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Modifying soil to enhance biological control of belowground dwelling insects in citrus groves under organic agriculture in Florida

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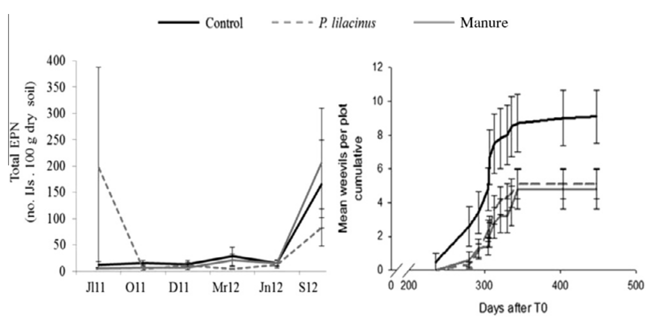
HIGHLIGHTS

- Manure and biocontrol fungi approved for organic citriculture were evaluated in the field.
- Impact on the citrus soil food web (weevil, nematodes, fungi and bacteria) were evaluated.
- Amendments did not produce major changes in the entomopathogenic nematode community.
- Both amendments marginally reduced the number of weevils emerging from soil.
- The two amendments might contribute to protection of citrus under organic production.

GRAPHICAL ABSTRACT



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ABSTRACT

An emerging organic citrus industry in Florida could benefit greatly from effective, non-conventional methods to mitigate losses from pests and diseases. We studied part of a soil food web in an organic orchard to learn ways to conserve and enhance biological control of insect pests by native entomopathogenic nematodes (EPNs). We evaluated two OMRI (Organic Materials Review Institute) approved cultural practices: (i) a mulch of commercially pelleted chicken manure, (ii) a commercial formulation of *Purpureocillium lilacinus*, and (iii) an un-amended control. Several soil nutrients (i.e. nitrogen, phosphate, and potassium) were affected by the amendments, but initial equilibrium values (T0) were restored by the last sampling time (T12). The plant parasitic nematode *Tylenchulus semipenetrans* increased in both treatments compared to the untreated control at T3 ($P < 0.05$). The oomycete *Phytophthora nicotianae* increased in the *P. lilacinus* plots at T1, marginally at T12, but decreased at T6 and T9. *Steinernema diaprepesi*, *Heterorhabditis indica* and *Heterorhabditis zealandica* were the only EPNs regularly detected in the orchard. Mulch increased numbers of *H. zealandica* at T6 and T9 ($P < 0.05$) and free living nematodes at T12 ($P < 0.01$). The nematophagous fungus (NF) *P. lilacinus* persisted in plots where it was augmented ($P < 0.05$), reaching a maximum level at T3 that was 17.5-fold greater than that in controls. Numbers of *Paenibacillus* sp. were directly related to both those of *S. diaprepesi* and *Acrobeloides*-group nematodes ($P < 0.01$), but inversely to the FLN counts ($P < 0.05$). The application of these two amendments did not produce strong changes in the EPN community but decreased the emergence from soil of adult *Diaprepes abbreviatus*, a root weevil pest. Thus, both amendments might contribute to citrus pest management under organic production.

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1. Introduction

Conventional agriculture produces high yields by means of large resource inputs and prophylactic, often unnecessary, plant protection treatments (Gutiérrez et al., 2005; Pimentel et al., 2005). This production system introduces into the ecosystem numerous synthetic substances with a broad spectrum of activity, substantial toxicity and long persistence in the environment, giving rise to serious concerns about public health and environmental pollution (Schuman, 1993; Pimentell, 2005; Pimentel et al., 2005). These substances can also have negative effects on agricultural production if they kill natural enemies of herbivores, or induce the selection of pesticide resistant pest species.

Social demand for quality food products, health and environment protection has induced national and international initiatives and even government regulations aimed at fostering more sustainable agricultural practices, such as reduced tillage and integrated pest management. So called organic production integrates appropriate cultural techniques (i.e. crop rotation, planting dates, etc.), biological pest controls and naturally occurring chemicals, to help manage pests while maintaining reasonable yield with a significantly reduced environmental impact (Rechcigl and Rechcigl, 2000; Horowitz and Ishaaya, 2004). Recent studies demonstrated that the reduced yield typical of organic compared to conventional production ranges from 5% to around 30% and is due variously to the type of crop, the local conditions (i.e. soil type) and the implementation of the crop management methods (Seufert et al., 2012). Organic management can mitigate the ecological disequilibrium induced by the culture of a single or very few plant species if it employs practices that promote evenness among natural enemies in addition to richness (Crowder et al., 2010). Hence, successful production under organic agriculture requires a strong core of knowledge about the complexity of the agro-ecosystem. The identification of key limiting factors related to the crop, its herbivores and their native natural enemies can help develop tactics to preserve and enhance those indigenous species that are capable of controlling pests and pathogens.

Entomopathogenic nematodes (EPN) of the genera *Steinernema* and *Heterorhabditis* are some of best non–chemical alternatives for managing insect pests in numerous crops worldwide (Georgis et al., 2006; Kaya et al., 2006; Dolinski et al., 2012). These nematodes are known for their ability to quickly kill their host thanks to the mutualistic enteric γ-Proteobacteria, carried by the infective juvenile (IJ) nematode (Boemare, 2002). This non-feeding IJ stage is naturally occurring in soils of natural and agricultural areas where it must survive in the environment until it infects a suitable host. Once inside the insect hemocoel the bacteria are liberated, the host is killed and both organisms reproduce until the resources become limiting (Boemare, 2002; Dillman et al., 2012). The lack of food induces the nematodes to arrest development at the IJ which store new bacteria and exit the cadaver in search of new hosts (Boemare, 2002).

EPNs are perfectly suited for organic agriculture because they occur commonly in soils throughout the world, making them potentially amenable to conservation for biological control (Adams et al., 2006). Conservation of native natural enemies can help avoid the introduction of exogenous organisms that might displace native populations (Hummel et al., 2002) or introduce chemical compounds needed for their commercial formulation. EPN activity is modulated by biotic and abiotic factors, and their efficacy depends on soil characteristics, agricultural management, and the multitrophic food web interactions occurring in the rhizosphere (Stuart et al., 2006). Numerous studies have reported the effects of soil properties on EPN biology (Barbercheck and Duncan, 2004; Hoy et al., 2008; Campos-Herrera et al., 2013a), whereas the impact of the soil community has only recently received much attention (Jabbour and Barbercheck, 2011; Hodson et al., 2012; Ulug et al., 2014; Zenner et al., 2014). Duncan et al. (2007) reported that composted manure mulches reduced the abundance of some nematophagous fungi (NF) in a citrus orchard, while increasing the numbers of buried sentinel weevil larvae that were killed by native EPNs (Duncan et al., 2007). The augmentation of EPNs in the orchard temporarily increased the numbers of sentinel weevils killed by EPN, but also increased the abundance of NF. Subsequently, the number of weevils killed by EPNs decreased below that in untreated trees, most likely the result of increased natural enemies of EPNs. This type of trophic cascade was reproduced in the laboratory where the survival of EPNs depended on the temporal responses of NF to nematode augmentation (El-Borai et al., 2007).

The recent development of new sampling technologies based on PCR has contributed to characterizing the structure and function of the citrus rhizosphere community (Campos-Herrera et al., 2013b). More than 20 species-specific molecular probes were employed in field experiments to demonstrate that a new citrus management system, developed to combat a devastating bacterial pathogen, altered the soil food web in ways that increased another serious pest–disease complex (Campos-Herrera et al., 2013c, 2014). The richness and species composition of EPN communities in different eco-regions were linked to different patterns of herbivory associated with the citrus root weevil *Diaprepes abbreviatus* and to specific soil properties (Campos-Herrera et al., 2013a).

Despite reports that habitats with rich EPN communities and high EPN activity against sentinel weevil larvae tended to have smaller weevil populations (Futch et al., 2005; Duncan et al., 2003, 2007), Campos-Herrera et al. (2013a) found no evidence of greater EPN density in eco-regions with fewer *Diaprepes* weevils. Therefore, if EPN affect the spatial pattern of root weevils in Florida, their role is likely modulated by factors other than their population size (e.g., EPN community richness or species composition, effects of edaphic properties). Several soil properties related to soil water potential (content of clay and organic matter, water holding capacity and depth to ground water) were associated with the species composition of EPN communities, and manipulation of water potential may be a means of enhancing EPN activity. Similarly, the addition of coarse sand to the planting holes of citrus trees in a poorly drained orchard helped conserve EPN activity and reduced weevil numbers compared to trees planted in the fine textured native soil (Duncan et al., 2013).

Amending soil with naturally occurring substances such as animal manure or biological control agents is one of the limited IPM tactics available in organic agricultural systems. The objective of this study was to characterize the effects of two soil amendments on the food web composed of native EPNs, some of their natural enemies and the herbivores *D. abbreviatus* and *Phytophthora* spp. which form a pest-disease complex that causes major economic losses in citrus in Florida and the Caribbean basin (Graham et al., 2003). We hypothesized that mulching with the recommended amount of chicken manure would reduce NF and increase EPNs, albeit less dramatically than reported by Duncan et al. (2007) who used higher than recommended amounts of manure. We also hypothesized that amending soil with the nematophagous fungus species *Purpureocillium lilacinus* would reduce certain plant parasitic nematodes that are exposed in egg masses on the root surfaces (Mittal et al., 1995; Parajuli et al., 2014), without affecting EPNs which reproduce within the insect host cadavers.

2. Material and methods

2.1. Experimental design, sampling procedures and chemical analyses

The experiment was conducted in a commercial citrus orchard located in the central ridge eco-region near Bartow (Florida,