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Lateral Movement of the Entomopathogenic Nematode, *Steinernema arenaria* (Artyukhovsky, 1967) (Rhabditida: Steinernematidae) in Sand at Different Temperatures

A. S. CSONTOS and M. NÁDASY

Department of Entomology, Faculty of Agriculture, University of Veszprém,
H-8360 Keszthely, Deák F. u. 57, Hungary

The lateral movement of the entomopathogenic nematode, *Steinernema arenaria* (Artyukhovsky et al., 1997) (Rhabditida: Steinernematidae) in sand was determined at 15, 20, 25 and 30 °C. The infective juveniles moved farther and quicker for the same time period as the temperature increased from 15 to 30 °C, but interestingly enough more infective juveniles moved the opposite way than toward its host. At 15, 20, 25 and 30 °C, an average of 0.2, 2.8, 18, and 21.8% of the infective juveniles moved toward the larvae, while 0.8, 17.3, 39.3 and 55.6% moved in the opposite direction. The movement of the infective juveniles at all temperatures was significantly different from each treatment at $P < 0.001$. The extraction efficiency decreased at all temperatures as time went by.

Keywords: Entomopathogenic nematodes, *Steinernema arenaria.*

The entomopathogen nematode *Neoaplectana anomali* (Artyukhovsky, 1967) was found in the Riazan and Voronez provinces of Central Russia in a dead larva of the chafer, *Anomala dubia* scop. by Kozodoi (1984). This species is reported only from the above-mentioned two central provinces of the former USSR. The synonymization of the *S. anomali* to *S. anomalae* was made by Curran in 1989 while in 1997 this species was redescribed (Artyukhovsky, 1997) and named as *S. arenaria.* The synonymization and the rediscription process was based on morphometrics, slide preparation, protein disc-electrophoresis, DNA isolation and analization and PCR amplification (Artyukhovsky et al., 1997). This species is very similar to *S. glaseri* both morphologically and biologically. There are some differences between the two species though: the male of *S. arenaria* has a swollen tip on its spicules whereas in *S. glaseri* males, the spicule tip is notched or even hooked. This character is the only consistent morphological difference that can be used to separate the adults of the two species since the quantitative morphological data are too variable and frequently overlap. Quantitative measurements of the infective juveniles demonstrated less variation and indicated that the distance between the head to the excretory pore can be used as a distinctive diagnostic character. The infective juveniles of the species *S. arenaria* are long and thick with a lateral field of 8 equal bands (9 lines). *S. glaseri* has a shorter distance from the head to its excretory pore. The body of the *S. arenaria* males has a yellowish color while the body of the females has a colorless gonads

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with yellowish intestine. Simultaneously, ratio D (distance from head to excretory pore divided by the distance from the head to the base of the pharynx) also shows very little overlap (Poinar and Kozodoi, 1988) *(Table 1).*

Table 1

Measurements (in μm) and ratios of the nematode *S. arenaria*

	Males*	Females*	Juveniles*	Juveniles**	Juveniles***
Number of specimen	50	50	50	10	15
Total length	1280–2320	2600–9720	930–1580	724–1408	928–1088
Greatest width	70–165	170–400	31–44	44–77	28–35
Distance: head to excretory pore	–	–	–	–	76–86
Distance: head to nerve ring	–	–	–	–	100–120
Distance: head to pharynx base	–	–	–	123–131	138–160
Oesophagus length	125–209	180–280	132–187	–	–
Tail length	14–40	40–105	65–95	77–84	64–77
Anterior end of testis flexure	230–690	–	–	–	–
Spicula	63–93	–	–	–	–
Gubernaculum	45–63	–	–	–	–
Anal diameter	–	–	–	–	16–22
A	12.1–23.4	9.7–38.9	24.7–39.6	17.2–25.6	26.4–34.4
B	8.5–15.5	11.8–37.4	5.9–9.2	5.9–10.8	5.9–7.0
C	38.4–126.7	39.4–140.9	12.2–17.9	9.4–16.9	13.2–15.0
D	–	–	–	–	0.52–0.59
D%	52–96	47–98	53–68	–	–
E	–	–	–	–	1.06–1.30
V%	–	48–60	–	–	–
H%	–	–	23–57	–	–

*A. K. Artyukhovsky et al. (1997)
**E. M. Kozodoi (1984)
***G. O. Poinar and E. M. Kozodoi (1988)

As it was stated earlier not only morphological differences can be found between the two species: differences in total proteins, malate dehydrogenase, esterases and acid phosphatase have been reported between *S. glaseri* and *S. arenaria* and could be used to separate these morphologically very similar species (Artyukhovsky et al., 1997). Both *S. arenaria* and *S. glaseri* develop rapidly in *Galleria mellonella* larvae than do other steinernematid species (Poinar, 1979). The development of *S. arenaria* is even slightly faster than that of *S. glaseri* (Artyukhovsky et al., 1997). Kozodoi (1984) stated that "The new species has very large invasion larvae and spermatozoa. Experiments on infesting the insects have shown that the new species is more active and develops at a greater rate than *N. feltiae.*" Both nematode species emerge from the insect cadaver as predauer or mature second stage juveniles instead of third stage juveniles. The second stage juveniles of the

nematode *S. arenaria* emerge from the host 132 hours after initial contact (Artyukhovsky et al., 1997). At the same time the final molt usually occurs 48–72 hours after emergence. Both species belong to the group of Steinernematids, which has longer than 1000 mm juveniles (Nguyen and Smart, 1996). Both species prefer to attack larvae of Scarabaediae in nature (Artyukhovsky et al., 1997). At the same time there are behavioral differences between those Steinernematid species having a smaller infective juvenile and between those having a bigger infective juvenile. The infective juveniles of the smaller Steinernematid species tend to search for their host on the soil surface and nictate waving back and forth, bending or even jumping from one to another soil particle. Larger species like *S. glaseri* or *S. anomali* tend to disperse in the soil searching their host moving actively among the soil particles.

Materials and Methods

Stock cultures of infective juveniles of *S. arenaria* were maintained by infecting fourth instar larvae of the greater wax moth *(Galleria mellonella).* The infective juveniles were collected daily from cadavers of wax moth larvae placed on White traps (White, 1924). The emerged infective juveniles were stored in Ehrlenmeyer flasks and maintained at 9–10 °C in deionized water at a concentration of ca. 1000 nematodes/ml. New nematode stocks were produced every 3–4 wk.

Builders sand (sand, 98.6%; silt, 0.7%; clay, 0.7%; pH, 6.5) was used in this experiment. The sand was autoclaved at 90 °C for 99 min. After cooling, the moisture content was measured and adjusted to 3 mass %.

PVC (polyvinyl chloride) pipe with an inside diameter of 5 cm was cut into 2.5 cm sections. Seventeen of these 2.5 cm sections were taped together to make a tube 42.5 cm long in which lateral movement of the nematodes was determined. A total of 20 tubes were filled with sand. The sections in a tube were numbered from 1 to 17. The central section (number 9) in each tube contained a hole through which the infective juveniles were injected into the soil. Two last instar *Galleria* larvae in wire cages were put 20 cm away in the 17th section. The tubes were placed in a computer regulated thermostat called Florida Reach-In Chamber (Walker et al., 1993) set at 15, 20, 25 or 30 °C and allowed to stabilize for 24 hours before 5000 infective juveniles were injected into each tube. Each of five replicates contained four tubes with infective juveniles extracted from one tube at 8, 16, 24 and 32 hours after they were injected into the tubes. Thus, the interval between nematode extractions was 8 hours. Every 8 hours infective juveniles from sections of 5 tubes (5 x 17 = 85 samples) were extracted. The infective juveniles were recovered for counting by washing the sand in water and twice decanting them in suspension through a 500-mesh sieve and then into a 50 ml centrifuge tube. The percentage of the nematodes recovered (extraction efficiency) from each section was recorded. The experiment was repeated five times giving a total of 340 samples. The infective juveniles recovered were counted in Petri dishes (9 cm diam.) with the aid of a Nikon SMZ-2T microscope at a x30 magnification.

Any dead *Galleria* larvae in section 17 were put onto a White trap to make sure that the cause of mortality was the nematodes. Live *Galleria* larvae were put into Petri dishes for further observation for 5 days. Dead *Galleria* larvae were not dissected, only external observations were taken.

Statistical analysis was performed using an SPSS program for Analysis of Variance (ANOVA).

Results

At 15 °C, 32 hours was not enough for the infective juveniles to move 20 cm and reach the two *Galleria mellonella* larvae so none of the *Galleria* larvae were dead. The number of infective juveniles that moved toward the larvae from 8 to 32 hours increased from 0% to 0.3%, while those that moved in the opposite direction increased from 0.1% to 2.7%. An average of 99.1% (3222) infective juveniles remained in the central section without showing any movement. The extraction percentage ranged from 57% to 75% and the overall extraction efficiency was 65% (3252 infective juveniles recovered out of 5000).

At 20 °C, 32 hours was not enough for the infective juveniles to move 20 cm and reach the two *Galleria mellonella* larvae so none of the *Galleria* larvae were dead. After 8 hours an average of 4.3% of the infective juveniles moved toward the *Galleria* larvae, and 6.2% moved in the opposite direction. An average of 89.5% of the infective juveniles remained in the central section. After 16 hours, an average of 0.8% of the infective juveniles moved toward the larvae, and 12.3% moved in the opposite direction. An average of 86.9% of the infective juveniles remained in the central section. After 24 hours, an average of 1.2% of the infective juveniles moved toward the larvae, and 23.2% moved in the opposite direction. An average of 75.6% of the infective juveniles remained in the central section. After 32 hours, an average of 5.1% of the infective juveniles moved toward the larvae, and 30.2% moved in the opposite direction. An average of 64.6% of the infective juveniles remained in the central section. The extraction efficiency ranged from 60% to 74% and the overall extraction efficiency was 66.6% (3329 infective juveniles recovered out of 5000).

At 25 °C, within 8 hours an average of 25.4 infective juveniles moved 20 cm and reached the two *Galleria mellonella* larvae and 8 out of 10 *Galleria* larvae were dead. After 8 hours an average of 17.6% of the infective juveniles moved toward the *Galleria* larvae, and 11.6% moved in the opposite direction. An average of 70.8% of the infective juveniles remained in the central section. After 16 hours, an average of 15.9% of the infective juveniles moved toward the larvae, and 30.5% moved in the opposite direction. An average of 53.6% of the infective juveniles remained in the central section. After 24 hours, an average of 22.6% of the infective juveniles moved toward the larvae, and 58.5% moved in the opposite direction. An average of 18.9% of the infective juveniles remained in the central section. After 32 hours, an average of 15.7% of the infective juveniles moved toward the larvae, and 71.3% moved in the opposite direction. An average of 13% of the infective juveniles remained in the central section. The extraction efficiency ranged from 38% to 59% and the overall extraction efficiency was 49.3% (2463 infective juveniles recovered out of 5000).

At 30 °C, within 8 hours an average of 186 infective juveniles moved 20 cm and reached the two *Galleria mellonella* larvae and all 10 *Galleria* larvae were dead. After 8 hours an average of 31.5% of the infective juveniles moved toward the *Galleria* larvae, and 35.5% moved in the opposite direction. An average of 32.9% of the infective juveniles remained in the central section. After 16 hours, an average of 18% of the infective juveniles moved toward the larvae, and 45.2% moved in the opposite direction. An average of 36.8% of the infective juveniles remained in the central section. After 24 hours, an average of 13.7% of the infective juveniles moved toward the larvae, and 80.2% moved in the opposite direction. An average of 6.1% of the infective juveniles remained in the central section. After 32 hours, an average of 21.2% of the infective juveniles moved toward the larvae, and 76.4% moved in the opposite direction. An average of 2.4% of the infective juveniles remained in the central section. The extraction efficiency ranged from 33% to 68% and the overall extraction efficiency was 52.6% (2629 infective juveniles recovered out of 5000).

The movement of the infective juveniles at all temperatures was significantly different from each other at $P < 0.001$ *(Table 2).* The extraction efficiency always decreased at all temperatures as time went by. The reason of the movement of the infective juveniles is not known. Maybe further investigations would need to answer this very interesting question.

Table 2

Statistical analysis of the data gained at 15, 20, 25 and 30 ºC.
The results calculated by the SPSS program (ANOVA)

Temp.	Sections 1–89 10–17	Mean	Standard deviation	Standard error	95% confidence interval		Significance
					Lower Bound	Upper Bound	
	1–8	25	32				
15 °C	9	3222	409	51	983	1185	P < .001
	10–17	5	4				
	1–8	559	307				
20 °C	9	2665	544	53	1003	1211	P < .001
	10–17	97	72				
	1–8	968	459				
25 °C	9	1053	770	51	720	922	P < .001
	10–17	442	115				
	1–8	1461	396				
30 °C	9	594	517	51	775	978	P < .001
	10–17	574	310				

Conclusions

The behaviour of the species *S. arenaria* was very interesting: the larvae were not attractive to the infective juveniles of that species and as temperature rose more and more infective juveniles moved to the opposite direction and not toward its host *(Fig. 1).* We can conclude the following from the data gained at 15, 20, 25 and 30 °.

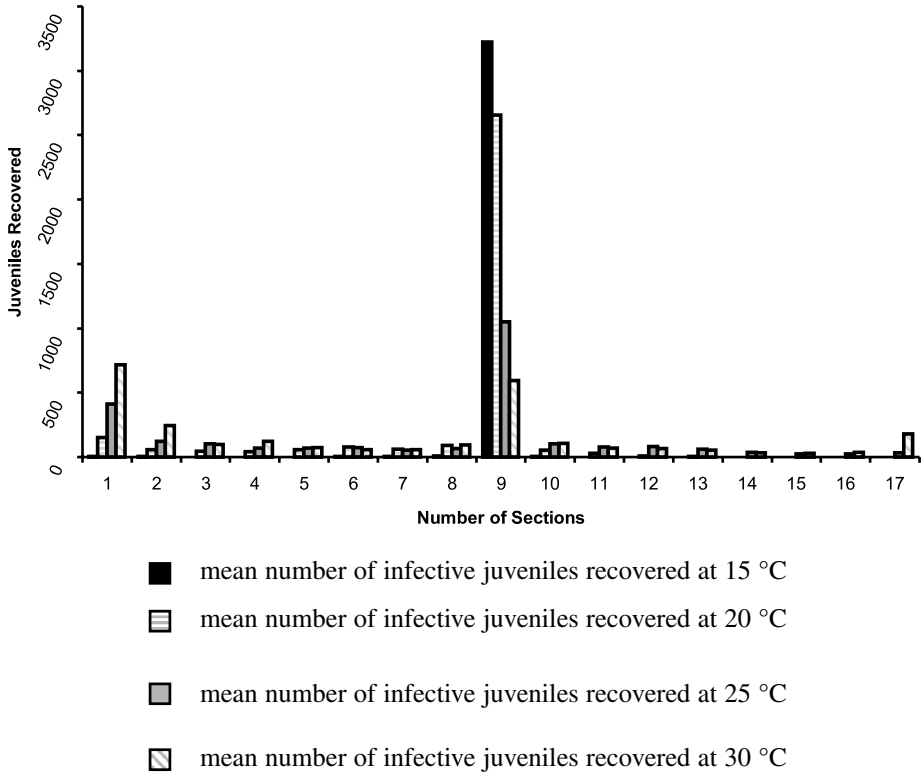

Fig. 1. The mean number of the infective juveniles recovered at 15, 20, 25 and at 30 °C (the data represent the mean of the four extractions made at each temperature)

At 15 °C, 32 hours was not enough for the infective juveniles of *S. arenaria* to locate and kill its host. It is noteworthy that an average of 99.1% (3222) infective juveniles remained in the central section without showing any movement.

At 20 °C, 32 hours was not enough for the infective juveniles to move 20 cm and reach the two *Galleria* larvae so none of the *Galleria* larvae were dead. An average of 79.8% (2657) infective juveniles remained in the central section without showing any

movement. After 16 hours, an average of 59 infective juveniles recovered from section 1, and 12.3% of the infective juveniles moved to the opposite direction and not toward the *Galleria* larvae.

At 25 °C, the movement of the infective juveniles was very quick, but interestingly enough more infective juveniles moved the opposite way than toward its host. Thirty-two hours after nematode injection an average of 15.7% of the infective juveniles moved toward the larvae, and 71.3% moved in the opposite direction.

At 30 °C, the movement of the infective juveniles was very quick: even 8 hours after injection all *Galleria* were dead, but interestingly enough more infective juveniles moved the opposite way than toward its host. Thirty-two hours after nematode injection an average of 21.2% of the infective juveniles moved toward the larvae, and 76.4% moved in the opposite direction.

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