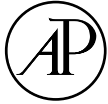

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Biological Control 24 (2002) 42–49

Biological Control

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Suppression of plant-parasitic nematodes by application of live and dead infective juveniles of an entomopathogenic nematode, *Steinernema carpocapsae*, on boxwood (*Buxus* spp.)

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Received 6 June 2001; accepted 4 December 2001

Abstract

Effects of live and dead (heat-killed) infective juveniles (IJs) of the entomopathogenic nematode *Steinernema carpocapsae* on nematodes associated with boxwood *Buxus* spp. were evaluated in field experiments during 1999 and 2000. Both living and dead IJs of *S. carpocapsae* were equally effective, causing more than 50% reduction in total populations of plant-parasitic nematodes relative to the control 15 and 30 days after treatment in both years. No significant differences were observed between reductions in populations of plant-parasitic nematodes following entomopathogenic nematode and chemical nematicide (ethoprop) treatments. In 2000, populations of *Criconemella*, *Hoplolaimus*, *Longidorus*, and *Rotylenchus* were significantly reduced in all treatments relative to the control 30 days after treatment. However, in 1999 these genera were unaffected by treatments even though total plant-parasitic nematode population was decreased. The population of *Tylenchorynchus* was significantly reduced in all treatments 30 days after treatment in 1999. Further, the population of *Tylenchus* was significantly reduced in ethoprop and dead *S. carpocapsae* treatments 15 days after treatment relative to the control whereas the population of *Aphelenchoides* was unaffected by these treatments in 1999. Live *S. carpocapsae* showed no significant effect on the population of both these genera either 15 or 30 days after treatment in 1999. No viable symbiotic bacteria were observed in the dead nematodes used in this study indicating that dead nematodes alone or dead nematodes in combination with dead symbiotic bacteria produced the suppressive effect. In contrast to the plant-parasitic nematodes, populations of non-stylet-bearing nematodes were not affected by the application of entomopathogenic nematodes. Although ethoprop reduced population of non-stylet-bearing nematodes relative to the untreated control 15 days after treatment in 2000, no significant differences were observed 30 days after treatment. These findings suggest that allelochemicals produced by dead or live nematodes and/or by their symbiotic bacteria may be selectively acting against plant-parasitic nematodes. Our results demonstrate that both live and dead infective juveniles of *S. carpocapsae* may provide a possible control strategy for plant-parasitic nematodes on boxwood. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Biological control; Entomopathogenic nematodes; Live and dead infective juveniles; Plant-parasitic nematodes; *Steinernema carpocapsae*; Boxwood

1. Introduction

Chemical nematicides are widely used to combat nematode pests. However, increasing awareness of environmental and human health concerns associated with chemical nematicides and removal of several efficacious products from the world market in recent years provide impetus for a search of environmentally compatible products for nematode management. Finding safer alternatives to chemical nematicides is especially urgent for turf and ornamentals due to their proximity to people, pets, and permanent dwellings. Although several fungal and bacterial biological control agents suppress plant-parasitic nematodes under laboratory and greenhouse conditions, they have rarely been developed as commercial products due to the difficulties in economic mass-production, formulations, and variability in field efficacy resulting from their sensitivity to environmental

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PII: S1049-9644(02)00004-X

stresses (Warrior, 2000). This provides impetus for developing biological control products based on non-living systems.

Several greenhouse studies have demonstrated suppression of plant-parasitic nematodes by application of entomopathogenic nematodes (Bird and Bird, 1986; Gouge et al., 1994; Grewal et al., 1999; Ishibashi and Choi, 1991; Ishibashi and Kondo, 1986; Lewis et al., 2001; Perry et al., 1998). Field trials in turfgrass also demonstrate potential of entomopathogenic nematodes to control plant-parasitic nematodes (Grewal et al., 1997; Smitley et al., 1992). These findings suggest the possibility of exploiting the antagonistic potential of entomopathogenic nematodes for biological control of plant-parasitic nematodes. Entomopathogenic nematodes are currently marketed worldwide for biological control of insect pests (Grewal and Georgis, 1998). Wide insect host range, high efficacy, lack of mammalian toxicity, and availability of techniques for economic mass-production have led to the rapid increase in use of these biological control agents in recent years (Grewal and Georgis, 1998; Kaya and Gaugler, 1993). Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* (Nematoda: Steinernematidae and Heterorhabditidae) are lethal insect parasites (Poinar, 1990). The infective stage juveniles (third-stage dauer juveniles) penetrate into the hemocoel of host insects through natural openings and release symbiotic bacteria (*Xenorhabdus* spp. for Steinernematidae and *Photorhabdus* spp. for Heterorhabditidae). Toxins produced by the developing nematodes (Burman, 1982) and bacteria (Akhurst and Boemere, 1990) cause septicemia and kill the insect host usually within 48 h after infection. Nematodes complete 2–3 generations inside the host. When the host cadaver is exhausted of resources, infective juveniles are produced and emerge from the cadaver in search of new hosts in soil.

The use of entomopathogenic nematodes for the control of plant-parasitic nematodes is of practical importance because they are already available as commercial products and can directly be used for nematode control, thus reducing the cost and time of product development. Recently, in a laboratory experiment, Grewal et al. (1999) observed that root penetration of plant-parasitic nematodes is suppressed by heat-killed entomopathogenic nematodes, but this finding needs to be tested under field conditions. Most field experiments evaluating the suppressive effects of entomopathogenic nematodes on plant-parasitic nematodes have focused on turfgrass and on the use of living entomopathogenic nematodes (Grewal et al., 1997; Smitley et al., 1992; Somasekhar et al., 2000). Therefore, the objective of this study was to compare the effects of live and dead *Steinernema carpocapsae* (Weiser) and a chemical nematicide ethoprop (Mocap 10G) on nematodes in the rhizosphere of *Buxus* spp. We hypothesized that application of dead entomopathogenic nematodes would be as effective as the live nematodes in suppressing the populations of plant-parasitic nematodes.

We selected boxwood because it is one of the popular woody ornamental plants grown in urban landscapes and home gardens. This plant is highly susceptible to several species of plant-parasitic nematodes (Haasis et al., 1961; Lehman, 1984). Infected plants usually decline over a period of time. Occasionally, plants may die suddenly under severe stress (Lehman, 1984). Although certain nematicides can reduce nematodes on boxwood (Benson, 1982; Haasis et al., 1961), their use is limited, mainly due to a lack of available products, bans and product withdrawals, and safety concerns of people, pets, and permanent dwellings.

2. Materials and methods

2.1. Entomopathogenic nematodes

Fresh batches of commercially produced infective juveniles of *S. carpocapsae*, all strain on sponge, were obtained from MicroBio, Cambridge, UK. Nematodes were stored at 4 °C until applied in the field. Dead infective juveniles used in this study were obtained by heating 250 ml nematode suspensions in a microwave (Panasonic Model NN-L728) oven at 1000 W for 2 min with final temperature of about 99 °C.

2.2. Design of experiment

Field experiments were conducted in 1999 (October–November) and 2000 (August–September) with 11–27 year-old Boxwood (*Buxus microphylla* L. 'Curleylocks' and *Buxus sempervirens* L. 'Asheville') plants maintained at the Secrest Arboretum, Ohio Agricultural Research and Development Center, Wooster, OH. The same plants were used in both years. The soil type is silt-loam with 53% clay, 11% silt, and 36% sand. The experiment was laid out in a completely randomized design with five replications for each treatment and each replicate consisting of two plants. The distance between the plants was 1 m. The treatments included: (i) live *S. carpocapsae*, (ii) dead *S. carpocapsae*, each applied at 2.5×10^9 infective juveniles/ha, and (iii) ethoprop (o-ethyl s, s-dipropyl phosphorodithioate) at 22.2 kg active ingredient (a.i.)/ha (recommended rate for turf and ornamental crops). Five replicates were maintained without any treatment as untreated controls. One day before application of treatments, a 1.17-m^2 area around each plant was cultivated to 15-cm depth manually with a trowel and irrigated with 10 liters of water. The following day, the appropriate numbers of live or dead nematodes in 10 liters of water were applied to 1.17-m^2 areas around each plant with a sprinkler can. Similarly,

the appropriate quantity of nematicide granules were mixed in the soil around each plant. All plants were irrigated immediately after application of the treatments. Similar cultural operations were performed on untreated control plants.

2.3. Sampling and extraction of nematodes

Soil sampling was done 1 day before application of treatments, and at 15 and 30 days after treatment. Since most of the feeding roots of boxwood plants were found to be present within 10 cm of soil depth (Larson, 1996), three 10 cm-deep × 2.5 cm-diam. soil cores were removed randomly from the treated area around each plant at each sampling time. Soil cores from each plant were mixed thoroughly to form a composite soil sample. All the soil samples were stored at existing field moisture levels at 4 °C until nematodes were extracted. Nematodes were extracted using the Baermann funnel technique from 10-g soil sub-samples taken from each composite sample (Flegg and Hooper, 1970). Nematodes were collected at 24 h intervals for 72 h and preserved in a 5% formalin solution. Total numbers of plant-parasitic, *Aphelenchoides* and *Tylenchus*, and non-stylet bearing nematodes in each sample were counted and plant-parasitic nematodes were identified to the genus level using an inverted compound microscope at 400× magnification. Data on *Aphelenchoides* and *Tylenchus* were recorded separately because of their uncertain feeding habits. Nematode population densities were expressed as nematodes/10 g of soil.

2.4. Viability of symbiotic bacteria in the heat-killed nematodes

Infective juveniles of *S. carpocapsae* were killed by heating the 250-ml nematode suspension in a microwave at 1000 W for 2 min. The homogenates of heat-killed and live infective juveniles were plated on separate MacConkey and NBTA (nutrient agar supplemented with 0.025 g bromophenol blue and 0.04 g 2,3,5-triphenyltetrazolium chloride per liter) agar plates (Kaya and Stock, 1997) and incubated at 25 °C to determine if the symbiotic bacterium *Xenorhabdus nematophilus* (Thomas and Poinar) was still alive. Homogenate of live infective juveniles was considered as a control treatment and three replicate plates were prepared for the homogenate of each heat-killed and live infective juveniles. This experiment was repeated twice and observations on bacterial growth were recorded 72 h after incubation.

2.5. Statistical analyses

Data on plant-parasitic, *Aphelenchoides* and *Tylenchus*, and non-stylet bearing nematode densities and density of dominant plant-parasitic genera were analyzed by analysis of variance (ANOVA) using General Linear Model procedure (SAS, 1998). Changes in population of both plant-parasitic and non-stylet bearing nematodes overtime (from 0 to 30 days) were compared using Tukey's mixed repeated measures analysis (SAS, 1998).

3. Results

3.1. Plant-parasitic nematodes

The population of plant-parasitic nematodes significantly decreased in all the treatments relative to the control 15 ($t = 6.5$; $df = 12, 32$; $P \leqslant 0.0001$ (1999); $t = 13.04$; $df = 12, 32$; $P \leqslant 0.0001$ (2000)) and 30 ($t = 7.93$; $df = 12, 32$; $P \leqslant 0.0001$ (1999); $t = 13.20$; $df = 12, 32$; $P \leqslant 0.0001$ (2000)) days after treatments in 1999 and 2000 (Figs. 1A and B). No differences

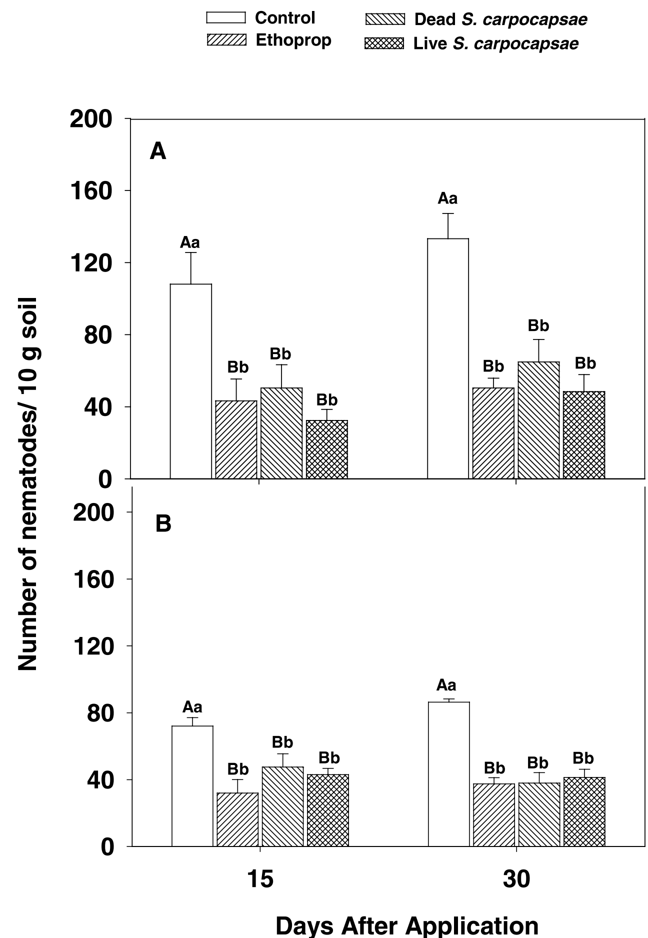

Fig. 1. Effect of live and dead *S. carpocapsae*, ethoprop (Mocap 10G) and water (control) on the total population of plant-parasitic nematodes in the rhizosphere of *Buxus* spp. in 1999 (A) and in 2000 (B). Bars (mean ± SE) in the same time interval with same lower case letter and between the time intervals with same upper case letter are not significantly different, according to Tukey's mixed repeated measures procedure ($P < 0.05$). A complete list of plant-parasitic nematode genera observed is given in Table 1.

Table 1
Mean relative abundance (%) of stylet-bearing plant-parasitic and non-plant-parasitic nematode genera in *Buxus* spp. rhizosphere prior to the treatment

Nematode genus	1999		2000	
	Mean	Range	Mean	Range
Aphelenchoides[a]	8.8	3.7–20.7	0.0	0.0–0.0
Criconemella	4.7	0.0–6.7	4.5	0.0–9.5
Helicotylenchus	15.0	10.3–18.8	30.3	27.5–32.4
Hemicycliophora	0.4	0.0–1.5	0.0	0.0–0.0
Hoplolaimus	0.9	0.0–2.9	16.9	12.3–22.1
Longidorus	0.3	0.0–1.1	0.9	0.0–2.9
Pratylenchus	9.2	0.6–16.8	3.7	1.7–5.2
Rotylenchus	41.8	33.9–50.4	35.9	29.7– 46.6
Trichodorus	1.6	0.0–3.6	2.2	0.0–5.6
Tylenchorynchus	1.6	0.0–5.5	1.6	0.0–3.8
Tylenchus[a]	15.6	2.3–27.2	0.0	0.0–0.0
Xiphinema	2.2	0.0–4.1	2.8	0.0–2.8
Unknown genera	1.9	0.0–6.2	3.1	1.1–6.7

[a] The feeding habit of these genera is uncertain as some species may feed on plants and others on fungi or both.

($P > 0.05$) in the populations of plant-parasitic nematodes were observed in live and dead *S. carpocapsae*, and ethoprop treatments either 15 or 30 days after treatments in both years (Figs. 1A and B). Also, no detectable increase in the population of plant-parasitic nematodes was observed between 15 and 30 days after application in all the treatments in 1999 and 2000 (Figs. 1A and B).

Eleven and 10 plant-parasitic nematode genera were recorded in the soil samples collected prior to the application of the treatments in 1999 and 2000, respectively (Table 1). The dominant genera found in 1999 were *Rotylenchus*, *Helicotylenchus*, and *Pratylenchus*, and in 2000 were *Rotylenchus*, *Helicotylenchus*, and *Hoplolaimus* (Table 1).

In 1999, the population of *Tylenchorynchus* was significantly ($P > 0.05$) reduced in live *S. carpocapsae* treatment and in all three treatments (dead *S. carpocapsae*, live *S. carpocapsae*, and ethoprop) relative to control 15 and 30 days after treatment, respectively. The population of *Trichodorus* was significantly decreased in control, dead *S. carpocapsae* and live *S. carpocapsae* treatments relative to ethoprop 30 days after treatment. However, the populations of other six genera including *Criconemella*, *Helicotylenchus*, *Hoplolaimus*, *Pratylenchus*, *Rotylenchus*, and *Xiphinema* were not affected by the application of dead or live *S. carpocapsae* or ethoprop 15 or 30 days after treatment (Fig. 2). Also, the population of all the plant-parasitic nematode genera except *Trichodorus* and *Tylenchorynchus* remained unchanged in all the treatments over time, i.e., from 15 to 30 days after treatment.

In 2000, populations of *Rotylenchus* [(dead *S. carpocapsae*, $t = 2.93$; $df = 12, 32$; $P = 0.006$), (live *S. carpocapsae*, $t = 2.28$; $df = 12, 32$; $P = 0.03$), (ethoprop, $t = 3.74$; $df = 12, 32$; $P = 0.0007$)] and *Xiphinema* [(dead *S. carpocapsae*, $t = 3.41$; $df = 12, 32$; $P = 0.0018$), (live *S. carpocapsae*, $t = 3.10$; $df = 12, 32$;

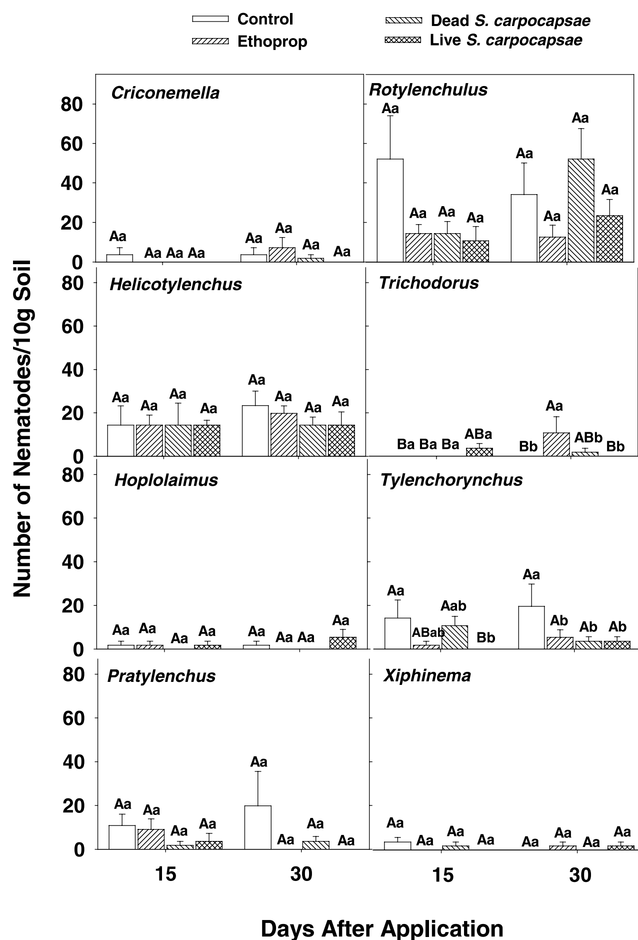

Fig. 2. Effect of live and dead *S. carpocapsae*, ethoprop (Mocap 10G) and water (control) on the populations of dominant plant-parasitic nematode genera in the rhizosphere of *Buxus* spp. in 1999. Bars (mean ± SE) in the same time interval with same lower case letter and between the time intervals with same upper case letter are not significantly different, according to Tukey's mixed repeated measure procedure ($P < 0.05$).

$P = 0.004$), (ethoprop, $t = 3.41$; $df = 12, 32$; $P = 0.0018$)] were significantly reduced in all the three treatments (dead and live *S. carpocapsae*, and ethoprop) relative to the control 15 days after treatment. However, the populations of other two genera, *Criconemella* and *Helicotylenchus* showed no differences in all the four treatments ($P > 0.05$) within 15 days of treatment (Fig. 3). Population of *Longidorus* was significantly reduced in dead *S. carpocapsae* and ethoprop treatments whereas the populations of *Hoplolaimus*, *Trichodorus*, and *Tylenchorynchus* were significantly ($P > 0.05$) reduced only in ethoprop treatment 15 days after application (Fig. 3). When considering the effects of all treatments 30 days after their application, populations of *Criconemella*, *Hoplolaimus*, *Longidorus*, and *Rotylenchus* were significantly ($P < 0.05$) reduced in all treatments relative to the control (Fig. 3). The population of *Helicotylenchus* was significantly ($P > 0.05$) reduced both in the live *S. carpocapsae* and ethoprop treatments whereas population of *Xiphinema* was significantly ($P > 0.05$) reduced only in the dead *S. carpocapsae* treatment relative to the control. Furthermore, the population of *Trichodorus* was significantly ($P > 0.05$) reduced in the entomopathogenic nematode (live and dead *S. carpocapsae*) treatments, whereas the population of *Tylenchorynchus* showed no detectable differences in all the four treatments 30 days after application (Fig. 3).

The population levels of four genera, *Helicotylenchus*, *Rotylenchus*, *Tylenchorynchus*, and *Xiphinema* remained unchanged over time (from 15 to 30 days) in all the treatments. The population levels of *Criconemella* and *Hoplolaimus* were considerably ($P > 0.05$) increased in the control 30 days after treatment as compared to 15 days after treatment (Fig. 3). However, the population

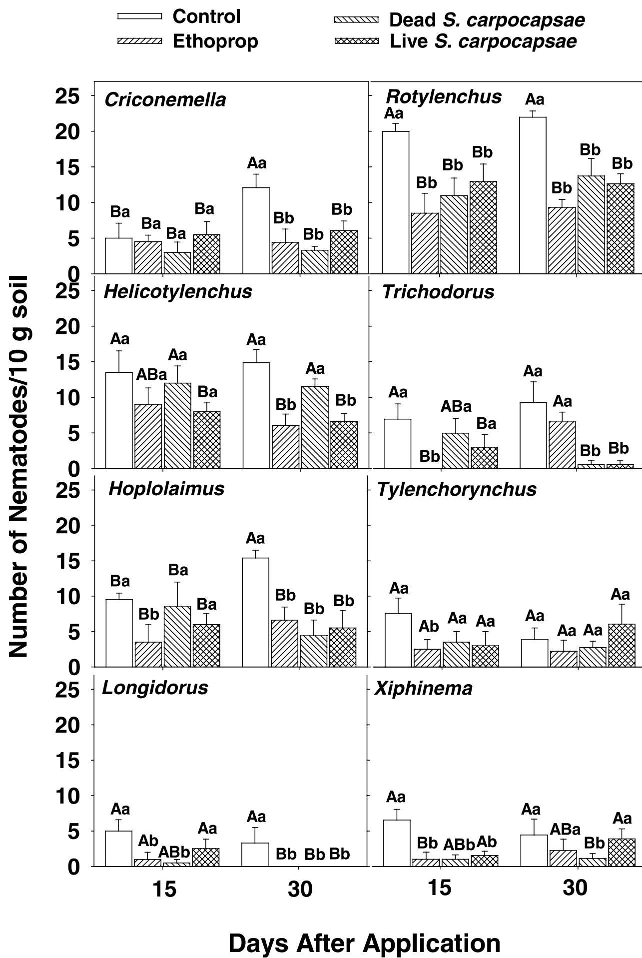

Fig. 3. Effect of live and dead *S. carpocapsae*, ethoprop (Mocap 10G) and water (control) on the population of dominant plant-parasitic nematode genera in the rhizosphere of *Buxus* spp. in 2000. Bars (mean ± SE) in the same time interval with same lower case letter and between the time intervals with same upper case letter are not significantly different, according to Tukey's mixed repeated measure procedure ($P < 0.05$).

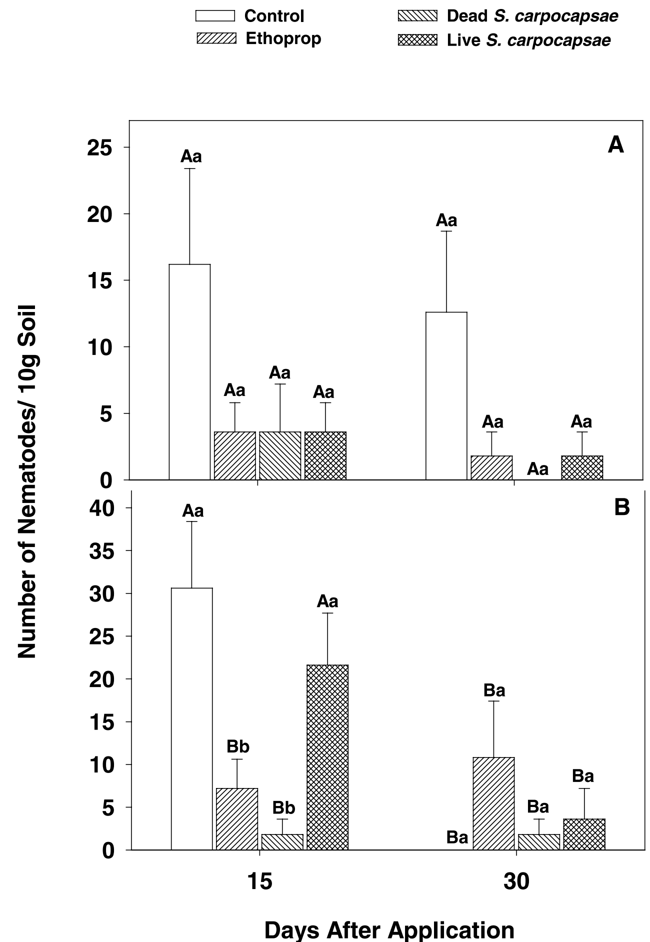

Fig. 4. Effect of live and dead *S. carpocapsae*, ethoprop (Mocap 10G) and water (control) on the population of *Aphelenchoides* (A) and *Tylenchus* (B) in the rhizosphere of *Buxus* spp. in 1999. Bars (mean ± SE) in the same time interval with same lower case letter and between the time intervals with same upper case letter are not significantly different, according to Tukey's mixed repeated measure procedure ($P < 0.05$). Both these nematode genera are grouped together because of their uncertain feeding habits.

levels of *Longidorus* and *Trichodorus* were affected by time in some of the treatments. For example, the population level of *Longidorus* was significantly lower in ethoprop, dead, and live *S. carpocapsae* treatments and the population level of *Trichodorus* was considerably higher in ethoprop treatment after 30 days of application as compared to 15 days after application.

3.2. Stylet bearing non-plant-parasitic nematodes

Two genera, *Aphelenchoides* and *Tylenchus*, were recorded from soil samples collected from the rhizospere of boxwood in 1999 but not in 2000 (Table 1). These two genera were grouped together as stylet bearing non-plant-parasitic nematodes because of their uncertain feeding habits (fungi and/or plants) (Fig. 4). Application of ethoprop ($t = 2.84$; $df = 12, 32$; $P \leqslant 0.007$) and dead *S. carpocapsae* ($t = 3.50$; $df = 12, 32$; $P \leqslant 0.001$) reduced the population of only *Tylenchus* but not of *Aphelenchoides* relative to the control treatment 15 days after treatment (Fig. 4). Live *S. carpocapsae* showed no detectable effect on the population of these nematodes either 15 or 30 days after treatment (Fig. 4). Population of *Aphelenchoides* remained unchanged in all the treatments even 30 days after treatment. However, populations of *Tylenchus* decreased significantly both in control and live *S. carpocapsae* treatments 30 days after treatment relative to the population recorded 15 days after the treatment (Fig. 4).

3.3. Non-stylet-bearing nematodes

The population of non-stylet bearing nematodes was unaffected by the application of both live and dead *S. carpocapsae* treatments 15 and 30 days after treatment both in 1999 (Fig. 5A) and 2000 (Fig. 5B). However, application of only ethoprop reduced the population of non-stylet bearing nematodes 15 days after treatment in 2000 ($t = 4.04$; $df = 12, 32$; $P \leqslant 0.0003$) but not in 1999 (Fig. 5A and B). No significant ($P > 0.05$) reduction in the population of non-stylet bearing nematodes was observed in ethoprop relative to the control 30 days after treatment in 1999 (Fig. 5A) or in 2000 (Fig. 5B). In 1999, overall population of non-stylet bearing nematodes remained unchanged whereas in 2000, except in the ethoprop treatment, the population of these nematodes significantly decreased in all the treatments 30 days after application relative to 15 days after treatment.

3.4. Viability of symbiotic bacteria in the heat-killed nematodes

No bacterial growth was observed on the MacConkey or NBTA agar plates inoculated with homogenate of heat-killed infective juveniles 72 h after incubation at 25 °C, but the bacterial colonies of *X. nematophilus* were

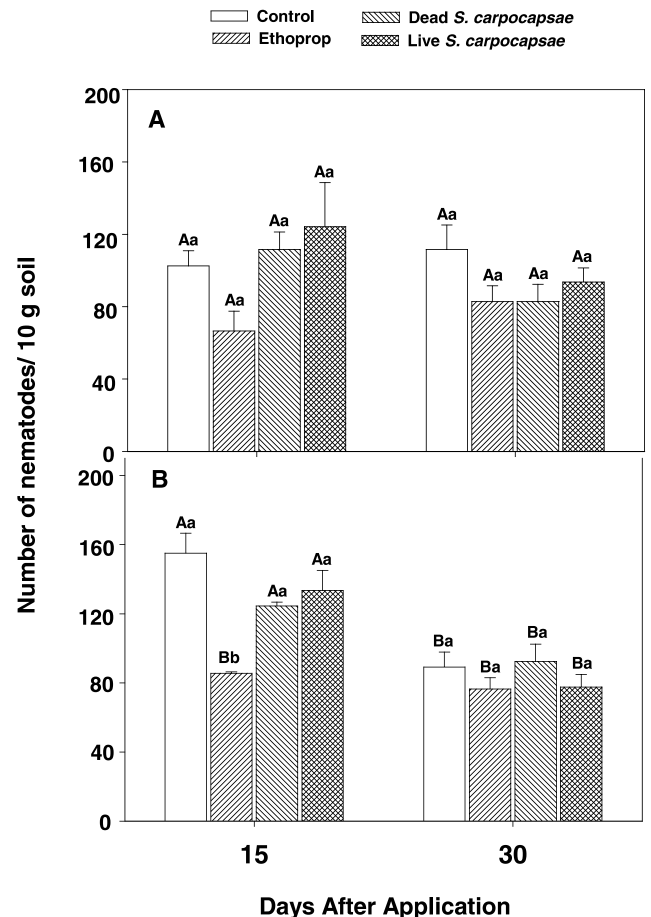

Fig. 5. Effect of live and dead *S. carpocapsae*, ethoprop (Mocap 10G) and water (control) on the total population of non-stylet bearing nematodes in 1999 (A) and 2000 (B) in the rhizosphere of *Buxus* spp. Bars (mean ± SE) in the same time interval with same lower case letter and between the time intervals with same upper case letter are not significantly different, according to Tukey's mixed repeated measure procedure ($P < 0.05$).

observed on the plates inoculated with homogenate of live infective juveniles of *S. carpocapsase*.

4. Discussion

Our results show that the application of both dead and live *S. caropocapsae* reduces total populations of plant-parasitic nematodes on boxwood. Further suppression of individual plant-parasitic nematode genera viz *Criconemella*, *Helicotylenchus*, *Hoplolaimus, Longidorus*, *Rotylenchus*, *Trichodorus*, *Tylenchorynchus*, and *Xiphinema* by dead and/or live *S. caropocapsae* indicates that its use may be expanded to other economically important crops that are affected by these nematodes. Although suppression of plant-parasitic nematodes by live entomopathogenic nematodes applied at similar or higher rates has been reported previously in several greenhouse (Bird and Bird, 1986; Gouge et al., 1994;

Grewal et al., 1999; Ishibashi and Choi, 1991; Ishibashi and Kondo, 1986; Lewis et al., 2001; Perry et al., 1998) and field studies (Grewal et al., 1997; Smitley et al., 1992; Somasekhar et al., 2000), this is the first report on suppressive effects of dead *S. caropocapsae* on plant-parasitic nematodes under field conditions.

Dead *S. caropocapsae* infective juveniles were as effective as live nematodes in controlling plant-parasitic nematodes. Grewal et al. (1999) reported that root penetration of plant-parasitic nematodes was suppressed by application of heat-killed entomopathogenic nematodes in a laboratory experiment. The results of this study demonstrate that a similar effect can be achieved under field conditions. These findings are of practical importance because the use of dead entomopathogenic nematodes may help in overcoming the difficulties in formulation, storage, and transportation associated with the use of living systems for biological control of nematodes.

The suppressive effects of entomopathogenic nematodes on plant-parasitic nematodes may be due to several factors. Bird and Bird (1986) demonstrated the attraction of *S. glaseri* (Steiner) to tomato roots and suggested that suppression of plant-parasitic nematodes by entomopathogenic nematodes may be due to the competition between the two nematode groups for space. However, this mechanism does not explain the suppression of plant-parasitic nematodes by application of dead *S. carpocapsae* observed in this study. Ishibashi and Kondo (1986) attributed the suppressive effects of entomopathogenic nematodes to the increased density of predators resulting from the application of nematode biomass to the soil. It is possible that application of live or dead *S. caropocapsae* in this study may have stimulated the growth of nematode antagonists in soil that suppressed the population of plant-parasitic nematodes. However, based on the observations of suppressing populations of plant-parasitic nematodes by entomopathogenic nematodes in sterile soil, and the suppression of root penetration of *Meloidogyne incognita* (Kafoid and White) Chitwood by heat-killed entomopathogenic nematodes, Grewal et al. (1999) suggested that behavioral response and increased natural enemies are unlikely to account for the entire effect observed in the field. More recent evidence (Grewal et al., 1999; Hu et al., 1999; Lewis et al., 2001; Samaliev et al., 2000) attributes the suppressive effects to the production of allelochemicals by the entomopathogenic nematode-symbiotic bacteria complex. Nematicidal properties of metabolites of symbiotic bacteria *Xenorhabdus* spp. associated with *Steinernema* spp. have been demonstrated in several laboratory/greenhouse studies (Grewal et al., 1999; Hu et al., 1999; Samaliev et al., 2000). Therefore, the suppressive effects of entomopathogenic nematodes observed in this and previous field studies (Grewal et al., 1997; Smitley et al., 1992; Somasekhar et al., 2000) may have been partly due to the release of allelochemicals produced either by living or dead entomopathogenic nematodes and their symbiotic bacteria. Further, the fact that the symbiotic bacteria carried by the nematodes were dead in the heat-killed nematodes used in this study indicates that the dead nematode body alone and/or its decomposition products or dead symbiotic bacteria and/or their decomposition products were able to cause the suppression of plant-parasitic nematodes.

In contrast to the plant-parasitic nematodes, populations of non-stylet-bearing nematodes remained unaffected by the entomopathogenic nematodes. Similar results were reported by Somasekhar et al. (2000) in turfgrass. These findings suggest that allelochemicals from the entomopathogenic nematode-symbiotic bacteria complex may act specifically against the plant-parasitic nematodes. This needs to be explored in future studies. Application of ethoprop significantly reduced the population of non-stylet-bearing nematodes relative to the untreated control 15 days after treatment in 2000. However, no significant differences in population of non-stylet-bearing nematodes were observed between ethoprop and the untreated control 30 days after treatment, indicating that it has a short-term suppressive effect in small plots.

Our results demonstrate that both live and dead infective juveniles of *S. carpocapsae* may provide a possible control strategy for plant-parasitic nematodes on boxwood. In this study, suppressive effects of *S. caropocapsae* were observed up to 30 days. However, the lower populations of *Aphelenchoides*, *Hemicycliophora*, *Pratylenchus*, *Rotylenchus*, and *Tylenchus* observed in 2000 as compared with 1999, suggest that suppressive effects of *S. caropocapsae* may be carried beyond 30 days. The duration of the suppressive effect and the depth at which it can influence plant-parasitic nematodes under woody plants need to be explored in future studies. This information may help in designing effective strategies for the control of plant-parasitic nematodes using entomopathogenic nematodes.

Acknowledgments

We thank Dr. Kenneth Cochran for the use of boxwood plants maintained in Secrest Arboretum, Ohio Agricultural Research and Development Center, Wooster, OH. Financial support from USDA Fund for Rural America and Ohio Agricultural Research and Development Center is acknowledged.

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