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Pathogenicity Tests on Nine Mosquito Species and Several Non-target Organisms with *Strelkovimermis spiculatus* (Nemata Mermithidae)

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Abstract: Nine species of mosquitoes and several species of non-target aquatic organisms were tested for susceptibility to the mermithid nematode, *Strelkovimermis spiculatus.* All species of *Anopheles, Aedes, Culex,* and *Toxorhynchites* exposed to *S. spiculatus* were susceptible. Of the nine mosquito species tested, *C. pipiens quinquefasciatus* had the greatest tolerance to initial invasion and the highest percent infection of those that survived. High levels of infection were also achieved with *Aedes taeniorhynchus* and *A. albopictus,* but these mosquitoes were significantly less tolerant to parasitism than *C. pipiens quinquefasciatus. Strelkovimermis spiculatus* did not infect or develop in any of the non-target hosts tested.

Key words: Aedes aegypti, Aedes albopictus, Aedes taeniorhynchus, Aedes triseriatus, Anopheles albimanus, Anopheles quadrimaculatus, biological control, *Chironomus* sp., Copepoda, *Corethrella brakeleyi,* Corixidae, Culicidae, *Culex pipiens quinquefasciatus, Culex restuans,* Diptera, Dytiscidae, entomopathogenic nematodes, Ephydridae, host range, Mermithidae, mosquito, nematode, Odonata, non-target organisms, safety testing, *Strelkovimermis spiculatus, Toxorhynchites rutilus septentrionalis.*

The mermithid nematode *Strelkovimermis spiculatus* Poinar and Camino was originally isolated and described from the flood-water mosquito, *Aedes albifasciatus* (Macquart) in Argentina (Poinar and Camino, 1986) and subsequently isolated from *Culex pipiens* (L.) (Garcia and Camino, 1990). *Strelkovimermis spiculatus* has a life cycle similar to other aquatic mermithids. Infective juveniles (preparasitic second-stage juveniles [J2]) hatch from eggs and actively seek and penetrate larvae of the mosquito host. The third-stage juvenile (J3) develops in the mosquito larva for 6 to 8 days, at which time the postparasitic fourth-stage juvenile (J4) emerges, killing the host. The J4 develop into adults, which mate and lay eggs in the aquatic substrate to complete the cycle.

Strelkovimermis spiculatus has demonstrated a tolerance for high levels of organic pollution and dissolved ions relative to other mermithids (Camino and Garcia, 1991) and is therefore being evaluated for introduction as a biological control agent of mosquitoes in polluted habitats. Determination of the mosquito host range and safety of *S. spiculatus* for non-target aquatic organisms is required prior to field-testing and is the subject of this paper.

MATERIALS AND METHODS

Cultures of *S. spiculatus* from *C. pipiens quinquefasciatus* Say used in this study were established from cultures collected in Buenos Aires Province, Argentina, and maintained in the laboratory using procedures previously described for the mass rearing of the mermithid nematode *Romanomermis culicivorax* Ross and Smith (Petersen and Willis, 1972). The following mosquitoes were obtained from the mosquito insectary at USDA ARS, Gainesville, Florida: *Aedes aegypti* (L.), *A. albopictus* (Skuse), *A. taeniorhynchus* (Wiedemann), *Anopheles quadrimaculatus* (Say), *A. albimanus* Wiedemann, *C. p. quinquefasciatus,* and *Toxorhynchites rutilus septentrionalis* (Dyar and Knab). *Culex restuans* Theobald was collected from the field. Preparasites of *S. spiculatus* were obtained by flooding sand cultures containing eggs with deionized water; preparasites used for the *A. taeniorhynchus* exposures were flooded with 0.1% NaCl solution. All mosquitoes were exposed to preparasites in groups of 100 except for *T. r. septentrionalis,* which were exposed in one group of 25. Mosquito larvae

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were exposed to *S. spiculatus* preparasitic J2 at a dose of 5 preparasites/larva in 100 ml of deionized water except for *A. taeniorhynchus,* which were exposed in 0.1% NaCl solution. An equal number of mosquitoes that were not exposed to preparasites served as controls. After 24 hours, larvae were transferred to 500-ml pans and reared to the fourth-instar stage. At this time, the fourth-instar larvae were transferred to well plates and held individually until emergence of the nematode. Mortality, percent infection, pupation, and average number of nematodes per larva were recorded. General Linear Models (SAS version 6.08, SAS Institute, Cary, NC) were performed with Tukey's comparison of the means using arcsine transformation of these data.

Corethrella brakeleyi (Coquillet) (Diptera: Chaoboridae) larvae (mixed ages, second to fourth instar) were collected from a flooded ditch in Alachua County, Florida. *Chironomus* sp. (Diptera: Chironomidae) larvae were collected from artificial cement containers at USDA ARS, Gainesville, Florida. All other non-target organisms were collected from secondary treatment ponds at a swine facility in Gainesville, Florida. All non-target organisms were exposed to a concentration of five *S. spiculatus* preparasites per individual. An equal number of non-target organisms, not exposed to nematodes, served as controls. A group of *C. p. quinquefasciatus* larvae was exposed as described above with each test to verify the infectivity of *S. spiculatus* preparasites. After 1 week, mortality and infection were recorded.

Results and Discussion

Strelkovimermis spiculatus successfully invaded and developed in each species of *Aedes, Anopheles, Culex,* and *Toxorhynchites* tested, but susceptibility varied considerably (Table 1). All mosquito larvae infected with *S. spiculatus* died as a result of emergence of the nematodes. Of the nine mosquito species tested, *C. p. quinquefasciatus* had the greatest tolerance to initial invasion (4% mortality of pre-fourth-instar larvae) and the highest infection among those fourth-instar larvae (88%). *Culex pipiens quinquefasciatus* also produced the largest number of nematodes per larva but was not significantly different from the other mosquitoes tested. High levels of infection also were achieved with *Aedes taeniorhynchus* and *A. albopictus,* but many more of these mosquitoes died as pre-fourth-instar larvae than did *C. p. quinquefasciatus.* Mortality of fourth-instar mosquito larvae was due to the emergence of nematodes from the mosquito; the cause of mortality in pre-fourth-instar larvae is unknown. *Strelkovimermis spiculatus* did not de-

TABLE 1. Susceptibility of nine mosquito species to *Strelkovimermis spiculatus.*

Species	Number of tests	Percent mortality of pre-fourth-instar larvae[1,2]	Percent infection of fourth-instar larvae[1,2]	Average number of nematodes per larva[1]
Culex pipiens quinquefasciatus	3	4.0 ± 2.3 d	88.1 ± 8.4 a	2.3 ± 0.2 a
Aedes taeniorhynchus	3	32.0 ± 4.6 bc	78.2 ± 9.9 ab	2.0 ± 0.4 a
A. albopictus	3	22.7 ± 2.4 c	67.8 ± 3.6 abc	1.9 ± 0.4 a
A. triseriatus	3	49.0 ± 1.7 ab	55.9 ± 5.0 bc	1.7 ± 0.1 a
A. aegypti	3	40.7 ± 7.3 abc	55.6 ± 6.0 bc	1.7 ± 0.3 a
Anopheles quadrimaculatus	3	57.3 ± 4.1 a	47.9 ± 5.3 bc	1.1 ± 0.2 a
A. albimanus	3	26.3 ± 1.2 bc	32.5 ± 3.4 c	1.4 ± 0.02 a
C. restuans	1	35	49	1
Toxorhynchites rutilus septentrionalis	1	20	50	1

[1] Means with the same letter within the same column are not significantly different (GLM, Tukey's grouping, $P \leq 0.05$).
[2] Arcsine-transformed means.

velop or cause mortality in any of the non-target hosts tested except for *Corethrella brakeleyi* (5% mortality) (Table 2).

Currently, 10 species of *Strelkovimermis* have been described from dipteran hosts within the families Chironomidae or Culicidae (Johnson and Kleve, 1996). The two species from mosquitoes are *S. peterseni* (Poinar) (Nickle, 1972; Poinar, 1979) and *S. spiculatus*. *Strelkovimermis peterseni* originally was isolated in southwestern Louisiana from *A. crucians* Wiedemann, *A. quadrimaculatus*, and *A. punctipennis* (Say) and later in northeastern New York from *A. punctipennis* (Molloy and Wraight, 1980). *Strelkovimermis peterseni* has been experimentally reared in the laboratory with *A. albimanus*, *A. atropos* Dyar and Knab, *A. barberi* Coquillett, *A. bradleyi* King, *A. freeborni* Aitken, and *A. stephensi* Liston (Molloy and Wraight, 1980; Petersen, 1973; Petersen and Chapman, 1970). Studies conducted by Petersen and Chapman (1970) demonstrated that *S. peterseni* was unable to parasitize any of the species of *Culex*, *Aedes*, and *Psorophora* tested, exhibiting specificity for anopheline mosquitoes. The present study indicates that *S. spiculatus*, which infected four different genera of mosquitoes, has a much broader mosquito host range than *S. peterseni*. In this respect, *S. spiculatus* is more like the mermithid *Romanomermis culicivorax*, which previously was shown to have an extremely broad mosquito host range (Petersen et al., 1968, 1969).

TABLE 2. Infection and mortality of non-target arthropods exposed to *Strelkovimermis spiculatus*.

Arthropods Tested	n	Percent mortality	Percent infected
Corethrella brakeleyi (Diptera: Chaoboridae)	50	5	0
Copepods (mixed species) (Crustacea: Copepoda)	200	0	0
Chironomus spp. (Diptera: Chironomidae)	50	0	0
Shore flies (Diptera: Ephydridae)	8	0	0
Water boatmen (Hemiptera: Corixidae)	50	0	0
Predaceous diving beetles (Coleoptera: Dytiscidae)	30	0	0
Damselfly larvae (Odonata: Zygoptera)	15	0	0
Dragonfly larvae (Odonata: Anisoptera)	10	0	0

Information on the safety of *S. peterseni* for non-target hosts is unavailable. However, extensive safety testing with *R. culicivorax* was conducted, and it was concluded that *R. culicivorax* is quite host-specific for mosquitoes (Ignoffo et al., 1973, 1974). The results of this study indicate that *S. spiculatus* is also specific for mosquitoes and poses little risk for non-target aquatic organisms.

Recent field studies with *R. culicivorax* have documented high levels of parasitism for several mosquito species in different habitats (Santamarina and Broche, 1991; Santamarina and Perez, 1997) and their ability to recycle in some of these habitats throughout the season (Santamarina and Perez, 1997). The strain of *S. spiculatus* used in this study was isolated from *C. pipiens* larvae developing in a eutrophic urban habitat (Garcia and Camino, 1990), and the nematode was shown to tolerate higher levels of organic pollution and dissolved ions relative to *R. culicivorax* (Petersen, 1985; Camino and Garcia, 1991). In addition, the information presented in this study indicated that the wastewater mosquito, *C. p. quinquefasciatus*, and the saltmarsh mosquito, *A. taeniorhynchus*, are both excellent hosts for *S. spiculatus*. Therefore, this strain of *S. spiculatus* has the potential to infect mosquitoes and recycle in habitats not suitable for *R. culicivorax*, particularly agricultural wastewater lagoons and possibly salt marsh habitats. Planned field releases will target these habitats for evaluation of *S. spiculatus*.

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