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Sending Mixed Messages: A Trophic Cascade Produced by a Belowground Herbivore-Induced Cue

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Abstract Plants defend themselves against herbivores both directly (chemical toxins and physical barriers) and indirectly (attracting natural enemies of their herbivores). Previous work has shown that plant roots of citrus defend against root herbivores by releasing an herbivore-induced plant volatile (HIPV), pregeijerene (1,5-dimethylcyclodeca-1,5,7-triene), that attracts naturally occurring entomopathogenic nematodes (EPNs) to *Diaprepes abbreviatus* larvae when applied in the field. However, the soil community is complex and contains a diversity of interspecific relationships that modulate food web assemblages. Herein, we tested the hypothesis that other nematode types beyond EPNs, as well as, nematophagous fungi are affected by the same HIPV that attracts EPNs to herbivore-damaged roots. We employed molecular probes designed to detect and quantify nematodes from the *Acrobeloides*–group (free-living bacterivorous nematodes, FLBNs), some of which compete with EPNs by 'hyperparasitizing' insect cadavers, and five species of nematophagous fungi (NF), which attack and kill EPNs. In two different agricultural systems (citrus and blueberry), we detected diverse species of nematodes and fungi; however, only the behavior of FLBNs was affected in a manner similar to that reported previously for EPNs. Although detected, NF abundance was not statistically affected by the presence of the belowground HIPV. We provide the first evidence showing subterranean HIPVs behave much the same as those aboveground, attracting not only parasitoids, but also hyperparasites and other food web members.

Keywords Herbivore-induced plant volatile · Entomopathogenic nematodes · Soil food webs · Belowground interactions · Real-time qPCR

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Introduction

Plants defend themselves both directly and indirectly. Chemical toxins and physical barriers make up much of what is designated as direct defense. Indirect defense is described by mechanisms that either attract natural enemies or improve their foraging in search of herbivores, thus facilitating control of herbivore populations (Karban and Baldwin 1997). While the manipulation of beneficial natural enemies is not a new subject, the notion has received renewed interest with the discovery of herbivore-induced plant volatiles (HIPVs) as attractants for natural enemies (Hare 2011; Kaplan 2012; Turlings and Wäckers 2004). Despite being an important concept, the study of indirect defense cues as a driver of ecological interactions has largely *i*) been restricted to only half of the plant, that is aboveground tissues (Hunter 2001), and *ii*) focused largely on the cue's ability to attract natural enemies of herbivores and/or improve their foraging success (Kaplan 2011; Turlings *et al.* 2012). As documented within the plant canopy, belowground herbivory also imparts significant natural selection favoring the evolution of root defenses and traits that not only mediate associations with the third trophic level, but an array of trophic interactions (Erb and Lu 2013). Understanding the cascading consequences of an HIPV among

competing species can reveal information that is crucial to interpreting the potential multiple roles of information-based induced plant defense.

Larvae of the *Diaprepes abbreviatus* (L.) root weevil (DRW) feed on the roots of more than 290 plant species including citrus, sugarcane, potatoes, strawberries, sweet potatoes, papaya, and non-cultivated wild plants (Simpson *et al.* 1996). Entomopathogenic nematodes (EPNs) are obligate parasites that kill their insect host with the aid of symbiotic bacteria (Dillman *et al.* 2012; Kaya and Gaugler 1993), and have been shown to be major pathogens of DRW in Florida citrus orchards (Duncan *et al.* 2003b). We recently have shown that a citrus cultivar (*Citrus paradisi* Macf. x *Poncirus trifoliata* L. Raf.) releases HIPVs in response to larval feeding by the weevil, *D. abbreviatus*, and that a specific HIPV, pregeijerene, attracts naturally occurring EPN species in both laboratory bioassays and field experiments, which demonstrated increased nematode-caused mortality of root herbivores (Ali *et al.* 2010, 2012).

The efficacy of EPNs, the only identified agents of belowground indirect HIPV defense to date, is mediated by biotic and abiotic factors (Ram *et al.* 2008; Strong 2002; Stuart *et al.* 2006; Stuart and El-Borai 2008). Yet, the extent to which plant belowground HIPVs modulate the interactions between EPNs and guilds encompassing EPN antagonists such as nematophagous fungi (NF) (Duncan *et al.* 2007; Jaffee and Strong 2005; Pathak *et al.* 2012) or free-living bacterivorous nematode (FLBN) competitors of EPNs (Campos-Herrera *et al.* 2012; Duncan *et al.* 2003a, b; Hoy *et al.* 2008) has not been considered.

Until recently, indirect cues (both above- and belowground) more often have been evaluated in the context of attracting beneficial natural enemies (Ali *et al.* 2011; Poelman *et al.* 2012; Rasmann *et al.* 2005; van Tol *et al.* 2001), yet given the diverse community in and around plants, the roles of these cues likely are complex. In laboratory experiments, it was found that an HIPV from roots could attract both beneficial and harmful nematodes (Ali *et al.* 2011). Because soil inhabitants must rely on chemical and tactile cues to communicate and respond, cues that roots exude in response to herbivory may be perceived by a diversity of organisms in the soil community that directly or indirectly influence the plant (Rasmann *et al.* 2012).

In field experiments, qPCR primers and probes were used to enumerate EPNs that responded to a belowground HIPV attractant in two distinct agroecosystems (citrus in Florida, USA and blueberry in New Jersey, USA) (Ali *et al.* 2012). Given the broad attractive effect of pregeijerene on multiple nematode species (Ali *et al.* 2011), and the natural associations reported between EPNs, NF, and FLBNs (Campos-Herrera *et al.* 2012; Pathak *et al.* 2012), the aim of this investigation was to identify additional members of the soil food web that respond to this HIPV. Here, we used species-specific probes and real time qPCR to further evaluate our samples from previous field experiments. Our hypotheses were that: *i*) FLBN would also be attracted by this HIPV, further complicating our interpretation of this cue as directly beneficial to plants, since it favors competition between the two nematode guilds; and *ii*) trapping NF would not be affected, because of their duality as saprobiont-nematophagous fungi; whereas, endoparasitic-obligate NF might be selectively attracted because of their dependence on nematodes as a resource. Overall, we examine ecological dynamics associated with information-based defense strategies, and investigate the larger role that HIPVs may have on additional trophic levels belowground.

Methods and Materials

Target Organisms: Free-Living Nematodes, Nematophagous Fungi The FLBN, *Acrobeloides maximum*, was recovered from soil in a citrus grove in the flatwood eco-region from a citrus orchard near Land O' Lakes, FL (82 28 28.42 W and 28 15 9.69 N) by using the *Galleria mellonella* bait system (Campos-Herrera *et al.* 2012). Morphological and molecular characterization confirmed nematode identity (Table 1). Laboratory experiments demonstrated the ability of these FLBNs to interfere with the development of *Steinernema diaprepesi, S. riobrave,* and *Heterorhabditis indica* by 'hyperparasitizing' larvae of the DRW (Campos-Herrera *et al.* 2012). Moreover, the distribution of natural populations of members of the *Acrobeloides*-groups in Florida is positively correlated with the occurrence of native EPNs (Campos-Herrera *et al.* 2012). *Acrobeloides maximum* was cultured on 1.5 % nutrient agar (NA; Difco, MD, USA) following protocols described by Duncan *et al.* (2003a, b). A total of 300 nematodes in suspension were saved as individual aliquots in 1.5 ml Eppendorf tubes at −20 °C for standard curve development (see details below).

Five nematophagous fungi that are regularly encountered in Florida citrus groves were evaluated (Table 1) (Duncan *et al.* 2013). Nematophagous fungi can behave either as predators of nematodes (trappers) or endoparasites, and their distribution includes a wide diversity of soil types and communities (Barron 1977; Gray 1983; Lopez-Llorca *et al.* 2007). In this study, we analyzed one endoparasitic NF, *Catenaria* sp., which is considered to be an obligate parasite. The motile zoospores of *Catenaria* sp. attach to the nematodes' cuticle and form a germ tube that penetrates into the nematodes' body cavity (Barron 1977). Additionally, two trapping NF were evaluated, *Arthrobotrys dactyloides* and *Gamsylella gephyropagum.* These fungi use various hyphal organs to capture soil nematodes, and they have the dual ability of living as saprophytes or predators, depending on biotic and abiotic conditions (Jaffee 1992). Two additional NF species also were investigated, *Hirsutella rhossiliensis* and *Paecilomyces lilacinus,* which also exist as saprophytes or predators that attack nematodes at various stages of development *via* spores that adhere to and penetrate the cuticle. Morphological and molecular characterization of all

Table 1 Species and source of free-living bacterivorous nematodes and nematophagous fungi employed in this study

Type of organism, species	Population	Material	Source
Free-living bacterivorous nematode			
Acrobeloides maximum	RT2	Live, nematodes	Authors
Nematophagous fungi			
Arthrobotrys dactyloides	H22	Genomic DNA from pure culture	Authors
Gamsylella gephyropagum	Mg-37	Genomic DNA from pure culture	Authors
Catenaria sp.	–	ITS rDNA sequence + pDrive	Authors
Hirsutella rhossiliensis	–	Genomic DNA from pure culture	R. A. Humber, USDA-ARS Ithaca, NY
Paecilomyces lilacinus	–	Genomic DNA from pure culture	R. A. Humber, USDA-ARS Ithaca, NY

fungi were performed (Pathak *et al.* 2012), and pure cultures were maintained in quarter-strength corn meal agar (CMA) at room temperature, except for *Catenaria* sp., which required fresh EPNs in sterile water for fungal reproduction (Pathak *et al.* 2012).

Herbivore Induced Plant Volatile Isolation, Application, and Field Experiment Design Protocols to isolate pregeijerene (1,5-dimethylcyclodeca-1,5,7-triene) were described in Ali *et al.* (2012). Briefly, Common Rue (*Ruta graveolens L.*) roots were shown to contain large amounts of the same herbivore-induced terpene (pregeijerene) that is released by citrus roots upon herbivore damage. Thus, pregeijerene extracted from the crushed roots of Common Rue could be purified using a series of solid phase extraction methods as described in Ali *et al.* (2012).

Previously, two field experiments were reported that evaluated the effect of pregeijerene on the belowground attraction of the target organisms, the EPN. Details of the experimental protocols and site descriptions are provided in Ali *et al.* (2012). Briefly, the first experiment was conducted in a sandy soil citrus orchard at the Citrus Research and Education Center, Lake Alfred, FL (28 07 26.84 N, 81 42 55.31 W). The experiment was placed within a section of mature orange trees that was irrigated with microsprinklers as described in Ali *et al.* (2012). A randomized design was used to place treatments between trees within eight adjacent rows. Cylindrical wire-mesh cages containing autoclaved sandy soil (10 % moisture) were buried 20 cm deep within the soil and beneath tree canopies (Duncan *et al.* 2003a, b). A replicate consisted of six cages placed equidistantly from one another in a circular pattern (48 cm diam.) for each treatment (N=10). All cages contained a single *D. abbreviatus* larva and were baited with one of two treatments per replicate: *i*) isolated pregeijerene dissolved in solvent 8 ng/μl (in 30 μl aliquots) or *ii*) 30 μl of blank solvent control. After 72 hr, eight soil core samples were taken from soil surrounding the treatment arena (an outside circle 4 cm from cages, Fig. 1), prior to removal of cages containing beetle larvae, to measure the number of nematodes and fungi attracted to the treatment arena. The soil within the six cages from each replication was combined (N=10), and nematode community was extracted (Jenkins 1964) for DNA analysis (Campos-Herrera *et al.* 2011). All baited traps contained sterilized soil that did not come from our field sites, thus any nematodes, fungi, and/or other organisms that were recovered were not present prior to field deposition, and represent a biological response to treatments.

The second experiment was conducted on a blueberry planting in Chatsworth, NJ, USA, using the blueberry root pest, *Anomala orientalis* (Waterhouse), and *Galleria mellonella* (L.), as additional sentinel larvae. The methods for these experiments were similar to those described above.

Identification and Quantification of Free-Living Nematodes and Nematophagous Fungi by Real-Time qPCR In order to describe key players affecting EPN efficacy in citrus and blueberry agro-ecosystems, we used real-time qPCR to quantify the attraction of naturally occurring FLBNs and NF that were isolated in association with nematodes extracted from the soil samples to the herbivore-induced volatile, pregeijerene (Ali *et al.* 2012). The FLBN, *Acrobeloides maximum*, recently was described as a competitor with EPN for the insect cadaver (Campos-Herrera *et al.* 2012). Because of the limited availability of ITS sequences corresponding to other species of FLBN, we used the SSU rDNA sequence to design primers and probes that identify organisms sharing>98 % similarity at this locus. Therefore, by using this molecular probe, we assessed the natural occurrence of nematodes from an *Acrobeloides*-group (Campos-Herrera *et al.* 2012). For standard curve preparation, 1.5 ml Eppendorf tubes containing 300 nematodes were prepared and saved individually as described above. Because these nematodes exist in soil in several stages of development (egg, juvenile and adult), we developed a standard curve by extracting genomic DNA of these nematodes and obtaining quantifications of DNA, diluted from 1 ng/μl to 0.1 pg/μl, as described by Campos-Herrera *et al.* (2012). Therefore, the quantifications were expressed as ng/μl for the nematodes from the *Acrobeloide*-group.

The species-specific primers and probe for the five NF were designed by using the ITS region, and were compared with multiples strains and species to assess specificity in the

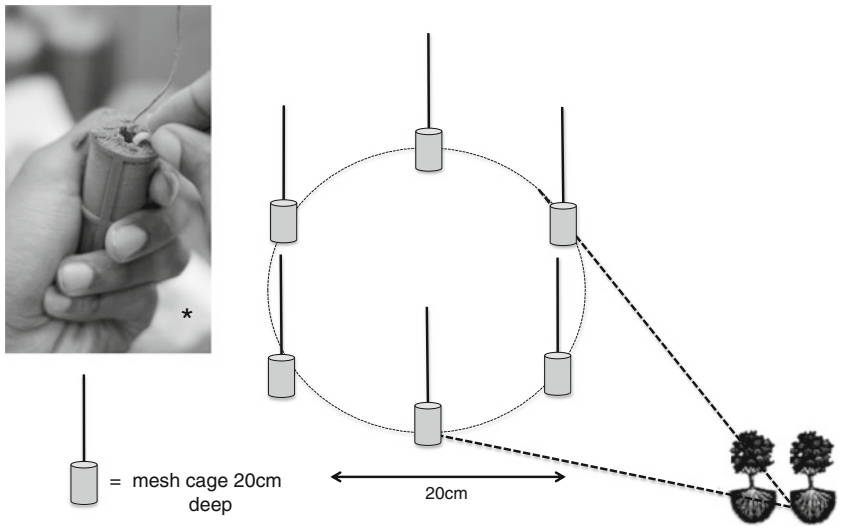

Fig. 1 Schematic diagram of the deployment and sampling procedure for field experiments in which sentinel traps with root weevils were deployed with or without HIPVs. Cages containing larvae, either with or without pregeijerene, were inserted 20 cm below the soils surface. *Photograph of mesh cage filled with sterilized soil receiving a sentinel larva, metal wire laced through the each cage was used to retrieve buried cages. One treatment replicate is depicted. Note: only organisms from sterilized soil within the cage were quantified

previous studies (Atkins *et al.* 2005; Pathak *et al.* 2012; Zhang *et al.* 2006). For the development of each of the five standard curves, genomic DNA extracted from pure cultures of all of the species were obtained (Pathak *et al.* 2012), except for the endoparasitic NF, *Catenaria* sp., for which a plasmid construction was used, including the entire ITS rDNA sequence described by Pathak *et al.* (2012). For all the NF, DNA was extracted, and the standard curve was constructed with serial dilutions from 1 ng/μl to 0.1 pg/μl, and values were provided as ng/μl.

The UltraClean™ Soil DNA Extraction Kit (MoBio) was employed to extract DNA following the protocol for maximum yield from each experimental nematode sample and from each of the corresponding tubes per isolate for development of the standard curves. All samples (experimental and positive controls) were evaluated for quality and quantity of DNA per duplicate using the Nanodrop System 1000 v.3.3.0 (Thermo Scientific, Wilmington, DE, USA). For the *Acrobeloides*-group standard curve, serial dilutions from 1 ng μl^{-1} to 0.1 pg μl^{-1} were performed, and quantifications were expressed as ng of DNA μl^{-1} (Campos-Herrera *et al.* 2012). The NF standard curve was also derived from 1 ng μl^{-1} to 0.1 pg μl^{-1}. In the case of the experimental samples collected from the field, all were adjusted to final dilutions according to FLBN (0.2 ng/μl, Campos-Herrera *et al.* 2012) or NF (10 ng/μl, Pathak *et al.* 2012) quantification.

Species–specific primers and probe sets for the FLBN *Acrobeloides*-group and all NF were reported in Atkins *et al.* (2005), Campos-Herrera *et al.* (2012), Pathak *et al.* (2012) Zhang *et al.* (2006). Primers and probes were synthesized by Integrated DNA Technologies Inc. (IDT, San Diego, CA, USA). All TaqMan® PCR probes were labeled at the 5′ end with a fluorogenic reporter (FAM), the 3′ end with a quencher (Iowa BlackTM FG), and included a specific molecule (ZEN) that provided increased stability (IDT, San Diego, CA, USA). Real–time, quantitative PCR (qPCR) (ABI Prism 7500, Applied Biosystem) was performed in optical 96–well reaction plates (USA Scientific, Orlando, FL, USA) in a final volume of 20 μl. Optimal primers and probe concentrations for each nematode and fungus were used in combination with 10 μl of the TaqMan Master Mix (Applied Biosystem, manufactured by Roche, Branchburg, NJ, USA). In addition, 400 nM of bovine serum albumin (BSA) (PROMEGA) was included in all runs to reduce possible interference with certain soil molecules (Torr *et al.* 2007). Annealing temperature and number of cycles were specific for each organism (Atkins *et al.* 2005; Campos-Herrera *et al.* 2012; Pathak *et al.* 2012; Zhang *et al.* 2006). Positive controls consisted of the corresponding standard curve, and the negative controls were the addition of sterile de–ionized water instead of DNA. Both positive and negative controls were included in all runs, and all samples (unknown and control) were run in duplicate. Data from the standard curves were log (x) transformed and a linear regression of FLBN or NF serial dilution on threshold cycle value (Ct) was performed after each run. This assessed the efficiency and accuracy of the qPCR experiment. To adjust the dilution used in each run, we used a correction factor based on total DNA estimation.

Statistical Analysis Quantifications of all target organisms were transformed before statistical analysis to adjust for disproportional representation of species. *Acrobeloides*-group quantification was square-root transformed. We estimated the NF 'infection/infestation rates' by dividing the DNA quantity of each NF species by the total amount of DNA in a sample, in order to provide a standard quantification independent of the nematode DNA per sample (Campos-Herrera *et al.* 2012; Duncan *et al.* 2013). We square-root transformed all of these values prior to statistical analyses. Additionally, to compare infection rates among all species of NF, the units of measure between species were standardized (0–

1) by dividing all data within a species by the highest measurement for that species (Rooij-van der Goes *et al.* 1995).

A *t-test* was used to compare nematode and fungal response between pregeijerene and control treatments within each experiment. Data were tested for unequal variance using *Levene's test*; data with unequal variance were analyzed using *Welch's t-test*. A two-factor analysis of variance (ANOVA) (NF species × treatment) was used to detect differences among NF species sampled in response to pregeijerene/control treatments. Spearman rank correlations were performed between percent larval mortality and FLBN abundance for both citrus and blueberry experiments to test for the possible influence of increasing larval mortality on the presence of FLBN, which could be a possible confounding factor. Although *P*-values reported for certain hypotheses tested were above the 0.05 level, the observed biological trends were consistent with those cases in which we observed *P*-values≤0.05. Therefore, we also acknowledged the possible biological importance of data where *P*-values obtained were≤0.10.

Results

Soil Samples from Experiment in Citrus Although the mean DNA of FLBN from the *Acrobeloides*-group was higher in soil samples that contained pregeijerene, as compared with the control, this result was only marginally significant (t_{18}=1.72, P=0.10) (Fig. 2a). There was an effect of the NF species present ($F_{4,99}$=2.63, P=0.03). We detected more DNA from *P. lilacinus* than from *Catenaria* spp., or *A. dactyloides*, while DNA from *G. gephyropagum* and *H. rhossiliensis* was not detected. Neither the treatment effect ($F_{1,99}$=1.40, P=0.23), nor the interaction between species and treatment were significant ($F_{4,99}$=0.59, P=0.67). Although *P. lilacinus* was detected at higher levels in our pregeijerene-treated samples than in the controls, this difference was not significant (t_{18}=0.56, P=0.58) (Fig. 2b).

Soil Samples from Experiment in Blueberry Mean DNA abundance of FLBN was higher in soil samples treated with pregeijerene than in the controls (t_{18}=2.047, P=0.05) (Fig. 3a). There was a marginally significant effect on NF species ($F_{4,99}$=0.37, P=0.08). We detected more DNA from *P. lilacinus* than from *Catenaria* spp., or *A. dactyloides*, and *H. rhossiliensis*; DNA from *Gamsylella gephyropagum* was not detected. Neither the treatment effect ($F_{1,99}$=0.19, P=0.66), nor the species by treatment interaction were significant ($F_{4,99}$=0.83, P=0.50). Again, *P. lilacinus* levels were higher in the pregeijerene-treated samples than in the controls, yet this difference was not statistically significant (t_{18}=0.314, P=0.75) (Fig. 3b).

Relationship with Larval Mortality and Nematode Abundance We found no significant relationships between larval mortality

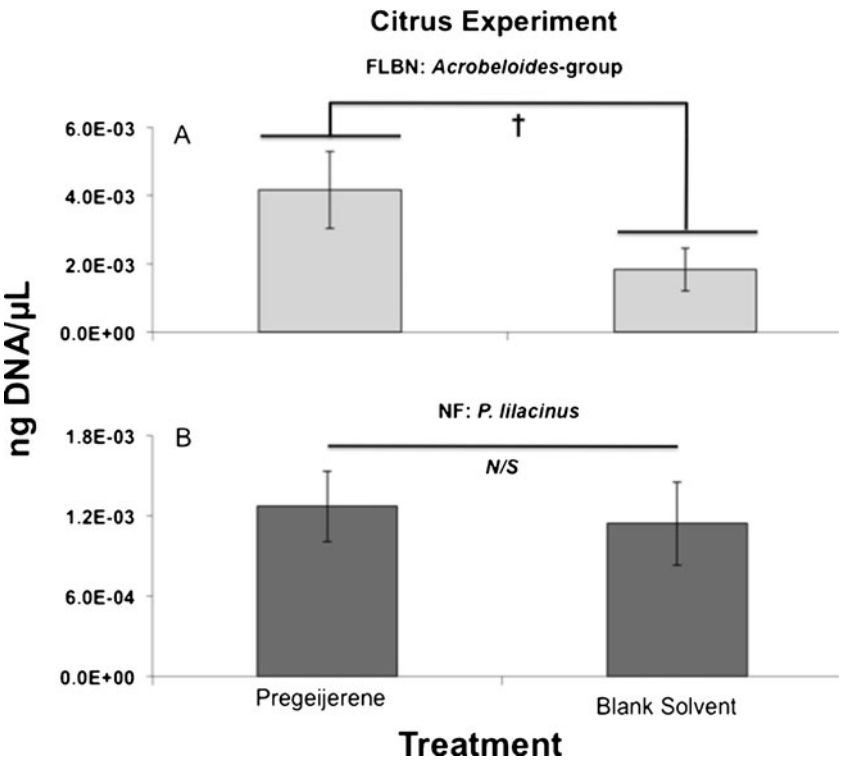

Fig. 2 **a** DNA representation of free-living bacterivorous nematodes (FLBN) from the Acrobeloides-group in soil collected from either sentinel traps containing pregeijerene or containing blank solvent in citrus. **b** DNA representation of nematophagous fungi (NF) in soil collected from sentinel traps treated with either pregeijerene or containing blank solvent (control) in citrus. †(marginal significance) = *P*-value≤0.10 but>0.05, n/s (non-significant) = *P*-value>0.05

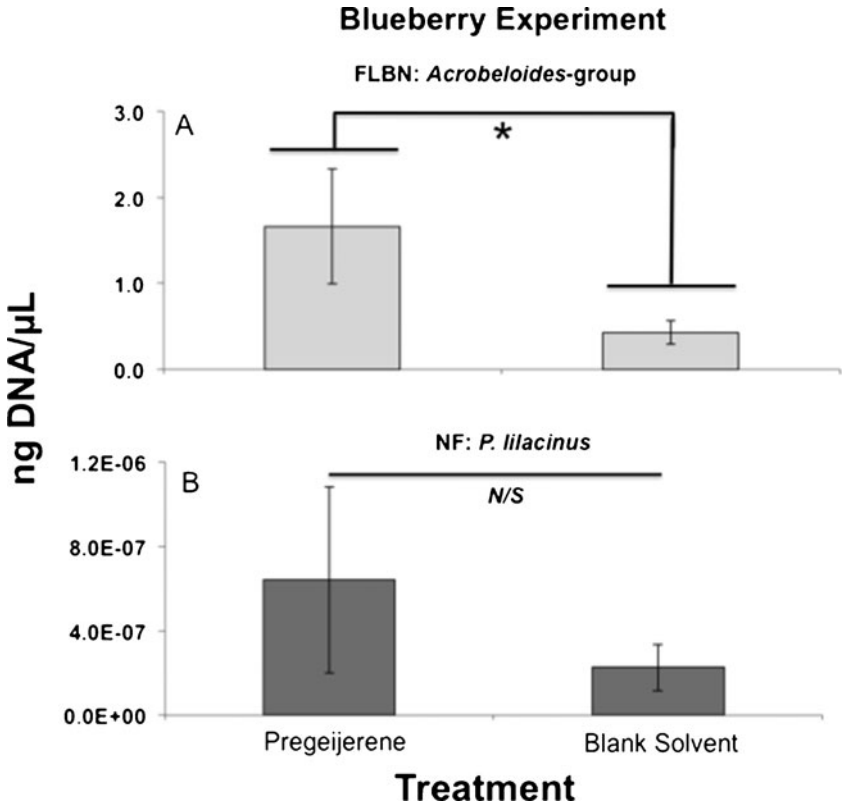

Fig. 3 **a** DNA representation of free-living bacterivorous nematodes (FLBN) from the Acrobeloides-group in soil collected from either sentinel traps containing pregeijerene or containing blank solvent in blueberry. **b** DNA representation of nematophagous fungi (NF) in soil collected from sentinel traps treated with pregeijerene or containing blank solvent (control) in blueberry. * = *P*-value≤0.05, n/s (non-significant) = *P*-value>0.05

and FLBN presence in either the citrus (Spearman's ρ_{18}=−0.06, *P*=0.79) or the blueberry field experiments (Spearman's ρ_{18}=0.25, *P*=0.28).

Discussion

This is the first report finding evidence that an herbivore-induced plant volatile (pregeijerene) attracts free-living nematode 'hyperparasites' living within the rhizosphere, in addition to previously reported attraction of entomopathogenic nematodes (Ali *et al.* 2012). Our previous work has demonstrated that citrus roots attract beneficial nematodes when they are fed upon by root herbivores (Ali *et al.* 2010). Thereafter, we evaluated whether this volatile cue also could attract plant parasites. We reported that plant parasites also were attracted by this same HIPV (Ali *et al.* 2011). However, these initial investigations consisted of laboratory bioassays and did not allow for analysis of multi-trophic interactions in a natural field setting.

The influence of plant cues on their surrounding community is a dynamic area of current research (Kaplan 2012). Although much effort has focused on induced plant volatiles as a result of plant damage, fewer studies have extensively examined the diverse impact of competing species occupying different trophic levels, particularly in studies of indirect defense (Kaplan 2012). Many studies have shown that HIPVs attract beneficial natural enemies of herbivores, yet quantification of plant fitness benefits as a result of such altered (induced) volatile profiles are rare (Allison and Hare 2009; Hare 2011; Kost and Heil 2008; but see Robert *et al.* 2013). Two mechanisms have been proposed to explain why fitness benefits are difficult to detect in information-based (*e.g.*, HIPVs) defenses. First, it may be that the attracted natural enemies do not immediately kill their hosts (*e.g.*, parasitoids), which results in continued damage to the plant (Kessler and Heil 2011). Second, complex interactions within the community may result in no net effect because of the lack of specificity of cue or signal perception and/or attractiveness (Kessler and Heil 2011; Orre *et al.* 2010; Robert *et al.* 2013). Both aboveground and belowground chemical ecology studies rarely take such competing factors into consideration simultaneously. Our findings indicate that a belowground HIPV can have diverse functions, and although plant fitness was not measured, we found evidence for complex community interactions.

Predators and parasitoids can have significant effects on herbivore performance and consequences on population density (De Moraes *et al.* 1998; Kessler and Baldwin 2001, 2004; Thaler 1999). However, there is less evidence that specialist natural enemies use more specific search cues than those used by generalists (Steidle and van Loon 2003). Investigating belowground, there are even fewer studies that examine cues that influence foraging strategies of natural enemies. Some

recent investigations have found evidence for differences in EPN response to HIPVs as a function of natural enemy (EPN) foraging strategy (Ali *et al.* 2011), specificity of belowground herbivore-induced volatile production, and associated natural enemy response (Rasmann and Turlings 2008), and influences of plant genotype on indirect defenses (Hiltpold *et al.* 2010; Rasmann *et al.* 2005). The behavior of EPNs to direct cues emitted from hosts demonstrates that parasitic nematodes have evolved specialized olfactory systems that likely contribute to appropriate host selection (Dillman *et al.* 2012). Furthermore, Hiltpold *et al.* (2010) observed "learning" behavior of an EPN in response to an HIPV. However, the FLBN 'hyperparasites', which were attracted to the HIPV in our system, have not been examined in this manner previously. Our results suggest that higher trophic levels may 'eavesdrop' on the same cue used by EPNs, thus enhancing host location.

One important aspect of the currently described results is the possibly reduced benefit of an HIPV to the plant given that this cue apparently attracts competitors of the beneficial natural enemies of the soil-borne herbivores. Although we found evidence for competition between herbivore natural enemies and other guilds, such as hyperparasites, the direct consequences on plant defense were not measured in this investigation. It is possible that these competitors may reduce EPN population growth rate; however, there is little evidence that they reduce the insecticidal efficacy of EPNs (Campos-Herrera *et al.* 2012; Duncan *et al.* 2003a, b). It may be inconsequential whether a cue increases competition between competing species occupying different trophic levels (parasites and their hyperparasites) if the result is reduced overall density of the herbivore species, despite this competition. However, if this competition reduces parasite species rapidly, due to the action of their hyperparasites, the herbivore population will likely soon rebound, leading to questions about the possible application of such HIPV cues for pest control in agricultural settings. In the currently described belowground system, we observed species interactions that may result in outcomes similar to those postulated by Poelman *et al.* (2012) aboveground, where the effect of HIPVs on natural enemy response may be attenuated due to simultaneous attraction of competitors or hyperparasites possibly resulting in overall negative consequences for plant survival. However, augmentative releases of EPN for biological control might counterbalance the negative effects of their natural enemies on pest mortality. Future work on how HIPVs influence hyperparasite-parasite-prey interactions within the subterranean environment are needed to fully explore induced plant defense, and may lead to practical application for pest management.

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