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RESEARCH ARTICLE

Ecological aspects of an isolate of *Steinernema diaprepesi* (Rhabditida: Steinernematidae) from Argentina

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Ecological aspects of *Steinernema diaprepesi* isolate SRC were studied to evaluate the species potential as biological control agent of insect pests. Under laboratory conditions, the following aspects were determined: the nematode life cycle, pathogenicity to several arthropods, reproductive capacity, tolerance to desiccation, effect of temperature on survival and infectivity of infective juveniles (IJs), and influence of soil texture and soil water potential on the isolate. The parasitic cycle on last-instar larvae of *Galleria mellonella* at 25°C was completed 8 days after infection. The nematode showed high virulence to lepidopteran larvae, being limited or nil in the remaining orders of arthropods evaluated. An acceptable offspring production of *S. diaprepesi* was confirmed in the species *G. mellonella* and *S. frugiperda*, suggesting that the isolate would have potential for control of lepidopteran larvae. Optimum temperature for reproduction was 20–25°C. IJs survived exposure to a range of temperatures between 10 and 40°C, with a significant reduction in the number of live IJs at 40°C. The nematodes remained infective at 20–40°C. IJ mortality was 100% on day 6 of exposure to 85% RH. The movement of IJs observed in the soil column experiments revealed that the isolate uses a cruiser-type search strategy. Soil texture and water potential significantly influenced IJ movement, search and penetration of *G. mellonella* larvae. The efficacy of this isolate was found to be favoured in sandy soils, regardless of the soil water potential.

Keywords: *Steinernema diaprepesi*; entomopathogenic nematode; ecology; biological control

1. Introduction

Entomopathogenic nematodes (EPNs) of the genus Steinernema (Nematoda: Steinernematidae) are insect parasites that can be used as biological control agents of pests for agriculturally important crops (Grewal, Ehlers, & Shapiro-Ilan, 2005). Infective juveniles (IJs) locate the insect host, penetrate into the haemocoel through natural openings or thin areas of the cuticle (Koppenhöfer, Grewal, & Fuzy, 2007; Peters & Ehlers, 1994) and release symbiotic bacteria carried in their intestines. The

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genus Steinernema is associated with bacteria of the genus Xenorhabdus; infected insects usually die due to septicemia approximately 48 h after infection (Forst & Clarke, 2002). The nematodes then feed on the bacteria and digested tissues to complete one to three generations inside the insect, before IJs emerge to the soil (Poinar, 1990).

Steinernema diaprepesi was described from larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) collected from a citrus plantation in Florida, USA (Nguyen & Duncan, 2002). The nematode was also reported in the Venezuela (Spiridonov, Reid, Podrucka, Subbotin, & Moens, 2004) and the Caribbean Islands of Martinique and Guadalupe (Tailliez, Pages, Ginibre, & Boemare, 2006). *S. diaprepesi* SRC was isolated from a commercial carrot crop in the locality of Santa Rosa de Calchines (Santa Fe, Argentina), thus becoming the first record for Argentina (Lax et al., 2011). However, the insect host could not be identified due to the procedure used to isolate EPNs from the soil (Bedding & Akhurst, 1975).

Most of the studies on EPNs include descriptions of new species and isolates of these organisms from soil samples for further taxonomic description, without considering the ecological characteristics (Koppenhöfer & Kaya, 1999). Increasing knowledge about the ecological characteristics of a given entomopathogenic species contributes to an accurate estimation of the potential of these organisms as biological control agents of an insect pest (Koppenhöfer & Fuzy, 2003; Koppenhöfer, Ganguly, & Kaya, 2000; Koppenhöfer & Kaya, 1999; Shapiro-Ilan, Stuart, & McCoy, 2005b).

For a nematode species to be considered a biological control agent, its pathogenicity and capacity to reproduce in the host should be demonstrated. The reproductive capacity is a measure of the ability of the nematode to produce progeny in a given host and is affected by temperature. At the same time, reproductive capacity allows us to estimate the number of IJs that a nematode will produce when it parasitizes an insect in a given environment and if the species will remain in the soil where it will be applied (Shapiro-Ilan, Dutcher, & Hatab, 2005a).

Soil temperature has a great influence on infectivity of EPNs (Grewal, Selvan, & Gaugler, 1994). Tolerance of these organisms to desiccation influences survival of IJs applied in a specific environment, varying markedly among species and isolates (Glazer, Klein, Navon, & Nakache, 1992). The proportion of sand, silt and clay in the soil and soil moisture content influences the movement of EPNs (Stuart, Barbercheck, Grewal, Taylor, & Hoy, 2006). Soil moisture affects nematodes because they need a water film around soil particles to move through them (Bardgett, 2005).

The objective of the present work was to explore ecological aspects of an Argentine isolate of *S. diaprepesi* to determine its potential as a biological control agent of insect pests. The life cycle of the nematode, its insect pathogenicity, reproductive potential, tolerance to desiccation and effect of temperature on IJs survival and infectivity were investigated. In addition, the influence of soil texture and soil water potential on the isolate was evaluated.

2. Material and methods

2.1. Study material

S. diaprepesi isolate SRC was obtained from soils of the Albardón Costero Santafesino (Lax et al., 2011), a sandy area adjacent to San Javier River (Santa Fe, Argentina). The nematodes were maintained at the EPN collection of the

Laboratorio de Zoología Agrícola (Facultad de Ciencias Agrarias-Universidad Nacional del Litoral) and multiplied on last-instar larvae of *Galleria mellonella* (Lepidoptera: Pyralidae), according to the procedure described by Kaya and Stock (1997). IJs were collected using modified White traps and stored at 16°C for 7–14 days until their use in the experiments.

Last-instar larvae of *G. mellonella*, *Tenebrio molitor* (Coleoptera: Tenebrionidae), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), *Colias lesbia* (Lepidoptera: Pieridae) and *Musca domestica* (Diptera: Muscidae) were obtained from laboratory-reared cultures at the Facultad de Ciencias Agrarias (Universidad Nacional del Litoral). *Periplaneta americana* (Blattodea: Blattidae) and *Cycloneda sanguinea* (Coleoptera: Coccinellidae) were obtained from laboratory colonies. *Gryllus argentinus* (Orthoptera: Gryllidae), *Scapteriscus borelli* (Orthoptera: Gryllotalpidae), *Diloboderus abderus* (Coleoptera: Scarabaeidae) and *Armadillidium vulgare* (Isopoda: Armadillidiidae) were collected from gardens in Esperanza, Santa Fe.

2.2. Observations of the life cycle

To determine the life cycle of *S. diaprepesi* SRC, 64 larvae of *G. mellonella* were infected. Larvae were individually placed in Petri dishes (100 mm) lined with filter paper (Whatman No. 1) and 100 IJs were applied in 1 mL of water. The dishes were kept in an incubator at 25°C throughout the experiment. To check for the presence of nematodes, four *G. mellonella* larvae were dissected every 12 h for 8 days using Ringer solution (Baliadi, Kondo, & Yoshiga, 2009). The nematodes obtained were fixed in cold formalin–acetic acid–alcohol (FAA) solution until microscope observations.

Since the detection of hermaphroditism in the genus *Steinernema* by Griffin, O'Callaghan, and Dix (2001), it has been necessary to determine the mode of reproduction of the species under study. For this purpose, one or two IJs were placed inside 3-mL Eppendorf tubes that had a perforated lid, containing 1.5 g of sterile sand (15% moisture). Then, a last-instar larva of *G. mellonella* was added to the tube, along with a small piece of cotton to ensure contact of IJs and host with the sand. Dead insect larvae were placed individually in White traps at 25°C. After 14 days, the cadavers were dissected to confirm the presence of *S. diaprepesi* SRC.

2.3. Pathogenicity tests

The pathogenicity of the isolate to various arthropods was evaluated by inoculating *S. diaprepesi* SRC at concentrations of 50 and 500 IJs (Koppenhöfer & Fuzy, 2003). Ten larvae of *G. mellonella*, *T. monitor*, *S. frugiperda* or *C. lesbia* and 1 mL of aqueous suspension containing the nematode inoculum were added to a Petri dish (100 mm) lined with filter paper. For the bioassays conducted with *M. domestica*, *P. americana*, *C. sanguinea*, *G. argentinus*, *S. borelli*, *D. abderus* and *A. vulgare*, the nematodes were applied in 50-mL plastic cups containing 10 arthropods in 40 g of moist sand.

Insect mortality was recorded daily for 7 days. The cadavers obtained were observed for other 14 days to determine whether or not the nematode reproduced inside them. The control treatments consisted of the addition of an equivalent volume of distilled water. The Petri dishes and cups were kept in an incubator at 25°C. Cadavers were dissected to confirm the presence of nematodes.

2.4. Effect of temperature on reproduction

Ten larvae of *G. mellonella* were placed in Petri dishes (100 mm diameter) lined with filter paper (Whatman No. 1) and exposed to 500 IJs for 48 h at 25°C, following the method of Shapiro-Ilan et al. (2009) with modifications. The cadavers were placed individually in White traps and incubated at 15, 17.5, 20, 22.5, 25, 27.5, 30, 35 and 40°C in the dark for 14 days to allow multiplication and emergence of all IJs. Evaluation temperatures were defined based on temperature conditions of the region where the nematode was isolated (mean low and high daily temperatures for the period 1982 to 2012 were 10.5 and 26.1°C, respectively) (Subsecretaría de Recursos Hídricos, 2012). IJs emerged from each trap were collected daily during 14 days and kept at 16°C. All the IJs obtained from each cadaver were kept in suspension by bubbling (Susurluk, 2005) in 100 mL of water, and three 1-mL aliquots were extracted to count nematodes under a light microscope using a counting chamber.

2.5. Effect of temperature on IJ survival and infectivity

The experiments were conducted following the method described by Morton and García-del-Pino (2009), with modifications. An aqueous suspension (20 µl) with 100 IJs was added to Petri dishes (50 mm diameter) containing 8 g of sterile sand (15% moisture). Petri dishes were sealed with Parafilm® and incubated in the dark at 10, 15, 20, 25, 30, 35, 40 and 45°C for 4, 8, 12 and 16 h. At the end of the incubation period, the dishes were maintained at 25°C for 24 h; then, the nematodes were extracted using the decanting-and-sieving method. Nematodes that were considered live (those that either were moving naturally or responded to probing with a needle) were counted under a stereomicroscope.

Another experiment was conducted to evaluate the effect of temperature on IJ infectivity. One larva of *G. mellonella* was placed in a Petri dish prepared as described above and preincubated at each temperature evaluated for 15 minutes. An aqueous suspension (20 µl) containing 100 IJs was applied on the surface of each Petri dish; dishes were then sealed with Parafilm® for incubation in the dark at 10, 15, 20, 25, 30, 35, 40 and 45°C. Mortality of *G. mellonella* larvae was recorded every 24 h for 6 days. Dead insects were dissected to confirm the nematode presence.

2.6. Tolerance to desiccation

Tolerance of *S. diaprepesi* SRC to desiccation was determined using the procedure described by Solomon, Paperna, and Glazer (1999). Two thousand IJs were placed in Petri dishes lined with a paper filter disc (Whatman No. 1) 55 mm in diameter. Excess moisture in the discs was removed through vacuum filtration. Filter paper discs containing nematodes were transferred to a desiccator set to 85% RH based on a saturated solution of KCl. After an incubation period of 3 and 6 days at 25°C, nematodes were rehydrated by immersion into Petri dishes containing 5 mL of sterile water at equal temperature during 24 h. Survival of IJs was immediately determined based on their movement or response when probed with a dissection needle.

2.7. Foraging behaviour

To determine the type of foraging strategy used by *S. diaprepesi* SRC, two experiments were conducted following the method proposed by Koppenhöfer and Fuzy (2003). The first experiment was conducted to determine if IJs could attach to a

mobile host. Approximately, 1000 IJs contained in 200 µl water were evenly distributed in Petri dishes (100 mm diameter) lined with a moist filter paper (Whatman No. 1) that was sprinkled with 0.5 g of sand. After 15 min, a larva of *G. mellonella* was incorporated and maintained in constant movement for 10 min (by disturbing it with a prod when necessary). Then the larva was rinsed with water in a Petri dish and the number of IJs present in the rinsing water was counted under a microscope. To determinate the behaviour of *S. diaprepesi* SRC, the cruiser species *H. bacteriophora* (Campbell & Gaugler, 1993) was used for comparative purposes.

The second experiment determined the EPN capacity to locate and infect a sedentary host (Koppenhöfer & Fuzy, 2003). The experiment was performed in vertical plastic columns assembled from three 5-cm sections (6 cm diameter), covered with Petri dish bottoms at both ends. The columns were filled 10.5 cm high with loam soil (41:40:19 of sand, loam and clay, respectively; – 10 kPa water potential). Nylon mesh (1 mm opening) containing a last-instar larva of *G. mellonella* was placed on the soil surface, or at 2, 5 or 10 cm soil depth. *S. diaprepesi* SRC IJs (200) contained in a 1 mL aqueous suspension were immediately applied on the surface of each column. After 96 h, the columns were disassembled and the larvae were collected. Both dead and live were rinsed with water and placed individually in the wells of 24-well plates with soil for an additional incubation period of 7 days. Dead larvae were dissected 2 days after their death to determine nematode establishment.

2.8. Effect of soil texture and soil water potential

The experimental units consisted of Poly (Vinyl chloride) (PVC) tubes of 6 cm internal diameter and 20 cm in height. The tubes contained soil of different texture (percent sand/silt/clay; pH; OM%): sandy (87:8:6, 7.1, 1.1), loam (41:40:19, 6.4, 1.6) and silt loam (10:65:25, 6.2, 2.4). A larva of *G. mellonella* was placed at the bottom of each tube. The assays were performed by adjusting each soil texture to −10, −100, −1000 and −3000 kPa of water potential, using the pedotransfer function of Imhoff et al. (2012), which was developed for the soils used in the present study. An aqueous suspension (200 µl) containing 1000 IJs was pipetted at the top of the column and the top was sealed to prevent moisture loss. The tubes were incubated at 25°C for 7 days. Larval mortality was recorded at the end of this period. Cadavers were dissected to confirm the presence of the nematode.

2.9. Statistical analyses

There were five replicates per treatment in each of four experimental repetitions, except for studies to determine the biological cycle of the isolate, which had 20 replicates per IJ rate in each of three experimental repetitions. The results obtained were analyzed with an analysis of variance (ANOVA) using INFOSTAT statistical software (Di Rienzo et al., 2010). A factorial analysis was performed to determine the effects of temperature, soil type and soil water potentials. The data obtained as percentages were arcsine square-root transformed before the statistical analysis (non-transformed means are presented in the figures). Differences among means were compared with the Tukey test at 5% probability level.

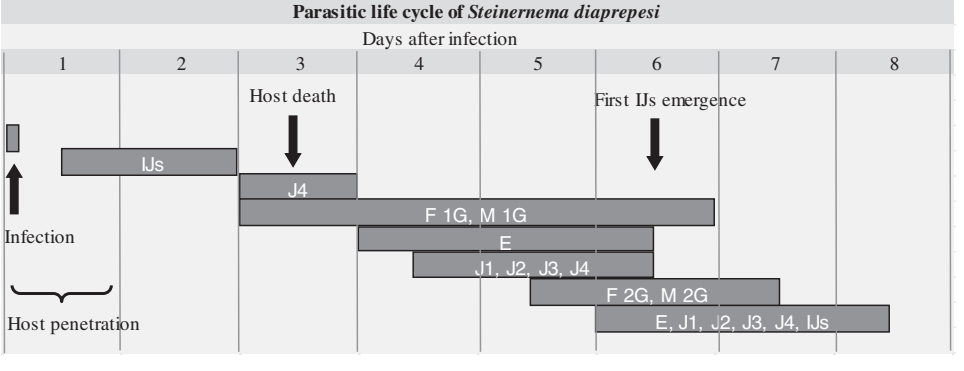
3. Results

3.1. Observations of the life cycle

S. diaprepesi SRC completed its parasitic cycle on last-instar larvae of *G. mellonella* at 25°C 8 days after infection (Figure 1). IJs penetrated the host within 24 h of inoculation. Host death occurred on day 3, along with the appearance of J3, J4 and first-generation females. On day 4, the appearance of males and second-generation females was observed. Emergence of IJs started on day 6 and extended up to day 8, when the parasitic cycle ended. Larvae of *G. mellonella* killed by the nematode showed a particular greyish coloration; the few first-generation females observed were of large size. The time elapsed between the start and end of IJ emergence was less than 48 h; when IJ emergence ended, there were practically no tissues inside the cadavers, but only the insect cuticle. The presence of females showing *endotokia matricida* (Poinar, 1990) was also observed. No progeny was observed in cadavers of *G. mellonella* exposed to one IJ. Of all *G. mellonella* larvae that were inoculated with two IJs, 17% died and progeny was generated, indicating that *S. diaprepesi* SRC is an amphimictic species.

3.2. Pathogenicity tests

The experiments showed that IJs of *S. diaprepesi* SRC are adapted to parasitize larvae of lepidopterans, generating low to nil pathogenicity in the remaining arthropod orders evaluated (Table 1). Inoculum concentration significantly affected mortality of arthropods and production of progeny (F = 11.07, df = 1.318, P = .0010; F = 4.76, df = 1.318, P = 0.0299, respectively), showing higher values at a higher IJ concentration. Among hosts, *G. mellonella*, *S. frugiperda* and *C. lesbia* were especially suitable at both inoculum concentrations (F = 194.13, df = 11.228, P < .0001; F = 243.6, df = 11.228, P < .0001, at 50 and 500 IJs, respectively). The mortality observed in the coleopteran species and in *P. americana* (16–32%) suggests that they may be considered intermediate or alternative hosts, despite their low



IJs = infective juveniles, J1-J4 = first through forthstage juveniles, E = eggs, F = females, M = males, 1G-2G = first and second generation.

Figure 1. Diagram of the life cycle of *S. diaprepesi* SRC on *G. mellonella* last-instar larvae at 25°C. IJs = infective juveniles, J1–J4= first through forth stage juveniles, E = eggs, F = females, M = males, 1G–2G= first and second generation.

Table 1. Infectivity of *S. diaprepesi* SRC to various arthropod species exposed to two nematode densities at 25°C.

Arthropod				% Mortality*		% Cadavers producing progeny	
Order	Family	Species	Stage	50 IJs	500 IJs	50 IJs	500 IJs
Lepidoptera	Pyralidae	*G. mellonella*	L5	94.5 ± 2.1 a	100 a	77 ± 3.17 a	85.5 ± 3.03 a
	Noctuidae	*S. frugiperda*	L4	86.5 ± 3.65 a	97.5 ± 1.23 a	61.5 ± 4.37 b	79.5 ± 3.66 a
	Pieridae	*C. lesbia*	L4	55 ± 2.76 b	82 ± 2.87 b	1 ± 0.69 c	33 ± 3 b
Coleoptera	Scarabaeidae	*D. abderus*	L3	3.5 ± 1.67 c	16 ± 3.03 d	0	1 ± 0.69 c
	Coccinellidae	*C. sanguinea*	L3	9 ± 2.4 c	32.5 ± 4.22 c	1.05 ± 0.72 c	5.5 ± 1.7 c
	Tenebrionidae	*T. molitor*	L5	4 ± 1.52 c	22.5 ± 0.99 cd	0	1 ± 0.69 c
Orthoptera	Gryllidae	*G. argentinus*	Adult	0	0	–	–
	Gryllotalpidae	*S. borelli*	Nymph	0	0	–	–
Blattodea	Blattidae	*P. americana*	Nymph	6 ± 1.97 c	19.5 ± 3.2 d	0.5 ± 0.5 c	2 ± 1.17 c
Diptera	Muscidae	*M. domestica*	L3	1.5 ± 0.82 c	5 ± 1.7 e	0	0
Isopoda	Armadillidiidae	*A. vulgare*	Adult	0	0	–	–

Means ± SEM followed by the same letter within a column are not significantly different ($P < .05$, Tukey).
*7 days post inoculation.

progeny production (1–5.5%). The remaining species were not suitable hosts, exhibiting low or nil mortality, and when they produced progeny, it was small.

3.3. Effect of temperature on reproduction

Mortality of *G. mellonella* larvae showed statistical differences (F = 396.56, df = 7.152, P < .0001). One hundred percent larval mortality occurred at between 20 and 35°C, with lower percentages being recorded at 17.5 and 40°C (37.5 and 72% mortality, respectively). No insect death was recorded at 15°C.

The reproductive potential of the *S. diaprepesi* SRC on *G. mellonella* larvae was significantly higher under temperate temperature conditions (20–25°C) than at 30 and 35°C (F = 39.22, df = 6.133, P < .0001) (Figure 2A). At 15 and 40°C, no multiplication of the nematode was observed. Statistical differences were observed between temperature and time at the start of IJ emergence (F = 111.32, df = 6.133, P < .0001) (Figure 2B).

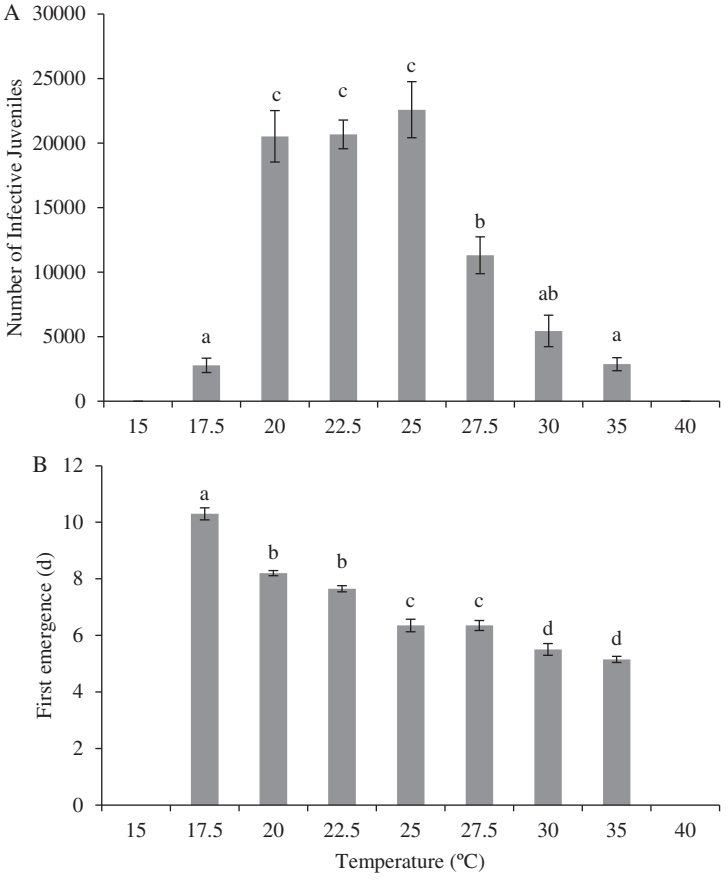


Figure 2. (A) Number of IJs of *S. diaprepesi* SRC emerged from last-instar larvae of *G. mellonella* exposed to different temperatures and (B) days elapsed from inoculation to first emergence of IJs of *S. diaprepesi* SRC from last-instar larvae of *G. mellonella* exposed to different temperatures. Different letters indicate significant differences among treatments, according to the Tukey test (P < .05).

3.4. Effect of temperature on survival and infectivity

IJs survived exposure to a range of temperatures between 10 and 40°C (Figure 3). A significant reduction in the number of live IJs was observed at 8, 12 and 16 h of exposure at 40°C ($F = 19.91$, df = 3.76, $P < .0001$). At 15°C, the number of live IJs was significantly higher after 4 h of exposure than after 16 h ($F = 3.1$, df = 3.76, $P = 0.0314$). A decrease in the number of live IJs was also observed at 16 h after exposure at 20°C ($F = 9.49$, df = 3.76, $P < .0001$).

At 8, 12 and 16 h of exposure, statistical differences were detected between the five lowest temperatures and 35 and 40°C ($F = 11.34$, df = 6.133, $P < .0001$; $F = 11.05$, df = 6.133, $P < .0001$ and $F = 26.69$, df = 6.133, $P < .0001$, respectively), with a lower number of live IJs being recorded at 35 and 40°C. The interaction between temperature and exposure periods was not significant ($F = 1.62$, df = 18.532, $P = .0518$). At 45°C no live IJs were detected.

The application of IJs at between 20 and 35°C produced 100% insect mortality, with variations in the time elapsed between the inoculation of IJs and death. At 25°C, 95% of *G. mellonella* mortality occurred on day 4 after inoculation. On day 2, 100% mortality was observed at 30°C. At 20, 35 and 40°C, time elapsed between inoculation and larval death exhibited more variation. No insect death was recorded at 10 or 45°C.

3.5. Tolerance to desiccation

Mortality of IJs under 85% RH was affected by exposure time ($F = 101.19$, df = 1.38, $P < .0001$), with 43.8 and 100% of IJs dying on days 3 and 6 of the experiment, respectively.

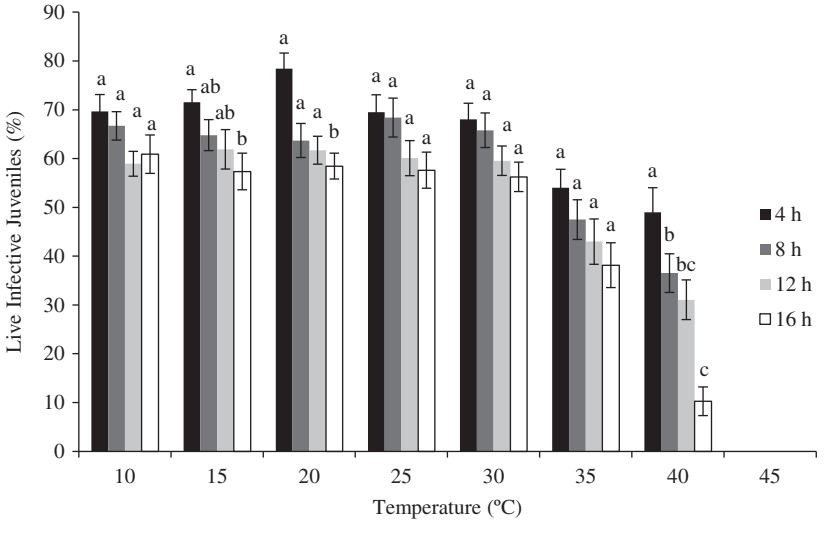


Figure 3. Effect of temperature on survival of IJs of *S. diaprepesi* SRC exposed to different temperatures for 4, 8, 12 and 16 hours. Different letters indicate significant differences among treatments at a given temperature, according to the Tukey test ($P < .05$).

3.6. Foraging behaviour

The attachment rate of *S. diaprepesi* SRC to mobile larvae of *G. mellonella* on sand-sprinkled filter paper was 4.25 ± 0.44 IJs. This was significantly higher than that recorded for *H. bacteriophora* (0.95 ± 0.21 IJs) ($F = 45.63$, df = 1.38, $P < .0001$). The experiments using vertical columns did not present statistically significant differences in host mortality percentage (70–95%) ($F = 3.96$, df = 3.12, $P < .0356$) or in the number of nematodes (12–27) established in the hosts ($F = 2.1$, df = 3.12, $P = .1536$).

3.7. Effect of soil texture and soil water potential

A significant effect of soil type ($F = 367.82$, df = 2.36, $P < .0001$) and soil water potential ($F = 107.26$, df = 3.36, $P < .0001$) on mortality of *G. mellonella* was observed, as well as a significant interaction between soil type and soil water potential ($F = 43.84$, df = 6.36, $P < .0001$). IJs applied to sandy soil killed 95–100% of *G. mellonella* larvae, regardless of the soil water potential. In loam soil, there were significant differences in insect mortality, with insects remaining alive (100%) in the −3000 kPa soil water potential treatment ($F = 127.18$, df = 3.12, $P < .0001$). In this soil type, IJs killed all the *G. mellonella* larvae at soil water potentials of −10 and −100 kPa, showing a slight reduction in mortality at −1000 kPa. In silt-loam soil, the insects remained alive at soil water potential of −1000 and −3000 kPa, with records of 15 and 70% mortality at −100 and −10 kPa, respectively ($F = 50.44$, df = 3.12, $P < .0001$) (Figure 4). At soil water potentials of −10, −100 and −1000 kPa, no significant differences in insect mortality were observed between sandy and loam soil, but there were differences between these two soil types and silty loam soil ($F = 80.95$, df = 2.9, $P < .0001$; $F = 111.34$, df = 2.9, $P < .0001$; $F = 120.41$, df = 2.9, $P < .0001$, respectively). In addition, at those soil water potentials mentioned, mortality was lower in silty loam soil. Larval mortality recorded at −3000 kPa was significantly higher in sandy soil than in loam and silty loam soil ($F = 157.54$, df = 2.9, $P < .0001$).

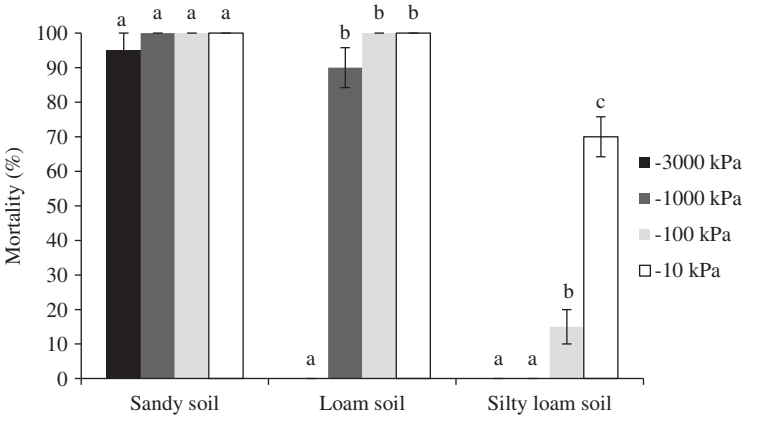


Figure 4. Effect of soil texture and soil water potential on mortality of last-instar larvae of *G. mellonella* caused by *S. diaprepesi* SRC. Different letters indicate significant differences among treatments, according to the Tukey test ($P < .05$).

4. Discussion

This study characterises ecological aspects of *S. diaprepesi* SRC, which are necessary for future implementation as a biological control agent of pests. Knowledge of the biotic and abiotic conditions of the agricultural ecosystem where EPNs are intended to be applied as well as the understanding of the ecological characteristics of these organisms will allow us to increase the possibilities of commercial success, avoiding unsuccessful applications or economic losses.

S. diaprepesi SRC completed its life cycle at 25°C over 8 days, a short period compared with other species. This is a desirable characteristic for a nematode species with potential for biological control because it would facilitate in vivo nematode multiplication and, if applied through infected cadavers, IJs would soon emerge in search of hosts.

The pathogenicity tests showed that *S. diaprepesi* SRC were more virulent to the lepidopteran species than to the remaining orders evaluated, suggesting the potential for control of lepidopteran larvae. In addition, an acceptable production of *S. diaprepesi* SRC progeny was confirmed only in *G. mellonella* and *S. frugiperda*. The low mortality and reproduction observed in the species of the orders Coleoptera and Blattodea suggest that they might be alternative hosts of the isolate, although they would restrict their reproduction. Studies conducted with other isolates of *S. diaprepesi* showed that the species had a lethal effect on the coleopteran *D. abbreviatus* (El-Borai, Stuart, Campos-Herrera, Pathak, & Duncan, 2012; El-Borai, Zellers, & Duncan, 2007; Nguyen & Duncan, 2002). In addition, Molina-Ochoa et al. (2009) confirmed the susceptibility of the tick *B. microplus* to this nematode.

Temperature affected nematode reproduction. The highest multiplication was obtained at 25°C, with progenies of 22,584 IJs per larva of *G. mellonella*. The isolate showed low reproductive capacity, especially compared with other species of the genus. For example, at the same temperature, *S. siamkayai* and *S. riobrave* generated more than 120,000 and 130,000 IJs per *G. mellonella* larva, respectively (Raja, Sivaramakrishnan, & Hazir, 2011; Shapiro-Ilan et al., 2005a). The low reproduction observed is correlated to the large size of IJs of *S. diaprepesi*.

At 15°C, no nematode reproduction occurred, indicating that the lower reproduction limit is between 16 and 17.5°C. *S. diaprepesi* SRC was able to reproduce at up to 35°C, although the number of IJs generated was significantly reduced. The reduction in the number of IJs in response to the increase in temperature was already mentioned for other EPN isolates (Del Valle, Dolinski, Souza, & Samuels, 2005; Gungor, Keskin, & Hazir, 2006). The time necessary for the start of IJ emergence decreased with increasing temperature. The high percentage of IJ survival at 30 and 40°C and the fact that they were infective to larvae of *G. mellonella* at 40°C make *S. diaprepesi* isolate comparable to *S. riobrave* and *S. siamkayai*, two species well known for their tolerance to high temperatures (>35°C) (Cabanillas & Raulston, 1996; Grewal et al., 1994; Raja et al., 2011).

The conditions of the environment where *S. diaprepesi* SRC was obtained determine that IJs have at least one mechanism to cope with changes in soil temperature and moisture (soil temperature at 5 cm depth usually exceeds 35°C in summer). Long periods of water deficit in the soil and high temperatures would affect survival of these organisms at the upper layers of the soil profile. Hence, the isolate would be tolerant to desiccation or able to migrate through the soil profile

following the moisture gradient in search of favourable conditions. *S. diaprepesi* SRC may use both strategies. Survival of IJs (56.2%) after 72 h under desiccation conditions showed that the isolate was more tolerant than *S. feltiae*, *S. riobrave* and *S. carpocapsae* (Shapiro-Ilan et al., 2005b; Shapiro-Ilan et al., 2009).

The movement of IJs observed in the soil column confirmed that *S. diaprepesi* SRC uses a cruiser-type search strategy (Lewis, Gaugler, & Harrison, 1992, 1993) and that it would respond to stimuli from the hosts. The behaviour found can be compared to that detected in the cruiser species *S. scarabaei* using the same evaluation technique (Koppenhöfer & Fuzy, 2003). The number of IJs of *S. diaprepesi* SRC attached to a mobile host was significantly higher than the number of attached IJs of the *H. bacteriophora*, which has a cruising-type strategy. However, Koppenhöfer and Fuzy (2003) counted 76 IJs of *S. carpocapsae* (ambusher-type strategy) attached to larvae of *G. mellonella*, a much higher value than that detected in *S. diaprepesi* SRC. It should be noted that *S. diaprepesi* SRC did not show nictation (Campbell & Gaugler, 1993; Ishibashi & Kondo, 1990) behaviour (Del Valle, personal observation).

Soil texture had a significant influence on IJ movement and on search of and penetration into larvae of *G. mellonella*. The efficacy of this isolate was found to be favoured in sandy soils, regardless of the soil water potential. Under laboratory conditions, Shapiro-Ilan, Stuart, and McCoy (2006) found that *S. diaprepesi* has greater survival capacity in sandy loam soil than other EPN species and isolates. In loam soil texture, insect mortality percentage was high at –10, –100 and –1000 kPa of soil water potential. However, no mortality was recorded in soil at –3000 kPa of water potential. In silt-loam soil, larval mortality was lower and strongly related to soil water potential, with no death recorded at –3000 or –1000 kPa of soil water potential. Reduced or no insect mortality with decreasing soil water content was already observed in previous studies and agree with our results. For example, Koppenhöfer and Fuzy (2007) observed significant differences in mortality of *Popillia japonica* larvae exposed to *H. bacteriophora*, *H. zealandica* and *S. glaseri* with soil water potential ranging between –1 and –3000 kPa.

Previous studies reported that infectivity of EPNs is affected by soil texture (Koppenhöfer & Fuzy, 2006, 2007). Movement and efficacy of several species are reduced in soils with high clay content (Kaspi et al., 2010; Lezama-Gutiérrez et al., 2006; Portillo-Aguilar, Villani, Tauber, Tauber, & Nyrop, 1999). El-Borai et al. (2012) documented that IJs of *S. diaprepesi* were more efficient in protecting citrus seedlings from damage induced by *D. abbreviatus* in coarse-textured soil than in fine-textured soil. Soils with lower sand proportion and, therefore, higher amount of silt and clay particles restrict EPN movement and are even harmful to them by reducing soil oxygen levels (Georgis & Poinar, 1983; Kung, Gaugler, & Kaya, 1990). *S. diaprepesi* is among the EPN species with the largest IJs and that IJs' size is likely to restrict movement in finer soils.

Up to the present, the symbiotic bacterium *X. doucetiae* was found to be associated with *S. diaprepesi*; this bacterium has greater tolerance to high temperatures than the remaining species of the genus (Tailliez et al., 2006). Maximum growth temperature was 40–42°C, which could be related to reproduction and infectivity temperatures of the studied isolate.

At present, it is difficult to associate an EPN species with a preferred habitat because studies focusing on this aspect are scarce for most species (Hominick, 2002).

Previous studies on *S. diaprepesi* and the present results suggest that the nematode would adapt to habitats with sandy soils and high temperatures.

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