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Geospatial patterns of soil properties and the biological control potential of entomopathogenic nematodes in Florida citrus groves

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ABSTRACT

Entomopathogenic nematodes (EPNs) are widely distributed in natural and managed ecosystems worldwide. Due to the cryptic nature of soil food webs, EPN ecology and their role in modulating insect population dynamics remain largely a matter of speculation. A weevil pest of citrus, *Diaprepes abbreviatus*, is less abundant in orchards on the central ridge (hilly topography, deep, coarse sand soils) than in the flatwoods (flat topography, fine sand soils with a high water table). We speculate that native EPNs are a key factor regulating these weevils and thus hypothesized that EPNs are most abundant and/or species diverse in central ridge orchards. In this study, we measured and analyzed the natural distributions of EPNs in these two regions concomitantly with those of selected abiotic and biotic soil components. Our objective was to identify physical properties that can potentially be manipulated to conserve native EPNs that serve to control *D. abbreviatus*. We used species-specific qPCR probes for *i*) 13 EPN species, *ii*) two species of *Paenibacillus* that are ectoparasitically associated with EPNs, *iii*) free-living bacteriophagous nematodes (*Acrobeloides*-group) that might compete with EPNs, and *iv*) oomycete pathogens of citrus roots, *Phytophthora nicotianae* and *Phytophthora palmivora*. Citrus orchards were surveyed in eco-regions categorized as central ridge (23 localities) and flatwoods (30 localities). EPNs and *Acrobeloides*-group were detected in all sites and the abundances of the two guilds were positively related. Heterorhabditids comprising two species occurred in more localities at higher numbers than did five steinernematid species. *Heterorhabditis indica* dominated flatwoods communities, whereas communities with abundant *Steinernema diaprepesi*, *Heterorhabditis zealandica* and *H. indica* occurred on the central ridge. Spatial patterns of *S. diaprepesi* were more aggregated than those of *H. indica* and other dominant species. The central ridge supported greater EPN evenness, diversity and species richness. For the first time, quantitative natural positive associations between EPNs and two species of *Paenibacillus* bacteria were assessed. The oomycete pathogen *P. palmivora* was only detected in the flatwoods, whereas *P. nicotianae* was widespread and equally abundant in both regions. Four variables that affect soil water potential (groundwater depth, available water capacity, clay and organic matter content) significantly contributed to explain the variability in a redundancy analysis of the selected soil communities. Management of soil water potential may aid in establishing and conserving diverse EPN communities that provide more effective control of *Diaprepes* root weevils.

1. Introduction

Florida citrus orchards are planted in eco-regions known broadly as central ridge and flatwoods based on soil texture and water drainage. The central ridge is comprised of deep, coarse

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sandy soils at higher elevation throughout inland areas of the peninsula. Flatwoods soils are more variable in texture, extending from the coasts to medium elevation inland areas. They are shallower and less well drained than soils on the central ridge. The root weevil *Diaprepes abbreviatus* L. (Coleoptera, Curculionidae) is one of the most important biotic threats to citrus (Dolinski et al., 2012). The subterranean larvae feed on roots causing extensive loss of cortical tissue and promoting infection by plant pathogenic *Phytophthora* spp. This pest-disease complex causes fruit loss and serious tree decline in orchards. The motile zoospores of *Phytophthora* spp. infect roots most readily in poorly drained soils, which occur more commonly in flatwoods than in ridge orchards (Graham et al., 2003). Moreover, weevil populations tend to be significantly larger and therefore more damaging in flatwoods orchards than in those on the central ridge (McCoy et al., 2000; Futch et al., 2005). The possible involvement of natural enemies in the *D. abbreviatus* spatial pattern was proposed by Duncan et al. (2003) who found that sentinel weevil larvae were killed at higher rates by a diverse entomopathogenic nematode (EPN) community in a central ridge orchard with few weevils, than in a flatwoods orchard with abundant weevils and just a single EPN species.

Entomopathogenic nematodes (EPNs) belonging to the genera *Steinernema* and *Heterorhabditis* are known to occupy all continents, except Antarctica (Hominick, 2002; Adams et al., 2006). EPNs have a mutualistic association with enteric γ-Proteobacteria carried by the migratory, third-stage infective juvenile (IJ) nematode (Boemare, 2002). The nematode acts as a vector for the bacteria to reach the insect hemocoel, and both organisms contribute to insect death (Dillman et al., 2012; Sugar et al., 2012). The nematodes and bacteria then begin parallel development in the cadaver, and the nematodes produce several generations until food and waste become limiting, at which time development arrests at the IJ stage. Infective juveniles incorporate bacteria in the anterior intestine or in a specific vesicle and leave the cadaver in search of new hosts (Boemare, 2002).

Citrus growers in Florida have used commercially formulated products containing various EPN species for more than two decades to reduce the numbers of *D. abbreviatus* larvae in soil (Georgis et al., 2006; Dolinski et al., 2012). During trials to characterize the effectiveness of these products against sentinel *D. abbreviatus* larvae buried in cages beneath trees, a significant presence of native EPNs became apparent. An unknown EPN frequently isolated in central ridge orchards was described as *Steinernema diaprepesi* (Nguyen and Duncan, 2002). *Heterorhabditis indica* and *Heterorhabditis zealandica* were also encountered regularly on the central ridge in association with *S. diaprepesi* (Duncan et al., 2003, 2007; Stuart et al., 2008). By contrast, *H. indica* was the sole EPN species recovered from sentinels in most flatwoods orchards, with the exception of a second undescribed *Steinernema* sp. *glaseri*-group species that was infrequently detected (Campos-Herrera et al., 2011a). The results of laboratory and greenhouse experiments indicated that both steinernematid species persisted in soil longer and protected citrus seedlings from weevil herbivory better than either heterorhabditid (El-Borai and Duncan, 2007; El-Borai et al., 2007, 2009, 2012).

Conservation of natural enemies in the citrus tree canopy is a primary objective of pest management strategies in Florida and other citrus growing regions (Browning, 1999; Michaud, 2002). However, despite the importance of subterranean insects such as *D. abbreviatus*, no tactics are available to conserve or enhance the natural enemies of arthropod pests in the rhizosphere. The complexity of soil and a profound ignorance of the structure and function of soil food webs impede the development of such tactics, although advances in molecular methods to study cryptic communities are helping resolve this problem (Torr et al., 2007; Campos-Herrera et al., 2011a, 2011b, 2012a; Pathak et al., 2012). The EPN communities in Florida citrus orchards appear ideal for studying the conservation of beneficial natural enemies in soil because EPN species and the rates at which they kill weevil larvae have been reported to differ measurably in different locations (Stuart et al., 2008; Duncan et al., 2013). Understanding the causes of these spatial patterns could reveal new methods to induce key EPN species to provide greater pest suppression in normally nonconducive habitats.

Here we describe the spatial patterns of EPN species recovered from citrus orchards across Florida's central ridge and flatwoods regions. We employed qPCR primers and probes to characterize these communities and several additional organisms likely to interact with EPNs. Ectoparasitic *Paenibacillus* bacteria can attach in large numbers to the EPN cuticle, thus impeding nematode motility and host finding (El-Borai et al., 2005). A free-living, bacteriophagous nematode (FLBN) in the *Acrobeloides*-group was reported to be a competitor of EPNs for nutrients in the cadaver (Campos-Herrera et al., 2012a). The oomycete root pathogens *Phytophthora nicotianae* and *Phytophthora palmivora* are more virulent in citrus roots damaged by weevil herbivory (Graham et al., 2003). Because orchards on the central ridge tend to have fewer *D. abbreviatus* larvae and healthier root systems (McCoy et al., 2000; Futch et al., 2005) we hypothesized that we would find more abundant and/or diverse EPN communities, fewer natural enemies of EPNs and less abundant levels of *Phytophthora* spp. on the central ridge than in the flatwoods. We also characterized the sites with respect to edaphic physical and chemical properties in order to explore relationships between soil properties and soil communities. Our objective was to identify specific physical properties that can potentially be manipulated to conserve populations of native EPNs in order to increase biological control of *D. abbreviatus*.

2. Materials and methods

2.1. Survey design, sampling methods and chemical analyses

A total of 53 Florida citrus orchards known to be infested by the *Diaprepes* root weevil were surveyed during summer and early autumn 2009–2010. In most orchards, two composite samples were recovered from the same 2–3 ha area. Each sample was composed of 30 single soil cores (2.5 cm dia. × 30 cm deep), collected individually from the under-canopies of 30 trees (i.e., ca. 1400 cm^3 soil per sample). The samples were transported to the laboratory in coolers and processed within 16–32 h. The localities were selected to be representative of the central ridge ($n = 23$) and flatwoods ($n = 30$) eco-regions (http://www.plantmaps.com/interactive-florida-ecoregions-l4-map.php) (Supplemental data 1). Sampling sites in each orchard were named for an owner or locality, georeferenced and the elevation determined from Google Earth® (data not shown). Estimates of groundwater depth (GWD) were recovered from the Web Soil Survey (USDA, Natural Resources Conservation Service, USA; http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx).

Each composite sample was gently mixed and nematodes were extracted by sucrose centrifugation from two 500 cm^3 subsamples (Jenkins, 1964). This method also recovers microorganisms present in or on the nematodes. Contents of the two independent extractions were then combined in a single test tube to provide a corresponding combined nematode extraction of 1 L per sample ($n = 2$ per locality, most of the cases). After nematodes settled in the tube, excess water was aspirated, nematodes were concentrated in 1.5 mL Eppendorf tubes and stored at −20 °C until DNA extraction procedures (Campos-Herrera et al., 2011a).