Biological characterization of the entomopathogenic nematode, *Steinernema innovationi*: a South African isolate

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Abstract

Entomopathogenic nematode species perform differently under different environmental conditions; therefore, the authors investigated the biological and environmental characteristics that could optimize performance of *Steinernema innovationi*. The authors studied the effect of temperature on infectivity and reproduction, the foraging behavior and host range. Thermal activity was optimal between 22 and 25°C. Highest infective juvenile (IJ) yields in last instar *Galleria mellonella* were observed at 22°C (333,014 IJs/g) and 25°C (354,165 IJs/g). An average of 26% of the IJs infected *G. mellonella* larvae at depths of 15 cm within 24 hr. *Steinernema innovationi* IJs raised greater than 95% of their body off the substrate and moved in a circular pattern, but did not jump. The hosts, *Acheta domesticus*, *Chilo partellus*, and *Plutella xylostella* showed the least susceptibility. All other hosts, [*Eldana saccharina*, *Sesamia calamistis*, *Tenebrio molitor*, *G. mellonella*, *Cydia pomonella*] suffered 100% larval mortality. Pupal mortality ranged from 47 to 68%. An LC50 and LC70 of 3 and 31 IJs/larva, respectively, were calculated for *Agrotis ipsilon*. The results indicate that *S. innovationi* is adapted to moderate temperatures. Furthermore, to the author's knowledge this is the first report of a nematode (particularly from '*glaseri* group') that shows cruiser-type behavior in movement, but also nictates.

Key words

Entomopathogenic nematode, Foraging behavior, Host range, nematode reproduction, Optimum temperature, Virulence.

A number of entomopathogenic nematode (EPN) species belonging to the families Steinernematidae and Heterorhabditidae have proven to be effective biocontrol agents. These nematodes offer several advantages over traditionally used chemical pesticides. These include: there is no development of resistance (Divya and Sankar, 2009); they pose no threat to humans, non-target organisms and the environment (Lacey and Georgis, 2012); EPNs can kill their insect host within 48 hr (Shapiro-Ilan et al., 2012); no withholding periods; exemption from registration requirements in most countries (Divya et al., 2011); and they can be mass produced easily (Shapiro-Ilan et al., 2012). Although EPNs attack over 200 insect hosts (Hazir et al., 2003), species performance in biological control differs greatly according to their biology, artificial manipulation (e.g., in vitro mass production and formulation) and the environmental conditions (temperature, moisture, soil particle size, UV, and pH) (Barbercheck and Duncan, 2004; Shapiro-Ilan et al., 2012). Entomopathogenic nematodes are more successful for control of soil dwelling insect pests, or insects that spend part of their life cycle in the soil (Shapiro-Ilan and Gaugler, 2002). Host susceptibility differs amongst species, with some EPN species only adapted to particular hosts. For example, *Steinernema scapterisci* (Nguyen and Smart) is a specialist against Orthoptera with insects in the orders Coleoptera, Lepidoptera, and Hymenoptera being poor hosts or non-hosts (Nguyen and Smart, 1992). Nevertheless,

relatively little information regarding species performance is available to accompany descriptions of new species (Koppenhöfer and Kaya, 1999; Koppenhöfer and Fuzy, 2003; Gungor et al., 2006; Morton and Garcia-del-Pino, 2009; Çimen et al., 2014).

Steinernema innovationi (Çimen, Lee, Hatting, and Stock) is an indigenous species isolated from the free state province in South Africa (Hatting et al., 2009). A cost-effective medium was developed for mass production of this species in South Africa to service a niche market (Ramakuwela et al., 2016). The ability of the nematode to find and infect hosts and withstand environmental conditions is crucial for its use as a biocontrol agent (Shapiro-Ilan et al., 2012). Hence, Koppenhöfer and Kaya (1999) suggested that the systematic description of a new species should be coupled with its ecological characterization. However, performance characterization was not done with the description of *S. innovationi*. Accordingly, the objectives of this study were to investigate the effect of temperature on infectivity and reproduction, the foraging behavior and host range of *S. innovationi* to characterize its performance: (i) For temperature, we determined the optimum temperature for infectivity, time-till-death, first day of progeny emergence from the cadaver and reproductive yield. (ii) For foraging behavior, the ability of the infective juvenile (IJ) to disperse and find its host in a sand column and their ability to nictate and jump was examined. (iii) For host range, we assessed efficacy of IJs against larvae and/or pupae of nine insect species representing three orders (Coleoptera, Lepidoptera, and Orthoptera).

Materials and methods

Nematode source and production

The nematode was originally isolated from a soil sample collected from the Free State province in South Africa (Hatting et al., 2009) using the insect baiting technique (Kaya and Stock, 1997). IJs of *S. innovationi* were maintained in aqueous suspension at 10°C. In vivo nematode production was done by passaging through last instar *Galleria mellonella* (Linnaeus, Lepidoptera: Pyralidae) larvae according to Kaya and Stock (1997). Larvae of *G. mellonella* were reared on a diet described by Mohamed and Coppel (1983) at the Agricultural Research Council-Small Grain, ARC-SG (Bethlehem, South Africa).

For in vitro production, IJs were produced using a media based on pureed larvae of the house fly, *Musca domestica* (Linnaeus, Diptera: Muscidae). The flies were reared at ARC-SG on a diet containing 2 kg bran, 300 g Nespray® milk powder, 6 g sodium benzoate, 20 g Brewer's yeast, and 3 L lukewarm water.

Last instar *M. domestica* were pureed using a hand blender. 5 g of a mixture of larval puree and 0.15 g canola oil was absorbed in 0.5 g of sponge cubes in a 100 ml Erlenmeyer flask, and autoclaved at 121°C for 15 min (Ramakuwela et al., 2016). After cooling, flasks were inoculated with 0.5 ml of a 48-hr-old unknown *Xenorhabdus* sp. isolated according to Ulug et al. (2015). The inoculated flasks were incubated at 27°C for 72 hr to establish growth of the symbiotic bacterium and then each flask was inoculated with 0.5 ml of a monoxenic nematode culture [inoculum prepared according to Lunau et al. (1993)] and incubated at 22°C for 28 d. Nematodes were harvested by washing the sponges with 50 ml distilled water six times. In vitro produced IJs were used for host range assays to assess effectiveness of the newly developed medium.

Effect of temperature on infectivity

The effects of temperature on infectivity were assessed using a sand-well bioassay system by filling wells of a 24-well tissue culture (TC) plate with 0.5 g sterile, air-dried loam sand (Ricci et al., 1996; Koppenhöfer and Kaya, 1999; Hazir et al., 2001). The plates with sand were incubated for 1 hr at 10, 15, 20, 25, and 30°C before introducing the nematode and control treatments (Bazman et al., 2008). Nematode suspensions and sterile water for the control were also incubated at the respective temperatures for 1 hr prior to inoculations. A total of 30 wells per temperature were inoculated by pipetting 50 IJs (produced in vivo) in 60 µl of distilled water into each well (Gungor et al., 2006; Ramalingam et al., 2011). The control treatment was inoculated with 60 µl of sterile distilled water only. One *G. mellonella* larva was added per well. Three replicates of 10 larvae were used per treatment, plus the control treatment. Each culture plate was placed inside a 330 × 215 mm Zip Seal bag (GLAD®, South Africa) to conserve moisture and was incubated at its appropriate temperature in total darkness. Plates were monitored for larval mortality every 24 hr for 10 d. Cadavers were removed daily and washed three times with distilled water to remove IJs from the cuticle and were then incubated for another 24 hr at 25°C followed by enzymatic digestion in pepsin solution (Mauleon et al., 1993; Bazman et al., 2008). Parameters measured were: percentage larval mortality, time-till-death, and number of established IJs per larva. The experiment was repeated on a different test date with a fresh batch of IJs.

Effect of temperature on reproduction

The effect of temperature on reproduction was assessed using the sand-well bioassay system described

above using a concentration of 100 IJs in 100 µl of distilled water per well (Fallon et al., 2004). *Galleria mellonella* larvae weighing between 250 and 300 mg (weight difference ≤ 50 mg) were used, 30 larvae in three replicates of 10 per temperature. Each culture plate was incubated at 25°C for 48 hr in the dark to ensure infection. Plates were monitored for mortality every 24 hr. Infected cadavers were individually transferred into modified White traps (Kaya and Stock, 1997) and incubated at temperatures of 18, 20, 22, 25, and 27°C where they were monitored for IJ development daily until emergence was less than 50 IJs in each White trap. The following parameters were recorded: first day of progeny emergence from the infected cadaver and the total number of IJs produced. First day of emergence was recorded as the day on which IJs were visible on the cadaver, filter paper or in the water of the White trap. For yield, emerging IJs were periodically harvested every 24 hr by emptying the White traps and filling them with fresh water until the number of emerging IJs was less than 50 IJs in a given White trap (Hazir et al., 2001). Harvested IJs in water suspension were stored in a 250 ml flat angled TC flask (Lasec SA, www.lasecsa.co.za) separately for each White trap. Three sub-samples per flask were counted to calculate the number of IJs produced per *G. mellonella* cadaver using the dilution method described by Kaya and Stock (1997). Yield was then converted to yield per gram of *G. mellonella* larvae using individual larval weights recorded at the beginning of the experiment (Barbercheck et al., 1995). The experiment was repeated twice on a different date with a fresh batch of IJs.

Movement behavior

Vertical plastic columns with a depth of 16 and 5 cm diameter were used to assess IJ movement in the sand profile and infectivity of *G. mellonella* larvae at different depths (1, 5, and 9 cm in experiment I; 3, 6, 9, 12, and 15 cm in experiment II). The columns were filled with moistened (10% w/w) sand in which one *G. mellonella* larva immobilized by enclosing it inside a gauze-type sachet was positioned horizontally at each depth (Grewal, Lewis, Gaugler, and Campbell, 1994). In experiment I, a total of 15 columns (5/depth, 1, 5, and 9) were used and assessed for EPN presence after 72 hr. The experiment was repeated with 125 columns (five columns per depth at 3, 6, 9, 12, and 15) for examination every 24 hr. Columns were inoculated with 100 IJs in 100 µl sterile distilled water dispensed onto the top surface of the column. Control columns were treated with 100 µl of sterile distilled water only. All columns were covered with

parafilm and incubated vertically at 25°C. Mortality and IJ establishment were assessed every 24 hr. Cadavers were washed three times with distilled water to remove IJs on the cuticle followed by enzymatic digestion in pepsin solution to count established IJs. Nematodes were counted using a hand-held counter under a dissection microscope at 40x magnification.

Standing and jumping behavior

A total of five plastic petri dishes (9 cm diameter) lined with double moistened filter paper and sprinkled with 0.5 g of sand particles were used in the experiment. Approximately 100 IJs were transferred to each petri dish, and the behavior of the IJs was monitored for 10 min using a dissecting microscope at 40x magnification (Ramalingam et al., 2011). The search behavior of IJs was classified according to Campbell and Gaugler (1993).

Host range

The host range tested comprised nine species: *Eldana saccharina* (Walker, Lepidoptera: Pyralidae)*, Sesamia calamistis* (Hampson, Lepidoptera: Noctuidae), *Chilo partellus* (Swinhoe, Lepidoptera: Pyralidae) (supplied by the South African Sugarcane Research Institute); *Tenebrio molitor* (Linnaeus, Coleoptera: Tenebrionidae), and *Galleria mellonella* (Linnaeus, Lepidoptera: Pyralidae) (reared at the ARC-SG Research Council-Small Grain); *Plutella xylostella* (Linnaeus, Lepidoptera: Plutellidae) (sourced from ARC-Plant Protection Research); *Agrotis ipsilon* (Hufnagel, Lepidoptera: Noctuidae) (supplied by ARC-Grain Crops); *Cydia pomonella* (Linnaeus, Lepidoptera: Tortricidae) (sourced from the Stellenbosch University), and *Acheta domesticus* (Linnaeus, Orthoptera: Gryllidae) (sourced from a local pet shop [Paw Pet] in Bethlehem, Free State). Except for *A. ipsilon*, all larvae (final instar) and pupae were exposed to a low and high dosage of 50 and 500 IJs, respectively (Koppenhöfer and Fuzy, 2003), in 60 µl distilled water per host including a control per host insect exposed to 60 µl of sterile distilled water only. Medium size *A. domesticus* were also exposed to a control treatment. An LC_{50} was determined for *A. ipsilon* by applying IJs at a rate of 0, 1, 5, 10, 25, and 50 IJs/larva (12 reps each). For all lepidopteran and coleopteran hosts, a Sandwell bioassay was used as per Gungor, et al. (2006). The bioassay of *A. domesticus* (adults) was performed in 100 ml plastic cups, half filled with 50 g of moistened (10% moisture) sand. The insects were fed pieces of potatoes *ad libitum*. In all assays, a total of 30 larvae or pupae (as single

replicates) were used per treatment and the control, for all insects. The TC plates and plastic cups were maintained individually inside a 330 × 215 mm Zip Seal bag (GLAD®, www.glad.co.za) and incubated at 25°C. Mortality was assessed on Day 3 for larvae (and nymphs of *A. domesticus*) and Day 7 for all insect pupae. Each cadaver was dissected to confirm nematode infection. The experiment was repeated on a different date with a fresh batch of IJs.

Statistical analysis

For mortality, time-until-death, establishment and first day of emergence results were analyzed using analysis of variance (ANOVA). The standardized residuals was normally distributed (Shapiro–Wilks test) and therefore the means of the significant mortalities were separated using Fisher's Unprotected t-test (least significant difference – LSD) at the 5% level of significance (Snedecor and Cochran, 1980). Yield was analyzed by ANOVA and the regression analysis of yield vs temperature was performed with TableCurve (SYSTAT Software Inc., 2002). Foraging behavior: the number of established IJs per depth was analyzed by ANOVA and the mean counts were separated using Fishers' unprotected t-test at the 5% level of significance (Snedecor and Cochran, 1980). Host range: a factor ANOVA was performed, with different treatments and insects. The experimental design was a randomized complete block with two replicates. The standardized residuals was normally distributed (Shapiro–Wilks test) and therefor the means of the significant mortalities were separated using Fisher's Unprotected t-test (least significant difference – LSD) at the 5% level of significance (Snedecor and Cochran, 1980). An LC_{50} for *A. ipsilon* was determined using TableCurve software (SYSTAT Software Inc., 2002). All data analysis were performed using SAS statistical software (SAS Institute, 1999).

Results

Effect of temperature on infectivity

There were significant differences in the performance of the IJs at temperatures that varied from 10 to 30°C and significantly lower infectivity was measured at 10°C (28%) ($F = 6.94$; $df = 4.5$; $P = 0.028$) (Fig. 1A). Time-until-death was the shortest at 30°C ($F = 740.75$; $df = 4,160$; $P < 0.0001$), followed by 25, 20, 15 then 10°C after 1, 2, 3, 7, and 8 d, respectively (Fig. 1B). Significantly higher mean numbers of established IJs ($F = 29.51$; $df = 5,139$; $P < 0.0001$) were recorded at 25°C (26.5 IJs/host) followed by established IJs r

ecorded at 20 and 30°C (19.6 and 21.2 IJs/host, respectively) and the least establishment was recorded at 10°C (2.6 IJs/host) (Fig. 1C).

Effect of temperature on reproduction

S. innovationi showed the earliest emergence at 25 and 27°C (mean of 5 d) and the slowest at 18°C (mean of 8 d) ($F = 52.08$; $df = 4,156$; $P < 0.0001$) (Fig. 2A). Compared to the other temperatures, the number of emerged IJs was significantly higher at 22°C (333,014 IJs/g) and 25°C (354,165 IJs/g), whereas the lowest number of IJs was observed at 18°C (119,453 IJs/g) ($F = 30.95$; $df = 4,155$; $P < 0.0001$) (Fig. 2B).

Foraging behavior

Overall, IJs of *S. innovationi* infected *G. mellonella* at all the tested depths. No differences were observed at 1 and 5 cm with significantly lower ($F = 29.61$; $df = 2.28$; $P = 0.010$) establishment at 9 cm depth in the first experiment (data not shown). In experiment II, IJs were able to infect *G. mellonella* at depths up to 15 cm within a period of 24 hr with significant differences in number of established IJs at different depths over a 72 hr period ($F = 41.69$; $df = 4.14$; $P = 0.042$) (Fig. 3). In this experiment, the number of established IJs was comparable at depths of 9, 12, and 15 cm, irrespective of time. By 24 hr post-inoculation, the number of nematodes establishing in hosts at 12 and 15 cm (24 and 26 IJs, respectively) did not differ from the numbers establishing at a depth of 3 cm (38 IJs) and no difference in this comparison was detected on subsequent days.

Standing and jumping behavior

We observed that *S. innovationi* IJs raised greater than 95% of their body off the sand particle and moved in a circular pattern displaying a phenomenon termed 'waving nictation' which is equivalent to 'pendulum swinging' and 'small waving.' Jumping which occur after the nematode twist the front portion of the body and pulling it away resulting in a force which pushes the IJ into the air was not observed.

Host range

Larval mortality at both 50 and 500 IJs was lowest for *A. domesticus* followed by *C. partellus* and *P. xylostella* (Max. 27, 47, and 92% at 500 IJs concentration, respectively). The rest of the hosts reached 100% mortality. *P. xylostella* was the only target pest that showed a significant increase ($F = 13.28$;
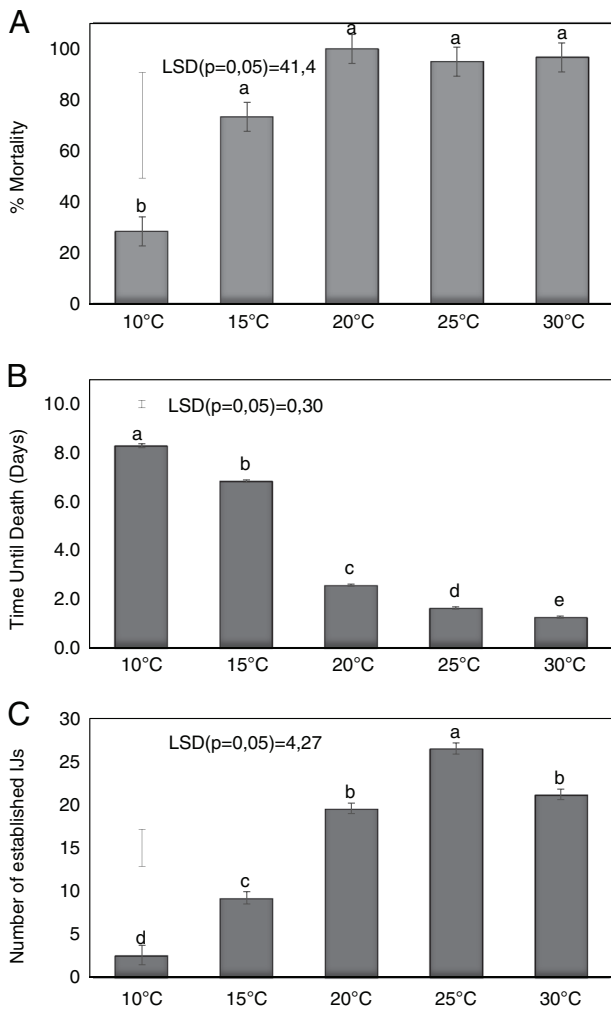
Figure 1: *Galleria mellonella* larvae inoculated with *Steinernema innovationi* (50 infective juveniles (IJs)/insect) after 10 d of incubation at different temperatures (10, 15, 20, 25, and 30°C). (A) Mean percentage mortality; (B) Number of days until death; (C) Number of IJ established in *G. mellonella* larvae. Standard error bars with the same lowercase letter indicate no significant difference.

df = 3.33; *P*<0.0001) in mortality from 52 to 92% at 50 and 500 IJs doses, respectively (Fig. 4). The number of pupae with nematode establishment was lower for *C. pomonella*, *E. saccharina*, and *S. calamistis* as compared to larvae. The number of *C. partellus* larvae vs pupae with nematode establishment was non-significant (*F* = 1.95; *df* = 11.17; *P* = 0.1041) at the two doses (Fig. 5). The black cutworm, *A. ipsilon*, was highly susceptible to *S. innovationi* with larval mortality

caused by an individual IJ. An LC_{50} was reached at a mean concentration of 3 IJs/larva increasing to a maximum LC_{70} at a calculated mean of 31 IJs/larva [logistic dose Response (LDR) equation, $Y = a/[1 + (x/b)^c)]$ (SYSTAT Software Inc., 2002).

Discussion

This study documents the intrinsic and extrinsic factors that affect performance of *S. innovationi* as well as its host range, which is crucial knowledge for its development and application as a biological pesticide. EPN species or even strains have been observed to have diverse temperature optima (Grewal, Selvan, and Gaugler, 1994; Gungor et al., 2006; Tarasco et al., 2015). The temperature experiments showed that *S. innovationi* has an optimum temperature between 22 and 25°C. The activity of IJs of most EPNs is arrested at temperatures below 8°C, and IJs are killed by temperatures above 38°C, depending on the species (Salma and Shahina, 2012). The optimum temperature of most EPN species fall between 20 and 30°C (Campos-Herrera et al., 2008; Mejia-Torres and Saenz, 2013; Shapiro-Ilan et al., 2014; Tarasco et al., 2015), which corresponds with the optimum temperature of this species.

It is believed that EPN isolates or strains are adapted to the environment from which they were isolated (Kung et al., 1991). *Steinernema innovationi* was isolated from a region with a climate defined as humid subtropical with summer rainfall, and is relatively cool (warmest month < 22°C) (Hatting et al., 2009). However, its optimum temperature is higher than the mean temperature of the region from where it was originally isolated. Similarly, *Steinernema anatoliense* (Hazir, Stock, and Keskin) has an optimum temperature outside the mean temperature of its region of origin (Gungor et al., 2006). Moreover, different strains are commercialized and used in different parts of the world for control of insect pests (Sharma et al., 2011). The presumed adaptability of *S. innovationi* to warmer temperature was also supported by its subsequent isolation from the warmer KwaZulu-Natal province, which is characterized by a humid subtropical climate with annual rainfall and its warmest month >22°C (Hatting et al., 2009). The higher optimum temperature may have been caused by adaptation to warm rearing temperatures (25°C) during short term preservation. Jagdale and Gordon (1998) demonstrated that the repeated use of a constant rearing temperature led to the adaptation of a strain to that temperature and improved its performance at temperatures close to the rearing temperature. These authors also showed that infectivity at low temperatures was en-
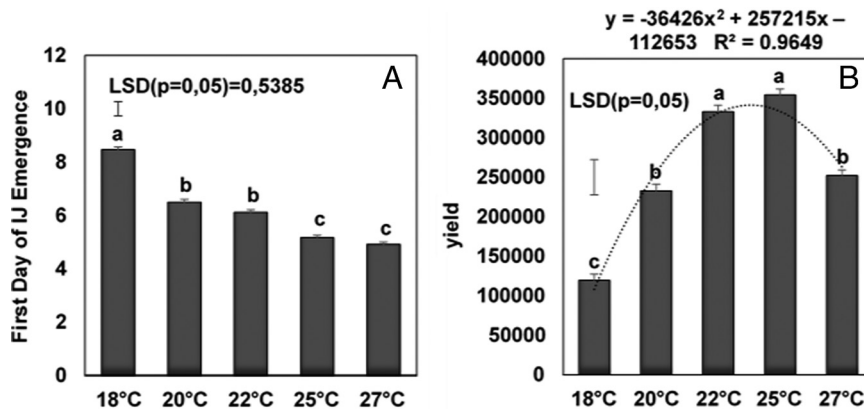
Figure 2: *Galleria mellonella* inoculated with *Steinernema innovationi* (100 infective juveniles (IJ)/ insect) and incubated to study nematode development at five different temperatures (18, 20, 22, 25, and 27°C). (A) Days until emergence; (B) Yield (number of IJ) per gram of *G. mellonella*. Standard error bars with the same lowercase letter indicate no significant difference.
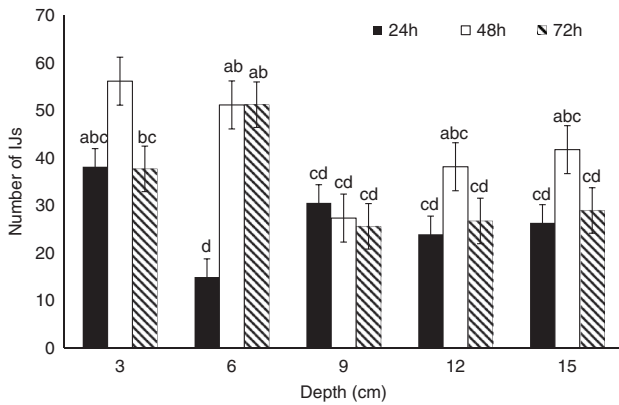

Figure 3: Mean number of infective juveniles (IJs) of *Steinernema innovationi* established in *Galleria mellonella* in sand columns at different depths after 24, 48, and 72 hr post inoculation with 100 IJs on the top surface of the sand column. Standard error bars with the same lowercase letter indicate no significant difference.

hanced by propagating nematodes at such temperatures, whereas infectivity at warm temperatures was enhanced by rearing under warm conditions.

Although *S. innovationi* reproduced at all temperatures tested, there were significant differences in yields at different temperatures, emphasizing that production outside the optimum temperature will have a negative effect on yield. Gungor et al. (2006) observed a similar pattern in that IJ yield of *S. anatoliense* dropped significantly at production temperatures below 20°C and above 25°C. While there was

no significant difference in yield at the two highest yielding temperatures (22 and 25°C), there was a significantly slower emergence at 22°C. The faster emergence at 25 and 27°C could be an advantage because a shorter cycle during mass production would allow for a greater cumulative yield in a short period of time. On the other hand, Hazir et al. (2001) cautioned that a lower temperature, and hence a slower rate of emergence, gives the nematode juveniles more time to feed and accumulate more lipid reserves, which are necessary for the survival of the non-feeding IJ stage. The tendency of sub-surface soil temperatures to be lower than ambient temperature would favor propagation at lower temperatures, which could enhance infectivity under field conditions.

The foraging behavior of EPNs has been classified as cruisers, ambushers, and intermediate foragers. The cruiser types actively search for hosts, whereas ambushers sit and wait for an insect to pass within close range (Lewis, 2002). The intermediate types fall between these two categories. Infective juveniles of *S. innovationi* demonstrated their capacity to locate and infect larvae of *G. mellonella* at different depths. This nematode species belongs to the 'glaseri group' (Çimen et al., 2014), which is classified as Clade V according to the phylogenetic analysis of the internal transcribed spacer (ITS) and the D2D3 region of the 28rRNA gene (Spiridonov et al., 2004). Entomopathogenic nematodes in this category are classified as cruisers (Shapiro-Ilan et al., 2009), which may suggest that *S. innovationi* is a cruiser. The cruiser-type behavior of *S. innovationi* is further supported by the observed rapid movement in sand columns. Wilson et al. (2012) argued that the ability of IJs to nictate depends on the substrate, with organic matter
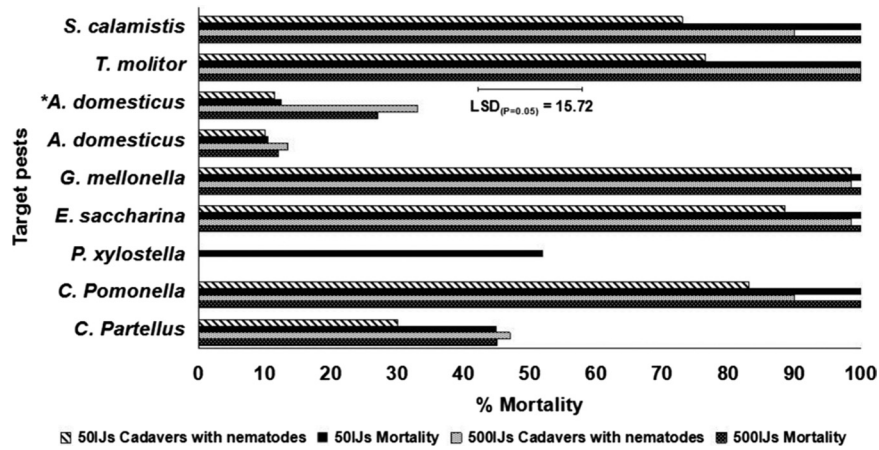
Figure 4: Mean mortality and confirmation of nematode development in different insect hosts post inoculation with 50 or 500 infective juveniles/host of *Steinernema innovationi*. Mortality was corrected using Schneider-Orelli's formula (Püntener, 1981). *7 days incubation time.
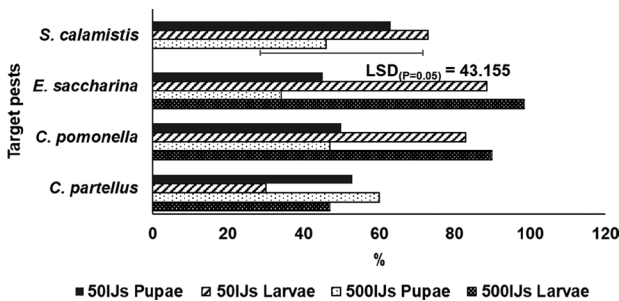

Figure 5: Comparison of mean mortality of larvae vs pupae per insect host following infection with 50 or 500 infective juveniles/host of *Steinernema innovationi*. Mortality was corrected using Schneider-Orelli's formula (Püntener, 1981).

promoting IJs to lift their body off the substrate to bridge large pores between particles. Furthermore, their hypothesis states that the ability to body wave in sandy soil is limited. The ability of *S. innovationi* to stand on its tail and moved in a circular pattern (waving nictation, an ambusher characteristic) on sand particles would suggest that this is an intermediate forager. To our knowledge, this is the first report of such a mixture in traits among EPNs in the *'glaseri* group'. Campbell and Gaugler (1993) observed increased host finding ability when *Steinernema carpocapsae* (Weiser) where able to nictate and declined when it was unable to nictate. Thus, being an intermediate forager, *S. innovationi* carries potential for increased host finding ability.

Rapid migration is important for the dispersal of IJs in the field following application. *Steinernema innovationi* managed to search, infect, and kill its host within 24 hr at all depths up to 15 cm. Mortality of the target insect larvae within 24 hr at all depths may have been enhanced by the relatively large particles of the loam sand (bulk density = $1.42 \, g/cm^3$). Jabbour and Barbercheck (2008) observed a rate of movement of 15.4 cm/day and 33.3 cm/day for *S. carpocapsae* in soils with bulk densities of 1.4 and $1.5 \, g/cm^3$, respectively. Similarly, Bilgrami et al. (2001) demonstrated targeted directional movement by *Steinernema glaseri* (Steiner) toward larvae of *G. mellonella* within 12 hr of incubation. Rapid migration is important for the dispersal of IJs in the field following application.

All insects tested were prone to infection by *S. innovationi,* although the level of susceptibility differed amongst hosts. Host susceptibility differs amongst species, with some EPN species only adapted to particular hosts. As in the case with *S. scapterisci* being highly specific to Orthoptera with insects in the orders Coleoptera, Lepidoptera and Hymenoptera being poor hosts or non-hosts (Nguyen and Smart, 1992). Variation in mortality against different species was also demonstrated by Athanassious et al. (2010) when they tested efficacy of three EPNs against four stored product pests of wheat. In the current study, the larvae of *T. molitor*, *G. mellonella*, *E. saccharina*, *S. calamistis*, and *C. pomonella* were the most susceptible (100% mortality). The reason for high mortality of *T. molitor* and *G. mellonella* could be attributed to their inherent high susceptibility to EPN and hence their wide use in laboratory experiments dur-

ing EPN studies (Shapiro-Ilan et al., 2012). The other three species, *E. saccharina*, *S. calamistis*, and *C. pomonella,* corresponded with *G. mellonella* in terms of susceptibility as they belong to the same order (Lepidoptera).

This study demonstrates (at 25°C) that nematode dose can be manipulated to increase mortality of a particular host. Mortality or percentage of hosts with nematode recycling increased for *P. xylostella* from 52 to 92% and for *T. molitor* from 77 to 100% at the higher concentration of 500 IJs/host. De Carvalho Barbosa Negrisoli et al. (2013) demonstrated a similar trend where mortality of *Anagasta (Ephestia) kueniella* (Zeller, Lepidoptera: Pyralidae) was increased by increasing the dose from 10 to 200 IJs/insect using five strains of *Heterorhabditis* and three strains of *Steinernema*. Similarly, mortality of *A. ipsilon* in this study increased with increased IJ concentration from 1 IJ/larva to reach a LC_{70} at 31 IJs/larva.

Different life stages have also been found to differ in susceptibility to EPNs. In particular, larvae have been found to be more susceptible than pupae (Theunis, 1998; De Carvalho Barbosa Negrisoli et al., 2013; Garcia-del-Pino et al., 2013; Malan et al., 2011). De Carvalho Barbosa Negrisoli et al. (2013) found that mortality of *A. kueniella* larvae was higher than that of pupae. They achieved maximum mortality of 96 and 80% with larvae and pupae, respectively. Similarly, in the current study, larvae of *E. saccharina*, *S. calamistis*, and *C. pomonella* were more susceptible than their pupal stages, at 3 and 7 d incubation for larvae and pupae, respectively.

This study demonstrated that the optimum temperature for the infectivity of *S. innovationi* is between 22 and 25°C. Host range results provide a guideline to indicate relative virulence of this new species against different hosts, suggesting its application for control of different pests. Furthermore, this means that the in vitro production medium/protocol (Ramakuwela et al., 2016) did not detrimentally affect the biology of the nematode and yielded virulent IJs. Being an intermediate forager, this strain carries potential for targeting insect pests near the soil surface as well as deeper into the soil.

Acknowledgments

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