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Susceptibility of neotropical mosquito larvae (Diptera: Culicidae) and non-target aquatic organisms to the entomoparasitic nematode *Strelkovimermis spiculatus* Poinar & Camino, 1986 (Nematoda: Mermithidae)

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Summary – Fourteen species of mosquito and several species of non-target aquatic organisms were tested for susceptibility to the neotropical mermithid nematode, *Strelkovimermis spiculatus*. All species of *Aedes*, *Anopheles*, *Culex*, *Isostomyia* and *Ochlerotatus*, plus one of the two *Psorophora* species exposed to *S. spiculatus* preparasites, were parasitised. None of the non-target aquatic organisms exposed to *S. spiculatus* were infected, with the exception of Chironomidae larvae in which nematode penetration was observed although host reaction in the form of nematode melanisation occurred 24-48 h post-infection, the nematodes dying within the host.

Keywords – host range, mosquitoes, non-target organisms, *Strelkovimermis spiculatus*.

The mermithid nematode *Strelkovimermis spiculatus* Poinar & Camino, 1986, a parasite of mosquito larvae, was first isolated from the neotropical floodwater mosquito *Ochlerotatus albifasciatus* (Macquart) in temporary ponds around La Plata, Argentina (Poinar & Camino, 1986). *Strelkovimermis spiculatus* has a life cycle similar to other aquatic mermithids (Camino & Reboredo, 1994) and is readily cultured under laboratory conditions (Camino & Reboredo, 1996). Previous studies have shown *S. spiculatus* to be a promising biocontrol agent of mosquitoes (Camino & García, 1991; Maciá *et al*., 1995; Camino & Reboredo, 2000). Little is known about the susceptibility of mosquitoes to *S. spiculatus* in Argentina (Garcia & Camino, 1990; García *et al*., 1994) and nothing at all about the susceptibility of non-target neotropical aquatic fauna to *S. spiculatus* parasitism.

Determination of the mosquito host range and effect of *S. spiculatus* on non-target aquatic organisms which share the same habitat as the target mosquito larvae is a prerequisite to field-testing *S. spiculatus* for mosquito biocontrol and is the subject of this paper.

Materials and methods

The preparasitic juveniles (J2) used in this study were obtained from a colony of *S. spiculatus* maintained at the Centro de Estudios Parasitológicos y de Vectores-Cepave, using procedures previously described by Camino and Reboredo (1996). Preparasites were obtained by flooding sand cultures containing eggs with dechlorinated tap water. Preparasites 24 h old were counted under a stereomicroscope.

The following mosquito species used in the experiments were field-collected from the environs of La Plata city (34°55′S, 57°57′?), Argentina: *Aedes aegypti* (Linné); *Anopheles albitarsis* Lynch Arribalzaga; *Culex apicinus* (Philippi); *C. castroi* Casal & García; *C. chidesteri* Dya; *C. dolosus* Lynch Arribalzaga; *C. maxi* Dyar; *C. pipiens* Linné; *C. renatoi* Lane & Ramalho; *Isostomyia paranensis* (Brethes); *Ochlerotatus albifasciatus* (Macquart); *O. crinifer* (Theobald); *Psorophora cyanescens* (Coquillett), and *P. ferox* (von Humboldt). Mosquito species were identified using the keys of Lane (1952) and Darsie and Mitchell (1985). *Anopheles* larvae were identified

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Table 1. *Susceptibility of mosquito species to* Strelkovimermis spiculatus.

Host species	Instar	N° exposed	% Infection (± SD)	Intensity (± SD)
Aedes aegypti	I	350	94.3 ± 11.3	2.8 ± 0.9
	II	165	97.1 ± 4.8	1.9 ± 0.6
	III	400	84.1 ± 8.8	2.1 ± 1.0
	IV	600	27.7 ± 8.0	1.1 ± 0.1
Anopheles albitarsis	II	10	12.5 ± 6.2	1
	IV	7	0	
Culex apicinus	I	75	100	4.4 ± 1.1
	II	149	78.7 ± 15.3	2.6 ± 1.9
C. castroi	I	15	70	1.2
	II	11	50	2
	III	5	0	
C. chidesteri	II	30	60	2
C. dolosus	II	58	86.2 ± 7.5	5 ± 1.6
C. maxi	IV	8	12.5	1
C. pipiens	I	100	95.5 ± 5.2	1.1 ± 0.2
	II	120	85 ± 13.8	2.1 ± 1.0
	III	300	67 ± 15.1	1.7 ± 0.4
	IV	300	5.6 ± 6.8	1.0 ± 0.0
C. renatoi	II	3	100	1
	IV	2	100	1
Isostomyia paranensis	I/II	5	100	6.5 ± 3.5
	III/IV	6	100	2.5 ± 0.8
Ochlerotatus albifasciatus	II	75	97.5 ± 5	3.0 ± 0.6
	IV	75	27 ± 5	1.3 ± 0.6
O. crinifer	II	105	60.5 ± 7.1	1.6 ± 0.5
	IV	45	2.3 ± 4.0	1
Psorophora cyanescens	IV	27	0	
P. ferox	I	5	100	3.5
	II	90	45.7 ± 5.0	1.3 ± 0.1
	IV	75	3.3 ± 5.7	1

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Some species of mosquito larvae were of limited availability and so the instars and numbers of hosts used varied (see Table 1). Bioassays were conducted in 250 ml plastic containers with 100 ml of dechlorinated tap water. Mosquito larvae exposed to *S. spiculatus* preparasites at a dose of ten preparasitic J2/larva are listed in Table 1. Each host species was exposed separately by instar and species type. Exposed larvae were reared for 48 h and fed daily with finely ground rabbit food. Treated larvae were examined with light microscopy and percent infection and average number of nematodes per larva (intensity) recorded.

Table 2. *Susceptibility of non-target organisms exposed to* Strelkovimermis spiculatus.

Non-target organisms	N° exposed	% Infection
Amphipoda (Crustacea)	60	0
Cladocera (Crustacea)	150	0
Ostracoda (Crustacea)	300	0
Cyclopoidea (Crustacea: Copepoda)	60	0
Calanoidea (Crustacea: Copepoda)	10	0
Hemiptera	12	0
Chironomidae (Diptera)	150	7
Psychodidae (Diptera)	45	0
Chaoboridae (Diptera)	4	0
Coenagrionidae (Odonata: Zygoptera)	17	0
Annelida	15	0
Cnesterodom decenmaculatus (Vertebrata: Pisces)	3	0

The non-target aquatic organisms tested are listed in Table 2. Non-target organisms for bioassays were collected from ponds around La Plata and were transported to the laboratory in water from the collection sites. Non-target organisms were exposed as groups at a dose of ten preparasites/organism in 250 ml plastic containers with 100 ml of dechlorinated tap water. Exposed individuals were reared for 10 days, then dissected and examined for nematode infection.

All bioassays were conducted at 26 ± 1°C and under a 12:12 (L:D) h photoperiod. At least two replicates were conducted for each test depending on availability of larvae. Control groups were used in all tests and handled in a similar manner, but without including nematodes. For each set of tests, *A. aegypti* larvae were exposed to *S. spiculatus* preparasites to confirm the viability of the nematode culture.

Results and discussion

Of the 14 mosquito species tested, 13 were susceptible to *S. spiculatus* infection when exposed to ten preparasites of *S. spiculatus* per larva (Table 1). Larvae of *P. cyanescens* were not infected by preparasites, although in this case only fourth instar larvae were exposed. The overall parasitism of the 13 susceptible species was 67.6%. The major difference in host susceptibility was the degree of infection by instar, which was substantially greater

in young larvae (first and second instars) with an overall mean of 79.6 (12.5-100)% whereas it was 37.8 (0-100)% in older larvae (third and fourth instars) (Table 1). The neotropical mosquito, *I. paranensis*, which breeds in phytotelmata habitats, according to the standard system for classifying larval mosquito habitats (Laird, 1988), was the most susceptible mosquito species tested with 100% infection at all four instar stages (Table 1). The lowest infection rates were recorded in *A. albitarsis* where 12.5 and 0% of the second and fourth instar exposed larvae were parasitised, respectively (Table 1).

The number of nematodes per mosquito larva (intensity) varied according to mosquito species and instar larvae infected. An overall mean of 2.25 nematodes per larva was determined for susceptible mosquito species. First and second instars harboured more nematodes per host (2.8 nematodes/larva) than third and fourth instars (1.4 nematodes/larva) (Table 1). Nematode melanisation as a form of host resistance was not observed in any of the mosquito species exposed to *S. spiculatus* preparasites.

To date, members of the genus *Strelkovimermis* have been described exclusively from dipteran hosts of the family Chironomidae and Culicidae (Johnson & Kleve, 1996). Two species of *Strelkovimermis*, *S. peterseni* (Poinar) and *S. spiculatus*, have been described from mosquitoes. *Strelkovimermis peterseni* was reported as highly specific to anopheline mosquitoes and unable to parasitise any of the species of *Aedes*, *Culex*, and *Psorophora* tested (Petersen & Chapman, 1970). The present study indicates that *S. spiculatus*, which parasitised 13 species belonging to six Culicidae genera, has a much broader mosquito host range than *S. peterseni*. In addition, Becnel and Johnson (1998) determined that a strain of *S. spiculatus* isolated from *C. pipiens* larvae collected in Argentina successfully invaded and developed in nine mosquito species from the genera *Aedes*, *Anopheles*, *Culex* and *Toxorhynchites*, the lowest infection rates being recorded on *Anopheles* larvae. The low infection rates found on *Anopheles* species, and the lack of susceptibility of *P. cyanescens* to *S. spiculatus* preparasites, are not well understood. Differences in susceptibility of the mosquito species are caused by physiological, physical, and/or behavioural characteristics (Petersen & Chapman, 1979). As a result, in order to determine the susceptibility levels more tests with larger number of larvae from these refractory species need to be conducted.

Natural *S. spiculatus* infections were found in mosquito larvae sampled from temporary and permanent ponds around La Plata City, Buenos Aires Province, Argentina (Poinar & Camino, 1986; García & Camino, 1990; García *et al*., 1994). Prevalence rates were substantially higher in populations of its natural host, *O. albifasciatus*, in which *S. spiculatus* usually reached epizootic levels with maximum infection rates ranging from 80-100% (Maciá *et al*., 1995; Micieli & García, 1999).

Strelkovimermis spiculatus infections were not recorded for the majority of non-target aquatic organisms exposed to the preparasites with the exception of a dipteran of the family Chironomidae in which nematode penetration was detected on 7% of the larvae exposed to preparasites (Table 2). All nematodes which penetrated Chironomidae larvae died 24-48 h post-infection due to host resistance in the form of melanisation. A previous study by Becnel and Johnson (1998) on the safety of aquatic non-target organisms exposed to *S. spiculatus* indicated that this nematode posed little risk for non-target organisms, including members of the Chironomidae. This is in accord with the results obtained in this study.

Strelkovimermis spiculatus is therefore a promising potential biological control agent because of its wide range of mosquito hosts and its inability to attack non-target aquatic organisms.

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