistance has developed in the laboratory colony of this species despite 5 years of using noninfected mosquitoes following exposures to *R. nielseni* to maintain host colonies.

No physiological resistance was noted in *C. tarsalis*, *C. p. pipiens*, or native *C. p. quinquefasciatus*. However, penetration was consistently higher 24 h after treatment in *C. salinarius*, and some resistance (about 10%) was evident at very late stages of host development. *Culex territans* Walker was the only species in which no parasite development was observed, even though the preparasites penetrated up to 67% of the larvae (a much lower incidence than in the control larvae). Obviously, the species was highly resistant, and the parasites died soon after penetration.

*Culiseta inornata* (Williston): *Culiseta inornata*, the most susceptible of the culicine species, was about 9.5 times more susceptible than *C. p. quinquefasciatus* at low levels of parasitism, and had no resistance to development of the parasite.

*Psorophora species*: *Psorophora confinnis* and *P. varipes* (Coquillett) were highly susceptible to penetration and development by *R. nielseni*. *Psorophora ferox* (Humboldt) was highly susceptible to penetration (infection levels were six to seven times higher than in corresponding controls), but was highly resistant to the development of *R. nielseni*; parasitic development never exceeded 13%.

## DISCUSSION

The data show that when the susceptibility of two species of mosquito hosts to *R. nielseni* is to be compared, the levels of infection

TABLE 1. Susceptibility to the parasitic development of the nematode *Reesimermis nielseni* of 18 species of mosquitoes compared with that of a laboratory strain of *Culex pipiens quinquefasciatus*.

| Mosquito species | No. of tests | Susceptibility index for test species when parasitism in control species occurred within range given[a] | | | | |
|---|---|---|---|---|---|---|
| | | 0 - 10% | 11 - 25% | 26 - 50% | 51 - 75% | 76 - 100% |
| *Anopheles* | | | | | | |
| *albimanus* | 5 | 9.1 | 2.7 | 1.6 | 1.7 | 1.1 |
| *freeborni* | 2 | 6.0 | 2.7 | 2.4 | 1.7 | 1.1 |
| *quadrimaculatus* | 3 | 7.4 | 1.9 | 2.2 | . . . | 1.1 |
| *Aedes* | | | | | | |
| *sollicitans* | 4 | . . . | 2.7 | 2.0 | 1.5 | 1.1 |
| *nigromaculis* | 4 | . . . | 2.3 | 1.7 | 1.1 | 1.1 |
| *taeniorhynchus* | 3 | . . . | 1.9 | 1.4 | 1.2 | 1.0 |
| *tormentor* | 3 | 2.4 | 1.4 | 1.3 | 0.8 | 1.0 |
| *aegpyti* | 3 | . . . | 1.3 | 0.8 | 0.9 | 0.6 |
| *triseriatus* | 3 | . . . | 0.1 | 0.2 | 0.1 | >0.1 |
| *Culex* | | | | | | |
| *tarsalis* | 5 | 6.2 | 2.8 | 1.5 | 1.5 | 1.0 |
| *salinarius* | 3 | 6.1 | 1.3 | 1.3 | 1.5 | 1.0 |
| *p. pipiens* | 3 | 1.8 | 2.4 | 1.6 | 1.5 | 1.1 |
| *p. quinquefasciatus* (native) | 3 | . . . | 1.1 | . . . | 1.1 | 1.0 |
| *territans* | 3 | 0 | 0 | 0 | 0 | 0 |
| *Culiseta inornata* | 3 | 9.4 | 2.2 | 1.7 | 1.3 | 1.1 |
| *Psorophora* | | | | | | |
| *confinnis* | 2 | . . . | 3.2 | 1.9 | . . . | 1.2 |
| *varipes* | 3 | . . . | 1.9 | 1.2 | 1.1 | 1.0 |
| *ferox* | 4 | . . . | . . . | . . . | . . . | 0.1 |

[a]Susceptibility index = $\frac{\text{mean \% parasitism of test species}}{\text{mean \% parasitism of control species}}$.

TABLE 2. Penetration and development of *R. nielseni* in 18 species of mosquitoes and the comparative susceptibility to penetration of these species with the "control" *Culex pipiens quinquefasciatus*.

| Species | Penetration (mean %) | Development (mean %) | Difference in penetration compared with "control" |
|---|---|---|---|
| *Anopheles* | | | |
| *albimanus* | 63 | 64 | 31 |
| *freeborni* | 77 | 79 | 26 |
| *quadrimaculatus* | 79 | 73 | 45 |
| *Aedes* | | | |
| *sollicitans* | 65 | 84 | 14 |
| *nigromaculis* | 66 | 72 | 15 |
| *taeniorhynchus* | 53 | 58 | 16 |
| *tormentor* | 62 | 69 | 1 |
| *aegypti* | 30 | 40 | −7[a] |
| *triseriatus* | 10 | 3 | −42[a] |
| *Culex* | | | |
| *tarsalis* | 71 | 68 | 23 |
| *salinarius* | 69 | 56 | 32 |
| *p. pipiens* | 76 | 81 | 24 |
| *p. quinquefasciatus* (native) | 44 | 58 | 2 |
| *territans* | 21 | 0 | −23[a] |
| *Culiseta inornata* | 75 | 72 | 28 |
| *Psorophora* | | | |
| *confinnis* | 77 | 79 | 33 |
| *varipes* | 72 | 69 | 13 |
| *ferox* | 90 | 6 | 29 |

[a]Negative value denotes a susceptibility to penetration less than that of *C. p. quinquefasciatus*.

achieved during testing can influence the results. For example, *Anopheles albimanus* was nine times more susceptible to *R. nielseni* than *C. p. quinquefasciatus* when the level of parasitism in the latter was below 10%, but less than two times more susceptible when the parasitism of the control species ranged from 50 to 75%. The differences in susceptibility between species at low levels of parasitism may be of significance in determining the relative success to be expected from field releases.

The ability of a given mosquito species to resist *R. nielseni* may be behavioral, physical, or physiological or some combination of these. Behavioral resistance (as high physical activity and the ability of the host to remove the attacking parasite) was indicated in *Aedes triseriatus*, *A. aegypti*, and *C. territans*, each of which had a lower incidence of invasion than the control species. *Aedes triseriatus* and *A. aegypti* are indeed extremely active species; conversely, the anopheline species, which are relative inactive species, were among the most susceptible.

Physical resistance resulting from apparent variations in the thickness of the cuticle of a given species has proved to be especially important in the penetration of older larvae (7), and may have been a factor in causing differences in susceptibility in species in this study.

Physiological resistance, the ability of a given species to prevent the normal development of the nematode once it has penetrated, was evident in *A. triseriatus*, *C. territans*, *C. salinarius*, and *P. ferox*. However, it was complete only in *C. territans*. Resistance to the development of the parasite is known to occur in *A. quadrimaculatus* (9); it was not, however, observed during the present study. Apparently, resistance in this species develops only in larvae infected in the late instars (9).

Some field data are available concerning the effectiveness of *R. nielseni* against *Anopheles* spp. (5, 8, 9), but with limited exceptions (6, 8), little is known concerning the effectiveness of the nematode against the culicine species. The present study provides information about the comparative susceptibility of various mosquito species to *R. nielseni*; however, such comparative data will have limited practical use until the effectiveness of this parasite can be determined in the field against several of these species.

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The JOURNAL OF NEMATOLOGY for April (7: 95-206) was issued 9 April 1975.