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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.

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 indundative 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,

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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 × 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

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

[a] HOC = height-of-cut.
[b] DAT = Days after treatment.