Research Article

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Designing the ideal habitat for entomopathogen use in nursery production

Anne L Nielsen* and Edwin E Lewis

Abstract

BACKGROUND: Greenhouse and nursery producers use entomopathogens (nematodes and fungi) to control soil pests. Although it is known that the physical and chemical properties of mineral soil significantly impact upon soil pathogens, the influence of soilless media used for plant production on entomopathogen performance is poorly understood.

RESULTS: Survival and foraging distance were differently affected by sand : peat, bark and sawdust media for entomopathogenic nematodes, but not for the immobile fungus *Metarhizium anisopliae*. Redwood sawdust medium consistently had a negative impact upon entomopathogenic nematodes. Dividing media into individual components supported the hypothesis that redwood sawdust reduced foraging and infection abilities of *S. riobrave* and *H. bacteriophora*. Physically altering the components by adding sand significantly improved foraging and infection success for *S. riobrave* in media not optimum for foraging.

CONCLUSION: This study is the first to highlight the importance of selecting the appropriate soilless media and pathogen species combinations to increase efficacy of biological control. *H. bacteriophora* was able to find hosts in a wider diversity of medium components than *S. riobrave*, although both nematode species performed well in peat moss and recycled plant material. These results suggest that peat moss, recycled plant material and hardwood bark are components amenable to EPN biological control programs.

Keywords: entomopathogenic nematode; *Metarhizium anisopliae*; potting soil; soilless media; below-ground pest; habitat manipulation; biological control

1 INTRODUCTION

Nursery and greenhouse production employs non-mineral planting materials known as potting media or soilless media for plant propagation and production. To manage arthropod pests, biological control agents are applied to these media in the nursery and greenhouse, but sometimes suffer from unpredictable efficacy. Owing to the easily malleable environmental conditions associated with nursery production, it may be possible to optimize biological control efforts by selecting soilless medium components and combinations known to enhance biological control agent survivorship and efficacy. The practice of conservation biological control works to maximize the impact of natural enemies by taking advantage of, or creating environmental conditions that are favorable to, biological control agents and their impacts.[1,2] Conservation biological control tactics have been successfully employed for entomopathogenic nematodes by timing applications appropriately and using application practices that favor survival and establishment.[3] Habitat manipulation takes this one step further by purposefully altering the habitat to enhance specific organisms. For example, the addition of animal manure and sand in citrus soils has been shown to increase survival and persistence of EPNs.[4] In the nursery and greenhouse industries, many operations, both small and large scale, create their own customized soilless media on site, providing the opportunity to develop mixes for anticipated pest problems.

Soilless media are composite mixtures of natural materials, such as sphagnum peat moss, coconut coir, compost, sand, bark, sawdust, vermiculite, perlite, bagasse or rice hulls, and are designed to retain and/or release water as well as provide structure for plant growth and weight. The available nutrients, percentage organic matter, pH, water-holding capacity and particle size vary greatly among soilless media and from those of mineral or composite soils.[5] Peat-based mixes were adopted by the greenhouse industry in the 1960s and are composed of peat mixed with materials to increase water retention, porosity and weight.[6,7] A sustainable alternative to peat moss, which is harvested from peat bogs, is coconut coir, the ground mesocarp of coconut (*Cocos nucifera* L.).[8] Materials such as sawdust, shredded bark or compost are often locally sourced and, like peat and coir, have good water retention capacity, can provide supplemental nutrients and are commonly the primary component in media. Components such as sand, vermiculite and perlite are typically added to media for water balance. Vermiculite is a micaceous material that can hold and release large quantities of water and minerals. Perlite is a neutral volcanic rock that can hold moisture. In addition to providing water drainage, sand is often added because it can provide structural support and weight.

Soil-dwelling stages of root weevils, scarab beetles, thrips and fungus gnats are targeted for control using entomopathogens.

* *Correspondence to: Anne L Nielsen, Rutgers University, RAREC, 121 Northville Rd., Bridgeton, NJ 08302, USA. E-mail: nielsen@aesop.rutgers.edu*

Department of Nematology, University of California, Davis, CA, USA

Two species of root weevils, *Otiorhynchus sulcatus* Fabricius and *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), are major pests of ornamental plants in nursery production. The entomopathogenic nematodes (EPNs) *Heterorhabditis bacteriophora, H. megidis, H. marelatus, H. indica* and *Steinernema riobrave* are recommended for their control, even in situations where *D. abbreviatus* is under quarantine restrictions.

Entomopathogenic nematodes in the families Heterorhabditidae (Rhabditida: Heterorhabditidae) and Steinernematidae (Rhabditida: Steinernematidae) are obligate parasites of insects that move through the water film in the pore spaces between soil particles, making moisture necessary for movement.[9] The third-stage infective juvenile (IJ) is the only stage that exists outside the host. IJs have species-specific foraging strategies ranging from 'cruiser' to 'ambusher'.[10] Cruiser nematodes move through the substrate to locate hosts and are most efficient at infecting sedentary larvae below ground. Ambusher nematodes employ a sit-and-wait strategy and are usually associated with insects that move on or near the substrate surface. Once a host is located, the IJs enter the host hemocoel via natural openings, after which they release symbiotic bacteria (*Xenorhabdus* spp. for *Steinernema* spp. and *Photorhabdus* spp. for *Heterorhabditis* spp.) causing host death usually within 48 h. Nematodes complete 2–3 generations within a host, with tens to hundreds of thousands of new IJs emerging after host resources are depleted. Their success or failure as biological control agents is heavily influenced by biological, chemical and physical characteristics of soil. This is one reason that the efficacy of EPNs can be enhanced through conservation biological control efforts in greenhouse production.

Entomopathogenic fungi in the order Hypocreales, such as *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae), are filamentous free-living soil fungi that reproduce inside insects via conidia. Conidia are found naturally in the soil and attach to the insect host cuticle where germination occurs. Once inside the host, the conidia form hyphal bodies, which produce toxic destruxins causing host death. Time to mortality is dose dependent but generally occurs within 48 h. After host death, the hyphae emerge from the cadaver and under high humidity produce conidia that are dispersed through wind or water.[11,12]

Interspecific differences in movement, dispersal and efficacy exist among nematode species and can be further impacted upon by soil characteristics. Mineral soil (natural or field soil composed of sand, silt and clay) influences efficacy of entomopathogens owing to physical characteristics of the soil environment, especially pH, moisture, particle size and salinity.[13–16] For example, acidic soils appear to limit the efficacy of EPNs,[14] whereas basic soils limit efficacy for fungi and bacteria.[16,17] Particle size and soil moisture are often related because, generally, greater particle sizes allow water to drain at quicker rates than smaller particles and are very important for entomopathogenic nematode movement. The water available to plants and animals is often measured by water potential in kilopascals (kPa), the amount of energy required to remove water from the soil. EPN efficacy is reduced in soils with either low (−1000 kPa) or excessive moisture (−1 kPa).[18,19] As water potential decreases, nematode movement becomes more difficult because the thickness of the water film surrounding the particle is diminished.

The relationship between water potential and entomopathogenic fungi and bacteria is not as clearly defined, although wet soils (in terms of gravimetric water content and matrix potential) tend negatively to influence *M. anisopliae* conidia survival and sporulation.[20–21] Two commercially available species of fungi are *M. anisopliae* and *Beauvaria bassiana* (Bals.) Viull (Hypocreales: Clavicipitaceae). While the efficacy of entomopathogenic fungi may be related to the abiotic/physical properties of the soil, there is evidence that both soil amendment and sterilization can alter efficacy and persistence through effects on available nutrients and fungistatic properties of soil.[17,20] The use of entomopathogenic fungi for control of soilborne insects is often inconsistent owing to limited spore redistribution and persistence.[22]

The conditions that influence the efficacy of entomopathogens in soil should have the same impact in soilless media. One advantage of soilless media is that they can be altered to meet grower's needs.[5] A new hypothesis of habitat specialization between EPN genera was suggested by the measurement of reduced attraction of *H. megidis* to hosts in peat soils compared with *S. carpocapsae* possibly owing to absorption of host volatiles and search strategies.[23] It is hypothesized that the primary factors impacting upon efficacy are chemistry (i.e. transmission of host volatiles), water potential and pore/particle size. The present objective was to test this hypothesis by measuring the influence of soilless media on the fate of entomopathogens commonly applied in greenhouse production. Two application methods for entomopathogens were evaluated, an aqueous drench with IJs of EPNs or conidia of *M. anisopliae*, and cadaver application for EPNs. The cadaver application employs two nematode-infected late-instar *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and has potential application in nurseries and greenhouse production systems. Studies have shown that IJs emerging from cadavers directly into soils have greater persistence and virulence than IJs applied in water.[24,25] The present authors tested the survival of pathogens under greenhouse conditions in common media and then evaluated the movement of nematodes through medium mixes to identify the influence of medium type on nematode foraging behavior. It was hypothesized that, if nematode survival or movement were reduced in any medium, it would be due either to chemical or to physical attributes of the medium components. Growers or manufacturers may utilize the results from this study to engineer the structural and chemical aspects of their medium.

2 EXPERIMENTAL METHODS

2.1 Nematodes and fungi

Steinernema riobrave (strain 355) and *Heterorhabditis bacteriophora* (Oswego strain) nematodes were cultured in last instars of the greater wax moth, *Galleria mellonella* (L.).[24] These EPN species were chosen because of their commercial availability, effectiveness against weevil pests and foraging behaviors. Infective juveniles were stored in dH_2O at 20 °C for no more than 14 days before testing. For all experiments, a block represents a temporally or spatially separate replicate of the bioassay, using different nematode stock solutions (separate *G. mellonella* infections).

Nematodes were applied as either an aqueous drench or in infected cadavers. Cadaver treatments were prepared by using a stock nematode solution, *S. riobrave* or *H. bacteriophora*, applied to an individual *T. molitor* larva at the rate of 600 IJs larva^{-1}.[25] *Tenebrio molitor* is a suitable choice for cadaver application because it is highly sclerotized (compared with *G. mellonella*) and can withstand handling. Each larva produces approximately 96 000 *H. bacteriophora* (Oswego) IJs; the IJs produced are thought to be healthier and more efficacious.[24,25] Single larvae were placed in a cell of a 24-well plate lined with filter paper. Infected *T. molitor* larvae were stored in an incubator at 25 °C for 10 days or until

IJs began emerging (as identified through white traps and visual inspection of selected individuals).

Metarhizium anisopliae, formulated as 'Tick-Ex', was donated by Novozymes (Bagsværd, Denmark) and was used for all fungus tests.

2.2 Soilless media

Potting medium mixes and individual components were obtained from commercial producers and suppliers. Cedar and pine bark mix ('bark') was developed by Monrovia in Woodlake, California; Redwood sawdust mix ('sawdust') was developed and used in Hines Nursery in Woodland, California; peat : sand mix ('peat : sand') was developed at the University of California and was obtained from the Horticultural Greenhouses at UC Davis. Redwood sawdust (without bark) was obtained from Four Winds Growers in Fremont, California. Sand, vermiculite and perlite were also obtained from the Horticultural Greenhouses at UC Davis. Mixes were exposed to 60 °C for a minimum of 10 h to kill harmful bacteria or fungi and to remove moisture.

Soilless media were analysed by the UC Soil Analytical Laboratory in Davis, California, for pH, total percentage carbon, total percentage nitrogen and organic matter. The salinity of the soilless media was analyzed as a 1 : 2 solution of medium : deionized water, which was then agitated and allowed to settle for 30 min. Salinity [measured as electroconductivity (EC) in dS m^{-1}] of the liquid was measured with a Hannah Instruments (Woonsocket, RI) HI 255 EC probe. Soil particle sizes of the three media were calculated after separation with a wet sieve shaker (WS Tyler RO-Tap model RX-29) designed to break aggregates of soil and characterize soil samples according to particle sizes. The sieve shaker uses a series of differently sized screens to trap soil particles as they are rinsed and shaken through the sieves. For each medium, samples were first run through a 2 mm sieve to isolate large materials or clumps. Samples were then dried in a forced air oven at 60 °C overnight or until completely dry. After cooling, 100 g samples were run through the sieve shaker. Particles separated by each sieve were again oven dried and then weighed to quantify the particle size composition.

Water potential was calculated (kPa) according to Kaya and Stock[26] using 55 mm diameter Whatman No. 42 filter paper (Kent, UK). To prepare media for water potential calculation, media were prepared under greenhouse conditions with half of the samples watered daily until dripping, half watered every other day and pots stored in a greenhouse (20–32 °C) under a 16 : 8 light regime.

2.3 Persistence

Entomopathogen persistence in potting media under nursery conditions was compared using a factorial experiment with medium, watering regime and pathogen treatment as factors. Watering regime was included because water is a valuable resource and a major cost of production, and water may influence the survival of the pathogen. The three potting media (bark, sawdust, peat : sand) were used to fill 1 gal pots (surface radius 7.75 cm^2). Each pot was randomly assigned an entomopathogen treatment (*H. bacteriophora* drench, *H. bacteriophora* cadaver, *S. riobrave* drench, *S. riobrave* cadaver or *M. anisopliae* drench). *Metarhizium anisopliae* was applied at 3 oz 1000 ft^{-2} (200 µL of a 10× solution applied in 50 mL of dH_2O; 8 × 10^8 spores). Nematode cadaver treatments were applied by placing two *T. molitor* cadavers that had been exposed to the appropriate EPN species ca 10 days prior to application. Drench applications of EPNs were applied at a rate of 54 IJs cm^{-2} (ca 10 206 IJs). Pots were not watered the day after treatment, after which one of three watering regimes was employed: (1) every day; (2) every other day; (3) delayed watering until 3 days after treatment, then every day thereafter. Each of the 45 factorial combinations (three media × five entomopathogen treatments × three watering regimes) was replicated 8 times and blocked in two separate greenhouses (1 — mean max 25.0/ min 18.9 °C; 2 — max 32.0/ min 20.0 °C) for a total of 720 weekly samples. Every 7 days, two soil cores (2.5 cm diameter) were taken from each pot and placed in 50 mL centrifuge tubes (Sarstedt, Nümbrecht, Germany). Each tube was baited with two *G. mellonella* larvae to indicate the presence or absence of the entomopathogen. Tubes were stored at room temperature and evaluated for the presence or absence of the entomopathogen beginning 4 days after collection until 7 days after collection. Cadavers with signs of infection were dissected to confirm the presence of nematode.

2.4 Foraging distance in soilless media

The ability of EPNs to locate their host in the various media was tested using a vertical column bioassay. PVC columns of 10.0 cm length and 5.08 cm interior diameter were divided into four 2.5 cm sections and filled with the selected soilless medium. Each medium was sieved through a 4 mm screen to remove large pieces of material and pebbles and then moistened to 15% moisture (v/v) with dH_2O. At the bottom of the column, an additional 2.0 cm ring with a <20 µm screen (Small Parts Inc., Lexington, KY) was glued to the top to prevent the entrance of IJs from the arena sections above. Three *G. mellonella* larvae were placed inside this bottom section, providing host cues to the foraging IJs so that IJ movement could be quantified. While the IJs were able to move down through the column sections to approach the hosts, the screen prevented host infection. To reduce evaporation, 60 mm petri plate lids were placed at the top and bottom of the column. One thousand IJs were added to the top of the column, and after 3 days the column sections were separated. Nematodes were extracted from each column section by washing the medium, and then the water was strained using a 43 µm mesh screen. Nematodes were then rinsed from the screen into a 50 mL beaker topped with a KimWipe (Kimberly-Clark Corp., Irving, Texas) and inverted onto a Baermann funnel to extract live nematodes. Samples were taken after 48 h and stored until counting. Each medium by nematode species combination (three media × two EPN species) was replicated 5 times and blocked temporally 2 or 3 times, with a total of 10 or 15 replicates for each treatment combination. Data did not meet assumptions of normality and were rank transformed before analysis.

2.5 Foraging distance in medium components

The individual components that make up soilless media were isolated to evaluate their impact upon nematode foraging and infection. The components were baked at 40 °C until dry; sand (Table 2) was washed 3 times to remove particles, salt and other contaminants that could negatively impact upon EPN foraging, and then baked at 100 °C until dry.[15] All materials were stored at 10 °C until use. The physical structure of each component was modified by adding sand at rates of 25 and 50% (v/v). On the day of use, medium components were moistened (percentage moisture (v/v): peat 50%, bark 20%, sawdust with bark 33%, sawdust without bark 30%, recycled plant material 27%, vermiculite 33%, perlite 10%, sand 10%) and mixed with sand, as described above. These moisture levels allowed the component to clump together without

becoming waterlogged.[27] Each component alone and all of the sand mixes were evaluated in the 10 cm PVC columns as described in Section 2.4. In this bioassay, the ability of nematodes to infect their host was also evaluated. A single *G. mellonella* larva was placed in the bottom section which had an aluminum insect window screen (Phifer Inc., Tuscaloosa, AL) attached to the top of the bottom, and filled with selected medium to prevent the larva from travelling up the column. Approximately 1000 IJs of *S. riobrave* or *H. bacteriophora* were added to the top of the column, then capped with a petri dish lid. To improve upon methods in Section 2.4 and provide optimum conditions for the EPNs, the medium-filled columns were stored in a sealed box to retain moisture at 25 °C for 3 days. After 3 days, the column sections were separated, the *G. mellonella* larva was isolated and the medium from each section was wrapped in a KimWipe (Kimberly-Clark Corp., Irving, TX) and individually placed in a Baermann funnel containing dH_2O to extract live nematodes. The Baermann funnel extraction method was chosen owing to higher extraction efficiency and for easier sampling processing. After 48 h, 15 mL of water was collected from each funnel and stored in a 50 mL centrifuge tube until counting. For each nematode species, all components with sand mixes were compared, replicated 3 times and temporally blocked 3 times.

2.6 Foraging distance index

Foraging efficacy in the 10 cm PCV columns was calculated by developing an index of distance based on the number of nematodes in each section as follows:

$$[(1.25 \times a) + (3.75 \times b) + (6.25 \times c) + (8.75 \times d)] \times N^{-1}$$

where a, b, c and d represent the number of nematodes in a given section, the constants (1.25, 3.75, 6.25, 8.75) indicate the distance from the top of the column to the midpoint of the section and N is the total number of live nematodes per column. This provides a weighted average of the distance travelled by nematodes within the column, with greater values indicating increasing proximity to the hosts.[15]

2.7 Data analysis

Survivorship for the persistence study was analyzed by pathogen with proportional hazards to identify interactions between factors, and Kaplan–Meier survivorship analysis to calculate median lifespan.[28,29] The foraging distance indices for medium mixes were compared with a one-way ANOVA. Foraging distance index and the number of IJs invading each larva were corrected with Abbott's formula[30] using the mean value of the sand treatments as the control. The corrected foraging distance indices were compared among treatments using a factorial ANOVA. The corrected numbers of IJs that invaded the *G. mellonella* larva were log transformed and then compared using a factorial ANOVA (components bioassay). If the level of sand significantly impacted upon foraging distance, a Pearson's correlation analysis was performed. Where appropriate, means were separated using Tukey's HSD at $P \leq 0.05$. All statistical analyses were performed in JMP v.8.0 (SAS Institute, Cary, NC) or in R v.2.12.0.

3 RESULTS

The three soilless media were analyzed for their chemical properties and particle sizes. All three had salinity values and pH levels within the acceptable range for EPNs and fungi. Where the media differed was in the percentage total nitrogen, carbon and organic matter. Bark had the highest percentage nitrogen compared with the other media, and both sawdust and bark had high percentage carbon and organic matter compared with the peat : sand medium (Table 1). Under the greenhouse conditions described above, all three media were saturated in terms of the water potential. Particle size differed between the media as well, and this was obvious visually. The bark was coarse, with the highest proportion of large material (≤3.96 mm). The sawdust had 75% of particles greater than 0.50 mm, whereas the peat : sand was the finest material with primarily medium-sized particles (Table 2). Regardless of watering regime, all three media had water potentials ranging from −1.0 to −1.5 kPa, classifying them as saturated.

3.1 Persistence

Entomopathogens *H. bacteriophora, S. riobrave* and *M. anisopliae* were sampled weekly for 4 weeks, and their persistence was calculated. There was no significant effect of watering regime ($df = 2$, $\chi^2 = 0.57$, $P = 0.75$), so this parameter was removed from all further analyses. Owing to differences in ambient temperature in the two greenhouses, there was a significant block effect ($df = 1$, $\chi^2 = 16.87$, $P < 0.0001$) and significant entomopathogen and medium main effects, plus a significant interaction between them ($df = 4$, $\chi^2 = 78.66$, $P < 0.0001$; $df = 2$, $\chi^2 = 64.64$, $P < 0.0001$; $df = 8$, $\chi^2 = 46.67$, $P < 0.0001$ respectively). The five entomopathogen treatments were *H. bacteriophora* drench and cadaver, *S. riobrave* drench and cadaver and *M. anisopliae* drench. Survival analysis of each pathogen treatment was performed separately (Fig. 1; Tables 3 and 4). Generally, persistence was longer in the cooler greenhouse. Infective juveniles in the peat : sand medium and the bark medium had higher persistence than the sawdust medium. *S. riobrave* persisted 1–2 weeks less in the sawdust medium, depending on temperature and application method, than in the other medium types. In the cooler greenhouse, log-rank tests found significant differences in survival between medium types for all of the nematode treatments ($P < 0.01$) and marginally significant differences for *M. anisopliae* drench ($P = 0.049$). In the warmer greenhouse, significant differences in nematode and fungus survival among medium types were observed for all treatments except for *H. bacteriophora* drench ($P = 0.093$) and *M. anisopliae* drench ($P = 0.351$). Mean survival was longer for all entomopathogens in the cooler greenhouse, regardless of medium type, except for *S. riobrave* cadaver application in a peat : sand medium.

3.2 Foraging distance in soilless media

The foraging distance index was calculated for each treatment, replicate and species to give an average distance per nematode travelled in the bioassay. There were no significant differences between blocks for either species, so data were combined. A significant effect of medium type was found for *H. bacteriophora* ($F = 31.24$, $df = 2, 43$, $P < 0.0001$) and *S. riobrave* ($F = 8.66$, $df = 2, 43$, $P = 0.0007$). For both nematode species, vertical movement was significantly lower in the sawdust medium than in the peat : sand or bark medium (Fig. 2).

3.3 Foraging distance in medium components

The mean foraging distance index was calculated for each treatment, block, replicate and species to give an average distance per nematode travelled in the bioassay. Sand is often considered to be the optimum medium for EPN movement. The present authors

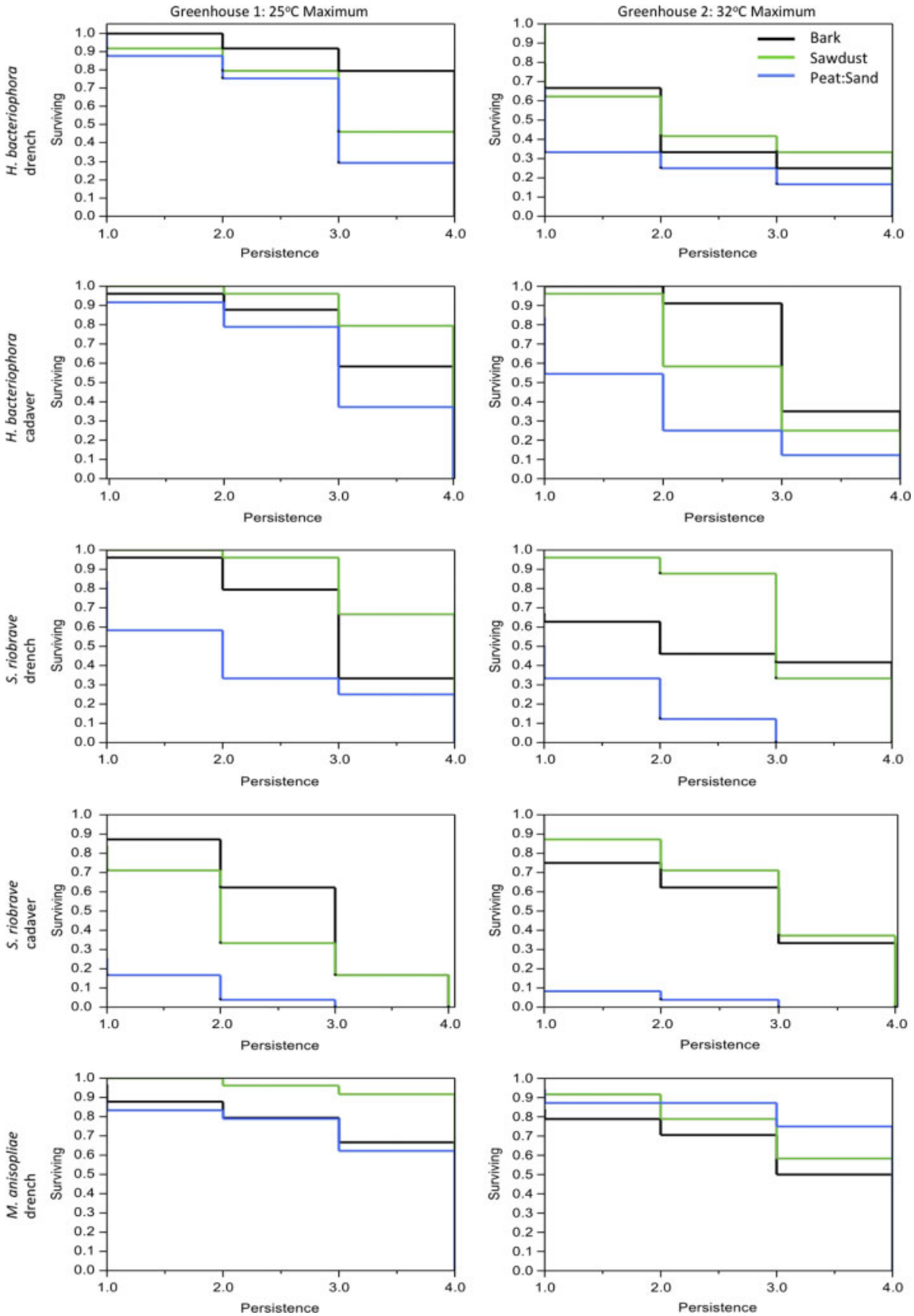

Figure 1. Survival (in weeks) of entomopathogens in soilless media under two greenhouse conditions. Survival indicates proportion of replicates in which persisting pathogen populations were detected via bioassay.

Table 1. Soilless medium chemical analysis

	Composition (v/v)	pH[b]	EC (dS m^{-1})[c]	N (total) (%)	C (total) (%)	Ash	OM
Bark	67% cedar/pine bark, 33% recycled plant material[a]	6.5	0.11	0.69	22.1	57.2	12.82
Sawdust	85% redwood sawdust (with bark), 15% sand	5.6	0.07	0.06	27.6	41.3	15.98
Peat : sand	50% peat moss, 50% UC sand	8.0	0.03	0.05	1.6	94.5	0.91

[a] Compost = recycled, piled plant material plus sand for a total of 8% sand.
[b] H_2O 1 : 5.
[c] Measured (H_2O 1 : 2) using HI 255.

Table 2. Particle size (proportion of total v/v) for each soilless medium

	Very fine (≥0.053 mm)	Fine (0.054–0.106 mm)	Medium (0.107–0.25 mm)	Coarse (0.26–0.50 mm)	Very coarse (0.51–1.00 mm)	Large material (≥3.96 mm)
Bark	0.07	0.13	0.12	0.13	0.18	0.38
Sawdust	0.01	0.07	0.17	0.27	0.41	0.07
Peat : sand	0.04	0.15	0.30	0.32	0.17	0.02
UC sand	0.02	0.14	0.25	0.35	0.22	0.00

used pure sand as a positive control treatment, and in order to dilute the individual components that were tested. There was no significant interaction between component and amount of sand for either EPN species (*H. bacteriophora*: $F = 1.35$, df = 12, 167, $P = 0.20$; *S. riobrave*: $F = 0.81$, df = 12, 165, $P = 0.63$). For *H. bacteriophora* there was a significant effect of component type, but not of sand mix (component: $F = 9.26$, df = 6, 167, $P < 0.0001$; sand: $F = 2.64$, df = 2, 167, $P = 0.07$). Owing to the high amount of variability in the distances travelled by IJs, Tukey's HSD did not identify differences between individual treatment. However, the greatest distance occurred in peat moss, and the shortest distance towards a host cue in both redwood sawdust and perlite (Fig. 3).

Steinernema riobrave was not able to forage in as diverse medium types as *H. bacteriophora*. There was a significant effect of component type and sand level, with increasing levels of sand generally increasing foraging distance (component: $F = 54.47$, df = 6, 166, $P < 0.0001$; sand: $F = 13.30$, df = 2, 166, $P < 0.0001$) (Fig. 3). IJs in recycled plant material and peat moss had the greatest foraging distance towards a host cue (Fig. 3). Unlike *H. bacteriophora, S. riobrave* did not forage efficiently in cedar/pine bark. Foraging distance was also significantly lowered in the redwood sawdust medium and perlite. However, there was a positive correlation between foraging distance and sand level for perlite (Pearson's correlation 0.42), vermiculite (0.44), redwood sawdust without bark (0.51), sawdust with bark (0.57) and cedar/pine bark (0.60).

In this bioassay, the IJs were allowed to penetrate the *G. mellonella* larva, providing an indication of the biological control efficacy that might be achieved in each medium component (Fig. 3). Again, there was no significant interaction between component type and sand level for either EPN species (*H. bacteriophora*: $F = 0.55$, df = 12, 164, $P = 0.88$; *S. riobrave*: $F = 1.66$, df = 12, 166, $P = 0.08$). Medium component significantly impacted upon the number of *H. bacteriophora* IJs that penetrated the hosts, but not the level of sand (component: $F = 2.73$, df = 6, 164, $P = 0.014$; sand: $F = 0.65$, df = 2, 164, $P = 0.52$). Recycled

Table 3. Mean (± SEM) time of survival (in weeks) of entomopathogen in soilless medium under cool greenhouse conditions

Entomopathogen	Application	χ^2	*P*-value	Bark	Sawdust	Peat : sand
H. bacteriophora	Drench	11.28	0.004	3.92 ± 0.06	3.62 ± 0.15	3.67 ± 0.16
H. bacteriophora	Cadaver	8.80	0.012	3.79 ± 0.14	3.62 ± 0.18	3.96 ± 0.05
S. riobrave	Drench	17.93	0.001	3.71 ± 0.14	2.75 ± 0.23	3.96 ± 0.04
S. riobrave	Cadaver	31.35	0.001	3.38 ± 0.21	1.46 ± 0.18	2.88 ± 0.22
M. anisopliae	Drench	6.00	0.049	3.63 ± 0.18	3.50 ± 0.22	3.96 ± 0.18

Table 4. Mean (± SEM) time of survival (in weeks) of entomopathogen in soilless medium under warm greenhouse conditions

Entomopathogen	Application	χ^2	*P*-value	Bark	Sawdust	Peat : sand
H. bacteriophora	Drench	4.74	0.094	2.79 ± 0.23	2.25 ± 0.24	3.04 ± 0.19
H. bacteriophora	Cadaver	19.21	<0.001	3.92 ± 0.06	2.63 ± 0.22	3.54 ± 0.12
S. riobrave	Drench	25.99	<0.001	2.75 ± 0.28	1.96 ± 0.23	3.80 ± 0.14
S. riobrave	Cadaver	36.31	<0.001	3.13 ± 0.27	1.21 ± 0.15	3.46 ± 0.21
M. anisopliae	Drench	2.09	0.351	3.33 ± 0.24	3.67 ± 0.26	3.62 ± 0.18

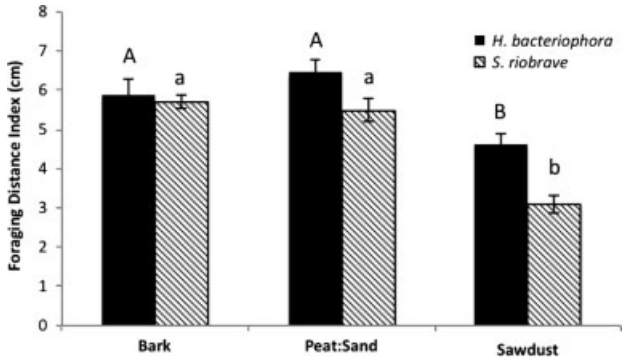

Figure 2. Foraging distance index (± SEM) of *Heterorhabditis bacteriophora* (black) and *Steinernema riobrave* (gray) in a 10 cm column. The foraging index provides a weighted average of the distance travelled by nematodes within the column, with greater values indicating increasing proximity to the hosts. Differing letters indicate significant difference between soilless media at $P \leq 0.05$.

plant material proved to be the component with the highest number of IJs infecting the host for both species, and *S. riobrave* infection was also efficient in peat moss. Owing to the high variability in the recycled plant medium and the large number of comparisons, Tukey's HSD did not reveal differences between sand mixes for *H. bacteriophora* infection (Fig. 4). *Steinernema riobrave* was unable to infect a host in either redwood sawdust media, which was expected due to the limited distance travelled (see above). For *S. riobrave* there was a significant effect of both component and sand mix on infection ability (component: $F = 9.41$, df = 6, 166, $P < 0.0001$; sand: $F = 7.42$, df = 2, 166, $P < 0.0001$). The highest level of sand positively influenced the number of IJs that penetrated the larva, with infection absent or low in components with less than 50% sand ($P < 0.05$) (Fig. 4).

4 CONCLUSIONS

Measuring the influence of soilless media on entomopathogen behavior and efficacy was the objective of these experiments. Although they are often treated as biological insecticides, entomopathogens require appropriate biotic and abiotic conditions for success.[3] The three soilless medium types evaluated were within the acceptable ranges of EPN survival for pH and salinity. There were differences in percentage organic matter and carbon content, with the sawdust and bark media having greater inherent carbon sources.

The watering method did not affect survival of entomopathogens; however, it may be a significant factor under warmer conditions or in outdoor nurseries where evaporation is greater. Survival of *M. anisopliae* was not affected by medium type, which is likely because it is a non-mobile organism, requiring the insect host to come into contact with it. Previous research has

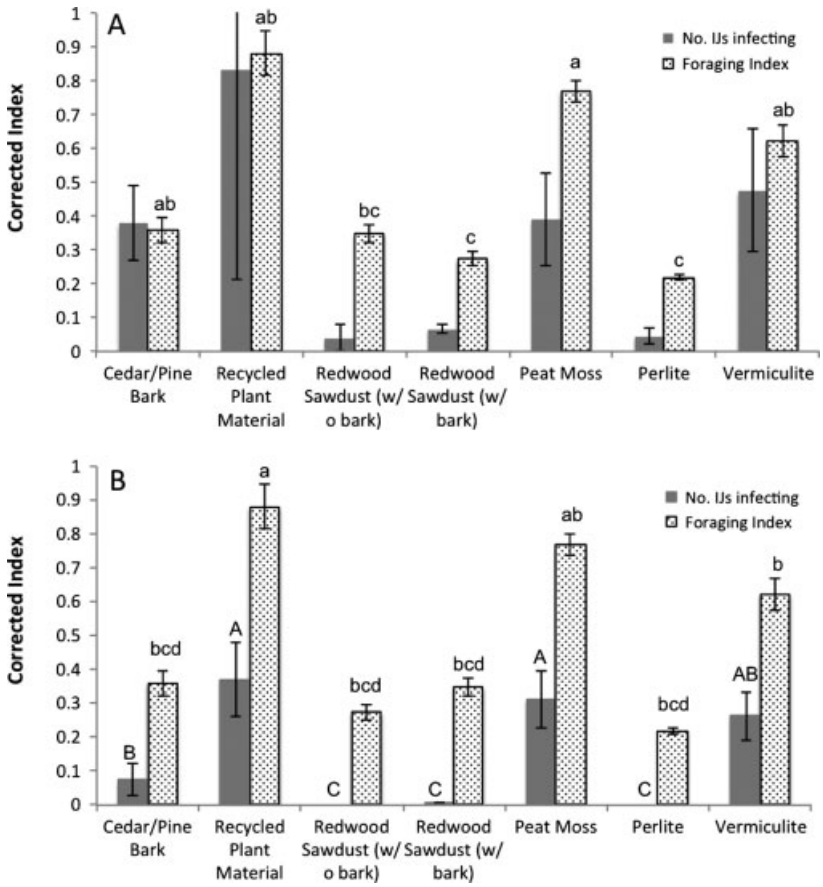

Figure 3. Corrected mean (± SEM) index for the number of IJs infecting a *Galleria mellonella* larva (gray bars) and the foraging distance index (± SEM) (hatched bars) of (A) *Heterorhabditis bacteriophora* and (B) *Steinernema riobrave* in individual soilless medium components. The foraging index provides a weighted average of the distance travelled by nematodes within the column, with greater values indicating increasing proximity to the hosts. Differing letters indicate significant difference between medium components at $P \leq 0.05$ (Tukey's HSD).

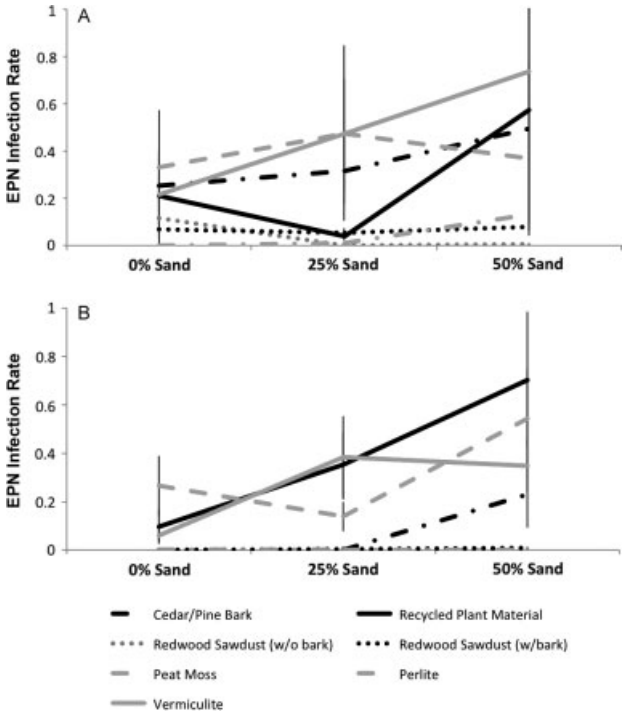

Figure 4. Effect of increasing sand mixture on EPN infection rates (mean ± SEM): (A) *Heterorhabditis bacteriophora*; (B) *Steinernema riobrave*.

shown that medium type does not impact upon *M. anisopliae* conidia formation until after 120 days, with peat-based media having significantly greater populations.[31] Temperature can significantly affect efficacy and sporulation of *M. anisopliae*, with warmer temperatures increasing infection by entomopathogens.[14,32] Entomopathogenic nematodes, however, are mobile organisms that are influenced by abiotic and biotic factors. Temperature was a significant factor in survival of IJs in soilless media, with greater persistence in the cooler greenhouse. *Steinernema* spp. have lower minimum temperature thresholds for activity with a wider range (3–14 °C) than most *Heterorhabditis* spp. (10–16 °C).[14] It is possible that the maximum temperature within the pots exceeded the maximum threshold temperature for both species. Both nematode species chosen in this study display 'cruiser' behavior, actively foraging within the substrate, although *S. riobrave* is classified as an intermediate forager, switching between cruising and ambushing host searching strategies.[10] Because of behavioral differences and the varying physical and chemical properties of soilless media, it is not unexpected that differences in survival would exist.

The behavior of EPNs was compared in each individual component to gain a better understanding of why some mixed media are better than others for EPN efficacy. In these soilless media, *H. bacteriophora* was able to find and infect insects in a greater range of habitats than *S. riobrave*, which differs from results found in other studies in 'natural' soil habitats.[23,33] *Heterorhabditis bacteriophora* travelled the greatest distance in peat moss, and a large number of IJs were able to penetrate the larvae in that component as well. Kruitbos *et al*.[23] suggested that *H. megidis* did not show horizontal movement towards hosts in peat moss owing to disruption of host volatiles, but this discrepancy between the present results and theirs could be species specific, related to orientation of host volatiles or IJ size (*H. megidis* is larger than *H. bacteriophora* at 768 µm versus 570 µm respectively). The number of infecting IJs was obscured for *H. bacteriophora* because of the large variance in the recycled plant material owing to its high heterogeneity compared with other components. Whether it was due to this heterogeneity or other factors such as pore size, *S. riobrave* outperformed in the recycled plant material and peat moss.

In spite of physical alteration of the structure of each component with sand, this did not significantly increase foraging distance or the number of IJs infecting hosts for *H. bacteriophora*. This is surprising, given that *Heterorhabditis* spp. are generally classified as coastal or marine species.[33,34] However, physically altering the texture of the medium with sand generally improved foraging distance for *S. riobrave*. Regardless of physical alteration, movement and infection did not occur in either redwood sawdust media, suggesting that other factors caused inhibition. A negative relationship was apparent between the redwood sawdust media and nematode foraging, as IJs travelled significantly less in the sawdust medium than in the peat : sand or the bark media. Redwood is known for its strong chemical properties, and it is hypothesized that these inhibited IJ movement towards a host by alteration of perception of host cues. In a thorough sample of various ecosystems in California, EPNs were not isolated in any of the redwood forests sampled, although they were commonly isolated from coniferous and oak forests.[33] Their absence in redwood systems may be explained by the inability to forage successfully for hosts in redwood.

The reduction in nematode survival, movement and efficacy by the sawdust media and to a lesser extent by the bark component (for *S. riobrave* only) deserves further study. *Steinernema riobrave* foraging distance was correlated with the amount of sand mixed with the cedar/pine bark component, suggesting that the physical structure of this component impacted upon foraging success. This may be due to the intermediate foraging behavior of *S. riobrave*, as both a cruiser and an ambusher, and the large texture of cedar/pine bark may have blocked or weakened host cues and initiated a switch to ambushing behavior. The reduced movement in perlite that was observed for both species is not unexpected in view of the physical structure of the perlite itself; it is very rough and does not retain water for long periods of time. Except for the production of cacti and succulents, perlite is used in such miniscule amounts in media that it is unlikely significantly to reduce efficacy.

How various potting medium components impact upon entomopathogen performance can be utilized by researchers and growers alike to customize potting soils to provide the optimum environment for biological control organisms. If weevil larvae are to be controlled by EPNs, a medium that is composed of bark and/or peat provided a suitable habitat for foraging and infection of larvae. Sand did not improve foraging of *H. bacteriophora*, but it did for *S. riobrave* and could be added for weight, depending on the plant requirements. Selecting the best medium for the plant requirements as well as forseeing pest problems will help to improve conservation biological control in nursery and greenhouse production.

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REFERENCES

1 Gurr GM, Wratten SD and Barbosa P, Success in conservation biological control of arthropods, in *Biological Control: Measures of Success*. Springer, Dordrecht, The Netherlands, pp. 105–132 (2002).
2 Landis DA, Wratten SD and Gurr GM, Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annu Rev Entomol* **45**:175–201 (2000).
3 Lewis E, Campbell JF and Gaugler R, A conservation approach to using entomopathogenic nematodes in turf and landscapes, in *Conservation Biological Control*, ed. by Barbosa P. Academic Press, London, UK (1998).
4 Stuart RJ, El-Borai FE and Duncan LW, From augmentation to conservation of entomopathogenic nematodes: trophic cascades, habitat manipulation and enhanced biological control of *Diaprepes abbreviatus* root weevils in Florida citrus groves. *J Nematol* **40**:73–84 (2008).
5 Nielsen AL, Spence KO and Lewis EE, Efficacy patterns of biopesticides used in potting media. *Biopestic Int* **4**:87–101 (2011).
6 Boodley JW and Sheldrake R, *Cornell peat–lite mixes for commercial plant growing*. *Information bulletin* No. 43, Cornell University, Ithaca, NY, 7 pp. (1973).
7 Ingram DL, Henley RW and Yeager TH, *Growth media for container grown ornamental plants*. *Bulletin* No. 241, University of Florida IFAS Extension, Gainesville, FL (1991).
8 Olson DL, Oetting RD and Van Lersel MW, Effect of soilless potting media and water management on development of fungus gnats (Diptera: Sciaridae) and plant growth. *Hort Sci* **37**:919–923 (2002).
9 Kaya HK and Gaugler R, Entomopathogenic nematodes. *Annu Rev Entomol* **38**:181–206 (1993).
10 Lewis EE, Campbell J, Griffin C, Kaya H and Peters A, Behavioral ecology of entomopathogenic nematodes. *Biol Control* **38**:66–79 (2006).
11 Goettel MS and Inglis GD, Fungi: Hyphomycetes, in *Manual of Techniques in Insect Pathology*, ed. by Lacey LA. Academic Press, London, UK, pp. 213–249 (1997).
12 Hanel H, The life cycle of the insect pathogenic fungus *Metarhizium anisopliae* in the termite *Nasutitermes exitiosus*. *Mycopathologia* **80**:137–145 (1982).
13 Villani MG and Wright RJ, Environmental influences on soil macroarthropod behavior in agricultural systems. *Annu Rev Entomol* **35**:249–269 (1990).
14 Barbercheck ME, Effect of soil physical factors on biological control agents of soil insect pests. *Fla Ent* **75**:539–548 (1992).
15 Nielsen AL, Spence KO, Nakatani J and Lewis EE, Effect of soil salinity of entomopathogenic nematode survival and behavior. *Nematology* **13**:859–867 (2011).
16 Quesada-Moraga E, Navas-Cortez JA, Maranhao EAA, Ortiz-Urquiza A and Santiago-Ivarez C, Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. *Mycol Res* **111**:947–966 (2007).
17 West AW, Burges HD, Dixon TJ and Wyborn CH, Survival of *Bacillus thuringiensis* and *Bacillus cereus* spore inocula in soil: effects of pH, moisture, nutrient availability and indigenous microorganisms. *Soil Biol Biochem* **17**:657–665 (1985).
18 Wallace HR, Movement of eelworms. *Ann Appl Biol* **48**:107–120 (1960).
19 Koppenhöfer AM and Fuzy EM, Soil moisture effects on infectivity and persistence of the entomopathogenic nematodes *Steinernema scarabaei*, *S. glaseri*, *Heterorhabditis zealandica*, and *H. bacteriophora*. *Appl Soil Ecol* **35**:128–139 (2007).
20 Lingg AJ and Donaldson MD, Biotic and abiotic factors affecting stability of *Beauveria bassiana* conidia in soil. *J Invertebr Pathol* **38**:191–200 (1981).
21 Jabbour R and Barbercheck ME, Soil management effects on entomopathogenic fungi during the transition to organic agriculture in a feed grain rotation. *Biol Control* **51**:435–443 (2009).
22 Storey GK and Gardner WA, Movement of an aqueous spray of *Beauveria bassiana* into the profile of four Georgia soils. *Environ Entomol* **17**:135–139 (1988).
23 Kruitbos LM, Heritage S, Hapca S and Wilson MJ, The influence of habitat quality on the foraging strategies of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis megidis*. *Parasitology* **137**:303–309 (2010).
24 Shapiro-Ilan D and Gaugler R, Production technology for entomopathogenic nematodes and their bacterial symbionts. *J Ind Microbiol Biotechnol* **28**:137–146 (2002).
25 Shapiro-Ilan DI, Lewis EE, Son Y and Tedders WL, Superior efficacy observed in entomopathogenic nematodes applied in infected-host cadavers compared with application in aqueous suspension. *J Invertebr Pathol* **83**:270–272 (2003).
26 Kaya HK and Stock SP, Techniques in insect nematology, in *Manual of Techniques in Insect Pathology*, ed. by Lacey LA. Academic Press, London, UK, pp. 281–324 (1997).
27 Kaspi R, Ross A, Hodson AK, Stevens GN, Kaya HK and Lewis EE, Foraging efficacy of the entomopathogenic nematode *Steinernema riobrave* in different soil types from California citrus groves. *Appl Soil Ecol* **45**:243–253 (2010).
28 Kaplan EL and Meier P, Nonparametric estimation from incomplete observations. *J Am Statist Assoc* **53**:457–481 (1958).
29 Breslow NE, Analysis of survival data under the proportional hazards model. *Int Statist Rev/Rev Int Statist* **43**:45–57 (1975).
30 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
31 Bruck DJ, Ecology of *Metarhizium anisopliae* in soilless potting media and the rhizosphere: implications for pest management. *Biol Control* **32**:155–163 (2005).
32 Studdert JP, Kaya HK and Duniway JM, Effect of water potential, temperature, and clay-coating on survival of *Beauveria bassiana* conidia in a loam and peat soil. *J Invertebr Pathol* **55**:417–427 (1990).
33 Stock SP, Pryor BM and Kaya HK, Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California, USA. *Biodiversity Conserv* **8**:535–549 (1999).
34 Griffin CT, Finnegan MM and Downes MJ, Environmental tolerances and the dispersal of Heterorhabditis: survival and infectivity of European Heterorhabditis following prolonged immersion in seawater. *Fundam Appl Nematol* **17**:415–421 (1994).

