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Short communication

Susceptibility of adults of *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae) to the entomopathogenic nematode *Steinernema rarum* (Doucet, 1986) Mamiya, 1988 (Rhabditida: Steinernematidae) under laboratory conditions

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Soybean is attacked by a great diversity of pests that cause economically important damage from seed germination through to maturity (Aragón *et al*., 1997; Aragón, 2002). The velvetbean caterpillar *Anticarsia gemmatalis*, a foliage-feeding insect, attacks the upper part of the plant. The peak stage of pest infestation extends from flowering to the start of grain maturation (Aragón, 2002). One of the methods employed in the control of *A. gemmatalis* and other soybean pests is the use of insecticides (Aragón & Flores, 2006). Several of them are pyrethroid formulations (alfamethrine, cypermethrin, betacyfluthrin, deltamethrin, lambdacyalothrin) and they are recommended in very small doses (Gamundi & Perotti, 2008). Pyrethroids are applied during the early stages of crop development (Massaro, 2008).

Biological control agents are also used against *A. gemmatalis*. Available bio-insecticides are based on *Bacillus thuringiensis* Berliner (Aragón & Flores, 2006), Baculovirus (Moscardi, 1999) and *Saccharopolyspora spinosa* (Aragón & Vázquez, 2000). Fungi, such as *Nomuraea rileyi*, and hymenopterous and dipteran insects have been found naturally associated with *A. gemmatalis* and they are considered as important naturally occurring antagonists controlling the populations of this pest (Aragón *et al*., 1997; Avalos *et al*., 2004). Entomopathogenic nematodes (families Steinernematidae and Heterorhabditidae) are another group frequently used in the control of agricultural pests, mainly lepidopterans, coleopterans, dipterans and orthopterans (Adams & Nguyen, 2002). Laboratory assays with *Steinernema rarum* have shown the marked susceptibility of insects belonging to different orders (Doucet *et al*., 2008) as well as the great virulence of the OLI strain, compared with other isolates known for the province of Córdoba, Argentina (Cagnolo *et al*., 2004).

The susceptibility of larvae and pupae of *A. gemmatalis* to *Heterorhabditis bacteriophora* (RIV and RN strains), *S. rarum* (NOE strain), and *S. feltiae* (LCHOR strain) has been demonstrated (Doucet & Giayetto, 1994; Doucet *et al*., 1999). However, the effect of nematodes on the adult stage of *A. gemmatalis*, has still not been studied. The objectives of this work were: *i*) to evaluate the susceptibility of *A. gemmatalis* adults to *S. rarum* (OLI strain) under laboratory conditions; *ii*) to investigate the length of the nematode life cycle inside the host insect; *iii*) to quantify the production of infective juveniles (IJ) at the end of the parasitic cycle; and *iv*) to analyse possible spatio-temporal synchrony between the life cycles of the pathogen and the insect.

Steinernema rarum (OLI strain) was detected in soil samples from a soybean field in the locality of Oliva, province of Córdoba (Agüera de Doucet *et al*., 1990). This isolate is permanently maintained in culture at the Laboratory of Parasitology by *in vivo* rearing on *Galleria mellonella* larvae (Lepidoptera: Pyralidae), following conventional techniques (Kaya & Stock, 1997). IJ that had emerged from *G. mellonella* larvae no longer than 30 days prior to the experiment were used. Adults of *A. gemmatalis* were collected from the locality of Río Ceballos, province of Córdoba, in late March 2008. Infections were performed in Petri dishes (5 cm diam.) by distributing the nematode suspension on a filter paper disc and adding

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one adult of *A. gemmatalis* per dish. The nematodes were stored at 23 ± 2°C prior to use. Three nematode doses were applied: 0 (control), 50 and 500 IJ host^{-1}. Fifteen insects per dose were evaluated. Experiments were conducted at 25°C. Insect mortality was recorded every 24 h. Dead insects were transferred individually to White traps 4 days after dying and incubated at 25°C until IJ emerged (Kaya & Stock, 1997). Emerging IJ were collected from each insect for the following 10 days and stored in plastic boxes at room temperature for counting. The IJ emerging from each infected insect were counted using the volumetric dilution technique and mean value and standard deviation were calculated (InfoStat, 2004). Insect cadavers from which no IJ had emerged within 10 days of being placed in the White traps were dissected under stereomicroscope to confirm nematode presence.

Data on mortality, time between nematode exposure and death and number of IJ produced in individual larvae were analysed using ANOVA ($P > 0.05$). *A posteriori* Fisher test was used to determine differences between treatments (InfoStat, 2004).

Mortality of *A. gemmatalis* adults caused by *S. rarum* OLI was 80% at a dose of 500 IJ insect^{-1} and 33.3% at 50 IJ insect^{-1}. No mortality was recorded in the untreated control. Differences in mortality between the three doses applied were significant ($F = 17.74$; $P < 0.0001$). Host death occurred within 3 days of the start of the experiment at the 500 IJ dose, and within 4 days at the 50 IJ dose. A higher percentage of insect death was observed at day 1 of the treatments, and no dead caterpillars were detected at day 2. Differences in the time until death occurred proved to be statistically different between the three doses tested ($F = 15.39$; $P < 0.0001$) (Fig. 1). Production of new IJ was recorded at the doses (66 622 ± 35 223 and 58 150 ± 20 188 for 500 and 50 IJ, respectively).

Adults of *A. gemmatalis* have nocturnal habits; during the day, they remain on the ground near soybean plants or among leaves. They feed at night with peak feeding from sunset to dusk. The primary food source of adults is flower nectar. Eggs are laid singly mainly on the under side of leaves. Then, larvae hatch and start to feed. They go through six larval instars and drop to the ground to pupate, penetrating the soil to a depth of 2 cm until the adult emerges. Based on the life cycle characteristics of this lepidopteron and the susceptibility observed at the different stages, different management strategies involving nematodes could be implemented. Foliar application of *S. rarum* could be used to control larvae that are located on the leaf surface. A good

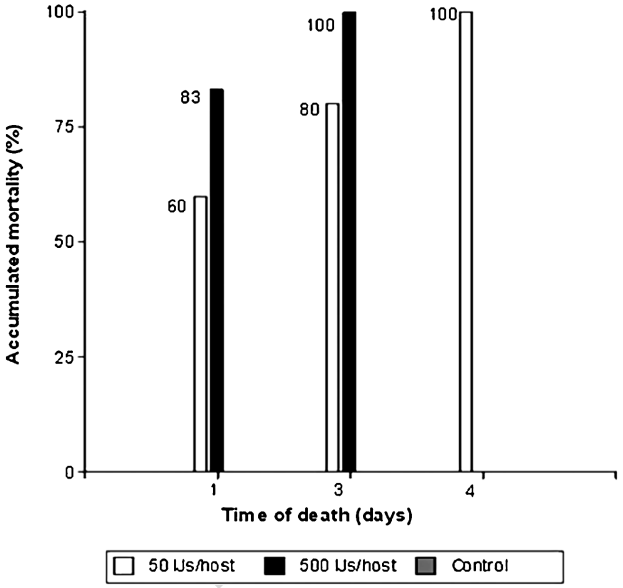

Fig. 1. *Mortality of adults of* Anticarsia gemmatalis *by* Steinernema rarum *(OLI).*

formulation might help the nematodes to survive on the plant but they also need to infect the larvae. The addition of some surfactants (*e.g.*, glycerol, oil-based antidesiccants, or non-ionic surfactants) can enhance the retention of droplets containing IJ on foliage (Wright *et al.*, 2005). Moreover, these agents could be applied to the soil to control pupae and adults. In the latter case, treatments should be performed at sunset, because with reduced evaporation nematodes would remain active for a longer period and their efficacy could be improved. As for foliar application, using antidesiccants and oil-based formulations and applying them at hours of low solar radiation would help to counterbalance conditions unfavourable for the nematode (Begley, 1990; Grewal, 2002).

It has been frequently suggested that native pests should be controlled with native enemies. This advice is based on the fact that native enemies are best adapted to local climate conditions (Bedding, 1990). Indigenous nematodes are exempted from registration in many European countries, Australia, and the USA, while in other countries they are subject to similar registration procedures as for a chemical pesticide (Hazir *et al.*, 2003). Accordingly, it should be noted that this nematode species was originally detected in soybean-cultivated soils in temperate areas and would therefore be adapted to this habitat. Studies on survival and infectivity to different temperatures of this isolate have demonstrated that both parameters are greatest

at 23 ± 2°C (Cagnolo & Campos, 2008). These aspects would be expected to enhance *S. rarum* biopesticide efficacy in the cultivated area.

Besides killing *A. gemmatalis* adults, the nematode developed inside them, *i.e*., the parasite's life cycle continued and new IJ were produced, as was reported for the isolate NOE in larvae of the same species (Doucet *et al*., 1999). The present work demonstrates that *S. rarum* can parasitise *A. gemmatalis* adults and reproduce inside them. In relation to the time until death of the larva, regardless of the dose used, the greatest percentage mortality was recorded within day 1 of exposure to the nematode. This short time until host death occurrence would be related to the high virulence shown by this isolate (Cagnolo *et al*., 2004).

The present results show that the adult of *A. gemmatalis* is a favourable host for *S. rarum* (OLI strain) since it provides the nematode with the necessary conditions to continue development and persist. Death of adults involves the interruption of the insect's life cycle, preventing the production of offspring and limiting population density. Furthermore, the emergence of a high number of IJ from the insect, naturally released to the environment, would increase the chances for the nematodes to persist in the habitat. If the capacity to persist in the environment is similar to the currently applied baculoviruses, *S. rarum* could become an equally cost effective and practical control method. Further field work is needed to improve the knowledge of this promising control agent of *A. gemmatalis*.

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