

Effect of White Grub Developmental Stage on Susceptibility to Entomopathogenic Nematodes

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ABSTRACT The pathogenicity of the entomopathogenic nematodes *Heterorhabditis bacteriophora* Poinar and *Steinernema scarabaei* Stock & Koppenhöfer against different developmental stages of the Japanese beetle, *Popillia japonica* Newman, and the oriental beetle, *Anomala (=Exomala) orientalis* Waterhouse, were studied under laboratory conditions. The efficacy of *S. scarabaei* did not differ between second and third instars in *P. japonica* or *A. orientalis* or between small (young) and large (older) third instars in *A. orientalis*. However, *H. bacteriophora* efficacy decreased from first over second to third instar and also from small third instars to large third instars in *A. orientalis* but did not differ significantly between *P. japonica* larval stages. Once *A. orientalis* third instars had purged their intestines in preparation for pupation, no significant mortality by *S. scarabaei* and *H. bacteriophora* was observed. In contrast, *P. japonica* susceptibility to both nematode species gradually decreased from stage to stage from actively feeding third instars to pupae. In two additional experiments, we found no difference in *Steinernema glaseri* (Steiner) susceptibility between second and third instars of *A. orientalis* but an increase in *S. scarabaei* susceptibility from the second to third instar of Asiatic garden beetle, *Maladera castanea* (Arrow). Our observations combined with those of previous studies with other nematode and white grub species show that nematode efficacy against white grub developmental stages varies with white grub and nematodes species, and no generalization can be made.

KEY WORDS Scarabaeidae, *Popillia japonica*, *Anomala orientalis*, *Steinernema*, *Heterorhabditis*

A COMPLEX OF WHITE GRUB species, root-feeding larvae of scarab beetles (Coleoptera: Scarabaeidae), are the most widespread and destructive insect pests in turfgrass in the United States (Potter 1998, Vittum et al. 1999). Key species are the accidentally introduced Japanese beetle, *Popillia japonica* Newman, throughout much of the eastern states, and native masked chafers, *Cyclocephala* spp., throughout the Midwest and western states. However, in the northeastern United States and along the eastern seaboard, the European chafer, *Rhizotrogus majalis* (Razoumowsky), and the oriental beetle, *Anomala (=Exomala) orientalis* Waterhouse, have become equally important as turfgrass pests as *P. japonica* (Alm et al. 1999; A.M.K., unpublished data). All the above-mentioned species have a very similar life cycle with one generation per year. At the latitude of New Jersey and Ohio, peak flight activity occurs in late June for *A. orientalis* and in early July for *P. japonica*. After mating, the females lay eggs among the roots of host plants, and the eggs hatch in 2-3 wk. The first and second instars each last ~3 wk so that by late August, third instars start to occur and by mid-September the majority of the larvae are in the third instar (Potter 1998; Vittum et al. 1999; A.M.K., unpublished data). After

overwintering below the frost line, the third instars resume feeding until pupation in late spring. The extensive feeding activity of the larger larvae can kill large areas of grass from mid-August into mid-October, especially under warm dry conditions (Potter 1998, Vittum et al. 1999). In addition, vertebrate predators can tear up the turf to feed on the grubs (Potter 1998, Vittum et al. 1999).

Although chemical insecticides are still the primary means for white grub control, the implementation of the Food Quality Protection Act of 1996 (Anonymous 1996) resulted in the loss of many insecticides for white grub control. Entomopathogenic nematodes (*Heterorhabditidae* and *Steinernematidae*) offer an environmentally safe and integrated pest management-compatible alternative to chemical insecticides for white grub control. When applied under favorable conditions, the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar has been as effective as chemical insecticides against *P. japonica* larvae (Georgis and Gaugler 1991).

Nematode efficacy against white grubs can be affected by a variety of interacting biotic and abiotic factors. Among the biotic factors are white grub species and nematode species. Thus, *Cyclocephala* spp. and particularly *A. orientalis*, *R. majalis*, or Asiatic garden beetle, *Maladera castanea* (Arrow), larvae

seem to be less susceptible to entomopathogenic nematodes such as *H. bacteriophora* and *Steinernema glaseri* (Steiner) than *P. japonica* (Simard et al. 2001; Cappaert and Koppenhöfer 2003; Koppenhöfer and Fuzy 2003a, b; Koppenhöfer et al. 2004). However, *Steinernema scarabaei* Stock & Koppenhöfer, recently isolated from *E. orientalis* and *P. japonica* larvae in turfgrass areas in New Jersey (Stock and Koppenhöfer 2003), has shown exceptionally high pathogenicity and efficacy against *P. japonica*, *A. orientalis*, *R. majalis*, and *M. castanea* (Cappaert and Koppenhöfer 2003; Koppenhöfer and Fuzy 2003a, b; Koppenhöfer et al. 2004). In addition to interspecific variation in nematode pathogenicity, different strains of the same nematode species also can vary considerably in their pathogenicity against a given white grub species as shown for *H. bacteriophora* in *P. japonica* and *C. borealis* (Grewal et al. 2002).

Most of the research on using entomopathogenic nematodes against white grub, particularly in turfgrass, has concentrated on the third larval stage because it is the most damaging stage and is the easiest to work with. However, observations in the European cockchafer, *Melolontha melolontha* L. (Deseö et al. 1990), *Maladera matrida* Argaman (Glazer and Goldberg 1989, 1993), *Phyllopertha horticola* L. (Smits et al. 1994), and *A. orientalis* (Lee et al. 2002) have not only shown differences in nematode susceptibility among different developmental stages but also indicated that the effect may vary with white grub species and nematode species. Therefore, the objective of the current study was to clarify the effect of white grub developmental stage on susceptibility to entomopathogenic nematodes with emphasis on the white grub species *A. orientalis* and *P. japonica* and the nematode species *H. bacteriophora* and *S. scarabaei*.

Materials and Methods

Nematodes, Insects, and Soil. *S. glaseri* (NC strain) and *H. bacteriophora* (TF strain) were cultured in late instar wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), larvae (Kaya and Stock 1997). *S. scarabaei* was cultured in *P. japonica* and *E. orientalis* larvae because its production in wax moth larvae was variable. It should be noted that *S. scarabaei* pathogenicity to third instars of *E. orientalis* did not differ when produced for one rearing cycle in wax moth larvae or in scarab larvae (Koppenhöfer and Fuzy 2003). The infective juvenile (IJ) stage nematodes emerging from infected larvae were harvested from emergence traps over 7 d and stored in water at 10°C for 7-30 d (Kaya and Stock 1997). The soil used in the laboratory and greenhouse experiments was a sandy loam (69% sand, 14% silt, 17% clay, and 1% organic matter, pH 6.4) that had been pasteurized (3 h at 70°C) and stored for at least 7 d before use. The soil moisture in all experiments was 12% (-7 kPa water potential). If not mentioned otherwise, all procedures and experiments were conducted at room temperature (22-25°C).

Larvae of the various white grub species used were obtained in two ways. Second and third instars of *P. japonica*, *E. orientalis*, and *M. castanea* were collected from turf areas at the Rutgers Research Farms in Adelphia, NJ, and East Brunswick, NJ. First, second, and third instars of *E. orientalis* and *P. japonica* also were reared from eggs on perennial ryegrass, *Lolium perenne* L., in clear 4-liter plastic boxes (30 by 17 by 11 cm) filled with 6 cm of sandy loam soil that were kept at room temperature under plant growth lights. Two hundred eggs were implanted into each box, and the development of the larvae was checked periodically by removing some soil and grass. The eggs were obtained by keeping adults in clear 4-liter plastic boxes filled with 5 cm of sifted moist sandy loam. Adult *P. japonica* were trapped with standard traps (Trécé, Salinas, CA), and adult *A. orientalis* were reared from field-collected larvae. For *P. japonica*, leaves of wild grape, *Vitis* spp., were added as a food source. Eggs were collected weekly by searching through the soil in the boxes.

Prepupae, pupae, and adult *P. japonica* and *A. orientalis* were reared from third instars in 30-ml plastic cups with soil and perennial ryegrass. The third instars had been collected in October or early May and had been stored at 10°C for 1-10 wk before being transferred to room temperature. To obtain sufficient numbers of the various life stages, excessive numbers of cups were examined twice per week until enough of the various stages were present to conduct a trial.

Laboratory Experiments. The experiments were conducted in 30-ml (10-cm²) plastic cups filled with 25 g of moist sandy loam with *L. perenne* seed added as food. After seed germination, individual life stages that had been held at room temperature for at least 24 h were released into the cups. Larvae that did not enter into the soil within 2 h were replaced. Prepupae and pupae were carefully embedded into a soil cavity and covered with soil. The cups were treated 1 d later. Treatments were applied in 0.5 ml of water; controls received water only. Treatments were replicated four times with nine to 12 cups per replicate. Mortality was assessed at 7 and 14 d after treatment (DAT).

Experiment 1 compared the *H. bacteriophora* susceptibility of first and second instars of *A. orientalis* and *P. japonica*. The larvae were reared in the laboratory. Because previous studies had shown a higher *H. bacteriophora* susceptibility in third instars of *P. japonica* than in third instars of *A. orientalis* (Koppenhöfer and Fuzy 2003), *P. japonica* were exposed to 50, 100, or 200 *H. bacteriophora* and *A. orientalis* to 100, 200, and 400 *H. bacteriophora*.

Experiments 2 and 3 compared the susceptibility to *H. bacteriophora* and *S. scarabaei* of field-collected second and third instars of *P. japonica* (experiment 2) and *A. orientalis* (experiment 3). Concentrations, based on previous observations, were 100 *H. bacteriophora* IJs and 15 *S. scarabaei* for *P. japonica* and 400 *H. bacteriophora* IJs and 20 *S. scarabaei* IJs for *A. orientalis*.

Experiment 4 compared the susceptibility to *H. bacteriophora* (400 IJs per larva) and *S. scarabaei* (20 IJs per larva) of field-collected small (50-114 mg) and

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large (≥ 175 mg) third instars of *A. orientalis*. The larvae were weighed directly before being placed into the experimental cups.

Experiments 5 and 6 compared the susceptibility to *H. bacteriophora* and *S. scarabaei* of actively feeding third instars, third instars that had purged their intestines in preparation for pupation, prepupae, and pupae of *P. japonica* (experiment 5) and *A. orientalis* (experiment 6). *A. orientalis* were exposed to 100 *S. scarabaei* or 400 *H. bacteriophora*, and *P. japonica* were exposed to 400 *S. scarabaei* or 400 *H. bacteriophora*.

Experiment 7 compared the susceptibility to *S. glaseri* (400 IJs per larva) of field-collected second and third instars of *A. orientalis*. Experiment 8 compared the susceptibility to *S. scarabaei* (20 IJs per larva) of field-collected second and third instars of *M. castanea*.

Greenhouse Experiments. For the greenhouse experiments, 1-liter pots (10 by 10 by 10 cm) filled with soil to a height of 9 cm were seeded with perennial ryegrass, *Lolium perenne* L. and watered every 3 d until the end of the experiment. The grass was allowed to grow for 5 wk and then cut by using scissors before introduction of five larvae per pot. The larvae were placed on the grass 3 d before the start of the experiment. Larvae that did not enter into the soil within 24 h were replaced. The greenhouse was maintained at 28°C/18°C (day/night; photoperiod of 14:10 [L:D] h), and the soil temperature in the pots averaged 23.1 \pm 1.6°C. Treatments were applied in 50 ml of water. Controls received 50 ml of water only. There were 10 and 12 pots per treatment for third and second instar treatments, respectively. After application, pots were arranged in a completely randomized design. The number of surviving larvae was determined at 14 DAT. Both second and third instars were exposed to 0, 1.25 \times 10⁹, and 2.5 \times 10⁹ *H. bacteriophora* per hectare (2.5 \times 10⁹ per hectare is 2,500 per pot).

Statistics. Experiments 1–6 were conducted twice; the remainder once. In experiments 1–6, the results in the two replications in time were combined for analysis with replication being a cofactor. Mortality data were corrected for control mortality (Abbott 1925) and arcsine square-root transformed before analysis. Analysis of variance and means separation with Tukey's test (SAS Institute 1996) were used to analyze comparisons. Comparisons with only two treatments were analyzed using *t*-test. Differences among means (\pm SE) were considered significant at $P < 0.05$.

Results

In experiment 1, comparing the *H. bacteriophora* susceptibility of first and second instars, control mortalities at 7 (14) DAT were 18% (36%) for *P. japonica* first instars, 25% (34%) for *P. japonica* second instars, 17% (26%) for *A. orientalis* first instars, and 13% (24%) for *A. orientalis* second instars. *P. japonica* mortality at 7 and 14 DAT was not affected by instar ($P \geq 0.2$); there was a significant effect of *H. bacteriophora* rate ($F \geq 9.8$; $df = 2, 45$; $P < 0.001$), but no effect of trial and no interactions among stage, trial, and rate ($P > 0.1$). *P. japonica* mortality did not differ between in-

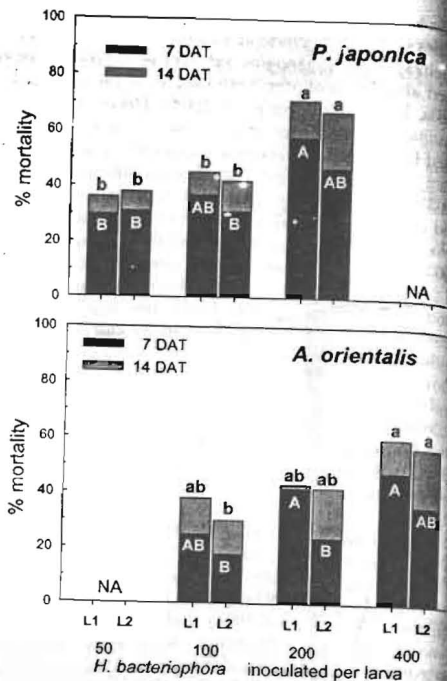


Fig. 1. Effect of treatment with three rates of *H. bacteriophora* on mortality of first (L1) and second (L2) instars of *P. japonica* and *A. orientalis* in 30-ml cups with soil and grass. Capital letters (lowercase letters) indicate significant differences in mortality at 7 DAT (14 DAT) ($P < 0.05$). NA, treatment was not included. Data shown are corrected for control mortality.

stars at any rate at 7 and 14 DAT (Fig. 1). *A. orientalis* mortality was higher in first than second instars at 7 DAT ($F = 14.4$; $df = 1, 46$; $P < 0.001$) but not at 14 DAT ($P = 0.5$). *A. orientalis* mortality was affected by *H. bacteriophora* rate at 7 and 14 DAT ($F \geq 6.7$; $df = 2, 45$; $P < 0.005$) and was affected by trial at 14 DAT ($F = 6.2$; $df = 1, 46$; $P < 0.05$), but not at 7 DAT, but there were no interactions among stage, trial, and rate at 7 and 14 DAT ($P > 0.2$). *A. orientalis* mortality was significantly higher in first instars than in second instars only at the medium *H. bacteriophora* rate at 7 DAT (Fig. 1).

In experiment 2, comparing the *H. bacteriophora* and *S. scarabaei* susceptibility of second and third instars of *P. japonica*, control mortalities at 7 (14) DAT were 5% (5%) for second instars and 5% (8%) for third instars. At 7 and 14 DAT, *P. japonica* mortality was not affected by instar ($P \geq 0.6$), 20 IJs of *S. scarabaei* caused significant higher mortality than 100 IJs of *H. bacteriophora* ($F \geq 75.7$; $df = 1, 30$; $P < 0.001$), but there was no effect of trial and no interactions among stage, trial, and rate ($P > 0.1$) (Fig. 2).

In experiment 3, comparing the *H. bacteriophora* and *S. scarabaei* susceptibility of second and third

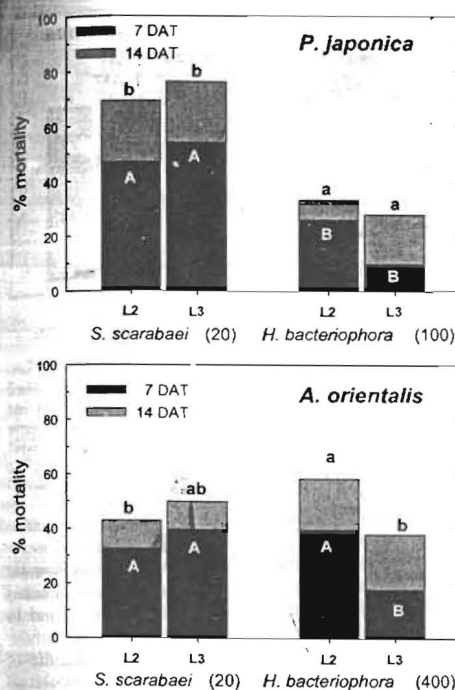


Fig. 2. Effect of treatment with *H. bacteriophora* or *S. scarabaei* on mortality of second (L2) and third (L3) instars of *P. japonica* and *A. orientalis* in 30-ml cups with soil and grass. Numbers in parentheses indicate number of infective juvenile nematodes inoculated per larva. Capital letters (lowercase letters) indicate significant differences in mortality at 7 DAT (14 DAT) ($P < 0.05$). Data shown are corrected for control mortality.

instars of *A. orientalis*, control mortalities at 7 (14) DAT were 3% (6%) for second instars and 3% (3%) for third instars. *A. orientalis* mortality was affected by instar at 7 and 14 DAT ($F \geq 4.1$; $df = 1, 62$; $P < 0.05$) and 20 IJs of *S. scarabaei* caused significantly higher mortality than 400 IJs of *H. bacteriophora* at 7 DAT ($F = 6.8$; $df = 1, 62$; $P < 0.05$) but not at 14 DAT ($P = 0.7$). There was a significant interaction of nematode treatment and instar at 7 and 14 DAT ($F \geq 15.9$; $df = 1, 62$; $P < 0.001$), but there was no effect of trial and no interactions between instar and trial and between nematode treatment and trial at 7 and 14 DAT ($P > 0.1$). At both 7 and 14 DAT, second instars were more susceptible than third instars to *H. bacteriophora* ($F \geq 4.4$; $df = 1, 56$; $P < 0.001$), but there was no instar effect for *S. scarabaei* (Fig. 2).

In experiment 4, comparing the *H. bacteriophora* and *S. scarabaei* susceptibility of small and large third instars of *A. orientalis*, control mortalities at 7 (14) DAT were 8% (15%) for small third instars and 3% (4%) for large third instars. Weights did not differ

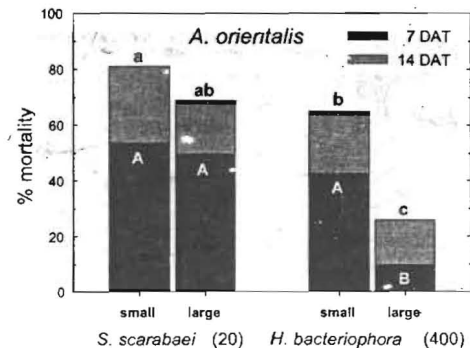


Fig. 3. Effect of treatment with *H. bacteriophora* or *S. scarabaei* on mortality of small (75–114 mg) and large (≥ 175 mg) third instars of *A. orientalis* in 30-ml cups with soil and grass. Numbers in parentheses indicate number of infective juvenile nematodes inoculated per larva. Capital letters (lowercase letters) indicate significant differences in mortality at 7 DAT (14 DAT) ($P < 0.05$). Data shown are corrected for control mortality.

among treatments within instar (small third instars: 80.5 \pm 1.0 mg, range 50–114 mg; large third instars: 222 \pm 2.2 mg, range 175–335). At 7 and 14 DAT, *A. orientalis* mortality was lower in large than in small third instars ($F \geq 20.0$; $df = 1, 30$; $P < 0.001$) and 20 IJs of *S. scarabaei* caused significant higher mortality than 400 IJs of *H. bacteriophora* ($F \geq 35.5$; $df = 1, 30$; $P < 0.001$). There was a significant interaction between nematode species and instar ($F \geq 6.3$; $df = 1, 30$; $P < 0.02$), but there was no effect of trial and no interactions between instar and trial and between nematode species and trial ($P > 0.1$). *H. bacteriophora* caused higher mortality in small than in large third instar at 7 and 14 DAT ($F \geq 43.1$; $df = 1, 14$; $P < 0.001$), but there was no size effect for *S. scarabaei* ($P = 0.58$ at 7 DAT; $P = 0.06$ at 14 DAT) (Fig. 3).

In experiment 5, comparing the *H. bacteriophora* and *S. scarabaei* susceptibility of *P. japonica* stages from actively feeding third instars through pupae, control mortalities for all stages were 3–8% at 7 DAT and 3–13% at 14 DAT. At 7 DAT, *P. japonica* mortality was affected by developmental stage ($F = 76.0$; $df = 3, 60$; $P < 0.001$) but not by nematode species or trial, and there were no interactions among trial, developmental stage, and nematode species ($P \geq 0.4$). At 14 DAT, *P. japonica* mortality was affected by stage ($F = 86.5$; $df = 3, 60$; $P < 0.001$), and, averaged across all stages, was higher for *H. bacteriophora* than *S. scarabaei* ($F = 6.6$; $df = 1, 62$; $P < 0.05$). There was a significant interaction between stage and nematode species ($F = 3.6$; $df = 3, 60$; $P < 0.05$), but no effect of trial and no interaction between trial and stage and between trial and nematode treatment ($P \geq 0.4$). At 7 and 14 DAT, mortality decreased significantly with stage for both nematode species ($F \geq 31.2$; $df = 7, 56$; $P < 0.001$), and both nematode species caused similar mortality in all stages (Fig. 4).

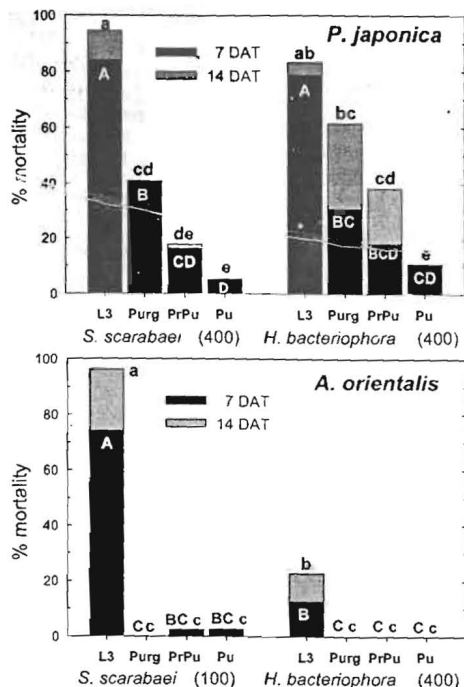


Fig. 4. Effect of treatment with *H. bacteriophora* or *S. scarabaei* on mortality of actively feeding third instars (L3), third instars with intestines purged in preparation for pupation (Purg), prepupae (PrPu), and pupae (Pu) of *P. japonica* and *A. orientalis* in 30-ml cups with soil and grass. Numbers in parentheses indicate number of infective juvenile nematodes inoculated per larva. Capital letters (lowercase letters) indicate significant differences in mortality at 7 DAT (14 DAT) ($P < 0.05$). Data shown are corrected for control mortality.

In experiment 6, comparing the *H. bacteriophora* and *S. scarabaei* susceptibility of *A. orientalis* stages from actively feeding third instars through pupae, control mortalities for all stages were 2.5–5% at 7 DAT and 2.5–10% at 14 DAT. At 7 and 14 DAT, *A. orientalis* mortality was affected by instar ($F = 63.5$; $df = 3, 60$; $P < 0.001$) and nematode species ($F \geq 38.8$; $df = 1, 62$; $P < 0.001$). On both observation dates, there was a significant interaction between instar and nematode species ($F \geq 23.8$; $df = 3, 60$; $P < 0.001$), but no effect of trial and no interaction between trial and instar or between trial and nematode species ($P > 0.5$). At 7 and 14 DAT, only third instars suffered significant mortality, and 100 *S. scarabaei* caused >4 times higher mortality than 400 *H. bacteriophora* ($F \geq 41.0$; $df = 7, 56$; $P < 0.001$) (Fig. 4). Purged third instars, prepupae, and pupae suffered <5% mortality, and not a single individual among these stages showed signs of nematode infection.

In experiment 7, comparing the *S. glaseri* susceptibility of second and third instars of *A. orientalis*, con-

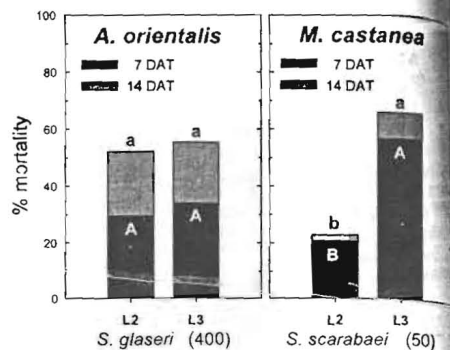


Fig. 5. Effect of treatment with *S. glaseri* against *A. orientalis* or *S. scarabaei* against *M. castanea* on second (L2) and third (L3) instars of *A. orientalis* in 1-liter pots with soil and grass. Numbers in parentheses indicate number of infective juvenile nematodes inoculated per larva. Capital letters (lowercase letters) indicate significant differences in mortality at 7 DAT (14 DAT) ($P < 0.05$). Data shown are corrected for control mortality.

control mortality at 7 (14) DAT was 8% (16%) for second instars and 5% (10%) for third instars. *S. glaseri*-caused mortality did not differ between instars at 7 and 14 DAT ($t \leq 0.27$, $df = 6$, $P \geq 0.8$) (Fig. 5).

In experiment 8, comparing the susceptibility of second and third instars of *M. castanea*, there was no control mortality, but *S. scarabaei* caused ≈ 3 times higher mortality in third instars than in second instars at 7 and 14 DAT ($t \geq 7.1$, $df = 6$, $P < 0.001$) (Fig. 5).

In the greenhouse experiment, *A. orientalis* control mortality at 14 DAT was 17% for second instars and 4% for third instars. *H. bacteriophora* caused higher mortality in second instars than in third instars ($F = 17.1$; $df = 1, 42$; $P < 0.001$), but there was no significant effect of *H. bacteriophora* rate ($P = 0.1$) and no interaction between instar and rate ($P = 0.6$). *H. bacteriophora* caused significantly higher mortality against second instars at the high rate than against third instars at both rates, with second instar mortality at the lower rate being intermediate ($F = 6.8$; $df = 3, 40$; $P < 0.001$) (Fig. 6).

Discussion

Our data show that developmental stage can have a significant effect on white grub susceptibility to entomopathogenic nematodes, but they also show that the effect varies with white grub species and nematode species. Thus, *S. scarabaei* pathogenicity did not differ between second and third instars in *P. japonica* or *A. orientalis* or between small (young) and large (older) third instars in *A. orientalis*, but it was considerably higher against third instars than second instars in *M. castanea*. In contrast, *H. bacteriophora* pathogenicity decreased from first over second to third instar and also from small third instars to large third instars in *A. orientalis*, but it did not differ sig-

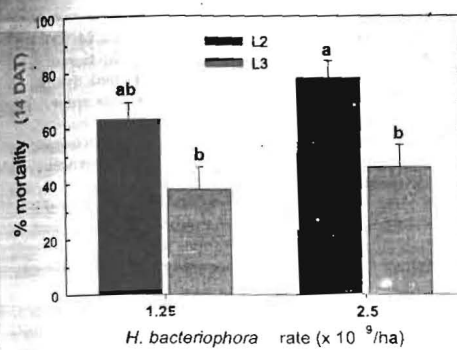


Fig. 6. Effect of treatment with two rates of *H. bacteriophora* on mortality at 14 DAT of second (L2) and third (L3) instars of *A. orientalis* in 1-liter pots with soil and grass. Numbers above bars indicate significant differences in mortality 14 DAT ($P < 0.05$). Data shown are corrected for control mortality.

nificantly among *P. japonica* larval stages. Finally, *S. glaseri* pathogenicity did not differ against second and third instars of *A. orientalis*.

Previous studies also showed effects of white grub larval stage on nematode efficacy that varied with white grub species and nematode species. Lee et al. (2002) observed higher mortality rates in second than in third instars of *A. orientalis* after exposure to *Heterorhabditis* sp. Gyeongsan, *S. carpocapsae* Weiser, *S. glaseri*, and *S. longicaudum* Shen & Wang. But, it has to be noted that the two stages were studied in separate experiments at different times. In the European cockchafer, *Melolontha melolontha* L., first and early second instars were more susceptible than third instars to *S. glaseri* and a *Heterorhabditis* sp. strain (Deseö et al. 1990). In *M. matrida* (Glazer and Golberg 1989, 1993), third instars were more susceptible to *H. bacteriophora* than first and second instars. And in *P. horticola*, instar susceptibility increased from first to third instar for *S. glaseri*, *Heterorhabditis* sp. NW-European group, and *H. bacteriophora*, but not for *S. arenarium* (= *anomali*) and *S. carpocapsae* (Smits et al. 1994).

Our observation also showed that susceptibility of *A. orientalis* and *P. japonica* to both *S. scarabaei* and *H. bacteriophora* further decreased from actively feeding third instars over third instars preparing for pupation and prepupae to pupae. In *P. japonica*, the decline in susceptibility was gradual with *H. bacteriophora* but rapid for *S. scarabaei*, especially when considering that the high mortality observed in the actively feeding third instars could have been achieved with <10% of the rate used for *S. scarabaei* (Koppenhöfer and Fuzy 2003a). In *A. orientalis*, no significant mortality by either nematode species was observed once the larvae had purged their intestines in preparation for pupation. It is possible that somewhat higher *A. orientalis* mortality would have occurred if higher but economically unfeasible nematode rates had been used. In

contrast to our observations, Lee et al. (2002) observed significant mortality of *A. orientalis* pupae after exposure to *S. longicaudum* (85%), *Heterorhabditis* sp. Gyeongsan (75%), *S. glaseri* (60%), and *S. carpocapsae* (25%) (all at 125 IJs per pupa). Similarly, Lacey et al. (2001) observed higher mortality in pupae than in third instars of *P. japonica* after exposure to *S. glaseri*. Because in all three studies similar experimental procedures were used, this difference in pupal susceptibility can only be explained by the use of different nematode species. It has to be noted that under natural conditions the earthen cells that larvae build for pupation (Potter 1996, Vittum et al. 1999) might restrict nematode access to pupae.

Differences in nematode susceptibility among developmental stages observed in various other insect groups also varied with nematode and insect species (e.g., Kaya and Hara 1980, Kaya 1985, Hudson and Nguyen 1989, LeBeck et al. 1993, Peters and Ehlers 1994, Jackson and Brooks 1995, Bélair et al. 1999, Shapiro et al. 1999). A variety of reasons are given to explain the observed differences, including size, immune response, and host behavior. For example, the typical portals of entry for nematodes may be too small in the younger stages (Jackson and Brooks 1995, Gaugler and Malloy 1981). Smaller hosts may be less attractive to nematodes because of the lesser amounts of attractants such as CO₂ or kairomones produced (Kaya 1985). However, older larvae also may become less susceptible if their immune system becomes stronger increasing their ability to eliminate invading pathogens (Watanabe 1987), or if their reduced feeding activity reduces nematode entry through the mouth, often the main portal of entry for nematodes into a host (Shapiro et al. 1999). Finally, differences in activity and location among host stage can affect their nematode susceptibility (Kaya 1985, LeBeck et al. 1993).

Mechanisms responsible for differences in nematode susceptibility among different white grub developmental stages have not been studied. Because instar susceptibility varies with white grub and nematode species, no generalization can be made. For example, because *H. bacteriophora* has been observed to penetrate in high number through the cuticle of third instars of *P. japonica* (Wang and Gaugler 1998), it is possible that the thicker cuticle in larger instars would be more difficult to penetrate for *H. bacteriophora*. But we only observed a decrease in *H. bacteriophora* susceptibility from second to third instar in *A. orientalis* and not in *P. japonica*. Smits et al. (1994) speculated that an increase in *P. horticola* susceptibility to various nematode isolates may be because the larger larvae may be more attractive to nematodes and the larger size of their natural openings would make nematode penetration easier. This would explain the increased susceptibility of *M. castanea* to *S. scarabaei*. One could argue that the lack of increase in *S. scarabaei* susceptibility observed for *P. japonica* and *A. orientalis* was related to the larger size ($\approx 50\%$) of larvae of the latter species. But then a similar effect should have been observed for *S. glaseri* in *A. orientalis* because *S. glaseri*

is ~50% larger than *S. scarabaei* (average maximum width 43 versus 31 μm ; Stock and Koppenhöfer 2003). Finally, the immune response also may differ among different larval stages.

Our study shows that no generalizations on nematode susceptibility to different white grub developmental stages are possible with the limited number of nematode-white grub combinations studied so far. Before recommendations about optimal timing of nematode application for a given white grub-nematode combination can be made, efficacy studies for that particular combination need to be conducted.

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