**Fine-scale spatial analysis of soil moisture and entomopathogenic nematode distribution
following release in wetting agent-treated turf**

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Abstract

Entomopathogenic nematodes (EPNs) are obligate insect parasites that have been widely studied for their use in the biological control of soil-dwelling pests. EPN persistence and pest impact in turfgrass systems may be hindered by management and construction practices favoring limited rootzone moisture. We sought to determine whether soil surfactants (“wetting agents”) could improve soil moisture uniformity on a fine-scale, and whether these changes improved post-application EPN persistence. Two EPNs with different foraging behaviors (*Steinernema carpocapsae*, *Heterorhabditis bacteriophora*) were studied in separate experiments in different turfgrass systems and seasons to which they would most appropriately be applied. Analyses using Spatial Analysis by Distance IndicEs (S.A.D.I.E) revealed that soil moisture patterns were dynamic on a fine scale, initially demonstrating random distributions, though becoming more aggregated over time. No significant improvements in nematode persistence or uniformity were found with a single wetting agent application at the time of EPN release. Both nematode species initially exhibited random distributions, though each became more uniform as population densities sharply declined over the observation periods. Surprisingly, little evidence was found linking EPN spatial pattern with that of moisture. Our findings suggest that short-term EPN survival is not correlated with soil moisture dynamics at a fine scale and that biotic factors may be more important in improving the post-application persistence and ultimately EPN performance in biological control programs in turfgrass.

Key words: Entomopathogenic nematodes; persistence; wetting agent; spatial distribution; S.A.D.I.E.

1. Introduction

Entomopathogenic nematodes (EPNs) of the family Steinernematidae and Heterorhabditidae are obligate insect parasites that may be found in many ecosystems around the world (Adams et al., 2006). They possess a mobile, non-feeding infective juvenile (IJ) stage that attack insects residing in the soil or in cryptic habitats. Once in contact with the insect, the IJ will penetrate its host either through natural openings (e.g. mouth, anus, spiracles) or directly through the cuticle (Bedding and Molyneux, 1982). The IJ releases a symbiotic bacterium into the insect's hemolymph that causes death of the host within 1-4 days (Kaya and Gaugler, 1993). The nematodes mature and reproduce within the liquefied interior of the cadaver for one to several generations depending on the species. Eventually, the cadaver will disintegrate and release potentially thousands to hundreds of thousands of IJs that may seek additional hosts.

EPNs show great promise as inundative biological control agents of insect pests that dwell in soils since they are mobile, cause rapid mortality, and can be applied through agricultural spray equipment (see review by Shapiro-Ilan et al., 2006). However, their widespread adoption in pest management may be limited by their lack of post-application persistence and the relatively high costs associated with reapplication. Poor post-application persistence has been attributed to several factors including exposure to ultraviolet light, predation by microscopic organisms (fungi, mites, and bacteria), soil and ambient air temperature, soil texture, and moisture (Kaya 1990, Smits 1996). Of these factors, soil moisture is believed to have the greatest influence on survival, spatial distribution, and efficacy of EPNs used in pest management since it plays a vital role in IJ survival and mobility when seeking hosts (Kung et al., 1990, 1991). Soil moisture optimums vary between EPN species, though in general, low to moderate (5-10 % volumetric water content) as well as excessively moist soil conditions (> 35%)

have shown to have negative effects on persistence of applied nematodes (Koppenhöfer et al., 1995; Grant and Villani 2003).

Turfgrass, which includes a diversity of systems ranging from intensely managed golf courses and athletic fields, to home lawns, parks, and roadside medians, is the largest irrigated crop in the United States (Milesi et al., 2005). Although EPNs have been successfully employed in turfgrass insect management from both a classical (Frank and Parkman, 1999, Frank and Walker, 2006) and augmentative biological control approach (see review by Georgis et al., 2006), their use in these systems is minimal in part due to the need for high application rates to overcome limited persistence (Koppenhöfer et al., 2015). EPN persistence in many turfgrass sites may be negatively affected by soil moisture management practices. The most intensely managed turf sites, such as golf courses, have the ability to precisely manipulate soil moisture though tend to be managed with minimal water inputs for economic and agronomic considerations. Additionally, it is advantageous to construct or amend fine turf soils with high percentages of sand to allow for adequate drainage and to better manipulate soil moisture content. Therefore, the cumulative effect of construction, irrigation, and conservation strategies involved in turfgrass culture may be at odds with optimal conditions for EPN survival and persistence.

Over the last decade, turfgrass managers have increasingly relied on soil surfactants to maintain consistent or uniform soil moisture in the sand-based rootzones. Soil surfactants or “surface active agents” are compounds that reduce the surface tension of water and the hydrophobicity of organic soil components to which they are applied (Turgeon 2012). Wetting agents are a class of surfactants that are widely used in turfgrass management to improve soil moisture characteristics. Wetting agents, though diverse in chemical structure and corresponding

use, are mainly non-ionic compounds (e.g. esters, ethers, and alcohols) which have the ability to wet soils that have a tendency to become hydrophobic. Soils, especially those with high percentages of sand, treated with wetting agents tend to have less localized dry spot and improved soil moisture uniformity in the rootzone (Kostka 2000; Zontek and Kostka, 2012).

The objectives of this study were to determine if a single wetting agent application could improve soil moisture spatial patterns sufficiently enough to promote EPN persistence following release in unirrigated turfgrass rootzones. Since soil moisture is critical to EPN survival and locomotion, we hypothesized that 1) a wetting agent application would improve EPN density and uniformity, and 2) that the association between nematode and moisture spatial distributions should strengthen as soils dry. In this study, we characterized the spatial distribution of soil moisture and two different EPN populations on two different scales, including a fine scale (30 cm $\times$ 30 cm plots) to better capture soil moisture and EPN population dynamics. Little is known about the mechanisms behind the population biology, dynamics, and structure of EPNs at a fine scale and the effects that these dynamics have on control of below-ground pests in the field (Stuart et al., 2006). A better understanding of the factors that influence the persistence and spatial dynamics would allow for greater predictability in biological control programs, and potentially serve to optimize release rates and strategies that could make EPNs more competitive with synthetic insecticides.

2. MATERIALS AND METHODS

2.1 Nematodes

Nematodes were obtained from Biologics Inc. (Willow Hill, Pennsylvania, USA) prior to field releases. Each nematode species was reared in late (6th- 7th) instar greater wax moth larvae

(*Galleria mellonella* L.) at room temperature (22–25°C) approximately 1 mo prior to the field trials (one generation). Wax moth larvae were supplied by Nature's Way (Harrison, OH, USA). White traps (100 × 15 mm Petri dish lined with filter paper floating on tap water) were used to harvest IJs as they emerged from nematode-killed insects over a 2-wk period (Kaya and Stock, 1997). IJs were then pooled and stored in 225-ml tissue culture flasks at 10°C at a concentration of 5000/mL until the date of application (maximum storage = 14 d). The concentration and viability of nematodes was estimated prior to application by diluting subsamples of the product in water, and calculating the number of live nematodes in ten 10-µl droplets. Final concentrations of EPNs were transported to the field in a cooler with an ice pack to maintain temperatures at approximately 20°C until application.

2.2 Field Sites

Field trials were conducted at Pennsylvania State University's Landscape Management Resource Center (LMRC) in University Park, Pennsylvania. The effects of wetting-agent applications on soil moisture content and EPN persistence (Experiment 1- see below) and spatial distribution (Experiment 2) were assessed in two separate experiments (Table 1). The experiments differed by the number of treatments, plot size, and sampling intensity relative to plot size. Each experiment was subdivided into two separate trials for different nematode species and turfgrass sites. Successful pest control with EPNs requires matching foraging strategies with pest biology (Lewis et al., 2006). Therefore the EPNs selected were applied to different turfgrass systems (close-cut golf turf vs. low maintenance lawn turf) to which they would be appropriately applied during the time of the year when major turfgrass pests in those environments were susceptible to

EPN infection. Different experiments with the same EPN species were run concurrently and separated by approximately 25 m.

Steinernema carpocapsae was observed in early summer (18 May to 15 June 2015) in Trials 1 (Experiment 1) and 3 (Experiment 2). This species was selected for its foraging behavior (“ambusher”) or activity against surface-active or stem and foliage feeding insects found in short-mown turfgrass (e.g. *Agrotis ipsilon* Hufnagel, *Listronotus maculicollis* Kirby) at this time of the year. Trials were conducted on *Agrostis stolonifera* L. maintained at mowing height of 1 cm. Soils were analyzed by Pennsylvania State University’s Agricultural Analytical Services Lab (University Park, Pennsylvania, USA) for physical and chemical properties. Pooled samples from both trials were characterized as a sandy loam (80% sand, 15% silt, 5% clay) with a near neutral pH (7.1), and low total nitrogen (0.134%) and organic matter (1.57 %).

Heterorhadtis bacteriophora, a soil “cruising” nematode, was observed in fall (8 to 22 September) when sedentary pest insects such as white grubs (Coleoptera: Scarabeidae *spp.*) are present. These trials (2 and 4) were conducted on turfgrass sites with higher mowing heights (*Poa pratensis* L.-*Lolium perenne* L. mowed at 5 cm) grown in native soils with greater percentages of fines (sandy clay loam; 75% sand, 22% silt, 3% clay), total nitrogen (0.34 %) and organic matter (5.3%), but lower soil pH (6.6).

Short-residual broad-spectrum insecticide applications (bifenthrin, Talstar®, FMC Corporation, Philadelphia, Pennsylvania USA; 1.46 L/ha) were applied to the turf in the trial areas 1 wk prior to EPN release to reduce the amount of surface-active insects that may otherwise serve as hosts for EPNs and thus affect estimations of persistence. Insecticide applications were made 1 wk prior to EPN release so as to not affect nematode survival.

2.3 Experiment 1: Nematode persistence

The ability of a wetting agent application to improve soil moisture content and EPN persistence (density) was evaluated in 1.52×1.52 m plots (Trial 1 and 2). One of three wetting agents (Aquatrols Inc., Paulsboro, NJ USA) or an untreated control (tap water; pH = 6.8- 7.4) was applied to the plot and immediately followed (< 30 mins) by EPN application. The wetting agents used in the study included an industry standard, methyl capped polymer (Revolution®) and two experimental polymers (ACA 2994, ACA 3232). Treatments were replicated six times, and arranged in a randomized block design (24 total plots). Plots were separated by 30 cm on each side to limit EPN movement into the neighboring plots. Plots and alleyways were marked with turf paint to easily locate the area for sampling. Paint was reapplied as necessary throughout the trial.

All plots were irrigated to the point of field saturation at the beginning of the trial to ensure uniform moisture across all treatments. Supplemental irrigation was withheld after the initial watering of the plots. Moisture readings were taken using a FieldScout TDR 300 (Spectrum Technologies, Aurora, Illinois USA). Average soil moisture values across the probe's depth (4 cm) was recorded. Each plot was probed 4 times to obtain an average moisture reading for the plot. Moisture readings were taken 3, 7, 14, 21 and 28 days after treatment (DAT) for *S. carpocapsae* treatments (Trial 1). A 0 DAT sampling was included in Trial 2 given the inherent variability of the native-soil rootzones and the difficulty in uniformly wetting rough-height turf plots.

EPNs and wetting agents were applied using a pressurized CO₂ backpack sprayer equipped with a flat fan nozzle (TeeJet VS 8002, Spraying Systems Co., Bellwood, Illinois USA). Screens were removed from the nozzle and the wand was operated at 137 kPa to ensure

uniform coverage and maximum viability. Wetting agents were applied prior to EPNs as a precaution or to minimize potential negative effects of extended contact between wetting agents and EPNs. However, preliminary laboratory assays with the experimental surfactants suggested no adverse effects with either viability or infectivity when mixed (label rates = 2% v/v; 5 days). Additionally, laboratory studies have shown that IJ infectivity and survival are not affected when combined in solution for as long as 12 days (Hector et al. 2013). Revolution® (19 L/ha), ACA 2994 (25.4 L/ha), or ACA 3162 (9.54 L/ha) were applied to the turf, followed by light irrigation (0.25 cm). Nematodes were applied ($\sim 2.5 \times 10^9$ IJs/ha) and followed with a light irrigation (0.25 cm) to wash them off of the canopy and to avoid exposure to UV light. All nematodes were applied early in the day ($\sim 06:00$ hr), and under cloudy conditions to minimize UV light exposure.

EPN populations were monitored in soil samples taken immediately after application (0 DAT) to assess the infectivity of applied nematodes, and repeated on 3, 7, 14, 21, and 28 DAT to assess persistence. The 21 and 28 DAT samplings were removed from Trial 2 to avoid the possibility of recycling occurring in resident white grub larvae that would otherwise be unaffected by contact insecticide treatments. Four soil cores were removed randomly from each plot with an Oakfield soil sampler (1.9 cm internal diameter $\times$ 15 cm deep) to a depth of 5 cm. The Oakfield sampler was rinsed with soapy water and wiped clean with 70% ethanol between soil samples. The samples were brought to the laboratory where the soil was pooled by replicate, thoroughly mixed, and placed into one deep Petri dish (100 $\times$ 25 mm) per sample. Nematodes in soil samples were estimated by exhaustively baited with *G. mellonella* larvae using standard soil aliquots (75 g) according to the methods by McGraw and Koppenhöfer (2009). Briefly, five larvae were added to each baiting dish and examined every three days for nematode infections

(e.g. color changes, flaccid appearance). Infected or diseased larvae were removed on every 3 days and replaced with fresh larvae. Soils were deemed “nematode-free” when no nematode infected-larvae were found for three consecutive baiting rounds.

2.4 Experiment 2: Fine-scale spatial distribution

Post-application EPN spatial dynamics were assessed for each nematode species and system in wetting agent-treated (Revolution®, 19 L/ha) and untreated microplots. Nematode density, spatial distribution, and volumetric water content was monitored in small plots (1.52 × 1.52 m) consisting of 25 individual microplots (30 × 30 cm). Each treatment was replicated four times (Blocks 1-4) to reduce the variability that may have occurred in a single plot. The application of wetting agent, nematodes, soil sampling, soil samples per plot, moisture monitoring, IJ estimations, and the marking of plots were the same as those described in Experiment 1. Samples were taken on 3, 7, 14, and 28 DAT in Trial 3 (*S. carpocapsae*) and 0, 3, 7, and 14 DAT for Trial 4 (*H. bacteriophora*).

2.5 Statistical analysis

Differences between treatments in EPN post-application persistence (Experiment 1) as measured by the number of insect cadavers killed by EPNs (untransformed) was analyzed by one-way analysis of variance (ANOVA) using Statistix 8.0 software package (Tallahassee, Florida, USA). Where significant differences were detected, pairwise comparisons were made using Tukey’s mean separation test ($\alpha = 0.05$).

The spatial distributions of EPNs and soil moisture were characterized using Spatial Distance by IndicEs (S.A.D.I.E.) freeware (v. 1.5; KF Conrad and IACR Rothamsted

Experimental Station, Harpenden, UK). S.A.D.I.E. characterizes the degree of clustering of counts by an index of aggregation (I) calculated through randomized permutations of x, y referenced data. For a complete description of the methodology, refer to Perry (1995, 1998). The program calculates the distance to regularity of the data (D), or the minimum total distance required to transport the counts to conform to a uniform distribution. The lower the D value, the more uniform the dataset is. The program then performs a randomization procedure to calculate the mean expected distance to regularity (E), where the sample counts are distributed at random throughout the sample area and their distance to regularity assessed. The overall index of aggregation (I_a) is calculated as $I_a = D/E_a$. I_a values equal to, greater than, and less than 1 indicate a random, aggregated, and uniform distribution, respectively. The randomization procedure allows for significance testing on the calculated index's departure from randomness (P_a). The null hypothesis, that the counts within the sampled area are randomly distributed with respect to each other, may be rejected when $P_a < 0.025$ (accept the alternative hypothesis for aggregation) or $P_a > 0.975$ (accept the alternative hypothesis for uniformity). The spatially referenced count data form clusters of either patches (large counts in close proximity to one another) or gaps (zeroes or small counts in close proximity). S.A.D.I.E. calculates each sample's contribution to local clustering through the unitless sub-indices v_i and v_j . The sub-indices were used to develop contour maps using SigmaPlot (v. 8.0; Systat Software, San Jose, CA, USA) to assist in the visualization of spatial arrangement of the counts.

We also tested whether volumetric water content and EPNs datasets were spatially correlated using the S.A.D.I.E. system (Perry and Dixon, 2002). The program generates an index of association (X) which describes the relatedness of two datasets at the same local spatial coordinates, along with an associated probability of the index's departure from randomness (P_x).

A local index (X_k) is calculated by examining the similarity of the count data between two populations with the same georeferenced (x, y coordinates) data at the k^{th} unit. The overall spatial association is calculated as: $X = \sum X_k / n$. X values significantly greater than zero are considered associated populations (patches corresponding with patches, gaps with gaps), significantly less than zero are considered dissociated populations (patches corresponding with gaps), and values not significantly different from zero are considered randomly distributed in respect to one another.

3. RESULTS

3.1 Experiment 1

3.1.1 Soil moisture

Despite small numerical differences, no statistical differences were detected in the volumetric water content between treated and untreated sand-based rootzones of Trial 1 (*S. carpocapsae*) (Figure 1). Without supplemental irrigation, average moisture levels across all treatments declined at a rate of 8.2% per day relative to initial moisture levels between application and 7 DAT. The one-time application of wetting agent did not result in significant improvements to average volumetric water content in any of the *S. carpocapsae* + wetting agent treatments ($df = 3$; $F > 0.52$; $P > 0.06$). However, Revolution® and ACA 3162 treatments were numerically higher (range 0.2 – 8.3%) than the untreated controls on all dates between 7 and 28 DAT

Light, natural rainfall may have hindered the ability to detect differences in volumetric water content of treatments in the fall-applied *H. bacteriophora* + wetting agent treatments (Trial 2). The fine-textured soils retained >30% volumetric soil moisture content throughout the

observation period (Figure 1). The inherent soil water holding capacity of the fine-textured soil likely contributed to the lack of significant differences in volumetric water content between wetting agent-treated and untreated plots ($df = 3$; $F > 0.15$; $P > 0.67$).

3.1.2 EPN persistence

The rate at which EPN populations declined was similar between species, despite great differences in environmental conditions (e.g. temperature, rainfall), EPN species, soil texture, turfgrass species and mowing heights, and volumetric water content (Figure 1). No significant differences were detected between population estimates in wetting agent-treated and untreated plots on any observation date for either *S. carpocapsae* ($df = 3$; $F > 0.21$; $P > 0.31$) or *H. bacteriophora* ($df = 3$; $F > 0.38$; $P > 0.37$). Both species experienced sharp population declines between the time of application and 3 DAT, averaging 14.6% day⁻¹ across all treatments for *S. carpocapsae* and 14.2% for *H. bacteriophora*. EPN population declines stabilized (< 10% day⁻¹) between 3-7 DAT, after which both population persisted at low densities through the end of each trial.

3.2 Experiment 2

3.2.1 Spatial distribution of soil moisture

Average volumetric water content rapidly declined in both surfactant-treated and untreated plots in *S. carpocapsae*-applied microplots (Trial 3), though trends in the soil moisture spatial patterns differed between treatments. At 3 DAT, wetting patterns in three of four untreated plots were randomly distributed ($I_a < 1.097$; $P > 0.17$), whereas three out of four treated plots were significantly aggregated ($I_a > 1.58$; $P < 0.003$). These trends continued for the remainder of the observation period with random distributions observed in 13 of 16 (81%)

untreated plots and aggregations in 11 of 16 surfactant-treated plots (68%). Despite the inability of the wetting agent to provide uniformity on a fine scale, the fewer random distributions observed in surfactant-treated plots suggest that wetting agents did improve the overall moisture retention capacity of treated soil. Surfactant-treated plots provided significantly greater soil moisture content on all dates ($df=1$; $F > 12.95$; $P < 0.0004$) despite only small numerical differences in volumetric water content (1.2 – 6% averaged across all blocks). Average daily declines in volumetric soil moisture between 3 and 7 DAT was rapid, yet similar for surfactant-treated plots and untreated plots (12 – 12.8 % per day). By 7 DAT both treatments were near permanent wilting points (~ 10% volumetric water content) and turf showed signs of stress (e.g. footprinting, discoloration). Rainfall received 13 DAT allowed all plots to rebound to levels approaching 0 DAT levels (average 26% volumetric water content), though treated plots demonstrated greater average moisture levels at 14 (24 vs 14%) and 28 DAT (28 vs 24%).

Review of contour maps of volumetric water content show that wetting patterns are dynamic (Figure 2). Of the wetting agent-treated blocks, only Block 1 approached uniformity after 3 DAT. All other blocks became significantly aggregated as soils dried, and remained significantly aggregated throughout the remainder of the study, despite increases in rainfall at 14 DAT. No consistent trends were detected in untreated plots. In general, the untreated blocks, though significantly lower in average volumetric water content, displayed more uniform wetting or lower I_a values, which may be the result of low overall soil moisture values. The contour plots show larger patches to be associated with most wetting agent-treated plots (vectors > 1.0), indicating benefit of wetting agent application.

Much higher average volumetric content of soils and consistent rainfall led to much less variability in the spatial patterns in fine-textured soil microplots (Trial 4). Moisture levels

ranged from 27 to 40% and 32 to 43% in untreated and surfactant-treated microplots, respectively. An additional 0 DAT soil sampling was added to the trial to potentially capture differences in wetting patterns that may have arisen from the start of the trial with a more heterogeneous, native-soil rootzones. Significantly greater moisture levels were observed in the average of the four wetting agent-treated microplots at 0 DAT ($df=1$; $F = 52.83$; $P < 0.0001$), as well as at 3 ($df=1$; $F = 12.24$; $P = 0.0006$) and 14 DAT ($df=1$; $F = 60.72$; $P < 0.0001$). No significant differences in soil moisture were detected 7 DAT ($df=1$; $F = 0.84$; $P = 0.36$).

Soil moisture patterns in Trial 4 were observed to be predominately random (I_a values nearing 1) and independent of treatment (Figure 3). Random distributions were observed in 13 of 16 wetting agent-treated datasets, and 15 of 16 untreated datasets. The remaining spatial patterns were significantly aggregated (I_a values > 1), including Block 1 (14 DAT; $I_a = 1.57$; $P_a = 0.004$) and 2 (0 and 7 DAT; $I_a > 1.39$; $P_a < 0.02$) in wetting agent-treated microplots, and Block 1 (3 DAT; $I_a = 1.42$; $P_a = 0.02$) in untreated microplots. Analysis of the contour mapping suggest that similar to the sand-based rootzones of Trial 3 spatial patterns, the wetting agent treated fine-textured microplots had greater patch areas or areas with high volumetric water content in close proximity to one another, leading to aggregations or the lack of uniform soil moisture patterns.

3.2.2 EPN spatial distribution

Fine-scale spatial analysis of nematode populations in microplots characterized dispersion patterns as either aggregated or randomly distributed (Figure 4). Statistically uniform spatial patterns were not observed on any sampling date for either species, including immediately following applications. In general, *S. carpocapsae* populations (Trial 3) were observed to be randomly distributed on most dates regardless of treatment. Random distributions were observed

in 18 of 20 treated and 16 of 20 untreated plots. Over time, *S. carpocapsae* populations became more uniform (I_a values less than 0) as IJ densities diminished. *S. carpocapsae* populations in wetting agent-treated microplots trended towards uniformity between 7 and 28 DAT, though the null hypothesis for randomness could not be rejected. Average I_a values in treated plots were not significantly different from untreated plots ($df=1$; $F = 0.45$; $P = 0.51$).

H. bacteriophora demonstrated similar spatial dynamics to *S. carpocapsae* when examined at a fine scale (Trial 4). However, *H. bacteriophora* displayed greater variability in aggregation values after release than *S. carpocapsae* and no discernable trends emerged as populations declined. All wetting agent-treated *H. bacteriophora* microplots were not significantly different from a random distribution, though most trended towards uniformity (average $I_a = 1.01$; $P \geq 0.04$). Three of 16 untreated datasets demonstrated significant spatial aggregations, including two of four 0 DAT datasets.

Although spatial analyses did not detect significant uniformity in EPN dispersion patterns for either species or treatment, a high percentage of EPN-positive soil samples were found between 0-7 and 0-14 DAT for *H. bacteriophora* and *S. carpocapsae*, respectively (Figure 4). *S. carpocapsae* was detected in more than 90% of the samples between application and 7 DAT, yet only a moderate percentage ($> 50\%$) of samples throughout the 28-day observation period. *H. bacteriophora* was detected in a similar high percentage of soil samples for half the amount of time as that of *S. carpocapsae* treatments, and after which populations became increasingly more variable between plots of the same treatment.

3.2.3 Spatial associations

Despite differences in the ability of wetting agents to provide greater average soil moisture across the microplots, few statistically significant associations were detected between the distribution of soil moisture and either nematode species. None of the *H. bacteriophora* spatial patterns were observed to be associated with that of moisture (Table 2), and only four of 32 *S. carpocapsae* (12.5 %) patterns were observed to be correlated with soil wetting patterns (Table 2). Three associations were positive, including two from untreated plots. One wetting agent-applied plot showed dissociation in spatial patterns between a significantly aggregated *S. carpocapsae* pattern with a random moisture pattern (Block 1, 7 DAT).

4. DISCUSSION

This study represents the first of its kind to describe changes in soil moisture patterns and its effects on released EPN persistence and distribution in turfgrass. Using spatially explicit models and a fine sampling grain we were able to assess the strength of associations between the distributions. Soil moisture is believed to be one of the most critical variables in the survival, persistence, and ultimately the successful use of EPNs mass released for the biological control of soil dwelling insects (Stuart et al., 2006). The findings from this study suggest that factors other than soil moisture, possibly those influencing nematode application, survival, and infectivity at the time of application or immediate hours following application, may have a greater influence on EPN persistence and spatial uniformity in turfgrass.

Contrary to our hypothesis, one-time applications of turfgrass wetting agents failed to improve the fine-scale distribution of soil moisture patterns in either sand-based or native soil turfgrass rootzones. Wetting agents have been long used in turfgrass management to ameliorate

water repellency and increase water infiltration rate (Kostka 2000). Additionally, secondary benefits including improved rooting and visual quality are believed to be a result of increased moisture content in the hydrophobic portion of the soils (Karnok and Tucker, 2001). Although evidence was found for improved moisture content with wetting agent application, we did not observe improved soil moisture uniformity at a fine-scale, which is likely to be due to several factors including the frequency of wetting agent application, degree of hydrophobicity of the soils, variability of soil moisture dynamics on a microscale, and/or a combination of these factors. Single applications are unlikely to make significant improvements to the overall moisture content of the soil, especially those where hydrophobicity is not severe. Karnok and Tucker (2001) hypothesized that the effect of a surfactant application to soils with little to no thatch and the absence of hydrophobic conditions would likely be minimal. Although we did not measure the degree of hydrophobicity of the soils, the inability to improve soil moisture uniformity in the sand-based rootzone (Trials 1 and 3) trials may account for the lack of a treatment effect, since these soils averaged less than 0.125 cm thatch and approximately 1.5 % organic matter. Greater thatch levels were observed in the fine textured, native soil plots (Trials 2 and 4), though uniformity was not improved. However, strong statistical differences were observed in the average overall volumetric content of fine-scale plots for both soil types. These improvements were independent of time of the year, environmental conditions, turfgrass canopy length, soil texture, and average moisture levels. This indicates that both soils had some degree of hydrophobicity and that sampling effort relative to the area sampled was a factor in detecting differences between treatments in short-term persistence trials.

Both EPN populations experienced substantial declines between 0 and 3 days after release. These declines were approximately 15% d⁻¹, which is similar to what has been reported

in other agricultural systems (Smits 1996). Abiotic factors including UV radiation, soil pH, texture, temperature, and moisture have all been shown to have detrimental effects on the short-term persistence and/or infectivity of EPNs. However, it is believed that immediate, sharp declines are attributable to desiccation and UV radiation (Curran 1992, Gaugler et al., 1992). As little as 30 or 60 mins of exposure to sunlight can inactivate *H. bacteriophora* and *S. carpocapsae*, respectively. However, studies conducted in tall fescue sod to assess UV degradation in the field did not find differences in *H. bacteriophora* persistence when applied on the turf surface or below-ground (Wilson and Gaugler, 2004). Extreme care was provided in our studies to minimize UV radiation, including early morning applications, and providing adequate carrier and post-application irrigation. Soil temperatures and moisture may have played a role in the decline of *S. carpocapsae* in the weeks following application, though these variables were not outside optimal ranges (< 10% volumetric water content; > 30° C) in the days following application (Grewal et al., 1994), nor do they explain the dramatic declines in *H. bacteriophora* populations. Edaphic conditions, including thatch, organic matter accumulation in the soil, and total porosity may have played a larger role in the *H. bacteriophora* declines, as these factors may restrict EPN movement and persistence as measured by infectivity of bait insects (Georgis and Gaugler, 1991).

Biotic factors may also influence the short-term persistence of EPNs. We did not measure the natural enemy assemblages present in the turf, but several studies have either made direct observations of predation or have inferred a negative relationship between natural enemies (e.g. mites, collembolans, nematode-trapping fungi) and EPN persistence (Epsky et al., 1988; Gillmore and Potter, 1993; Wilson et al., 2003; Ulug et al., 2014). Other biotic factors, such as the energetic demands placed on EPNs, may work in concert with abiotic factors to reduce post-

application performance. Fine-textured soils have much smaller pore spaces compared to the sand-based rootzones, and therefore may create anoxic conditions when filled with water. EPNs exposed to poor aeration as a result of being released in fine-textured soils consume stored carbohydrates and thus potential expire quicker than in a well-aerated soil (Croll and Matthews, 1977). Native soil (*H. bacteriophora*) trials experienced extended periods with high average soil moisture content. EPNs may be limited in their ability to disperse in soils with relatively high soil moisture contents, although it is believed that *H. bacteriophora* may be less affected than other nematode species (Portillo-Aguilar et al., 1999). A similar interaction between abiotic and biotic factors may also limit short-term persistence of *S. carpocapsae*. The nictating foraging behavior of the nematode is likely to lead to frequent local extinctions, given its activity at the soil surface and exposure to UV and desiccating environments. However, its potential to infect a wide variety of mobile, surface-dwelling insects likely allows for greater dispersal, recolonization, and persistence at low densities.

The spatial distributions of EPNs following application is of equal importance as persistence in inundative biological control programs given their relatively small size and inability to move independently over great distances (< 1 and 5 mm min^{-1} *S. carpocapsae* and *H. bacteriophora* on agar, respectively) (Campbell and Gaugler, 1993). Our understanding of EPN population spatial dynamics in turfgrass is limited, with most studies focusing on endemic populations in low-maintenance systems (e.g. home lawns, grasslands). Endemic populations are inherently patchy, with their spatial distribution arising from high reproductive outputs following the infection of a host and their limited dispersal capabilities. Previous work by Campbell et al. (1995, 1996, 1998) has determined that EPN populations in turfgrass are patchy at a field scale, and independent of neighboring populations. Fine-scale studies of *Steinernema*

spp. in grassland pastures also support patchiness, though the degree of aggregation changed with IJ age (Spiridonov et al., 2007). Our study of released EPNs not only supports EPN patchiness at a fine-scale over time, but more importantly adds support to the growing evidence of the difficulties of generating and/or maintaining a uniform spatial pattern using agricultural spray equipment. Nozzle type, agitation/circulation machinery, pressure, and droplet size may all contribute to the difficulties in uniformly applying nematodes to a surface (Georgis 1990; Lello et al., 1996; Brusselman et al., 2012; Beck et al. 2013). Poor deposition may occur when larger nematodes are applied through nozzles with small orifices (Beck et al., 2013). However, much of this research has been conducted on crops with greater vertical plant architecture than turfgrass where foliar-application uniformity is dependent on nematodes attaching to a substrate.

Although both *H. bacteriophora* and *S. carpocapsae* population densities were patchily distributed at a fine-scale, a high percentage of positive samples (i.e. at least one infected bait insect per sample) was found throughout much of the observation period. The relative number of cadavers killed in adjacent plots between 0-7 and 0-14 DAT for *H. bacteriophora* and *S. carpocapsae* (respectively) varied greatly, and likely led to inability to detect uniformity through S.A.D.I.E. analyses. Thus, an even finer grain than our sample unit (30 × 30 cm) may be needed in future studies, especially those aimed at understanding movement of populations from released sites. Improving uniformity of releasing thousands of microscopic organisms per square meter is likely to be a challenge, given the sheer number and inherent variability of populations. However, new approaches including split applications (Fenton et al., 2002) and improvements through classical selection (Shields 2015) have shown promise in improving biological control efficacy in other systems.

5. Conclusions

EPN population dynamics are influenced by numerous biotic and abiotic factors. A greater understanding of these factors could lead to optimization of biological control programs, and possibly improve competitiveness with synthetic insecticides. Wetting agent applications to turfgrass soils at the time of nematode release failed to produce meaningful improvements in the persistence of EPN populations, despite improvements in average soil moisture content. Our fine-scale analysis of moisture patterns and EPN population dynamics challenge current understanding regarding the associations between EPN persistence and soil moisture, and highlight the need for more research on improving uniformity through application technologies and a thorough understanding of the biotic factors influencing nematode survival immediately after release.

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Figure 1: Environmental conditions following release of *Steinernema carpocapsae* (A) or *Heterorhabditis bacteriophora* (B), average number of entomopathogenic nematode-killed bait insects (C-D) and average volumetric water content (E-F) in 1.52 x 1.52 m turfgrass plots treated with soil wetting agents (Trials 1 and 2). *Steinernema carpocapsae* (A, C, E) was released into short-mown golf course turf (*Agrostis stolonifera* at 1.25 cm) grown in a sandy loam on 18 May, 2015. *Heterorhabditis bacteriophora* (B, D, E) was released into low-maintenance lawn turf (*Lolium perenne-Poa* at 5 cm) grown in a clay loam on 8 September, 2015.

Figure 2: The spatial distributions of volumetric content in sand-based rootzone (Experiment 2: *S. carpocapsae*) plots as determined by S.A.D.I.E. analyses. The contour map was generated from 25 regular sample points from within a 1.52 × 1.52-m sample area by plotting local clustering values from SADIE analyses. Values >1.5 (areas in red) indicate clusters and contribute to local aggregations or patches of large counts in close proximity. Values < -1.5 indicate gaps (blue) or zeroes and low counts in close proximity to one another. I_a and P_a values beneath each map indicate the aggregation value of the overall spatial pattern and the associated significance test of the spatial pattern's departure from randomness. I_a values indicate an aggregated (>1), uniform (<1), and random distribution (close to 1). Significance of the spatial aggregation is assumed at $P < 0.025$.

Figure 3: The spatial distributions of volumetric content in fine-texture rootzone (Experiment 2: *H. bacteriophora*) plots as determined by SADIE analyses. The contour map was generated from 25 regular sample points from within a 1.52 × 1.52-m sample area by plotting local clustering values from SADIE analyses. Values >1.5 (areas in red) indicate clusters and contribute to local

aggregations or patches of large counts in close proximity. Values < -1.5 indicate gaps (blue) or zeroes and low counts in close proximity to one another. I_a and P_a values beneath each map indicate the aggregation value of the overall spatial pattern and the associated significance

Figure 4: Spatial Analysis by Distance Indices (S.A.D.I.E.) aggregation values (I_a) and the percentage of soil samples that were capable of killing *Galleria mellonella* bait insects in *S. carpocapsae* (May 2015) and *H. bacteriophora* (September 2015) microplot studies. Each data point represents a value from a 1.52 x 1.52 m plot, subdivided into 25- 30x30 cm subplots or samples. I_a values indicate an aggregated (>1), uniform (<1), and random distribution (close to 1).

Table 1: Summary of experiments including wetting agent treatments, individual plot size, entomopathogenic nematode species and release date, site characteristics, and frequency of EPN population sampling.

Experiment	Plot Size	Treatments	EPN	Date	Turfgrass (HOC) ¹	Soil texture (% sand/silt/clay)	EPN monitoring ²
Experiment 1: Nematode persistence	1.52 × 1.52 m	Revolution® (Aquatrols Inc.)	<i>Steinernema carpocapsae</i> (Trial 1)	18 May 2015	<i>Agrostis stolonifera</i> (1 cm)	Sandy loam (80/15/5)	0, 3, 7, 14, 21, 28 days DAT
		ACA 2994 (Aquatrols Inc.)					
		ACA 3162 (Aquatrols Inc.)					
		Untreated					
			<i>Heterorhabditis bacteriophora</i> (Trial 2)	8 September 2015	<i>Poa pratensis-Lolium perenne</i> (5 cm)	Sandy clay loam (75/22/3)	0, 3, 7, 14 DAT
Experiment 2: Fine-scale spatial distribution	30 × 30 cm	Revolution®	<i>Steinernema carpocapsae</i> (Trial 3)	18 May 2015	<i>Agrostis stolonifera</i> (1 cm)	Sandy loam (80/15/5)	3, 7, 14, 28 DAT
		Untreated					
			<i>Heterorhabditis bacteriophora</i> (Trial 4)	8 September 2015	<i>Poa pratensis-Lolium perenne</i> (5 cm)	Sandy clay loam (75/22/3)	0, 3, 7, 14 DAT

¹ HOC = height-of-cut

² DAT = Days after treatment

Table 2: Spatial relatedness (X) between entomopathogenic nematode species (*S. carpocapsae*, *H. bacteriophora*) and volumetric water content and the associated probability level (P_a) in microplots. X values greater than 0 are considered significantly associated when $P < 0.025$ and significantly dissociated with negative X values and when $P > 0.975$. Bolded figures represent significant spatial patterns.

		0 DAT			3 DAT		7 DAT		14 DAT		28 DAT	
		Block	X_a	P_a	X_a	P_a	X_a	P_a	X_a	P_a	X_a	P_a
<i>S.</i> <i>carpocapsae</i>	Wetting-agent	1	-	-	0.093	0.3413	-0.437	0.9787	-0.058	0.5974	-0.293	0.9207
		2	-	-	-0.163	0.7573	0.338	0.0805	-0.355	0.9287	1.268	0.6820
		3	-	-	0.562	0.0071	-0.278	0.8936	0.065	0.3964	-0.302	0.9196
		4	-	-	-0.304	0.9036	-0.045	0.5649	-0.173	0.7485	-0.339	0.9113
	Untreated	1	-	-	-0.130	0.5033	0.251	0.1052	-0.027	0.5423	0.370	0.2580
		2	-	-	0.058	0.3910	-0.026	0.5627	-0.165	0.7556	-0.904	0.6964
		3	-	-	0.177	0.1872	0.468	0.0102	-0.020	0.5511	0.024	0.4879
		4	-	-	0.167	0.2448	-0.109	0.6925	0.117	0.3079	0.146	0.2509
<i>H.</i> <i>bacteriophora</i>	Wetting-agent	1	0.239	0.844	-0.125	0.711	0.050	0.441	-0.267	0.854	-	-
		2	0.085	0.358	0.402	0.027	-0.176	0.782	0.095	0.376	-	-
		3	0.278	0.901	-0.233	0.853	0.091	0.339	-0.331	0.908	-	-
		4	0.016	0.533	-0.008	0.516	0.189	0.268	-0.013	0.532	-	-
	Untreated	1	0.157	0.268	0.145	0.257	-0.035	0.575	0.343	0.067	-	-
		2	0.128	0.292	0.204	0.193	0.166	0.253	0.285	0.098	-	-
		3	0.223	0.198	-0.174	0.753	-0.065	0.598	-0.245	0.801	-	-
		4	-	-	-	-	-	-	-	-	-	-
	4	0.218	0.826	0.202	0.171	0.047	0.427	0.197	0.208	-	-

Untreated

Wetting-Agent Treated

Untreated

Wetting Agent-Treated

0 DAT

3 DAT

7 DAT

14 DAT

S. carpocapsae *H. bacteriophora*