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Susceptibility of the European Chafer (Coleoptera: Scarabaeidae) to Entomopathogenic Nematodes (Rhabditida: Steinernematidae, Heterorhabditidae)[1]

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Abstract: The European chafer *Rhizotrogus majalis* (Razoumowsky) feeds on turfgrass roots, causing major damage in the urban areas of northeastern North America. A laboratory study was conducted to determine the susceptibility of third-instar chafer larvae to four species of entomopathogenic nematodes (*Steinernema glaseri, S. feltiae, S. carpocapsae,* and *Heterorhabditis bacteriophora*). Only *S. glaseri* was virulent, and only at high numbers (LD_{50} = 294 nematodes/larva). Prolonged contact with nematodes increased host mortality. Optimal conditions for *S. glaseri* with respect to soil type and soil moisture were determined. Maximum larval mortality was obtained with 7% moisture and a 50/50 mixture of chernozem and sand. These results suggest that these nematodes currently have little potential for the biological control of the European chafer in turfgrass.

Key words: biological control, entomopathogenic nematodes, European chafer, turfgrass pest.

Several species of white grub (Coleoptera: Scarabaeidae), including the common June beetle (*Phyllophaga spp.* Harris), the European chafer (*Rhizotrogus majalis* Razoumowsky), and the Japanese beetle (*Popillia japonica* Newman), are major pests of turfgrass and ornamental plants in North America (Potter, 1998; Rochefort et al., 1999). In urban areas, turfgrass promotes their development, and damage is considerable because the grubs feed on roots up to the soil surface, depriving the grass of water and nutrients. The economic significance of white grubs has been described and quantified in recent studies (Potter, 1998).

In Quebec, Canada, lawn surveys conducted during the summers between 1996 and 1999 revealed species distribution and extent of economic losses due to white grubs (Rochefort et al., 1999). The European chafer is the most common and destructive white grub species. Its geographical distribution is currently limited to southwestern Quebec. The Japanese beetle is also found in this region and can severely damage urban lawns. The common June beetle is distributed throughout Quebec, but larval field populations are low and rarely cause notable damage in turfgrass (Simard, unpubl. data). The current principal control method for the chafer is pesticides. However, citizens are increasingly anxious to reduce the use of chemicals, especially when applied near their homes. In addition, municipal authorities are placing greater restriction on the use of these products.

Biopesticides based on a nematode-bacteria complex have shown promise as a method of controlling several turfgrass pests (Alm et al., 1992; Kard et al., 1988; Villani and Wright, 1988; Wright et al., 1988). These biopesticides have many advantages in an urban setting. Entomopathogenic nematodes are environmentally friendly and do not harm vertebrates. Nematodes kill pests quickly, and they can be introduced in a water solution using the conventional sprayers used by turf-maintenance firms. In addition, nematodes can be used concurrently with a variety of pesticides without loss of survival and virulence (Rovesti and Deseö, 1990).

Studies have shown the potential of entomopathogenic nematodes in controlling the Japanese beetle (Alm et al., 1992; Yeh and Alm, 1992; Shetlar et al., 1988; Villani and Wright, 1988; Wright et al., 1988), but little information is available on the susceptibility of the European chafer. Wright et al. (1988) found mortality of 46 to 59% in chafer larvae due to *S. glaseri, H. heliothidis,* and *Heterorhabditis* sp. at a concentration of 385 nematodes/cm^2. These results are comparable to the mortality obtained with two chemical treatments—isofenphos and chlorpyrifos.

The aim of our study was to assess the virulence of four species of entomopathogenic nematodes (*Steinernema glaseri, S. carpocapsae, S. feltiae,* and *Heterorhabditis bacteriophora*) to third-instar larvae of the European chafer. This instar causes the greatest damage to turfgrass in late summer. Based on their host foraging strategy, *Steinernema feltiae, S. glaseri,* and *H. bacteriophora* are ''cruiser'' species that actively seek out a potential host; *S. carpocapsae* is an ''ambusher,'' which remains mainly immobile and waits for a host to pass by (Campbell and Gaugler, 1993). The three *Steinernema* species each have a different associated bacterial symbiont belonging to the genus *Xenorhabdus,* while *H. bacteriophora* is associated with *Photorhabdus luminescens* (Gaugler and Kaya, 1990). All four nematode species are commercially available.

The specific objectives of the present study were to (i) determine LD_{50} values for the four nematode species and (ii) identify the optimal soil type, soil moisture content, and contact time for nematode effectiveness against the European chafer.

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Materials and Methods

In August 1998, third-instar larvae of the European chafer were harvested from a park in a residential neighborhood in Laval, Quebec (45°28′N; 73°45′O). For 3 weeks, cohorts of 100 larvae were held in containers of grass (Vert Éternel, 50% Kentucky bluegrass, 30% creeping red fescue, 20% perennial ryegrass) planted in an organic mixture. Only injury-free larvae were used for the tests.

Steinernema carpocapsae, S. feltiae, and *H. bacteriophora* were obtained commercially from Nic Inc. (Stevensville, Ontario), Microkill Inc. (Laval, Quebec), and Integrated BioControl Systems Inc. (Aurora, IN), respectively. *Steinernema glaseri* was obtained from A. M. Koppenhöfer at the University of California at Davis. All nematodes were reared on larvae of the greater wax moth (*Galleria mellonella*) using the method described by Dutky et al. (1964). Nematodes emerging from the wax moth larvae were harvested twice a week. Depending on the number of nematodes harvested, two storage methods were used. Small quantities of nematodes were harvested in a layer of distilled water at the bottom of a flat-bottomed culture flask, and large quantities were harvested in a thin sponge. Nematodes were refrigerated at 6 °C except for *S. glaseri,* which was maintained at 10 °C for a minimum of 2 weeks before use.

Twelve hours before inoculation with the nematodes, transparent plastic Solo Cup containers (30 cm^3) were prepared, unless otherwise stated, by adding three grains of rye, one third-instar chafer larva, 15 cm^3 of sand (soil texture analysis provided in following section), and then three more grains of rye. Soil moisture was adjusted to 7%, and containers were closed with an air-tight lid and placed in the dark at 23 °C.

The nematodes were introduced with a pipet at the desired concentration in 0.6 ml distilled water. The nematodes were placed on the soil surface in the center of the container. A preliminary test was made to check whether this method of introducing the nematodes would affect chafer mortality. There was no significant difference in chafer mortality among the following methods of introduction: (i) on the soil surface at the center of the container; (ii) on top of the soil with equal volumes in each of the four corners of the container; and (iii) in the middle of the volume of soil, followed by mixing with the soil (ANOVA; $F = 0.45$, $df = 11$, $P < 0.722$).

The containers were then placed in the dark in a cabinet at 23 °C for 4 days. After 4 days, the chafer larvae were removed and transferred to another Solo Cup containing a moistened cotton swab to avoid dehydration and promote nematode development. Larval mortality was recorded after 48 and 72 hours. Dead larvae were held in the dark at 23 °C for 10 days and then dissected to check for presence of nematodes.

For each experiment, the virulence of each nematode species was checked by inoculating wax moth larvae, using the same protocol as was used for the chafer larvae. In all cases, the mortalities of the wax moth larvae were between 90% and 100% at a concentration of 25 nematodes/larva/container.

Virulence: The virulence of the four nematode species was assessed on sand (soil texture analysis provided in section below) using concentrations of 0, 1,000, 2,000, and 5,000 nematodes per insect larva. For each treatment, 10 third-instar chafer larvae were used and the experiment was conducted three times. The LD_{50} was determined only for *S. glaseri* because of the low mortality rates found with the other three species during the first step. For the LD_{50}, concentrations of 25, 50, 100, 250, 500, 1,000, 2,000, and 5,000 nematodes per insect larva were used. For each treatment, 30 third-instar chafer larvae were used and the experiment was conducted three times.

Influence of soil texture, moisture content, and larval exposure time: Six soil types were tested to evaluate the virulence of *S. glaseri* on chafer larvae: sand (95% sand, 2% silt, 2% clay), sandy loam (74% sand, 10% silt, 16% clay), loam (50% sand, 37% silt, 13% clay), clay (24% sand, 33% silt, 43% clay), chernozem, and 50/50 (v/v) chernozem/sand. The soil moisture for each soil type was adjusted to the following levels, empirically referred to as good for seed bed germination: 8% in sand, 14% in sandy loam, 16% in loam, 22% in clay, 215% in chernozem, and 110% in 50/50 (v/v) chernozem/sand. Soil textural characteristics were determined by the chemistry laboratory of the Horticultural Research and Development Center (Agriculture and Agri-Food Canada at St-Jean-sur-Richelieu, Quebec). Each soil type was tested using 10 third-instar chafer larvae inoculated with 1,000 nematodes per larva. The experiment was conducted three times.

The effect of soil moisture content on the virulence of *S. glaseri* was studied in sand only because of the low larval mortality found in the other soil types tested. Four moisture contents (0, 7%, 14%, and 21%) were considered. As above, each treatment consisted of 10 individual larvae inoculated with 1,000 nematodes per larvae, the experiment was conducted three times. Five exposure times also were studied on sand at the 7% moisture content using the same procedures and design as for the soil moisture studies.

Statistical analysis: In the virulence experiment, an ANOVA procedure with nested factors (concentration), followed by a protected LSD test for differences among means, was used to differentiate the nematode species. The LD_{50} value for *S. glaseri* was determined using the Probit procedure (SAS Institute, 1987). Statistical analysis of the abiotic and biotic factors was carried out with the GLM procedure ANOVA (SAS Institute, 1987). Significant differences between treatments were identified using a protected LSD test for differences among means. Normality and homogeneity were

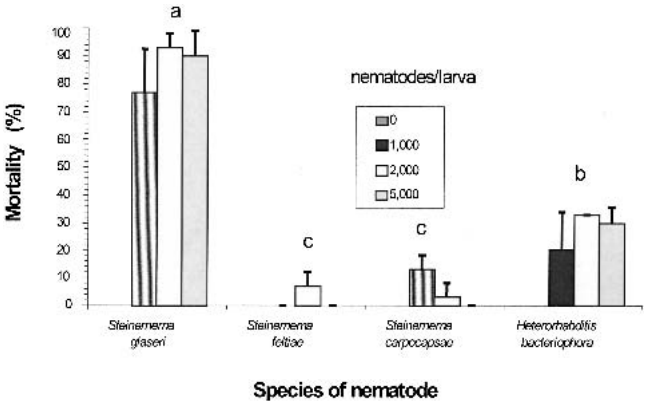

FIG. 1. Mortality of third-instar larvae of the European chafer (*Rhizotrogus majalis*) infected by four species of nematode *Steinernema glaseri, S. feltiae, S. carpocapsae,* and *Heterorhabditis bacteriophora.* The visible portion of the error bar is equal to half the standard deviation of the mean. Treatments marked with the same letter are not significantly different (ANOVA followed by LSD test; $\alpha = 0.05$).

checked by the Univariate procedure and a Residual plot, respectively (SAS Institute, 1987).

RESULTS

Of the four species tested, *S. glaseri* caused the highest mortality of third-instar European chafer larvae ($F = 240.85$, df = 3, $P < 0.001$) (Fig. 1). An LD_{50} value of 294 nematodes/larva was found, with a confidence interval ($\alpha = 0.05$) of 235 to 366 (Slope 1.07 ± 0.08; $X^2 = 194.49$; $P < 0.0001$; Intercept −2,65 ± 0.20). *Heterorhabditis bacteriophora* was significantly more virulent (LSD; $\alpha = 0.05$) than either *S. feltiae* or *S. carpocapsae,* which caused low mortalities.

The virulence of *S. glaseri* was affected by soil type ($F = 21.73$, df = 29, $P < 0.0001$) (Table 1). Mortality was highest (70%) in a 1:1 chernozem/sand mixture and lowest (7%) in clay, at a concentration of 1,000 nematodes/insect. Virulence was also affected by soil moisture in sand ($F = 4.25$, df = 11, $P < 0.0452$) (Table 2). Low virulence occurred when the moisture content was zero, and virulence was highest at moisture content of 7%. Host mortality increased with contact time (Fig. 2) and reached 90% after 5 days' exposure to *S. glaseri* ($F = 19.69$, df = 4, $P < 0.0001$).

TABLE 1. Mortality (%) of third-instar larvae of the European chafer (*Rhizotrogus majalis*) infected by the nematode *Steinernema glaseri,* as influenced by soil type.

Soil type	Mortality (%) ± SD[1]	Mortality (%) in control ± SD[1]
Chernozem/sand (1:1)	70.0 ± 3.2 a	0.0 ± 0.0e
Sand	50.0 ± 0.0 b	0.0 ± 0.0e
Chernozem	20.0 ± 0.0 c	3.3 ± 1.1e
Sandy loam	13.3 ± 2.1cd	0.0 ± 0.0e
Loam	13.3 ± 1.1cd	0.0 ± 0.0e
Clay	6.7 ± 1.1de	0.0 ± 0.0e

[1] Treatments marked with the same letter are not significantly different (ANOVA followed by LSD test; $P = 0.05$).

TABLE 2. Mortality (%) of third-instar larvae of the European chafer (*Rhizotrogus majalis*) infected by the nematode *Steinernema glaseri,* as influenced by soil moisture content in sand.

Moisture(%)	Mortality (%) ± SD[1]
0	23.3 ± 1.1 b
7	66.7 ± 1.1 a
14	43.3 ± 3.8ab
21	40.0 ± 3.7ab

[1] Treatments marked with the same letter are not significantly different (ANOVA followed by LSD test; $P = 0.05$).

DISCUSSION

Of the four nematode species tested, *S. glaseri* was the most virulent against third-instar European chafer larvae. Others have reported similar results with this host-nematode combination (Wright et al., 1988; Gerritsen et al., 1997). The ability of *S. glaseri* to infect its hosts is often attributed to its searching strategy, in that it ''cruises'' for immobile hosts such as white grubs (Thurston et al., 1994). However, in view of the restricted size of the containers used for these experiments and the relatively high concentrations of nematodes used, the searching strategy was not likely a factor in virulence because the nematodes quickly distribute themselves throughout the entire volume of soil (Gaugler and Kaya, 1990) and the grubs cannot escape nematode-infested areas (Schroeder et al., 1993). In this study, the higher mortality of chafer larvae was probably due to the greater ability of *S. glaseri* to penetrate and overcome its host's defenses (Gaugler et al., 1994; Wang et al., 1995). Recently, Wang and Gaugler (1999) suggested that the layer of protein covering *S. glaseri* allows it to counteract host immune responses for a period sufficiently long to allow the release of bacteria, which then cause the host's death through septicemia. Among other possible explanations, *S. gla-*

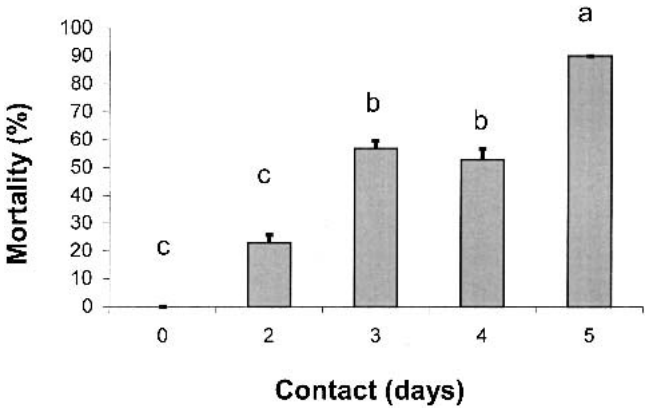

FIG. 2. Mortality of third-instar larvae of the European chafer (*Rhizotrogus majalis*) infected by the nematode *Steinernema glaseri,* by contact time. The visible portion of the error bar is equal to half the standard deviation of the mean. Contact times marked with the same letter are not significantly different (ANOVA followed by LSD test; $\alpha = 0.05$).

seri may be attracted to this host very strongly compared to the other nematode species.

Hosts are usually infected through the mouth, anus, or spiracles (Gaugler and Kaya, 1990). In the Scarabaeidae, a membrane prevents the nematodes from entering through the spiracles (Gaugler and Kaya, 1990). In a preliminary test with and without grains of rye in the experimental container, we observed that the presence of food fostered infection of third-instar European chafer larvae by *S. glaseri* (One-way ANOVA, $F = 51.57$, df = 5, $P < 0.0020$). Presumably, the act of eating facilitated entry of the nematode through the mouth. However, the presence of food might also enable the nematode and its bacterial symbiont to become established by modifying chemical conditions inside the chafer's body.

As entomopathogenic nematodes become adapted to a host, mortality levels rise. Gerritsen et al. (1997) have shown that the mean length of *S. glaseri* doubles after 8 generations on larvae of *Melolontha melolontha* (Coleoptera: Scarabaeidae) and mortality levels rise, as well. Another study (Gouge and Hague, 1995) reported similar results with *S. feltiae*. The nematode species used in our study were propagated for 5 to 6 generations on wax moths. However, had they instead been reared on European chafers, mortalities may have been higher because there would have been an opportunity for the nematodes to be better adapted to infecting the chafer larvae.

Our results concerning the impact of soil texture and moisture, and exposure time, generally confirm earlier work. *Steinernema glaseri* was more virulent to chafer larvae in sandy soil (Gaugler and Kaya, 1990). Portillo-Aguilar et al. (1999) obtained similar results with wax moths. The larger spaces in sandy soil likely make it easier for the nematode to move about and find its host, and allow oxygenation. Insufficient oxygen in the soil means that significantly fewer nematodes will be present (Gaugler and Kaya, 1990). In our study, the highest chafer mortality was found with a 1:1 chernozem/sand mixture. Presumably, a chernozem soil is more favorable to nematode survival because it reduces abrasion and injury to the cuticle during movement (Gaugler and Kaya, 1990). Low soil moisture is correlated with reduced chafer mortality because of desiccation of the majority of the nematodes (Gaugler and Kaya, 1990). Our results showed reduced chafer mortality at high moisture levels, as well. It is possible that when the soil is almost saturated, oxygen is less available and nematode movement is restricted (Gaugler and Kaya, 1990).

Our study shows that very high concentrations of entomopathogenic nematodes are necessary for acceptable levels of European chafer control. In urban settings, the diversity of soil types and soil moisture levels makes the use of nematodes even more difficult. Controlled irrigation following introduction of nematodes appears to be essential (Shetlar et al., 1988). Also, variable climatic conditions can preclude achievement of necessary contact times when nematodes are used. All these factors, including the low susceptibility of the European chafer, indicate that at this time the use of entomopathogenic nematodes will not be economically competitive with chemical methods of insect control.

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