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Susceptibility of *Hoplia philanthus* (Coleoptera: Scarabaeidae) larvae and pupae to entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae)

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ABSTRACT

Biological control potential of nine entomopathogenic nematodes, *Heterorhabditis bacteriophora* CLO51 strain (HbCLO51), *H. megidis* VBM30 strain (HmVBM30), *H. indica*, *Steinernema scarabaei*, *S. feltiae*, *S. arenarium*, *S. carpocapsae* Belgian strain (ScBE), *S. glaseri* Belgian strain (SgBE) and *S. glaseri* NC strain (SgNC), was tested against second-, and third-instar larvae and pupae of *Hoplia philanthus* in laboratory and greenhouse experiments. The susceptibility of the developmental stages of *H. philanthus* differed greatly among tested nematode species/strains. In the laboratory experiments, SgBE, SgNC, HbCLO51 and HmVBM30 were highly virulent to third-instar larvae and pupae while SgBE was only virulent to second-instar larvae. Pupae were highly susceptible to HbCLO51, HmVBM30, SgBE and SgNC (57–100%) followed by *H. indica* and *S. scarabaei* (57–76%). In pot experiments, HbCLO51, SgBE and *S. scarabaei* were highly virulent to the third-instar larvae compared to the second-instar larvae. Our observations, combined with those of previous studies on other nematode and white grub species, show that nematode virulence against white grub developmental stages varies with white grub and nematode species.

1. Introduction

The white grub, *Hoplia philanthus* (Füessly) (Coleoptera: Scarabaeidae), causes significant root damage to turfgrass, pastures and ornamentals in Belgium (Ansari, 2004; Ansari et al., 2006b). This insect has a 2-year life cycle (Ansari et al., 2006b). Adults emerge from soil in early June and feed on the foliage of various ornamental plants. Most grubs reach the second-instar by the middle of September and continue feeding up to November of the first year. Before the soil surface freezes, larvae move downwards into the soil for overwintering during December–January. Most economic damage is caused in the second year, after the larvae have molted from the second to the third-instar in June. In late October and early November of the second year, third-instar larvae migrate deeper into the soil. In late March of the third year, overwintering larvae become active and move upwards to the soil surface for a brief feeding period. Minor turf damage can occur as the mature larvae soon complete their feeding to initiate pupation in May. The pupae do not form pupal cells and are found between 10 and 20 cm below the soil surface (Ansari et al., 2006b). Although several insecticides are available for larval control, their application is discouraged because of the effect on the environment and human health. In addition, the movement of these chemical insecticides through the thatch layer is quite difficult which affect their efficacy (Vlug, 1989; Ansari et al., 2006c).

The entomopathogenic nematodes (EPNs), *Heterorhabditis bacteriophora* Poinar and *Steinernema glaseri* (Steiner), occur naturally as pathogens of *H. philanthus* (Ansari et al., 2003a, 2005) and are potential alternatives to chemical insecticides. EPNs are used for the biocontrol of insect pests including white grubs with varying degrees of success (Ansari et al., 2003b; Grewal et al., 2005; Georgis et al., 2006). Species and strains of EPNs demonstrate significant differences in virulence to white grub species (Koppenhöfer et al., 2004, 2006) as well as between their developmental stages (Lee et al., 2002; Koppenhöfer and Fuzy, 2004; Ansari et al., 2006a). For example, Lee et al. (2002) reported that second-instar larvae and pupae of *Exomala orientalis* Waterhouse (Coleoptera: Scarabaeidae) were more susceptible to *Heterorhabditis* sp. Gyeongsan, *S. longicaudum* Nonsan, *S. glaseri* and *S. carpocapsae* (Weiser) compared to the third-instar larvae. Similarly, Deseö et al. (1990) found that first- and second-instars of May chafer, *Melolontha melolontha* L. (Coleoptera: Scarabaeidae) were also more susceptible to *S. glaseri* and a strain of *Heterorhabditis* than third-instar larvae. In contrast, Smits et al. (1994) reported that the susceptibility of *Phyllopertha horticola* L. (Coleoptera: Scarabaeidae) to *H. bacteriophora*, *H. heliothidis*

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Kahn, Brooks and Hirschmann, *H. megidis* Poinar, Jackson and Kelin, *Heterorhabditis* sp. NLH-E87.3 and *S. glaseri* increased with larval development. Ansari et al. (2003b) demonstrated that the third-instar larvae of *H. philanthus* were also highly susceptible to both *H. megidis* and *S. glaseri*, but the susceptibility of different life stages (second-instar and pupa) of this insect to several other EPN species has not been studied. Accordingly, we evaluated the virulence of nine EPN species/strains to second- and third-instar larvae and pupae of *H. philanthus* in the laboratory. We further provide information obtained in pot experiments conducted in a greenhouse to determine the susceptibility of different larval stages of *H. philanthus* to selected nematode species.

2. Materials and methods

2.1. Sources of nematodes

Entomopathogenic nematode species/strains and their abbreviations used in this study are listed in Table 1. All species were cultured in last instar of *Galleria mellonella* (L.) (Kaya and Stock, 1997), except *Steinernema scarabaei* Stock and Koppenhöfer, which was cultured in *H. philanthus* larvae due to its inconsistent production in *G. mellonella* (A.M. Koppenhöfer, unpublished data). Dead *G. mellonella* and *H. philanthus* larvae were placed on modified White traps (White, 1927) and emerging infective juveniles (IJs) were collected and stored in distilled water at 10 °C. Nematodes used in experiments were less than 15 days old.

2.2. White grubs

The second- and third-instars and pupae of *H. philanthus* were collected from a heavily infested private lawn in Lokeren, Belgium. No natural occurrence of EPN was detected by baiting soil samples with *G. mellonella*. As far as we are aware, these sites had not been previously treated with any insecticides. In order to remove the diseased and weak grubs from the experiments, larvae were stored individually in moist sand in 24-well plates (Falcon No. 3047, Hamburg, Germany) at 15 °C for 1 week before use. The pupae were used on the day of their collection.

2.3. Laboratory experiments

2.3.1. Second- and third-instar

The experiments were conducted in 25-ml plastic cups (4.5 cm diameter. 3 cm height; surface area: 15.9 cm^2) containing 20 g sterilized sandy loam soil (84.2% sand, 12.6% loam, 3.2% clay and 2.5% organic matter) with a carrot slice as a food source for the larvae. A single larva was released into each cup, and larvae that did not enter the soil within 2 h were replaced. The virulence of *H. bacteriophora* CLO51 strain (HbCLO51), *H. megidis* VBM30 strain (HmVBM30), *H. indica* Poinar, Karunakar and David, *S. scarabaei*, *S. feltiae* (Filipjev), *S. arenarium* (Artyukhovsky), *S. carpocapsae* BE strain (ScBE), *S. glaseri* BE strain (SgBE) and *S. glaseri* NC strain (SgNC) to larvae was determined by pipetting 200 µl of distilled water containing 50, 200, 500 or 1000 IJs onto the soil surface of each cup. Control cups received same amount of water only. The moisture content of the soil was 12%. The cups were kept in an incubator at 22 ± 1 °C in the dark. Larval mortality was assessed at weekly intervals for 4 weeks and dead larvae were dissected under the stereomicroscope to confirm that the mortality resulted from nematode infection. The dead larvae were also kept on White traps to observe nematode emergence from nematode-killed insects. Each treatment was replicated three times with 10 cups/replicate (30 cups/treatment) and repeated twice over time.

2.3.2. Pupae

The virulence of the nematode strains to pupae was determined using a protocol similar to one described above. Individual pupae were carefully transferred to cups containing moist soil with no food. Due to the lack of a dose response, only three concentrations of IJs were used against pupae. Each cup was inoculated with 200 µl of distilled water containing 200 IJs (low concentration), 400 IJs (medium concentration) or 800 IJs (high concentration) of the EPN species/strains. Because of the higher susceptibility of pupae to the EPN, mortality was assessed at weekly intervals for 2 weeks.

2.4. Pot experiments

In the laboratory experiments, strains HbCLO51 and SgBE were identified as highly virulent against second- and third-instar *H. philanthus*. Though, *S. scarabaei* was only moderately virulent against *H. philanthus* larvae, this nematode was also included in the greenhouse experiment because of its proven efficacy against other scarab larvae (Koppenhöfer et al., 2004). Pots (1.5 l and 14-cm diameter.), filled with 1.4 l of autoclaved sandy soil (see above) were seeded with perennial ryegrass (*Lolium perenne* L.) and watered every 2–3 days until the end of the experiment. The ryegrass was allowed to grow for 6–8 weeks before larvae were introduced; it was cut (3–4 cm high) at 15 day intervals to stimulate a denser root growth. Nine second- or third-instar larvae were placed on top of the grass in each pot. Larvae that did not enter the soil within 2 h were replaced with healthy ones. The air temperature in the greenhouse averaged 23.5 °C (range 18–27 °C) and soil temperature at 7 cm depth of the pots averaged 21 °C (range 18–24 °C). In all experiments, 50 ml of water containing 4000 ± 50 or 8000 ± 50 IJs (= 2.5–5.0 × 10^9 IJs/ha) was applied to each pot followed by 50 ml of water to wash nematodes into the soil. Controls received the same amount of water only. Each combination of nematode species and concentration was replicated in 12 pots and arranged in a randomized block design. Four pots were randomly selected from each combination of concentration and nematode strain at 14, 28 and 42 days after treatment (DAT), and

Table 1
Sources of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) used in this study

Nematode species/strain	Abbreviation	Source
Heterorhabditis bacteriophora CLO51	HbCLO51	Institute for Agricultural and Fisheries Research, Merelbeke, Belgium
H. megidis VBM30	HmVBM30	Institute for agricultural and Fisheries Research, Merelbeke, Belgium
H. indica	Hi	Institute for Phytopathology, Christian-Albrechts-University, Germany
Steinernema feltiae MA40	SfMA40	Institute for agricultural and Fisheries Research, Merelbeke, Belgium
S. arenarium	Sa	Institute for Phytopathology, Christian-Albrechts-University, Germany
S. scarabaei	Ss	Department of Entomology, Rutgers University, USA
S. carpocapsae Belgium	ScBE	Institute for agricultural and Fisheries Research, Merelbeke, Belgium
S. glaseri Belgium	SgBE	Institute for agricultural and Fisheries Research, Merelbeke, Belgium
S. glaseri NC	SgNC	S.E. Spiridonov, Russian Academy of Sciences, Moscow, Russia