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# Virulence of the entomopathogenic nematodes *Heterorhabditis bacteriophora*, *Heterorhabditis zealandica*, and *Steinernema scarabaei* against five white grub species (Coleoptera: Scarabaeidae) of economic importance in turfgrass in North America

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**Abstract**

We compared the virulence of the entomopathogenic nematodes *Steinernema scarabaei*, *Heterorhabditis zealandica*, and *Heterorhabditis bacteriophora* (GPS11 and TF strains) against third instars of the Japanese beetle, *Popillia japonica*, the oriental beetle, *Anomala* (= *Exomala*) *orientalis*, the northern masked chafer, *Cyclocephala borealis*, the European chafer, *Rhizotrogus majalis*, and the Asiatic garden beetle, *Maladera castanea*, in laboratory and greenhouse experiments. The virulence of the nematode species relative to each other differed greatly among white grub species. *H. bacteriophora* and *H. zealandica* had similar modest virulence to *P. japonica*, *A. orientalis*, *C. borealis*, and *M. castanea*. But against *R. majalis*, *H. zealandica* showed low virulence with a clear concentration response whereas *H. bacteriophora* caused only erratic and very low mortality. In contrast, *S. scarabaei* had modest virulence against *C. borealis*, but was highly virulent against *R. majalis*, *P. japonica*, *A. orientalis*, and *M. castanea* with *R. majalis* being the most susceptible and *M. castanea* the least susceptible.


*Keywords:* *Anomala orientalis*; *Cyclocephala borealis*; *Maladera castanea*; *Popillia japonica*; *Rhizotrogus majalis*; Scarab; *Heterorhabditis*; *Steinernema*; Insect–parasitic nematodes; Biological control

## 1. Introduction

The root-feeding larvae of scarab beetles (Coleoptera: Scarabaeidae), also referred to as white grubs, are important pests of many agricultural and horticultural plants in the USA. The Japanese beetle, *Popillia japonica* Newman, is a major pest of turfgrass and ornamentals throughout much of the eastern states, and native masked chafers, *Cyclocephala* spp., are major pests of turfgrass and ornamentals throughout the Midwest and western states (Potter, 1998; Vittum et al., 1999). In the Northeast and along the eastern seaboard, larvae of the oriental beetle, *Anomala* (= *Exomala*) *orientalis* Waterhouse, the European chafer, *Rhizotrogus majalis* (Razoumowsky), and the Asiatic garden beetle, *Maladera castanea* (Arrow), along with the Japanese beetle have become important as turfgrass and ornamental pests (Alm et al., 1999; Koppenhöfer, unpublished data). All these species have an annual life cycle (Potter, 1998; Vittum et al., 1999). Between late spring and midsummer, adult beetles emerge, mate, and the females lay eggs in the soil among the roots of the host plants. By late summer/early fall most larvae have developed into the last of three instars. After overwintering the larvae resume feeding in spring until pupation in late spring. In turfgrass areas the extensive feeding activity of the larger larvae can kill large areas of grass especially under warm, dry conditions in late summer through mid-fall and, less commonly, in spring. In addition, vertebrate predators can tear up the

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turf to feed on the larvae even at relatively low larval densities.

Synthetic insecticides are still the primary means of managing white grubs in the USA. However, the implementation of the Food Quality Protection Act of 1996 (FQPA) (Anonymous, 1996) has led to the loss of many insecticides for curative white grub control in turfgrass. In addition, public opinion and local ordinances increasingly restrain the use of the remaining chemical products. Much of the newer chemistry (e.g., halofenozide, neonicotinoids) is used preventively over large areas. Preventive applications of these compounds are expensive, increase the chances of resistance development, and may ultimately increase dependency on chemical control. Their high efficacy against many turfgrass pests combined with their large-area applications is likely to reduce predators, parasitoids, and pathogens of white grubs and other insect pests by depriving them of prey/hosts.

Entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) have been studied extensively for the control of white grubs (Klein, 1990, 1993) and offer an environmentally safe and IPM compatible alternative to chemical insecticides for the control of white grubs. While the results of nematode field applications have been highly variable, an analysis of 82 field trials showed that nematodes can be as effective as synthetic insecticides against *P. japonica* larvae when applied under favorable conditions and using well-adapted nematode species/strains (Georgis and Gaugler, 1991). However, larvae of other white grub species including *Cyclocephala* spp., *A. orientalis*, *R. majalis*, or *M. castanea* appear to be less susceptible to the commonly used entomopathogenic nematodes (Cappaert and Koppenhöfer, 2003; Grewal et al., 2002; Koppenhöfer et al., 2000a,b, 2002, 2004; Koppenhöfer and Fuzy, 2003a,b; Shapiro-Ilan et al., 2002; Simard et al., 2001).

More recent research has focused on elucidating factors affecting nematode efficacy and the discovery and evaluation of new species and strains with improved white grub control potential. Grewal et al. (2002, 2004) showed that *Heterorhabditis bacteriophora* Poinar (GPS11 strain) and *Heterorhabditis zealandica* Poinar (X1 strain) were superior to numerous other *H. bacteriophora* strains and other nematode species in virulence and field efficacy against *P. japonica* and northern masked chafer, *Cyclocephala borealis* Arrow, larvae. The recently discovered *Steinernema scarabaei* Stock & Koppenhöfer has shown high virulence and excellent field efficacy against several white grub species (Cappaert and Koppenhöfer, 2003; Koppenhöfer and Fuzy, 2003a,b; Koppenhöfer et al., 2004). Our objective was to further characterize and directly compare the virulence and efficacy of these new species/strains *H. bacteriophora* Poinar (GPS11 strain), *H. zealandica* Poinar (X1 strain), and *S. scarabaei* against five important white grub species in turfgrass in the eastern USA, *P. japonica*, *A. orientalis*, *R. majalis*, *C. borealis*, and *M. castanea*.

## 2. Materials and methods

Field-collected third-instar larvae were used in all experiments. *A. orientalis*, *P. japonica*, *C. borealis*, and *M. castanea* were collected in turf areas at the Rutgers University Research Farms (Adelphia, NJ; North Brunswick, NJ). *R. majalis* were collected in turf areas at the University of New Hampshire Research Farm (Durham, NH). None of the sites had been treated with insecticides during the previous year. Larvae were kept individually at 10 °C for 1–10 weeks in a mixture of organic compost and loamy sand. *H. bacteriophora* (TF strain), *H. bacteriophora* (GPS11 strain), and *H. zealandica* (X1 strain) were cultured in last instars of the greater wax moth, *Galleria mellonella* (L.). *S. scarabaei* was maintained and cultured in *A. orientalis* and *P. japonica* larvae because its production in wax moth larvae is unreliable (Koppenhöfer and Fuzy, unpublished data). Koppenhöfer and Fuzy (2003a) showed that using scarab larvae vs. wax moth larvae as hosts did not significantly affect the virulence of *S. scarabaei* or *H. bacteriophora* to *A. orientalis* larvae. The emerging infective juveniles (IJs) were harvested over a period of 10 days from modified White traps (Kaya and Stock, 1997) and stored in tap water at 10 °C for 6–21 days and 8 –32 days for the *Heterorhabditis* spp. and *Steinernema* spp., respectively, before use. The soil used in the laboratory and greenhouse experiments was a sandy loam (61% sand, 27% silt, 12% clay, and 2.3% organic matter, pH 5.9) that had been pasteurized (3 h at 70 °C) and air-dried before use.

### 2.1. Laboratory experiment

A laboratory experiment was conducted to determine the concentration response of *S. scarabaei*, *H. zealandica*, and the GPS11 and TF strains of *H. bacteriophora*, each against *P. japonica*, *A. orientalis*, *C. borealis*, *R. majalis*, and *M. castanea*. The experiment was conducted at room temperature (22–24 °C) in 30-ml plastic cups (10 $cm^2$) filled with 25 g of moist sandy loam with perennial ryegrass, *Lolium perenne* L., growing in the soil as a food source for the larvae. After 3 days, individual larvae that had been held at room temperature for 24 h were released into the cups. Larvae that did not enter into the soil within 2 h were replaced. The cups were treated 1 day later. Treatments were applied in 0.5 ml water (final soil water potential −20 kPa = 13% w/w soil moisture). Controls received water only. There were 20 cups per treatment and the experiment was conducted twice. Larval mortality was assessed at 14 days after treatment (DAT). Nematode rate ranges and number of rates varied with the extent of already existing knowledge about the virulence of the four nematode species/strains to the five white grub species (e.g., Grewal et al., 2002; Koppenhöfer and Fuzy, 2003a,b). Thus, rates ranged from 4 to 3,200 IJs/larva, and between four and eight different rates were selected (see Fig. 1 for rates). For *M. castanea* only three rates were selected due to a limited number of larvae available.