Biological characterisation of *Heterorhabditis atacamensis* and *Steinernema unicornum* (Nematoda: Rhabditida), entomopathogenic nematodes from Chile

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Accepted for publication 14 November 2011

Summary. This is a bionomic study of the entomopathogenic nematodes (EPN) *Heterorhabditis atacamensis* and *Steinernema unicornum*, from the Atacama Desert and Tierra del Fuego in Chile, respectively. At 20°C, *H. atacamensis* infective juveniles took 22 days to emerge from cadavers, *S. unicornum* took 24 days. The thermal infectivity range for *H. atacamensis* was wider than *S. unicornum* (10-35°C *vs* 10-30°C). Exposure to 37°C for 60 min killed 100% *H. atacamensis* and 57% *S. unicornum*. *Heterorhabditis atacamensis* caused 53% insect mortality at the lower end of the soil moisture range, *S. unicornum* caused 100%; both caused ≥ 85% mortality at the upper end (near saturation point). *Heterorhabditis atacamensis* killed three of six insect species; however, mortality was relatively low for each (< 35%). *Steinernema unicornum* killed five of six species, with mortality consistently > 50%; mortality of the fruit tree weevil *Naupactus xanthographus* was highest (90%).

Key words: Desert, ecology, Heterorhabditidae, Nematoda, Steinernematidae, temperature.

Surveys on all continents and from a wide range of habitats, tropical rainforests (López-Núñez *et al*., 2007), deserts (Stock *et al*., 2009), high altitudes (Parsa *et al*., 2006), polar territories (Haukeland *et al*., 2006) *etc*., coupled to improved identification techniques, have increased our knowledge of the diversity of entomopathogenic nematodes (EPN). Exploring EPN diversity can uncover isolates with physiological and behavioural adaptations to local conditions, and hence, adaptations of value if/when using these organisms as biological control agents in the field.

In Chile, a country in which reducing the use of chemical pesticides is a part of National Policy, the local EPN fauna had been largely unexplored until a survey published in 2010 revealed 101 EPN isolates of four species (three of which were new, *Steinernema australe* Edgington, Buddie, Tymo, Hunt, Nguyen, France, Merino & Moore, 2009, *S. unicornum* Edgington, Buddie, Tymo, France, Merino & Hunt, 2009 and *Heterorhabditis atacamensis* Edgington, Buddie, Moore, France, Merino & Hunt, 2011), from a wide range of habitats, including the more ecological extremes of the Atacama Desert, southern Patagonia and high Andean altitudes (Edgington *et al*., 2010). The wide variety of ecosystems that the Chilean EPN isolates represent gives reason to suggest an indigenous EPN community with a wide range of environmental adaptations.

Key to the success of an EPN biological control is an understanding of ecological adaptations of the EPN; an EPN ill-equipped for the local temperature, soil type, moisture levels, host target, *etc*., will be severely limited (Koppenhöfer & Kaya, 1999; Georgis *et al*., 2006). Edgington & Gowen (2010) presented bionomic information of *S. australe* as a complement to the species description, using modifications of a protocol suggested by Koppenhöfer & Kaya (1999); the study gave information on host range, foraging strategy and a number of abiotic profiles for this EPN.

This present study presents bionomic information on two other species from Chile, *S. unicornum* and *H. atacamensis*, described in 2009 and 2010 (Edgington *et al*., 2009, 2011), and uses a similar protocol as that used for *S. australe*. The study includes tests on heat-shock tolerance, host

range and the effect of moisture and temperature on the EPN species. *Steinernema unicornum* was discovered in Tierra del Fuego, in the far south of Chile (53° 28′ 2.7″ S, 70° 11′ 26″ W) and is the first EPN from sub-Antarctic territory; it was discovered in a relatively dry, loamy-sand soil, within a native forest of Antarctic beech (*Nothofagus antarctica*). *Heterorhabditis atacamensis* was discovered in the Atacama Desert (23° 11′ 16.7″ S, 67° 59′ 31.6″ W), one of the driest places on earth in which some areas have had no recorded rainfall for decades (Conley *et al*., 2006); it was discovered in soil taken from a sandy bank at 2499 m above sea level (a.s.l.), in a location some 4000 km north of *S. unicornum*.

MATERIALS AND METHODS

Nematode culture. The nematodes used in the study were cultured in late instar waxmoth (*Galleria mellonella* L.) larvae (obtained from Live Foods Direct, Sheffield, UK or, cultured at the Instituto de Investigaciones Agropecuarias (INIA), Chillán, Chile). Emergent infective juveniles (IJ) were collected in modified White traps (White, 1927) and stored at 8 ± 2°C for *S. unicornum* and 13 ± 2°C for *H. atacamensis*, in tap water, prior to the trials; these temperatures were found to be suitable storage temperatures for the respective species. Only IJ collected within 10 days of first emergence were used in the trials and IJ in storage for > 20 days were discarded. All experiments were done in the laboratory at CABI and/or INIA.

Life cycle. Twenty late instar waxmoth larvae, in a Petri dish (9 cm diam.) lined with moistened filter paper, were exposed to a concentration of approximately 50 IJ larva^{-1} of each EPN species (in 1 ml sterilised tap water) and kept at 20 ± 2°C. There were three Petri dishes in total, *i.e*., 60 waxmoths. Every 24 h up to 240 h following inoculation (with the exception of 144 and 168 h), five waxmoth larvae were washed in tap water and dissected in 0.5% saline solution (NaCl). Larval mortality and *in vivo* EPN development were recorded. Five cadavers were transferred to modified White traps to monitor nematode emergence.

Temperature profile. Each chamber (1.5 cm^3) of a 25-chamber bioassay plate was partially filled with 0.5 g sterilised, air-dried sand (medium sized particles, mesh designation – 30+50). The test temperatures were 10, 15, 20, 25, 30 and 35 ± 2°C, with IJ and waxmoth larvae equilibrated for 1 h at these temperatures before testing. Fifty IJ in 50 µl sterilised tap water were transferred into each chamber, followed by one waxmoth larva. Control chambers received 50 µl sterilised tap water without IJ. Plates were placed in plastic bags with moistened tissue paper to reduce desiccation and then maintained at the test temperatures. Larval mortality and time to first IJ emergence were recorded every 24 h for 20 days (with the exception of 168 h), then every 48 h thereafter for another 20 days. The trial was done twice.

Heat-shock tolerance. The heat-shock tolerance of *S. unicornum* and *H. atacamensis* was assessed by examining survival after exposure to a water bath at 37°C. Plastic tubes (1.5 ml volume), containing 900 µl sterilised tap water, were submerged in a water bath at 37 ± 1°C for 1 h. Infective juveniles in sterilised tap water (approx. 2000 IJ ml^{-1}) were kept at 20 ± 2°C for 1 h. Each plastic tube in the water bath received 100 µl of the IJ suspension (*i.e.*, 200 IJ) and the tubes re-submerged at 37 ± 1°C. Five tubes of each species were removed from the water bath after 0, 15, 30, 45 and 60 min, the IJ left to recover on a counting chamber at room temperature for 2 h and a count of live and dead IJ made. Nematodes that did not respond to gentle probing with a needle were counted as dead. The trial was set up as a randomised block design with five blocks; each block contained five tubes of each species, with one tube taken from each block at each time. Control treatment consisted of IJ maintained in plastic tubes as above but kept at 10°C instead of 37°C. The trial was done twice.

Effect of soil moisture. Approximately 50 IJ in 50 µl sterilised tap water were placed at the bottom of a plastic tube (8 cm height, 1.5 cm diam., 28 ml volume), which was then part-filled with pre-moistened sand (mesh designation – 30+50) to a height of approx. 3 cm, the column being gently compacted by tapping the tube on the bench. The moisture contents (MC) tested were (w/w) 0, 5.6, 11.3 and 17.3% MC. Moisture contents were assessed using a HG53 Mettler Toledo Moisture Analyser. A disc of wire mesh was placed on the top surface of the sand onto which one late instar waxmoth larva was placed. The tubes were sealed and maintained at 20 ± 2°C for 120 h. Control treatment consisted of larvae maintained on top of the moistened sand column but without the addition of IJ. Mortality of waxmoth larvae was assessed after 120 h, with cadavers dissected in 0.5% NaCl to count the number of IJ that had penetrated. There were five tubes per treatment at each moisture level. The trial was done twice.

Laboratory host range. Seven insect species, all at the immature stage, and representing two orders (Coleoptera and Lepidoptera), were used to assess the laboratory virulence of *S. unicornum* and *H. atacamensis.* All insects were obtained within Chile

and tested at INIA (Region VII). Late instar larvae of *G. mellonella* (Lepidoptera: Pyralidae) and codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) were obtained from laboratory colonies at INIA; late instar blackmoth larvae *Dallaca pallens* (Blanchard) (Lepidoptera: Leporidae) were obtained from natural pastures in Region X in the south of Chile (approx. latitude 41°S); late instar larvae of the fruit tree weevil *Naupactus xanthographus* (Germar) (Coleoptera: Curculionidae), rose beetle *Naupactus cervinus* (Boheman) (Coleoptera: Entiminae), raspberry weevil *Aegorhinus superciliosus* (Guérin) (Coleoptera: Curculionidae) and carpenter worm *Chilecomadia valdiviana* (Philippi) (Lepidoptera: Cossidae) were obtained from various fruit crops in Regions VI to X (approx. 34 to 41°S). Each insect was placed into a chamber (1.5 cm high × 3 cm diam.) lined with moistened filter paper. Infective juveniles were applied at doses of 0, 50 and 500 IJ insect^{-1} in 100 µl sterilised tap water, and were then left at 20 ± 2°C. Insect mortality was monitored every 24 h for 15 days. The filter paper in each chamber was moistened occasionally with sterilised distilled water. There were 60 insects per treatment, arranged in four blocks of 15 insects each.

Data analysis. In all experiments the results from the repeat trials were similar and were therefore combined. When appropriate, mortality data was corrected for control mortality using Abbott's formula (Abbott, 1925). Any percentage data was arcsine transformed before significance testing (Dytham, 2003) (the data presented in the paper is pre-transformed data). Analysis of variance with appropriate factors was used to analyse treatment effects in all tests, with Tukey's test and Student's *t*-test used to analyse differences and relationships between treatments (Genstat 11th Edition, VSNI). Differences between treatment means (± SE) were considered significant at $P < 0.05$.

RESULTS

Life cycle. Mortality of waxmoth larvae at 20°C was observed after 72 h for both EPN species, 24 h later all larvae were dead. Hermaphrodite adults of *H. atacamensis* and first generation adults of *S. unicornum* were observed 96 h following inoculation, second generation adults 168 h later. The mean time from inoculation until IJ emergence from cadavers was 528 and 576 h (*i.e.*, 22 and 24 days) for *H. atacamensis* and *S. unicornum*, respectively. Second generation adults and non-infective juveniles of *S. unicornum* were observed outside the waxmoth cadaver 480 h following inoculation.

Temperature profile. *Steinernema unicornum* caused 100% waxmoth mortality at 10-25°C, 28% mortality at 30°C and 0% at 35°C; *Heterorhabditis atacamensis* caused 100% mortality at 10-25°C, 93% mortality at 30°C and 26% at 35°C (Fig. 1A). There was no waxmoth mortality in the control treatment at each test temperature; however, at 30 and 35°C a number of waxmoths had pupated by 6 days. Temperature had a significant effect on mean time until waxmoth death for both EPN species (*H. atacamensis*: $F = 564.0$; $df = 5,514$; $P < 0.05$; *S. unicornum*: $F = 64.3$; $df = 4,423$; $P < 0.05$); for *H. atacamensis*, waxmoth mortality was quickest at 25°C and slowest at 10°C (2 ± 0.0 and 6.2 ± 0.98 days until death, respectively) and for *S. unicornum* was quickest at 25°C and slowest at 30°C (2.8 ± 0.01 and 4.5 ± 0.4 days until death, respectively) (disregarding the 35°C treatment for *S. unicornum*) (Fig. 1B). For *H. atacamensis*, progeny emerged from 100 and 78% of cadavers at 20 and 25°C, respectively, but there was no emergence at any other temperature; for *S. unicornum*, progeny emergence was highest at 20°C (58% of cadavers), followed by 15, 10 and 25°C (44, 6 and 4%, respectively); no progeny emerged at 30 and 35°C (Fig. 1C). For *H. atacamensis*, temperature had a significant effect ($t = 9.1$; $df = 137$; $P < 0.05$) on the time until first nematode emergence with IJ observed outside the host 18.5 (± 0.3) and 22.4 (± 0.2) days after inoculation at 25 and 20°C, respectively (IJ emerged from only two treatments). For *S. unicornum*, temperature had a significant effect ($F = 37.6$; $df = 3,108$; $P < 0.05$) on the time until first nematode emergence, with IJ observed outside the host 15.4 (± 2.2), 22.3 (± 0.8), 33 (± 1.0) and 36 (± 1.8) days after inoculation at 25, 20, 15 and 10°C, respectively (Fig. 1D).

Heat-shock tolerance. At the control conditions (10 ± 2°C) IJ mortality was < 8% after 60 min exposure. For both species, 60 min exposure to a water bath at 37°C caused significant IJ mortality (*H. atacamensis*: $t = -133.0$; $df = 18$; $P < 0.05$; *S. unicornum*: $t = 24.2$; $df = 18$; $P < 0.05$), with IJ mortality increasing from 4.9 ± 0.58% at 0 min, 43.6 ± 2.4% at 30 min to 100% at 60 min for *H. atacamensis* and from 1.8 ± 1.2% at 0 min, 22.2 ± 1.6% at 30 min to 56.6 ± 1.2% at 60 min, for *S. unicornum* (Fig. 2).

Effect of soil moisture. Results from the soil moisture study can be seen in Table 1. At 0% MC neither species killed waxmoths. For both species there was a significant effect of increasing MC on waxmoth mortality (*H. atacamensis*: $F = 29.6$, $df = 3,8$, $P < 0.05$; *S. unicornum*: $F = 76.0$, $df = 3,8$, $P < 0.05$); *H. atacamensis* killed 53 ± 3.3% of waxmoths

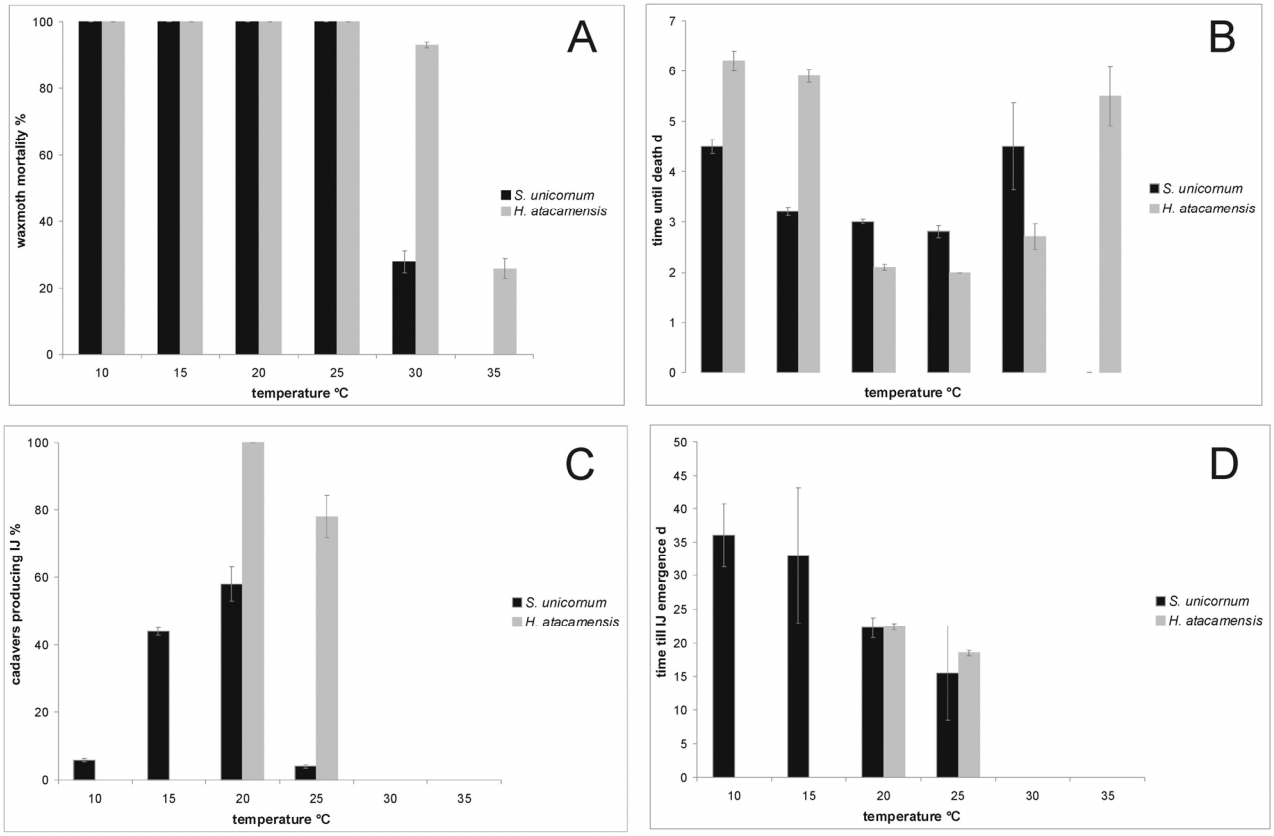

Fig. 1. A: Waxmoth larvae killed by *Steinernema unicornum* and *Heterorhabditis atacamensis* (%); B: Time until waxmoth death (d); C: Cadavers producing IJ (%); D: Time until first emergence (d) at test temperatures 10, 15, 20, 25, 30 and 35°C. Waxmoth larvae were kept in chambers partially filled with sand and exposed at a concentration of 50 IJ larva^{-1}; for a total of 40 d. Bars indicate means and vertical lines represent the 95% confidence intervals.

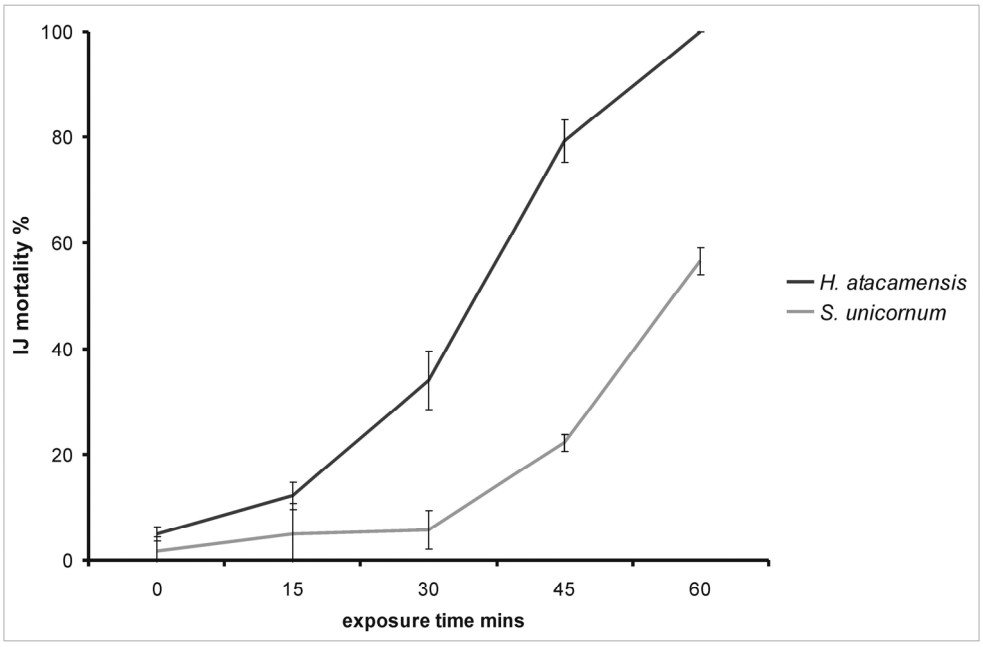

Fig. 2. Mortality (%) of *Steinernema unicornum* and *Heterorhabditis atacamensis* following exposure to 37°C for 0, 15, 30, 45 and 60 min. The points indicate means and vertical lines represent the 95% confidence intervals.

at 5.6% MC, increasing to 85 ± 7.6% at 17.3%; *S. unicornum* killed > 91% of waxmoths at 5.6, 11.3 and 17.3% MC. The mean number of *H. atacamensis* juveniles observed in cadavers was < 2.5 EPN cadaver^{-1} for all MC (*i.e.*, penetration levels < 5% of the original IJ inoculum); penetration of *S. unicornum* was highest at 11.3% MC, namely 14.4 ± 2.5 EPN cadaver^{-1} (approx. 30% of inoculum), with 10.4 ± 1.8 and 7.2 ± 1.4% of inoculum penetrating at 5.6 and 17.3% MC, respectively ($F = 12.5$, $df = 3,36$, $P < 0.05$).

Laboratory host range. Mortality of target insects can be seen in Table 2. There was significant mortality of all insects when treated with *H. atacamensis* ($P < 0.05$), with the exception of *C. pomonella* at 50 IJ insect^{-1} and *D. pallens* and *A. superciliosus* at both 50 and 500 IJ insect^{-1}, however (excepting *G. mellonella*), mortality levels were relatively low throughout the study (≤ 35%, at the highest inoculum level). There was significant mortality of all insects when treated with *S. unicornum* at 50 IJ insect^{-1}, ranging from 25 (*N. cervinus*) to 83% (*C. valdiviana*) ($P < 0.05$). When the dose of *S. unicornum* was increased from 50 to 500 IJ insect^{-1}, a significant increase in mortality was observed for just one insect, *D. pallens* (from 27 to 60%).

Table 1. Waxmoth mortality (%) and EPN penetration by *Heterorhabditis atacamensis* and *Steinernema unicornum* in a 3 cm column of sand of 0, 5.6, 11.3 and 17.3% moisture content (MC) (w/w). Waxmoths were exposed at a concentration of 50 IJ larva^{-1} for 120 h, with EPN placed at the bottom of the sand column and waxmoths on the top. Means followed by the same letter within a column, for each EPN, are not significantly different ($P > 0.05$)

EPN	Moisture content % w/w	Waxmoth mortality % (± SE)	EPN penetrating (± SE)
H. atacamensis	0	0 [a]	0 [a]
	5.6	53.3 (± 3.3) [bc]	1.2 (± 0.3) [b]
	11.3	33.3 (± 8.3) [b]	1.8 (± 0.4) [bc]
	17.3	85.0 (± 7.6) [c]	2.4 (± 0.3) [c]
S. unicornum	0	0 [a]	0 [a]
	5.6	100 [b]	10.4 (± 1.8) [bc]
	11.3	91.7 (± 8.3) [b]	14.4 (± 2.5) [c]
	17.3	100 [b]	7.2 (± 1.4) [b]

Table 2. Mortality (%) of insect species from Chile by *Heterorhabditis atacamensis* and *Steinernema unicornum*. Insects were exposed at a concentration of 0, 50 and 500 IJ insect^{-1} for 15 d. All insects were at the late-instar larval stage. Means ± SE followed by the same letter within a row, for each EPN, are not significantly different ($P > 0.05$). The data presented is pre-transformed data

Order	Family	Species	*Heterorhabditis atacamensis*			*Steinernema unicornum*		
			0 IJ	50 IJ	500 IJ	0 IJ	50 IJ	500 IJ
Lepidoptera	Pyralidae	*Galleria mellonella*	6.7 ± 2.0 [a]	90.0 ± 1.0 [b]	93.3 ± 1.4 [b]	1.7 ± 0.0 [a]	96.7 ± 4.1 [b]	100.0 ± 1.0 [b]
	Tortricidae	*Cydia pomonella*	6.7 ± 1.4 [a]	15.0 ± 2.2 [a]	35.0 ± 4.9 [b]	3.3 ± 1.0 [a]	63.3 ± 1.7 [b]	80.0 ± 5.4 [b]
	Leporidae	*Dallaca pallens*	1.7 ± 0.9 [a]	3.3 ± 1.0 [a]	3.3 ± 1.0 [a]	3.3 ± 1.0 [a]	26.7 ± 2.4 [b]	60.0 ± 2.4 [c]
	Cossidae	*Chilecomadia valdiviana*	1.7 ± 0.9 [a]	33.3 ± 6.4 [b]	32.0 ± 4.4 [b]	6.7 ± 2.4 [a]	83.3 ± 2.2 [b]	98.3 ± 0.9 [b]
Coleoptera	Curculionidae	*Naupactus xanthographus*	0 ± 0.0 [a]	10.0 ± 1.7 [b]	21.7 ± 2.6 [b]	3.3 ± 0.0 [a]	70.0 ± 4.1 [b]	90.0 ± 1.0 [b]
	Curculionidae	*Naupactus cervinus*	1.7 ± 0.9 [a]	15.0 ± 0.9 [b]	25.0 ± 2.9 [b]	5.0 ± 0.9 [a]	25.0 ± 4.1 [b]	41.7 ± 2.6 [b]
	Curculionidae	*Aegorhinus superciliosus*	5.0 ± 1.6 [a]	11.7 ± 1.6 [a]	18.3 ± 3.3 [a]	8.3 ± 1.6 [a]	40.0 ± 6.4 [b]	50.0 ± 2.2 [b]

DISCUSSION

The results from this study provide baseline information on the biology and ecology of *H. atacamensis* and *S. unicornum*, complementing the species descriptions by Edgington *et al.* (2011) and Edgington *et al.* (2009). The methodologies used follow profiling suggestions by Koppenhöfer & Kaya (1999) and provide information that will assist research into these EPN as biological control agents.

The thermal ranges for EPN infectivity were 10-35°C and 10-30°C for *H. atacamensis* and *S. unicornum*, respectively. Both species caused 100% mortality at 10°C, in < 7 days, quicker than a number of EPN sourced from cold regions, including *Heterorhabditis* sp. from the Peruvian Andes (12 days) (Parsa *et al.*, 2006) and *S. australe* from Chilean Patagonia (8 days) (Edgington & Gowen, 2010). The ability of *H. atacamensis* and *S. unicornum* to kill relatively quickly at low temperatures may reflect adaptations to their type-localities, in which daily temperatures in the Atacama Desert and Tierra del Fuego drop to 0-5°C during most of the year. Cold tolerance/activity of a

biological control agent would be useful in protecting young plants that are sensitive to insect attack in early spring. The higher upper thermal limit for infectivity of *H. atacamensis vs S. unicornum* (35° *vs* 30°C) may reflect higher average temperatures in the Atacama Desert compared to Tierra del Fuego, where the highs in summertime are 26 and 14°C, respectively. The thermal ranges for EPN reproduction were narrower than for host kill, namely 20-25°C for *H. atacamensis* and 10-25% for *S. unicornum*. A narrower temperature range for reproduction compared to kill is a feature not unusual for EPN (Koppenhöfer *et al*., 2000; Edgington & Gowen, 2010; Gungor *et al*., 2006). A failure of EPN to reproduce inside a cadaver may be due to inhibited growth of the symbiotic bacteria (Wright, 1992). Host mortality may be less dependent on bacteria growth and indeed can proceed with little reliance, if any, on the bacteria (Ciche *et al*., 2006). However, the narrow range for *H. atacamensis* reproduction within a cadaver may be cause for concern if a control strategy is reliant on recycling of the control agent.

On occasions second generation adults and non-infective juveniles of *S. unicornum* were observed outside the cadaver, where they survived for several days. Although the IJ stage has morphological and physiological adaptations to survive outside the host (O'Leary *et al*., 1998) and would generally be regarded as the soil-inhabiting stage of EPN, emergence of non IJ stages has now been observed with a number of EPN, including *S. affine* (San-Blas pers. comm.), *S. australe* (Edgington & Gowen, 2010) and *S. glaseri* (Lewis & Gaugler, 1994), albeit under artificial conditions. Stimulants for emergence include food conditions inside the cadaver (Strauch *et al*., 1994) and moisture levels outside (Koppenhöfer *et al*., 1997). In the present study, it is not clear whether the emergence of adults and non-infective juveniles of *S. unicornum* was a response to conditions inside and/or outside the cadaver.

Sudden rises in temperature beyond the thermal ranges, namely heat-shocks, can be fatal for EPN (Griffin *et al*., 2005). *Heterorhabditis atacamensis* was able to locate and kill waxmoths at 35°C but suffered 100% mortality when exposed for just 60 min to 37°C. By contrast, although *S. unicornum* did not kill the target host at 35°C, there was significant IJ survival following 60 min exposure to 37°C. Tolerance to heat-shock may reflect selection pressures from the local environment, *Heterorhabditis* spp. for example, from regions in Israel characterised by high summer temperatures, showed > 70% survival after 6 h exposure to 37°C, whilst *S. australe* from much cooler Chilean Patagonia suffered 50% mortality after just 30 min at 37°C (Edgington, 2010). The results therefore, of the present study, are somewhat surprising, with the sub-polar EPN showing greater heat-shock tolerance than the desert EPN. Tolerating hot temperatures will be a useful characteristic in the field if either *H. atacamensis* or *S. unicornum* are to be used as biological controls, in the more northerly latitudes of Chile for example, in and around the Atacama Desert, and also during periods of heat-stress whilst being transported. Furthermore, activity at temperatures ≥ 37°C may be of interest regarding the environmental impact and registration of a biological control product, *i.e*., whether the organism can survive at human body temperature. Mechanisms of surviving heat-shock, some of which have high heritable values amongst many groups of organisms including EPN, *e.g*., the production of both heat-shock proteins and trehalose, can be enhanced by careful genetic selection, mutagenesis and culturing techniques in the laboratory (Burnell & Dowds, 1996).

Optimum soil MC for penetration and mortality by EPN will vary according to EPN species and soil type (Shapiro-Ilan *et al*., 2006). Although both *H. atacamensis* and *S. unicornum* infected over a wide range of soil MC, mortality and establishment by *S. unicornum* was generally higher at the drier end of the range, compared with *H. atacamensis*. Koppenhöfer *et al*. (1995) observed a similar adaptation by *S. carpocapsae* to drier conditions and suggested this was essential as the IJ showed a behavioural preference for the soil surface. Whether the greater adaptation to drier conditions of *S. unicornum vs H. atacamensis* reflects a behavioural preference for drier, upper levels of soil is unknown (of note, both EPN species were recovered at a depth of 5-20 cm). At the opposite end of the moisture range, at 17.6% MC (near saturation point of the soil), *S. unicornum* and *H. atacamensis* both caused high mortality of the target host (*S. unicornum* caused 100% mortality, *H. atacamensis* 85%). Koppenhöfer *et al*. (1995) suggest the effect of soil moisture on EPN infectivity correlates with IJ size and that larger IJ are better adapted to move through thicker films (*i.e*., through wetter soils), as smaller nematodes may start floating. However, although both *S. unicornum* and *H. atacamensis* caused high mortality at the upper end of the moisture range, indeed close to saturation point, neither can be considered as large, at body lengths (ranges) 732 (654-786) and 611 (578-666) µm, respectively and body widths 29 (25-33) and 22 (19-26) µm, respectively; although, the higher mortality

by *S. unicornum* in the wetter soil (100 *vs* 85%) may reflect its slightly larger size.

The natural host ranges of *H. atacamensis* and *S. unicornum* are unknown as these EPN were isolated from soil using waxmoth baiting. *Steinernema unicornum* infected a wider range of insect hosts in the laboratory, compared with *H. atacamensis*. In fact, at laboratory conditions *H. atacamensis* was relatively ineffective at killing all target pests (mortality levels ≤ 35%). The ability of an EPN to kill an insect is controlled by a combination of host finding, host acceptance and host suitability, which in turn relies on the host, EPN, bacterial symbiont and environmental conditions (Lewis *et al*., 2006), *i.e*., it is a complex process. Laboratory host ranges of EPN are well documented. Temperate strains of *S. rarum*, *S. feltiae*, *S. australe* and *S. monticolum* could be regarded as generalist feeders, infecting a total of 11 insect orders between them, however when mortality at the family level is examined the EPN show clear host specificity (de Doucet *et al*., 1999; Koppenhöfer *et al*., 2000; Edgington & Gowen, 2010).

The results on the bionomics of *H. atacamensis* and *S. unicornum* presented here can assist researchers in assessing the use of these EPN as biological control organisms. The results complement both the formal description of *H. atacamensis* and *S. unicornum* and EPN research being carried out by staff at INIA investigating biological and ecological adaptations of EPN isolates from extreme habitats and their value as biological control agents.

ACKNOWLEDGEMENT

The authors wish to thank Dave Moore (CABI) and Andrés France (INIA) for their help in obtaining the EPN from the field and for reviewing the manuscript, the insectary team at INIA for providing insect cultures and CABI for supporting the work.

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S. Edgington, L. M. Merino. Биологические особенности энтомопатогенных нематод *Heterorhabditis atacamensis* и *Steinernema unicornum* (Nematoda: Rhabditida) из Чили.

Резюме. Исследованы биологические особенности чилийских энтомопатогенных нематод *Heterorhabditis atacamensis* и *Steinernema unicornum* из пустыни Атакама и Огненной Земли, соответственно. При 20°C инвазионным личинкам *H. atacamensis* требуется 22 дня для завершения развития и миграции из трупов, тогда как инвазионным личинкам *S. unicornum* требуется 24 дня. Температурный диапазон инвазионности был шире у *H. atacamensis*, чем у *S. unicornum* (10-35°C *vs* 10-30°C). Температура 37°C при 60-минутной экспозиции убивала 100% личинок *H. atacamensis* и 57% *S. unicornum*. Нематоды *Heterorhabditis atacamensis* вызывали 53% смертность насекомых при низких значения влажности почвы (минимальные значения влажности для мест их обитания), тогда как *S. unicornum* при таких условиях вызывали 100% смертность. Оба вида приводили к более чем 85% смертности при высоких значениях влажности почвы (близ точки насыщения). *Heterorhabditis atacamensis* убивали три из шести видов насекомых, использованных в эксперименте, хотя смертность была всегда довольно низкой (< 35%). *Steinernema unicornum* убивали 5 из 6 видов насекомых при постоянно высокой смертности (> 50%), причем смертность фруктового долгоносика *Naupactus xanthographus* была самой высокой (90%).