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Entomopathogenic nematodes, root weevil larvae, and dynamic interactions among soil texture, plant growth, herbivory, and predation

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ABSTRACT

Greenhouse experiments were conducted to assess the influence of soil texture on the persistence, efficacy and plant protection ability of entomopathogenic nematodes (EPNs) applied to control larvae of the *Diaprepes* root weevil (DRW), *Diaprepes abbreviatus*, infesting potted citrus seedlings. Seedlings were grown in pots containing either coarse sand, fine sand, or sandy loam. Three DRW larvae were added to each of 80 pots of each soil type. 24 h later, 20 pots of each soil type that had received weevil larvae were inoculated with EPN infective juveniles (IJs) of one of the following species: *Steinernema diaprepesi*, *Steinernema riobrave* and *Heterorhabditis indica*. Pots of each soil without EPNs were established as controls with DRW and controls without DRWs. Subsequently, pots with larvae received three additional larvae monthly, and the experiment continued for 9 months. Plant root and top weights at the end of the experiment were affected by both soil ($P \leqslant 0.0001$) and nematodes ($P \leqslant 0.0001$), and nematode species protected plants differently in different soils (interaction $P \leqslant 0.0001$). Soil porosity was inversely related to plant damage by DRW, whether or not EPNs were present; and porosity was directly related to the level of plant protection by EPNs. Mortality of caged sentinel weevil larvae placed in pots near the end of the experiment was highest in pots treated with *S. diaprepesi*. In a second, similar experiment that included an additional undescribed steinernematid of the *Steinernema glaseri*-group, soil type affected root damage by DRW and root protection by EPNs in the same manner as in the first experiment. Final numbers of *S. diaprepesi* and *Steinernema* sp. as measured by real-time PCR were much greater than those of *S. riobrave* or *H. indica* in all soils. Across all treatments, the number of weevil larvae in soil at the end the experiment was inversely related to soil porosity. In all soils, fewer weevil larvae survived in soil treated with *S. diaprepesi* or *Steinernema* sp. than in controls with DRW or treatments with *S. riobrave* or *H. indica*. The results of these experiments support the hypothesis that EPNs provide greater protection of seedlings against DRW larvae in coarse textured soil than in finer textured soil. However, less vigorous growth of the control without DRW seedlings in the two finer textured soils suggests that unidentified factors that stressed seedlings in those soils also impaired the ability of seedlings to tolerate weevil herbivory.

1. Introduction

Florida citrus growers have employed various tactics to manage the *Diaprepes* root weevil (DRW), *Diaprepes abbreviatus*, since it was first detected in the state in 1964 (McCoy, 1999; Shapiro-Ilan et al., 2002; Woodruff, 1964). DRW larvae cause major damage to citrus trees by feeding on the cortex of structural roots. Besides greatly reducing the cortical tissue, particularly in the crown area, the wounds can enhance infection by the plant parasitic fungi, *Phytophthora parasitica* and *Phytophthora palmivora*, and the resulting *Phytophthora–Diaprepes* complex has the potential to kill large numbers of trees annually (Graham et al., 2003; Stuart et al., 2004).

DRW occurs in all citrus growing areas of the state but its economic impact is greater in some areas than in others (Futch et al., 2005; McCoy et al., 2002, 2004; Stuart et al., 2008). Florida's citrus growing regions can be broadly categorized based on soil type and soil depth. An elevated 'central ridge' of deep, well drained, coarse sandy soil runs north and south through most of the central peninsula. The central ridge soils typically contain between 96% and 98% sand and a majority of the sand particles are medium to coarse in size (>0.25 mm wide). The remaining citrus growing regions surrounding the central ridge are broadly termed 'flatwoods'. The flatwoods soils vary in texture and drainage, but tend to be shallow, often requiring the construction of raised planting beds in order

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to allow sufficient root growth above the water table. Sandy flatwoods soils containing more than 90% sand are common, but contain less medium-to-coarse sized particles than soils of the central ridge. Economic losses to DRW are greatest in flatwoods orchards, particularly those with fine textured soil or poorly drained sites conducive for *Phytophthora* spp. to infect and cause root damage (Graham et al., 2003). Greater tree damage in the flatwoods is also an apparent function of larger weevil populations in flatwoods groves than in ridge groves. A 3-year survey of adult weevils reported average densities to be an order of magnitude higher in central and coastal flatwoods orchards compared to those on the central ridge (Futch et al., 2005).

DRW spatial patterns in Florida might depend at least in part on the conduciveness of soils to larval predation by entomopathogenic nematodes (EPNs) (Duncan et al., 2003; Stuart et al., 2008). Soil porosity was found to be positively associated with EPN predation rates in a number of studies (Barbercheck, 1992; Barbercheck and Kaya, 1991; Campos-Herrera and Gutiérrez, 2009; Hara et al., 1991; Hazir et al., 2003; Kaspi et al., 2010; Kung et al., 1990; Liu and Berry, 1995; McCoy et al., 2003; Portillo-Aguilar et al., 1999; Rueda et al., 1993; Stuart et al., 2006; Zhang et al., 1992), and predation of DRW by EPNs during 2 years was much greater in a central ridge orchard on coarse sand than in a flatwoods orchard on finer textured, sandy loam soil (Duncan et al., 2003). In contrast, Shapiro et al. (2000) demonstrated greater efficacy by two EPN species against DRW in fine textured compared to coarse textured soils at a standard water potential and speculated that greater water content might have favored EPNs in the finer soil. They suggested the need to evaluate the effect of soil texture across a range of water potentials typically encountered in the field. Thus, relationships and interactions between soil texture, other physical variables and EPN predatory behavior are complex and poorly understood. Moreover, the effects of soil type on tritrophic interactions among citrus, DRW and EPNs have not been studied under controlled conditions. In this paper we compare the growth of citrus seedlings in three Florida soils, with or without DRW larvae, and either inoculated or not inoculated with various EPN species. Our hypotheses were that, (1) soil type does not affect DRW damage to citrus seedlings but that (2) predation by EPNs on DRW and (3) plant damage mitigation afforded by EPNs are directly related to the porosity of the soils.

2. Materials and methods

2.1. Greenhouse experiment 1

Swingle citrumelo (*Citrus paradisi* Macf. × *Poncirus trifoliata* L. Raf.) rootstock is the most common variety used in commercial citrus production in Florida. The variety is known to be tolerant to blight, citrus tristeza virus, the plant parasitic nematode *Tylenchulus semipenetrans*, and *Phytophthora* spp., as well as cold tolerant (Stover and Castle, 2002). All plants were grown and maintained in a greenhouse (26 °C, and 60–80% RH) at the CREC in Lake Alfred, FL, USA. Three hundred, 4-month-old citrus *cv*. Swingle citrumelo seedlings were transplanted singly into plastic pots (10 × 10 × 30 cm) filled to a depth of 25 cm with soil that consisted of either coarse sand, fine sand, or sandy loam in equal numbers. The three soil types were collected from Florida citrus orchards near Lake Alfred (CREC, Polk County, coarse sand), Arcadia (DeSoto County, fine sand) and Poinciana (Kelley Block, Osceola County, sandy loam). Prior to use, soils were autoclaved and then spread in thin layers on cardboard to be air dried for 1 month. Soil properties from samples taken 6 months prior to collecting the soil used in the experiments were analyzed (Waters Agricultural Laboratories, Inc. Camilla, GA) and are shown in Table 1. Three EPN species (*Steinernema diaprepesi* (Sd), *Steinernema riobrave*, (Sr) and *Heterorhabditis indica* (Hi)) were isolated from caged sentinel DRW larvae buried in a commercial citrus orchard near Bartow, Florida. *S. riobrave* is a descendent of commercially formulated EPNs that were applied periodically to manage weevils in the orchard whereas the other EPN species are natives. Soils were packed by continually tapping the pots and pressing on the soil surface during the filling process, in order to recreate bulk densities similar to those in the field. Four weeks after planting, three DRW larvae were buried 3 cm deep in 80 pots of each soil type and, 24 h later, 50 IJs cm^{-2} (=5000 IJs per pot) of one of the EPN species in 5 mL water were pipetted onto the soil surface of 20 pots of each soil type. The insect *D. abbreviatus* larvae were reared on artificial diet developed by Beavers (1982) using procedures described by Lapointe and Shapiro (1999). The larvae were obtained from a mass culture maintained at Division Plant Industry Sterile Fly facility in Gainesville Florida, USA. Larvae used in all experiments were in the 5–7th instar. EPN were maintained in the laboratory by periodically infecting *Galleria mellonella* larvae on moist filter paper and allowing IJs to emerge in White traps (White, 1927). IJs were stored in tap water in transfer flasks at 15 °C for 1–5 days before use in experiments. In addition to the EPN treatments, a control without DRW (no weevils and no EPN; 20 replicates for each soil type) and a control with DRW (weevils but no EPN; 20 replicates for each soil type) were included.

After adding the initial weevil larvae and the IJs, the pots were arranged in a randomized block design on a central bench in the greenhouse. Pots were suspended above the bench surface and separated from each other by being held in alternating spaces in a metal grid held in a wooden frame to reduce the known propensity for movement of IJs between pots in irrigation water on bench surfaces (El-Borai et al., 2007). The pots were fertilized every second week with 9:3:6 (N:P:K) liquid fertilizer plus micronutrients (Growers Fertilizer, Lake Alfred, FL). Plants were individually watered as needed, judged by ambient temperature, soil dryness or signs of incipient wilt. Although desirable, it was impractical to use instrumentation to help standardize the soil water potential deficit at which pots were watered (Duncan and El-Morshedy, 1996; Duncan and McCoy, 2001), because herbivory caused extreme variability in plant size and evapotranspiration within and between the treatments. Greenhouse temperature was 25.5 ± 4 °C. Three DRW larvae (5–7th instar) were added to each pot (except the control without DRW or pots in which plants died) monthly for 9 months to simulate natural larval recruitment. After 6 months, the occurrence of EPN species in each pot was estimated with caged sentinel weevil larvae as follows. A single DRW larva (5–7th instar) was placed in a sand-filled cylindrical cage constructed from inline filters and plastic snap-on lids (Duncan et al., 2003) and buried in each pot except those of controls or pots in which plants had died. After 7 days, larvae were recovered and cadavers were placed on moistened filter paper in 5-cm diameter plastic Petri dishes sealed with Parafilm®. Cadavers were observed periodically for up to 30 days, and emerging IJs were identified with a compound microscope (×1000) (Nguyen and Hunt, 2007; Nguyen et al., 2007).

The experiment was terminated 9.5 months after adding EPNs. Plant top and root dry weights were determined after drying 72 h at 70 °C. DRW (adults, larvae, and pupae) remaining in the soil in pots containing live plants were recovered and enumerated.

2.2. Greenhouse experiment 2

We repeated the experiment in essentially the same manner, but with an additional EPN species and minor modifications. Seedlings were planted as previously described and 14 replications of each treatment combination of the 3 soil types and 4 EPN species