Virulence and Reproduction of Entomopathogenic Nematodes Isolated from a Single Mexican Locality[1]

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Abstract   Entomopathogenic nematodes (EPNs) are important biological control agents of insect pests. Strains or isolates obtained in specific regions and utilized in situ might contribute to the success of EPNs, since they are adapted to local abiotic conditions. We isolated and identified three isolates each of *Heterorhabditis bacteriophora* Poinar and *Steinernema carpocapsae* (Weiser) from soils at Saltillo, Coahuila State, Mexico. These six isolates were bioassayed against larvae of *Tenebrio molitor* L., using six concentrations of infective juveniles (IJs) per larva (0, 10, 25, 50, 100, 200). EPN-induced mortality of the isolates ranged from 15.2% to 100%. The designated M5 isolate of *S. carpocapsae* caused 100% mortality after 72 h at concentrations ≥25 IJs/larva with a median lethal concentration of 4.99 IJs/larva. Comparison of mortality levels induced at the same concentration of the six EPN isolates indicated that the *Steinernema* isolates, in general, induced higher mortality levels than the *Heterorhabditis* isolates. IJ production in *T. molitor* larvae also was greatest with the M5 isolate of *S. carpocapsae* following exposure of larvae to a concentration of 100 IJs/larva with a mean of 17,320 IJs/larva. Based on induced mortality and IJ production, the *S. carpocapsae* M5 isolate appears to be a viable candidate for further study and possible development for use in insect pest management programs. This study indicates that naturally coexisting local nematode isolates possess different attributes critical for their use as biocontrol agents.

Key Words   biological control, *Heterorhabditis*, *Steinernema*, insect pest management

Entomopathogenic nematodes (EPNs) in the families Heterorhabditidae and Steinernematidae are effective biological control agents in the management of a variety of economically important insect pests (Agazadeh et al. 2010). In their invasion of healthy insects, these nematodes are symbiotically associated with insect-pathogenic bacteria in the Enterobacteriaceae: *Photorhabdus* is associated with Heterorhabditidae and *Xenorhabdus* with Steinernematidae (Campos-Herrera et al. 2015). The third-instar infective juvenile (IJ) is the only free-living stage that lives in soil in search of an insect host (Wang et al. 2014).

EPNs are ubiquitous in cultivated and noncultivated soils throughout the world (Stuart et al. 2006). The importance of interspecific and intraspecific variation has

been recognized, and this variation accounts for differences in efficacy among species and strains/isolates of these entomopathogens.

There are different factors that regulate EPN effectiveness in biocontrol (e.g., the habits of the target insect) and environmental factors (e.g., temperature, humidity and soil texture, and pesticide use). The use of native strains or isolates of EPNs collected from, and adapted to, specific regions might contribute to the success of these agents against pests in those locales (Garcia-del-Pino et al. 2013, Parada Domínguez et al. 2019).

There are several reports on the presence of EPNs in central and southern Mexico (Morfín-Arriaga and Cortez-Madrigal 2018, Parada Domínguez et al. 2019) and northeastern Mexico (Treviño-Cueto et al. 2021). In the latter study, the larvicidal activity of local isolates of *Heterorhabditis* and *Steinernema* against larvae of the yellow fever mosquito, *Aedes aegypti* (L.), was determined in laboratory bioassays. General biological attributes of those isolates, however, were not the subject of that study. Several of those isolates, therefore, are the subject of the study reported herein. Attributes of newly isolated EPNs include virulence against selected target insects and production in the laboratory, both of which are essential for their utilization in biological control programs. In addition, production of IJs in susceptible hosts is a relevant consideration in that distribution of nematode-killed insect-containing IJs in soils is an effective method for deployment of these biocontrol agents (Shapiro-Ilan et al. 2006). Furthermore, collection, evaluation, and utilization of locally isolated biocontrol agents are relevant when considering restrictions to the importation and use of exotic biocontrol agents (Jaronski et al. 2013).

In order to contribute to the development of regional biological control programs utilizing local strains or isolates of beneficial organisms, our objective in this study was the general characterization of isolates of EPNs isolated from one locality in Saltillo, Mexico. Specifically, we determined (1) the mortality induced by each local isolate larvae of on yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), following exposure to concentrations of IJs; and (2) the numbers of IJs produced per host larva by each isolate in the laboratory.

Materials and Methods

Our study was conducted starting in May 2017 at the experimental fields and the Biological Control Laboratory, Universidad Autónoma Agraria Antonio Narro (UAAAN), Saltillo, state of Coahuila, Mexico.

EPN isolation. Twenty-eight soil samples were obtained at the Universidad Autónoma Agraria Antonio Narro, with differences in habitat and soil use as described by Treviño-Cueto et al. (2021). Each sample was a 1-liter volume of soil taken at a depth of 15 cm using hand shovels. The shovels were rinsed with 70% ethanol between samples. In the laboratory, nematodes were isolated from soil samples using the insect bait technique described by Kaya and Stock (1997) and Sanchez-Peña et al. (2011). Each sample was split among five 200-ml containers, and water was added to each so that the soil was slightly moistened but not saturated or muddy. Five last-instar *T. molitor* larvae obtained from a laboratory colony were added to each container. The containers were covered, placed upside down to bury the insect larvae, and incubated in bioclimatic chambers at 23 ± 2°C. After 7 to 9 d, parasitized larvae were recovered, identified by their typical symptoms of color change

(e.g., reddish brown/purple for heterorhabditids and ochre/brown/black for steinernematids). Cadavers were placed in modified White traps (Kaya and Stock 1997) for emergence of IJs. The emerged IJs were recovered from the water suspension in the trap and washed by gentle decantation in several changes of purified water to remove insect remains. All isolates were stored in purified water in petri dishes at 15–20°C.

EPN identification. The nematodes were identified to family based on morphological characteristics and appearance of infected larvae (Kaya and Stock 1997, Poinar 1979). Further identification was by the methods described by Maafi et al. (2003) and Treviño-Cueto et al. (2021). From each tentative nematode family, DNA was extracted from several dozen individuals. Genetic regions amplified were: internal transcribed spacer (ITS)-rRNA gene, D2-D3 region of 28S rRNA gene. For steinernematids, the D2-D3 region of the 28S rRNA gene was sequenced. Voucher specimens were deposited at the Plant Pest Diagnostics Center, California Department of Food and Agriculture, Sacramento, CA.

Bioassay of insecticidal activity of EPNs. Nematode IJs were harvested from the White traps and resuspended in 10 ml of purified water. The number of IJs per milliliter was determined and adjusted using the Neubauer chamber and microscopic observation. Six isolates included in the bioassay were three *Steinernema carpocapsae* (Weiser) isolates designated as M5, M6, and B7, and three *Heterorhabditis bacteriophora* Poinar isolates designated M18, MZ9, and MZ6. Last-instar *T. molitor* larva were exposed to nematodes.

The bioassay was performed in experimental units consisting of petri dishes (100 × 15 mm) each lined on the bottom with a circle of rinsed, moistened brown paper. Suspensions of either 0, 10, 25, 50, 100, or 200 IJs/larva were evenly distributed on the moistened paper, and 10 last-instar *T. molitor* larvae were placed in each dish. The IJs used were less than a month old. There was a total of six isolates with six concentrations of IJs for each isolate. Each isolate/concentration was replicated 10 times. Larval mortality was recorded every 24 h up to 72 h, verifying that larvae did not show mobility when poked with a probe.

IJ production in *T. molitor* larvae. Nematode development and IJ production in *T. molitor* larvae were quantified for each EPN isolate using the bioassay testing method but only with one concentration of IJs (e.g., 100 IJs/larva) and five EPN isolates (the M6 isolate of *S. carpocapsae* was not available for this test). After 7–9 d, dead larvae were randomly selected from each EPN isolate treatment, identified by the color change described (reddish brown/purple for heterorhabditids and ochre/brown/black for steinernematids), and placed in modified White traps (Kaya and Stock 1997) for emergence of IJs. The IJs in the White trap were transferred individually to petri dishes (10-cm diameter) containing 20 ml of sterile distilled water. Total IJ production was determined from dilutions using the Neubauer chamber.

Analysis of data. Mortality data were not corrected since mortality in the control was zero. An analysis of variance was performed for mortality, and Tukey's test was applied to separate the means in the R Studio program. Probit analysis (Finney and Tattersfield 1952) and estimation of the median lethal concentration (LC_{50}) were performed with the StatPlus (2022) statistical program. The production of IJs was compared by analysis of variance followed by Bonferroni's test for mean separation in the R Studio program.

**Table 1. Mean (±SD) percentage mortality of _T. molitor_ larvae caused by
S. carpocapsae M5 isolate at 24, 48, and 72 h postexposure.***

Concentration (IJs/larva)**	24 h Postexposure	48 h Postexposure	72 h Postexposure
10	30.75 ± 19.04c	66.55 ± 20.41b	83.94 ± 13.15b
25	56.80 ± 17.97a	80.33 ± 16.86ab	100.0 ± 0.00a
50	40.44 ± 9.41bc	92.44 ± 10.47a	100.0 ± 0.00a
100	45.49 ± 17.42bc	84.66 ± 12.76a	100.0 ± 0.00a
200	72.16 ± 17.16c	98.00 ± 4.21a	100.0 ± 0.00a
Control	0.00 ± 0.00d	0.00 ± 0.00c	0.00 ± 0.00c

* Within columns, means followed by different lowercase letters are significantly different as determined by Tukey's Honestly Significant Differences ($P \leq 0.05$).
** IJs, infective juveniles.

Results

EPN isolation. Six EPN isolates were obtained from the 120 samples collected from University soils. The six were determined to belong to the genera _Heterorhabditis_ and _Steinernema_ (Table 1) based on the characteristic coloration of the _T. molitor_ cadavers (Woodring and Kaya 1988). A reddish-dark wine color of larvae killed by EPNs is caused by the bacterium _Photorhabdus_ associated with _Heterorhabditis_; whereas _Xenorhabdus_, associated with _Steinernema_ spp., induces a brownish-dark color in EPN-killed larvae. We did not observe the reported fluorescence in our putative _Heterorhabditis_ isolates (Woodring and Kaya 1988).

EPN identification. Sequences of the ITS-rRNA and D2-D3 of 28S rRNA genomic regions of the _Heterorhabditis_ isolates showed 100% identity to many isolates of _H. bacteriophora_ from Mexico (e.g., MK421440.1), Argentina, the Middle East, and India (Machado et al. 2019), underscoring the cosmopolitan distribution of this species (Campos-Herrera et al. 2012). Sequences of _H. bacteriophora_ obtained herein are deposited in GenBank with the 28S D2-D3 rRNA region as accession MN191509 and the ITS-rRNA region as MN191510. The D2-D3 region of 28S rRNA gene sequence of the _Steinernema_ isolates matched with 100% identity that of isolates of _S. carpocapsae_ from different areas of the world (e.g., Colombia, Iran, Switzerland, and Mexico) (Noujeim et al. 2011). It also matched strain DD-136 of _S. carpocapsae_ cited in earlier studies (Welch and Bronskill 1962). The sequence of the 28S D2-D3 rRNA region of _S. carpocapsae_ derived from this work is deposited in GenBank (accession MN503268).

Bioassay of insecticidal activity of EPNs. The pathogenicity and virulence of entomopathogenic nematodes isolated from Saltillo, Coahuila, Mexico, on _T. molitor_ larvae were determined in laboratory bioassays. Control mortality was very low (0–1%) in these tests; therefore, mortality correction was not required (Abbott 1925). When insects were exposed to each isolate at varying concentrations ranging from 25 to 200 IJs/larva, the evaluated isolates induced mortality ranging from 15.16% to 100% in a lapse of 24 to 72 h (Tables 1–6). In general, within isolates, mortality did not increase

Table 2. Mean (±SD) percentage mortality of *T. molitor* larvae caused by *S. carpocapsae* M6 isolate at 24, 48, and 72 h postexposure.*

Concentration (IJs/larva)**	24 h Postexposure	48 h Postexposure	72 h Postexposure
10	24.02 ± 14.25a	58.83 ± 17.83b	77.09 ± 19.11ab
25	35.36 + 16.20a	76.14 ± 13.61ab	87.19 ± 9.18ab
50	31.33 ± 18.15a	79.41 ± 14.94ab	95.55 ± 7.76a
100	47.52 ± 15.12a	89.66 ± 10.79a	97.00 ± 4.83a
200	36.19 ± 27.31a	59.97 ± 38.87b	65.55 ± 37.30b
Control	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c

* Within columns, means followed by different lowercase letters are significantly different as determined by Tukey's Honestly Significant Differences ($P \leq 0.05$).
** IJs, infective juveniles.

markedly when larvae were exposed to increasing EPN concentrations. The lowest percent mortality values were observed in isolates such as *Heterorhabditis* MZ9 with 15.16% *T. molitor* larval mortality at a concentration of 50 IJs/larva after 24 h, or the *Heterorhabditis* MZ6 isolate with <63% larval mortality with 200 IJs/larva after 72 h (Tables 4 and 6).

A comparison of mortality levels induced by the six isolates observed at 72 h postexposure is included in Table 7. Again, mortality induced by some isolates (e.g., M6, B7, MZ6) exhibited a weak response to increasing IJ concentrations, for example, mortality levels consistent and at levels similar to those observed after exposure to the lowest concentrations tested (Table 7). Comparisons of mortality levels induced at the same concentration among the six isolates indicated that, in general, the *Steinernema* isolates induced significantly higher mortality levels than the *Heterorhabditis* isolates.

Table 3. Mean (±SD) percentage mortality of *T. molitor* larvae caused by *S. carpocapsae* M7 isolate at 24, 48, and 72 h postexposure.*

Concentration (IJs/larva)**	24 h Postexposure	48 h Postexposure	72 h Postexposure
10	43.91 ± 12.48a	66.27 ± 15.52a	71.72 ± 13.85a
25	48.00 ± 14.05a	65.16 ± 19.33a	69.38 ± 18.25a
50	53.00 ± 18.16a	60.55 ± 23.23a	71.13 ± 22.98a
100	40.19 ± 17.29a	57.33 ± 24.93a	60.55 ± 22.78a
200	52.66 ± 25.77a	67.33 ± 29.72a	69.05 ± 30.52a
Control	0.00b	0.00b	0.00b

* Within columns, means followed by different lowercase letters are significantly different as determined by Tukey's Honestly Significant Differences ($P \leq 0.05$).
** IJs, infective juveniles.

Table 4. Mean (±SD) percentage mortality of *T. molitor* larvae caused by *H. bacteriophora* MZ9 isolate at 24, 48, and 72 h postexposure.*

Concentration (IJs/larva)**	24 h Postexposure	48 h Postexposure	72 h Postexposure
10	45.16 ± 17.95a	58.03 ± 19.28a	64.16 ± 14.79b
25	36.36 ± 19.11ab	50.02 ± 13.44b	69.94 ± 15.84b
50	15.16 ± 21.32b	57.80 ± 18.65b	73.6 ± 15.53b
100	28.52 ± 22.01ab	82.72 ± 13.87a	92.52 ± 7.03a
200	21.91 ± 15.75ab	85.94 ± 7.89a	92.41 ± 7.44a
Control	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c

* Within columns, means followed by different lowercase letters are significantly different as determined by Tukey's Honestly Significant Differences ($P \leq 0.05$).
** IJs, infective juveniles.

The mortality response observed at 72 h postexposure was used for estimating the LC_{50} of each isolate (Table 8). The lowest LC_{50} among the six isolates was with the *S. carpocapsae* M6 isolate (4.99 IJs/larva). The least virulent isolate (i.e., exhibiting the highest LC_{50}) was the *H. bacteriophora* MZ6 isolate with an LC_{50} of 45.91 IJs/larva. Although the confidence limits of intervals were broadly overlapping, the analysis indicated important differences in the LC_{50}s among the isolates. These values ranged from 4.99 to 45.91 IJs/larva, which is almost a 10-fold difference among the isolates. We saw no evidence of statistically significant higher virulence for one nematode genus over the other in these assays, although there was a very general trend for *Steinernema* isolates being more pathogenic (Tables 7 and 8).

Table 5. Mean (±SD) percentage mortality of *T. molitor* larvae caused by *H. bacteriophora* M18 isolate at 24, 48, and 72 h postexposure.*

Concentration (IJs/larva)**	24 h Postexposure	48 h Postexposure	72 h Postexposure
10	22.88 ± 12.39a	24.88 ± 15.00a	32.47 ± 15.02a
25	19.77 ± 18.46a	25.25 ± 15.66bc	30.36 ± 15.78c
50	39.49 ± 21.38a	44.94 ± 21.03bc	50.72 ± 21.80bc
100	33.36 ± 24.71a	48.50 ± 24.06b	59.19 ± 21.41bc
200	70.77 ± 13.66b	75.00 ± 13.88c	80.61 ± 13.25a
Control	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00d

* Within columns, means followed by different lowercase letters are significantly different as determined by Tukey's Honestly Significant Differences ($P \leq 0.05$).
** IJs, infective juveniles.

Table 6. Mean (±SD) percentage mortality of *T. molitor* larvae caused by *H. bacteriophora* MZ6 isolate at 24, 48, and 72 h postexposure.*

Concentration (IJs/larva)**	24 h Postexposure	48 h Postexposure	72 h Postexposure
10	40.16 ± 19.00a	51.61 ± 30.30a	56.83 ± 17.95a
25	44.33 ± 19.52a	50.66 ± 24.70a	55.02 ± 19.11a
50	42.05 ± 21.56a	62.33 ± 26.18a	70.44 ± 21.23a
100	44.55 ± 18.60a	56.11 ± 24.89a	72.02 ± 22.01a
200	42.63 ± 14.82a	53.08 ± 23.09a	62.69 ± 15.75a
Control	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b

* Within columns, means followed by different lowercase letters are significantly different as determined by Tukey's Honestly Significant Differences ($P \leq 0.05$).
** IJs, infective juveniles.

IJ production in *T. molitor* larvae. The IJ production by the different EPN isolates in *T. molitor* ranged from 2,227 to 17,320 IJs/larva (Table 9). The isolate exhibiting the greatest IJ production was the *S. carpocapsae* M5 isolate, which also was the isolate with the lowest LC_{50}.

Discussion

Labaude and Griffin (2018) indicated that laboratory screening is essential to detect EPN strains of low virulence, while noting that the factors controlling virulence have not been identified with complete certainty. Virulence and reproductive capabilities are essential traits in the measurement of the fitness of EPNs. Rolston et al. (2009) found a wide range of virulence among isolates of *Steinernema feltiae* (Filipjev) collected from a small Irish island (Bull Island). Likewise, we found a range of lethal responses of *T. molitor* larvae to the isolates we tested. The lowest LC_{50} values we observed were with the M5 (4.99 IJs/larva) and M6 (6.23 IJs/larva) isolates of *S. carpocapsae*, and the MZ9 isolate of *H. bacteriophora* yielded an LC_{50} of 11.18. These values are similar to those reported by Doucet et al. (1992) with LC_{50} values of 4 to 9 IJs/larva for *H. bacteriophora* tested against *Galleria mellonella* L. larvae.

Bedding et al. (1983) evaluated several isolates of *Heterorhabditis* and *Steinernema* against several species of soil insects and *G. mellonella* larvae. They found no trend indicating higher virulence of one genus over the other, and the virulence of nematode species and strains towards insect species was variable. In their study, no one nematode taxon or isolate was the most infective for all insect targets. On the other hand, several studies indicate higher activity of *Steinernema* than *Heterorhabditis* against specific insect species (Molyneux 1986, Tarasco et al. 2015). Our comparison of mean mortality values indicated a similar trend (Table 7). These and other results emphasize the importance of evaluating several nematode species or isolates against specific insect targets before development and deployment as biological control agents.

Regarding IJ production of IJs in *T. molitor* larvae, the *S. carpocapsae* M5 isolate produced greater numbers of IJs per larva than the other isolates tested. The M5 isolate

Table 7. Mean (±SD) percentage mortality of *T. molitor* larvae 72 h postexposure to entomopathogenic nematode (EPN) isolates collected from soils from Saltillo, Mexico.*

IJs/larva**	*S. carpocapsae* M5	*S. carpocapsae* M6	*S. carpocapsae* B7	*H. bacteriophora* MZ9	*H. bacteriophora* M18	*H. bacteriophora* MZ6
0	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa
10	83.94 ± 13.15Ab	77.09 ±19.11ABbc	71.72 ± 13.85ABb	64.16 ± 14.79ABCDb	32.47 ± 15.02Eb	56.83 ± 17.95BCDb
25	100.0 ± 0.0Ac	87.19 ± 9.18ABbc	69.38 ± 18.25BCb	69.94 ± 15.84BCb	30.36 ± 15.78Eb	55.02 ± 19.11CDb
50	100.0 ± 0.0Ac	95.55 ± 7.76ABc	71.13 ± 22.98CDb	73.6 ± 15.53BCDb	50.72 ± 21.80Dbcd	70.44 ± 21.23CDb
100	100.0 ± 0.0Ac	97.00 ± 4.83ABc	60.55 ± 22.78Db	92.52 ± 7.03ABCc	59.19 ± 21.41Dbcde	72.02 ± 22.01Cb
200	100.0 ± 0.0Ac	65.55 ± 37.30Bb	69.05 ± 30.52BCb	92.41 ± 7.44ABCc	80.61 ± 13.25ABCe	62.69 ± 15.75BCEb

* Means within rows followed by the same uppercase letter are not significantly different (Tukey's; $P \leq 0.05$); means within columns followed by the same lowercase letter are not significantly different (Tukey's; $P \leq 0.05$).
** IJs, infective juveniles.

Table 8. Median lethal concentration (LC$_{50}$) of _T. molitor_ 72 h postexposure to entomopathogenic nematode (EPN) isolates collected from soils from Saltillo, Mexico.

EPN Isolate	LC$_{50}$ (95% CL*) (IJs/larva)**	Slope	Intercept	SE	P
S. carpocapsae M5	4.993 (3.881–6.062)	3.516	2.544	0.569	0.903
S. carpocapsae M6	6.230 (0.254–152.860)	1.022	4.187	0.597	0.000
H. bacteriophora MZ9	11.184 (3.319–23.328)	1.368	3.565	4.009	0.000
S. carpocapsae B7	15.056 (1.921–117.982)	0.7529	4.113	12.218	0.000
H. bacteriophora MZ6	22.074 (0.2292–239.553)	0.8732	3.8748	35.450	0.000
H. bacteriophora M18	45.918 (25.337–94.209)	1.141	3.092	11.189	0.010

* 95% confidence interval.
** IJs, infective juveniles.

was also the most active against _T. molitor_ larvae in our study (Tables 1–8). Treviño-Cueto et al. (2021) also found that M5 was the most pathogenic among the same isolates against _A. aegypti_ larvae, and it ranked second among those isolates in IJ production in _A. aegypti_ larvae.

Prabowo et al. (2019) cultivated in vivo _S. carpocapsae_ in _T. molitor_ larvae, obtaining 3,620, 6,290, 11,300, and 15,400 IJs/larva using EPN concentrations of 100, 200, 300, and 400 IJs/larva. Our results were comparable for insect larvae exposed to 100 IJs with mean production within a range of 1,650 to 17,320 IJs/larva across isolates from both species tested. For _S. carpocapsae_, the values obtained herein were 1,650 and 17,320 IJs/larva.

Shapiro-Ilan et al. (2008) indicated that a key factor for the in vivo reproduction of EPNs is the nutritional content of the host's diet in determining the reproductive capacity and quality of the IJs produced. The numbers they reported ranged from 100,000 to 200,000 IJs per _G. mellonella_ larva. It must be mentioned that

Table 9. Mean (±SE) number of entomopathogenic nematode (EPN) infective juveniles (IJs) produced per _T. molitor_ larva following 72 h postexposure to 100 IJs/larva of EPN isolates collected from soils from Saltillo, Mexico.*

EPN Isolate	IJs/larva
S. carpocapsae M5	17,320 ± 538.88a
H. bacteriophora M18	10,980 ± 4,482.56b
H. bacteriophora MZ6	7,990 ± 2,772.82c
H. bacteriophora MZ9	5,630 ± 1,619.37c
S. carpocapsae B7	1,650 ± 90.83d

* Means followed by different lowercase letters are significantly different (Bonferroní́s test, $P \leq 0.05$).

last-instar larvae of this insect are considerably larger and heavier than last-instar *T. molitor* larvae, thus explaining in part the higher numbers of IJs obtained by Shapiro-Ilan et al. (2008).

In conclusion, our results exhibit considerable variability in virulence and reproductive potential among the isolates we evaluated. These observations indicate that there are large differences, likely caused by only partially known underlying mechanisms, among coexisting, sympatric EPN isolates and that these differences must be considered when choosing local EPN isolates for development in biological control programs.

Acknowledgments

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