SCIENTIFIC NOTE
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Susceptibility of *Spodoptera frugiperda* and *Helicoverpa gelotopoeon* (Lepidoptera: Noctuidae) to the entomopathogenic nematode *Steinernema diaprepesi* (Rhabditida: Steinernematidae) under laboratory conditions

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Spodoptera frugiperda Smith and *Helicoverpa gelotopoeon* (Dyar) are important agricultural pests of several crops. The aim of the present work was to evaluate the susceptibility of larvae of both insects to an isolate of *Steinernema diaprepesi* Nguyen & Duncan under laboratory conditions, as well as the capacity of the nematode to multiply on these lepidoterans. Larvae (n = 15) were exposed to 0 (control), 50, and 100 infective juveniles (IJs) per Petri dish. Mortality was evaluated every 24 h during 6 d, and emerging IJs were counted. Mortality of *S. frugiperda* was 93% and 100% with 50 and 100 IJs dosage, and 87% and 93% in *H. gelotopoeon*, respectively. The production of IJs was significantly different between doses ($P \leq 0.05$) for *S. frugiperda* (11 329 with 50 IJs *vs*. 27 155 with 100 IJs) but not for *H. gelotopoeon* (19 830 *vs*. 26 361, respectively). This is the first study evaluating the susceptibility of these lepidopterans to *S. diaprepesi*. These results encourage the possibility of using this nematode for biological control of both pests.

Key words: Infective juveniles, biological control.

INTRODUCTION

The family Noctuidae is the most diverse group within Lepidoptera and includes the highest number of species of agricultural importance (Specht et al., 2004). The fall armyworm *Spodoptera frugiperda* Smith is a polyphagous insect that causes losses in several crops, such as peanut, sugarcane, cotton, soybean, alfalfa and particularly corn (Casmuz et al., 2010). Its wide distribution extends from Argentina and Chile to the southeast of Canada (Ashley et al., 1989). It is the most important lepidopteran pest of maize in northern Argentina and different countries of the Neotropical region (Casmuz et al., 2010).

The bollworm, *Helicoverpa gelotopoeon* (Dyar), damages mainly pods and seeds of soybean and other legumes. It also affects cotton, onion, and sunflower, among other crops (Specht et al., 2004). It is present in Argentina, Brazil, Chile, Uruguay, and Paraguay (Pastrana, 2004; Fichetti et al., 2009). In central-western Córdoba Province (Argentina), it can cause great damage to soybean seedlings.

The use of synthetic pesticides to control Lepidoptera worldwide has contributed to the development of resistance to insecticides in several species (Bloem and Carpenter, 2001), and generated environmental pollution. Hence, alternative management strategies are currently being explored, such as: the use of viruses (Escribano et al., 1999), fungi (Lacey et al., 2001) and transgenic cultivars with toxins of *Bacillus thurigiensis* (Romeis et al., 2006). Also, entomopathogenic nematodes (EPNs) (Heterorhabditidae and Steinernematidae) are used against insect pests (Divya and Sankar, 2009). The infective juveniles (IJs) penetrate the insect hemocoel and release their symbiotic bacteria (*Xenorhabdus* spp. in Steinernematidae and *Photorhabdus* spp. in Heterorhabditidae), which multiply and generate metabolites that kill the insect and serve as source of food for nematodes (Dowds and Peters, 2002). Susceptibility of *S. frugiperda* to EPNs has been frequently reported (Molina Ochoa et al., 1996; Doucet et al., 1999; García et al., 2008; Andaló et al., 2010), but to present, no studies have evaluated the effect of *Steinernema diaprepesi* Nguyen & Duncan. Furthermore, infectivity of EPNs on *H. gelotopoeon* has still not been reported. The aim of the present work was to evaluate the susceptibility of *S. frugiperda* and *H. gelotopoeon* larvae to an *S. diaprepesi* isolate under laboratory conditions, as well as the capacity of the parasite to multiply on the pests.

MATERIALS AND METHODS

An *S. diaprepesi* isolate (strain SRC) obtained from soil samples from Santa Rosa de Calchines, Department of Garay (Santa Fe Province, Argentina) (Lax et al., 2011) was

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used. Nematodes were maintained on larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae) in the laboratory, following the technique of Kaya and Stock (1997). The IJs were collected using White (1927) traps and kept in water at 10 ± 2 ºC until use, for no longer than 21 d.

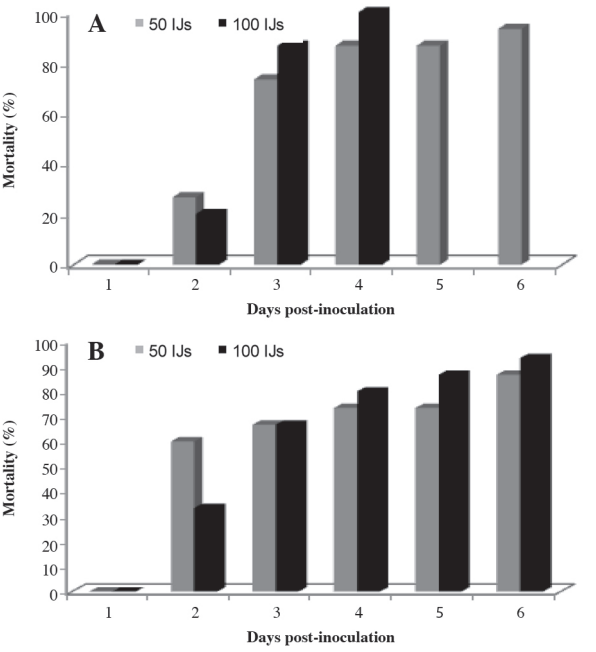
Same size last-instar of *S. frugiperda* and *H. gelotopoeon* larvae, provided by Laboratorio Líder (Córdoba Province, Argentina), were used. Infections were performed individually in Petri dishes (35 mm diameter) by applying IJs immersed in 0.5 mL water at 50 and 100 IJs per dish onto double filter paper. The same volume of water without nematodes was used as control. Each treatment contained 15 larvae; insects were maintained in the dark at 25 °C and mortality was evaluated every 24 h for 6 d. Four days after death, larvae were individually transferred to White traps and kept at 25 °C. Emerging IJs were collected the following 10 d and counted. For this, suspensions were increased to a volume of 10 mL and homogenized by bubbling; three 1-mL aliquots were taken from each suspension and placed for observation in a counting chamber under light microscope.

Insect accumulated mortality was compared between dosages in each day of observation and between species on day 6. For analysis, generalized linear models with binomial distribution and logit link function were performed with SPSS (2000). The number of IJs produced per larva was subjected to ANOVA ($P \leq 0.05$); differences between treatments were determined with a Fisher test (InfoStat, 2002).

RESULTS AND DISCUSSION

The isolate of *S. diaprepesi* used was able to infect and reproduce on both insect species. Larvae death started on day 2 in all treatments (Figure 1). At the end of the experiment, mortality of *S. frugiperda* was 93% at 50 IJ dosages, whereas the higher dosage reached 100% mortality on day 4. Mortality of *H. gelotopoeon* was 87% (50 IJs) and 93% (100 IJs). Control treatments had no mortality. No differences in mortality between insect species occurred in the 6 d analyzed or in accumulated mortality on day 6. The production of IJs was significantly different between dosages for *S. frugiperda*, but not for *H. gelotopoeon* (Table 1). No differences in IJ production occurred between insect species inoculated with the same EPN concentration.

This is the first study evaluating the susceptibility of *S. frugiperda* to *S. diaprepesi*. This insect has been previously found to be susceptible to other EPN species at different doses. In the laboratory, Andaló et al. (2010) studied the efficiency of 17 EPN populations on *S. frugiperda* at various concentrations; at the lowest dose (100 IJs), mortality reached 40-85%, with a population of *Heterorhabditis* sp. being the most efficient, not significant differences in mortality occurred with an increased dose of that EPN isolate. Molina Ochoa et al. (1996) analyzed



IJs: infective juveniles.

Figure 1. Accumulated mortality of *Spodoptera frugiperda* (A) and *Helicoverpa gelotopoeon* (B) larvae during 6 d post-inoculation with *Steinernema diaprepesi* strain SRC.

six EPN species against the same lepidopteran; at 100 IJs, the highest mortality (81%) occurred with an isolate of *Steinernema carpocapsae* (Filipjev), whereas an isolate of *Heterorhabditis bacteriophora* Poinar caused only 65% mortality.

Although there are no published studies on the effect of EPNs on *H. gelotopoeon*, other species of the genus have been studied. Mortality of *Helicoverpa armigera* (Hübner) larvae inoculated with 50 and 100 IJs of three EPN species ranged between 11% and 51% after 5 d (Kary et al., 2012). Five days post-inoculation (DPI) with IJs of *Steinernema riobravis* Poinar & Raulston at 40 and 100 IJs, Cabanillas and Raulston (1994) observed that prepupae of *Helicoverpa zea* (Boddie) exhibited similar susceptibility to that obtained herein with *H. gelotopoeon* (85% and 100%, respectively).

It can be assumed that the final EPN population varies with the number of IJs inoculated (Koppenhöfer and Kaya, 1995). Herein, differences in final population between dosages were detected in *S. frugiperda* but not in *H. gelotopoeon*. Several unknown factors affect

Table 1. Final population of *Steinernema diaprepesi* strain SRC collected 10 d after death of *Spodoptera frugiperda* and *Helicoverpa gelotopoeon* larvae (n = 15) under two infective juvenile (IJ) dosages.

Treatments (IJ dosages)	*S. frugiperda*	*H. gelotopoeon*
50	11 329 ± 7473Aa	19 830 ± 27 691Aa
100	27 155 ± 8855Ba	26 361 ± 26 926Aa

Different uppercase letter indicates significant differences between rows (dosages) and the same lower-case letter indicates non significant differences between columns (species) according to Fisher's test ($P \leq 0.05$).

nematode production *in vivo*, and these factors interact in unpredictable ways (Dolinski et al., 2007). This may help explain the high variability in the number of IJs produced per larva, especially in *H. gelotopoeon*. The amount of IJs obtained with both insects was lower than that observed in other studies with different hosts. On *Anticarsia gemmatalis* Hübner adults, Cagnolo et al. (2011) obtained 66 600 and 58 000 IJs of *Steinernema rarum* (Doucet) at 500 and 50 IJs, respectively, 10 d after insect death. As in our study, no significant differences in final IJ population occurred between dosages. At a higher dosage (200 IJs) of *Heterorhabditis baujardi* Phan, Subbotin, Nguyen & Moens on *G. mellonella*, Dolinski et al. (2007) obtained 165 000 IJs after recovering emerging nematodes for 7 d. Although these yields were higher than those observed herein, the importance of our results lie in that they confirm the capacity of the *S. diaprepesi* isolate to complete its life cycle successfully in both hosts evaluated.

The IJ production of *S. riobravis* per *H. zea* larva peaked 375 000 with 40 IJs, with a reduction observed with higher dosages (Cabanillas and Raulston, 1994). Likewise, with *G. mellonella* larvae, the final population (32 000 IJs) of *Heterorhabditis megidis* Poinar, Jackson & Klein 5 d after the start of IJ emergence was obtained by inoculating 300 IJs, and decreased with an increase in dosage (Boff et al., 2000). This lower final population with an increased IJ inoculum might be attributed to a higher intraspecific competition among nematodes (Selvan et al., 1993). In our work, this trend was not observed; however, this should be considered when the optimal dose of inoculum is estimated for a host species.

Besides, the effect of the isolate needs to be evaluated in the field, considering other variables, such as nematode capacity to find the host, influence of temperature and humidity (Rohde et al., 2010), and interaction with other pathogens (García et al., 2008). In addition, the site of the host plant where each stage of the insect life cycle occurs should be taken into account to develop an effective EPN application strategy.

CONCLUSIONS

Our results show that both lepidopterans, *Spodoptera frugiperda* and *Helicoverpa gelotopoeon*, were suitable hosts for *Steinernema diaprepesi* strain SRC. The nematode completed its life cycle and produced a high number of IJs, showing potential for biological control within an integrated pest management.

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