

## Research

# Drought Stress Impairs Communication Between *Solanum tuberosum* (Solanales: Solanaceae) and Subterranean Biological Control Agents

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Subject Editor: Chris Weldon

Received 24 May 2019; Editorial decision 30 August 2019

## Abstract

The attraction of entomopathogenic nematodes (EPNs) to herbivore-injured plant roots has been documented recently to be a common tritrophic interaction. Belowground tritrophic interactions are especially subject to modulation by many abiotic factors including drought. In this study, complementary greenhouse experiments were conducted to understand how drought stress might affect a potato plant's impact on EPN behavior. In separate trials, the responses of the EPN *Steinernema diaprepesi* (Nguyen and Duncan) (Rhabditida: Steinernematidae) to root herbivory by larvae of the weevil *Diaprepes abbreviatus* (Linnaeus) (Coleoptera: Curculionidae), in well-watered and drought stressed potato plants, were measured using soil-matrix olfactometers with two arms. The drought treatments were initiated in 30-d old plants. Drought-stressed potato plants received water when potentiometers read approximately 20 kPa, while for well-watered plants, the number was 8 kPa. Four weeks after initiating the treatments, 400 ml water was added to all pots, immediately before starting the experiments. The experiments revealed that *S. diaprepesi* infective juveniles (IJs) did not migrate preferentially toward drought-stressed or well-watered plants when neither were subjected to herbivory [ $t_{(21)} = 1.13$ ,  $P = 0.269$ ]. However, plants with roots damaged by herbivory attracted more *S. diaprepesi* IJs if they were well watered than if they were drought stressed [ $t_{(24)} = 3.19$ ,  $P = 0.004$ ]. If both plants in the olfactometers were drought stressed, EPNs moved preferentially toward those with root herbivory than those with undamaged roots [ $t_{(23)} = 3.19$ ,  $P = 0.004$ ]. No difference was detected in gas chromatography profiles between droughted and well-watered plant roots subjected to herbivory [ $F_{(24, 336)} = 0.68$ ,  $P = 0.87$ ]. GC analysis showed that three compounds, including 3-nonanone [ $t_{(6)} = 4.83$ ,  $P = 0.003$ ], artemisyl ketone [ $t_{(7)} = 6.21$ ,  $P = 0.000$ ], and benzoic acid, 4-ethoxy-, ethyl ester [ $t_{(7)} = -4.22$ ,  $P = 0.004$ ] were significantly higher in drought stressed than control plant roots. These results indicate that potatoes, like other plants, can recruit EPNs in response to root herbivory, and that drought stress dampens this tritrophic interaction where choice is involved. Additional research that resolves the mechanisms of these interactions may provide insights to exploit EPNs for crop protection.

**Key words:** entomopathogenic nematode, tritrophic interaction, herbivory, potato, drought

Entomopathogenic nematodes (EPNs) in two families, Steinernematidae and Heterorhabditidae, are insect-specific parasites that live in soil (Hazar et al. 2016). EPNs have been used in classical and augmentation biological control programs to suppress population density of pest insects in cryptic habitats in a variety of agroecosystems (El-Borai et al. 2007, Denno et al. 2008). The infective juvenile (IJ) stage of EPNs, the only free-living stage, relies on

endosymbiotic bacteria (*Xenorhabdus* spp. in steinernematid nematodes and *Photorhabdus* spp. in heterorhabditids) to kill their prey after penetrating the insect's body through natural openings or the cuticle. The IJs release the bacteria into the insect's hemocoel, resulting in septicemia, with death of the insect usually in 24–48 h. The nematodes utilize the cadaver to complete one to three generations. Development stops at the IJ stage when host nutrients are depleted

and the IJs begin to leave the cadaver in order to search for new prey (Grewal et al. 2005, Vega and Kaya 2012).

Searching by EPNs for cryptic hosts is challenging and costly; as a result, EPNs adopt different searching strategies and rely upon various chemical and physical host cues, changes in pH and plant roots and root emitted cues to locate insects in the complex soil matrix (Burman and Pye 1980; Pye and Burman 1981; Grewal et al. 1993; Boff et al. 2001, 2002; van Tol et al. 2001; Lewis 2002; Rasmann et al. 2005; Hiltbold and Turlings 2008; Ali et al. 2011; Demarta et al. 2014). Plant roots can affect the dispersal and host seeking of EPNs, because roots alone are attractive and even more so when they are damaged mechanically or by herbivory (Wang and Gaugler 1998, van Tol et al. 2001, Rasmann and Turlings 2008, Ennis et al. 2010). Roots of some plants damaged by insect feeding release herbivore-induced plant volatiles (HIPVs), which play an important role in guiding EPNs to the offending insect (Boff et al. 2001; van Tol et al. 2001; Lewis 2002, Rasmann et al. 2005; Ferry et al. 2007; Hiltbold and Turlings 2008; Rasmann and Turlings 2008; Ali et al. 2011, 2013; Rasmann et al. 2011; Turlings et al. 2012; Demarta et al. 2014; Tonelli et al. 2016).

It has been shown that the inducibility of volatile cues in plants may be affected by nutrient availability and water status (Erb and Lu 2013), thus, this belowground tritrophic interaction may be influenced by abiotic factors such as drought. Under natural conditions, plants often simultaneously experience multiple stresses, abiotic and biotic, each of which could weaken or boost the effects of the other (Copolovici et al. 2014). Global warming, changes in rainfall patterns, and water limitation are common concerns for many natural and agricultural systems. Current climate change models predict that crops will experience longer, more frequent, and more intense drought events in several geographic regions. Drought stress affects the plant's morphology, physiology, and biochemistry, which can have severe impacts on plant growth, development, and productivity, as well as defense. These changes can have ecological consequences for plant-insect-natural enemy interactions (Gutbrodt et al. 2011, Aslam et al. 2012, Erb and Lu 2013, Tariq et al. 2013, Weldegergis et al. 2015). Therefore, it is crucial to better understand how plants and their associated natural enemies will be affected by changing environments. In the case of drought, studies suggested that mild water stress increases HIPV emissions or has no effect, while severe drought decreases emissions (Niinemets 2010, Peñuelas and Staudt 2010, Becker et al. 2015, McCormick 2016, Martini and Stelinski 2017). The comparative effects of well-watered and drought-stressed plants on EPN behavior have, to our knowledge, not been addressed experimentally. Drought impacts on belowground tritrophic interactions can result from numerous effects on the survival or behavior of plants, root herbivores, and/or their natural enemies (Guyer et al. 2018). Understanding whether events such as drought, alone or with herbivory, might lead to independent or synergistic effects on plants due to modulation of EPN behavior is essential to predict the potential impacts of climate change on natural and agricultural systems.

Potato, *Solanum tuberosum* Linnaeus, ranks as the fourth most important food crop in terms of production and the world's most important non-grain food crop (FAO 2011). Potato plants are attacked by a large number of insects such as aphids, leafhoppers, beetles, and lepidopterous pests. These pests can damage the plant, either directly, through feeding on tubers and spoiling the harvest, or indirectly, by feeding on leaves, stems, and roots, or transmitting pathogens. Farmers worldwide use pesticides heavily to manage potato pests. Increasingly, these growers must deal with public environmental concerns that lead to the reduction of pesticide usage, while also confronting the rapidly rising world demand for food. Such a

challenge requires new management strategies including greater use of biological control agents (Mauceri et al. 2005, Cameron 2007, Alyokhin et al. 2012, Lacey et al. 2015). A number of studies have been conducted on the efficacy of EPNs against some potato insect pests (Parsa et al. 2006, Wraight et al. 2007, Shapiro Ilan et al. 2017, Hassani Kakhki et al. 2018). Furthermore, one study showed that damaged potato tubers release substances such as nonanal, octanal, and decanal, which showed different degree of attraction to EPNs in laboratory assays (Weissteiner 2010, Laznik and Trdan 2016). In preliminary experiments, we also found that *Steinernema diaprepesi* Nguyen and Duncan (Rhabditida: Steinernematidae) responded to herbivore-damaged roots of different potato varieties (Hassani Kakhki et al. 2018).

In this study, we conducted complementary greenhouse experiments to understand how drought-stressed potato plants modulates EPN behavior. Our study system included potato plant, citrus root weevil larvae, *Diaprepes abbreviatus* Linnaeus (Coleoptera: Curculionidae), a generalist herbivore, and the EPN *S. diaprepesi*. We conducted two-choice experiments to address the following questions: 1) do drought and/or herbivory influence EPN response to potato roots? and 2) do the chemical profiles of drought-stressed and well-watered plants differ from one another following herbivory?

## Materials and Methods

### Plants

The commercial potato cultivar Atlanta was used in all experiments. Pieces of sprouted seed tubers (80 g fresh mass) were planted in 2-L plastic pots (14 cm base diameter, 18 cm top diameter, and 18 cm height) containing autoclaved, sandy soil with an average pH of 6. Each pot had a 7-cm-diameter hole in the sidewall covered with a piece of plastic. These holes were later used to connect two pots using both ends of soil-filled, PVC T-joints to form two-choice olfactometers. Before planting, a potentiometers (Watermark; Irrometer Company, Inc., Riverside, CA) were installed 12 cm deep to monitor the soil moisture in each pot. Additional nutrients (15:30:15 N:P:K) were supplied via irrigation every week. The plants were grown and maintained in a greenhouse at the Citrus Research and Education Center (CREC) in Lake Alfred, FL at  $28 \pm 2^\circ\text{C}$ , and 60–80% RH.

### Insect

Post-neonate larvae of *D. abbreviatus* were obtained from the USDA, ARS Horticultural Research Laboratory insectary (Fort Pierce, FL) and subsequently reared on potato pieces. For all experiments, we used third- to sixth-instar weevil larvae.

### Nematodes

IJ *S. diaprepesi* were obtained from cultures maintained for more than a decade at the CREC in Lake Alfred, FL. The nematodes, originally isolated from a citrus orchard near Bartow, FL, were reared using last instar larvae of greater wax moth, *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) (Kaya and Stock 1997). IJs recovered from White traps (White 1927) were kept in shallow water in transfer flasks at  $15^\circ\text{C}$  until used in experiments within 2 wk of recovery.

### Experiment 1: Effect of Drought on EPN Response to Potato Roots

In the first experiment, we compared the response of *S. diaprepesi* to drought-stressed and well-watered plants. The drought treatments were initiated in half of the plants, chosen randomly, 30 d

after planting. Drought-stressed potato plants received 400 ml water based on observed plant wilting, usually after 5 d when potentiometers read approximately 20 kPa. Well-watered plants were irrigated with 400 ml of water, usually twice a week, when potentiometers read 8 kPa. The drought treatments continued for 4 wk. Just before starting the choice experiments, 400 ml water was added to all pots, thereby equalizing the soil moisture between treatments. A two-choice experiment comparing drought-stressed plants to well-watered plants was initiated by connecting the respective pots to one another through the PVC T-joints to form an olfactometer. Soil, the same as pot's soil, in the T-joints was adjusted to 7% moisture. Treatments were replicated 14 times (14 pairs). Because of directional movement of EPNs in response to electromagnetic fields, or other gradients, treatments in half the replications were assigned either facing north or south. Thirty hours after connecting the pots, 5,000 IJs in 5 ml of water were applied in the center part of the T-joint. Forty hours after EPN application, the olfactometer was disassembled. The soil from each pot was transferred to a larger container. The plant roots were cut into small pieces, added to the soil, and thoroughly mixed. IJ EPNs were extracted from a 300 g subsample of the soil-root mixture using sugar centrifugation, and then counted (Jenkins 1964).

#### Experiment 2: Nematode Responses to Drought-Stressed and Well-Watered Plants Subjected to Herbivory

In a second two-choice experiment, well-watered plants and drought-stressed plants were both subjected to weevil herbivory. The experimental and sampling procedures were identical to those described for experiment 1, except that six weevil larvae were placed on the soil surface of the pots and allowed to feed on potato roots for 30 h after connecting the respective pots to one another through the PVC T-joints for both drought-stressed and well-watered plants. If a larva did not enter the soil after 4 h, it was replaced with another larva. The treatments were replicated 14 times.

#### Experiment 3: Nematode Responses to Drought-Stressed Plants With and Without Herbivory

Both plants in each olfactometer were subjected to drought stress, as described in experiment 1. Six insects were added to one of the pots in each pair, and permitted to feed freely on roots. For each control pot, six 2-ml plastic microtubes containing individual *D. abbreviatus* larvae were added to the soil at the depth of 8 cm. The microtubes had pores drilled into them to allow insect volatile diffusion, while preventing insects from feeding on roots. The experimental procedure was as described above.

#### Experiment 4: Chemical Profiles of Droughted and Well-Watered Plant Roots Following Herbivory

In this experiment, well-watered and drought-stressed potato plants ( $n = 5$ ) were subjected to herbivory as described in experiment 2. After 70 h of weevil feeding, each plant was carefully removed from the pot and excess soil was shaken off, and the root system was rinsed with water. In the laboratory, after cutting into small pieces, potato roots were frozen in liquid nitrogen and then pulverized in a mortar similar to the procedure in Rasmann et al. (2005). A  $100 \pm 2$  mg sample was transferred to a 2-ml tube with 1 ml of *n*-hexane. Tubes were vortexed for 30 s, kept on ice for 10 min, and then repeated three times. Samples were centrifuged at 12,000 rpm for 1 min. The supernatants were collected and stored at  $-20^{\circ}\text{C}$  until

GC analysis. For the GC-MS analysis, we followed Ali et al. (2010) protocol. Nonyl acetate used as an internal standard. Briefly, a 1  $\mu\text{l}$  of the sample extract was injected into the GC-MS gas chromatograph (HP 6890) equipped with 30-m  $\times$  0.25-mm ID, 0.25- $\mu\text{m}$  film thickness DB-5 capillary column (Quadrex, New Haven, CT), interfaced to a 5973 Mass Selective Detector (Agilent, Palo Alto, CA). GC-MS chromatograms were analyzed using TurboMass software version 5.4.2 (Perkin Elmer). The abundances of these compounds were normalized according to the internal standard (nonyl acetate). Peak identifications were achieved using NIST (National Institute of Standards and Technology) and Wiley 9th edition (John Wiley and Sons, Inc., Hoboken, NJ) mass spectra database libraries, linear retention index (LRI), and compared with those of authentic compounds on the DB-5 column, when available.

#### Statistical Analysis

For analysis in experiments testing the response of EPNs toward different treatments in the two-choice olfactometer, paired *t*-tests were used (Minitab version 16, Minitab Inc., State College, PA). For volatile extracts, data were normally distributed. Differences between the quantities of profiled chemicals in insect-infested, well-watered, and drought-stressed plants were analyzed by one-tailed *t*-tests (Minitab version 16, Minitab Inc.).

### Results

#### Olfactometer Assays

In the first olfactometer trial, no differences were detected [ $t_{(21)} = 1.13$ ,  $P = 0.269$ ] between numbers of EPNs that moved toward drought-stressed plants (mean = 7.62) compared to well-watered plants (mean = 5.46) (Fig. 1A). Despite the uniform IJ inocula between all experiments, fewer total nematodes were recovered in this trial than in subsequent trials that involved herbivory.

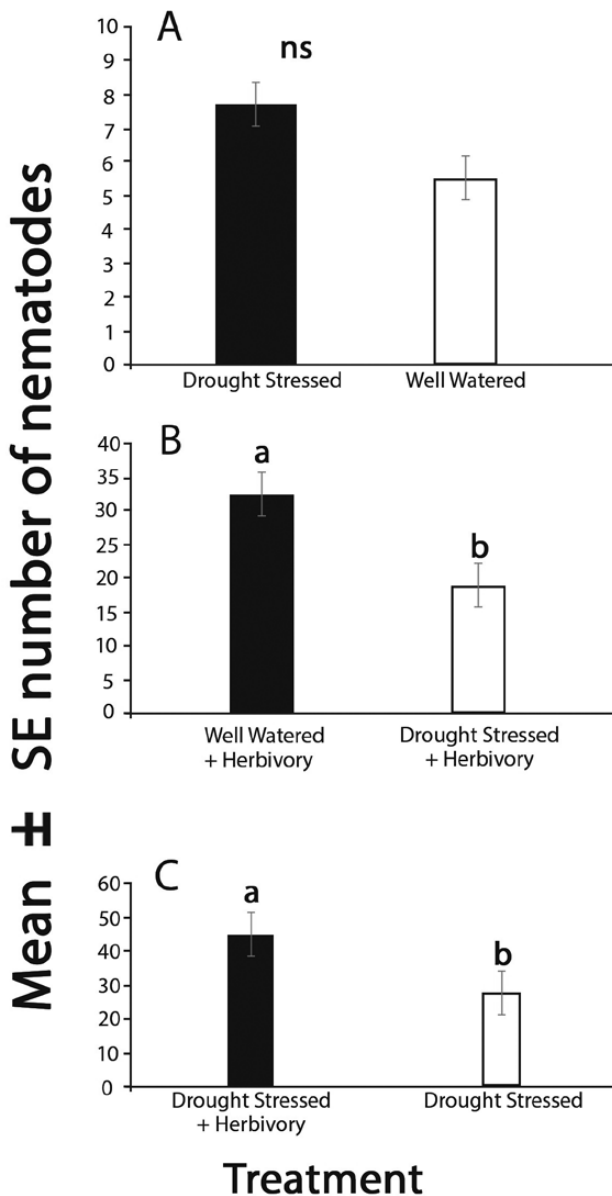
In the second experiment, well-watered plants with herbivory (mean = 32.1) attracted more *S. diaprepesi* [ $t_{(24)} = 3.19$ ,  $P = 0.004$ ] than did drought-stressed plants with herbivory (mean = 18.43) (Fig. 1B). In the final two-choice trial, more IJs [ $t_{(23)} = 3.19$ ,  $P = 0.004$ ] moved toward drought-stressed plants with herbivory (mean = 44.3) than to non-damaged, drought-stressed plants (mean = 28.4) (Fig. 1C).

#### GC-MS Analysis

Significant differences were detected between chemical profiles of herbivore infested, drought-stressed and well-watered roots (Fig. 2A–C). Twelve different compounds were detected and identified in the *n*-hexane extract of potato roots (Table 1). The relative amounts of hexane-extracted volatiles from potato roots of drought-stressed compared to well water plants ( $n = 5$ ) are shown in Table 1. Three compounds, including 3-nonanone [ $t_{(6)} = 4.83$ ,  $P = 0.003$ ], artemisyl ketone [ $t_{(7)} = 6.21$ ,  $P = 0.0001$ ] and benzoic acid, 4-ethoxy-, ethyl ester [ $t_{(7)} = -4.22$ ,  $P = 0.004$ ], were significantly higher in drought-stressed than control plant roots (Fig. 2C). In herbivore infested, drought-stressed plants, the relative amount of benzoic acid, 4-ethoxy-, ethyl ester was about 2.5 times higher than that in herbivore infested, well-watered plants.

### Discussion

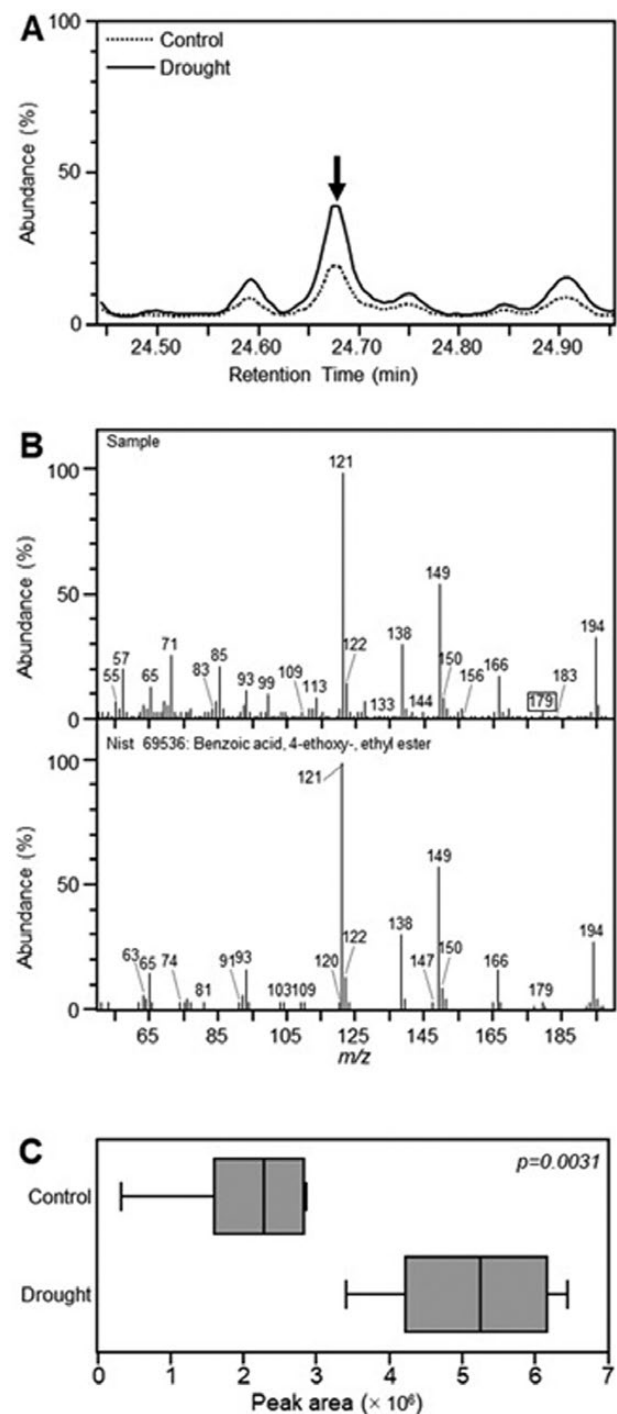
To our knowledge, this is the first report showing that drought stress modulates interactions between plants and EPNs. Whereas drought stress had no measurable effect on the attraction of *S. diaprepesi* to intact potato roots, herbivory induced attraction in intermittently



**Fig. 1.** Mean number of *Steinerema diaprepesi* Nguyen and Duncan (Rhabditida: Steinernematidae) that moved toward drought-stressed plants versus well-watered control plants (A), well-watered plants with herbivory versus drought-stressed plants with herbivory (B), or drought-stressed plant without herbivory compared to drought-stressed plant with herbivory (C). Each panel represents a separate experiment ( $n = 13$ ) conducted in a 2-arm olfactometer. Different letters indicate statistical significance at  $P = 0.05$ , whereas "ns" indicates  $P > 0.05$ .

drought-stressed plants, albeit less than if compared directly to that in plants with no experience of drought. Although the simultaneous effects of soil desiccation and herbivory on EPN response were decoupled in these experiments, the data indicate that drought modulates the ability of potatoes to communicate with these beneficial nematodes.

Presumably, the foraging behavior of *S. diaprepesi* was influenced by modifications in volatile production by plants and/or the herbivores. Additional investigations comparing nematode response to insects fed previously on well-watered versus drought-stressed potatoes will be necessary to address how this stress affected herbivore quality. Regarding plant response, previous investigations suggest that



**Fig. 2.** Detection of benzoic acid, 4-ethoxy-, ethyl ester, a major volatile organic compound found in potato roots. (A) Overlapped GC chromatograms for benzoic acid, 4-ethoxy-, ethyl ester of *Diaprepes abbreviatus*-infested drought-stressed and well-watered potato roots. (B) Mass spectrum of benzoic acid, 4-ethoxy-, ethyl ester in potato root samples and in NIST library for comparison. Note the increase in the amount of benzoic acid, 4-ethoxy-, ethyl ester in *n*-hexane extract of herbivore-infested drought-stressed potato roots. (C) Peak areas of benzoic acid, 4-ethoxy-, ethyl ester of *Diaprepes abbreviatus*-infested drought-stressed and well-watered potato roots. Horizontal thick lines indicate the medians, black/white dots indicate the means, boxes show the interquartile ranges including 25–75% of the values, and whiskers reflect the highest and the lowest data points.  $P$ -value  $< 0.05$  indicates statistically significant differences among treatments using two-tail  $t$ -test.



**Table 1.** Relative amounts of hexane-extracted volatiles from potato roots of drought-stressed compared to well-watered plants ( $n = 5$ )

	Retention time	Compound	P value	Fold change
1	8.44	3-Hexanol	0.85	0.9
2	8.50	2-Hexanol	0.30	1
3	9.60	3-Methyl-cyclopentanol	0.444	1
4	9.8	(Z)-2-Heptene	0.12	1.2
5	11.52	3-Nonanone	0.003**	1.4
6	12.66	Artemisyl ketone	0.000***	1.75
7	12.76	2-Pentanone	0.192	1.3
8	14.4	1-Phenyl-3-buten-1-ol	0.24	0.6
9	20.43	1-Eicosanol	0.32	1.1
10	20.6	2-Isopropyl-5-methyl-1-heptanol	0.263	1.2
11	24.6	Benzoic acid, 4-ethoxy-, ethyl ester	0.004**	2.4
12	28.27	Hexanedioic acid, bis(2-ethylhexyl) ester	0.294	0.5

Peak areas were normalized by internal standard (nonyl acetate). The relative amount of each compound was obtained by calculating its peak area. Compounds were identified by comparing their retention time and mass spectra with library. Fold change for each compound was calculated by dividing the average of peak area of drought stressed by the well watered.  $P$ -value shows significance levels for the treatments, \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Compounds were identified using NIST and Wiley libraries based on hits greater than 700.

multiple stresses, occurring simultaneously or sequentially, may cause either 1) an overlap and/or 2) cross-talk between signaling pathways. Infection of citrus by a phloem-limited bacterium induced the same, overlapping HIPV in roots as did feeding by *D. abbreviatus*, obviating this EPN recruitment mechanism (Rivera et al. 2017). However, the first experiment reported here revealed no evident mechanistic overlap by drought stress and herbivory. Cross-talk between different HIPV emissions can have additive or suppressive effects on natural enemy foraging behavior, compared to that induced by a single stress (Holopainen and Gershenzon 2010, Aslam et al. 2012, Tariq et al. 2013, Weldegergis et al. 2015, Nguyen et al. 2016). In our experiments, the combined stresses may have resulted in additional volatiles that counteracted the attractant to some extent; however, drought-stressed, insect-damaged potato roots may have simply released less HIPV than did the well-watered counterparts. Tariq et al. (2013) found when the host plant *Brassica oleracea* (Brassicaceae) was exposed to drought and root herbivory by *Delia radicum* Linnaeus (Diptera: Anthomyiidae), *Aphidius colemani* Viereck (Hymenoptera: Braconidae), an aphid parasitoid, preferred well-watered plants that produced greater overall emission of HIPVs. Multiple stresses might significantly limit plant nutrients and energy, thereby reducing its ability to respond to all stress factors simultaneously (Holopainen and Gershenzon 2010). The preponderance of evidence suggests that the effect of drought on HIPV emissions can largely be attributed to a dose-dependent response, with some variation due to plant species, drought duration, timing, and method used to measure drought; severe drought generally decreases the HIPV emissions, while mild drought may increase emissions or have no effect (Niinemets 2010, Peñuelas and Staudt 2010, Becker et al. 2015, McCormick 2016). For example, *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) showed preference for headspace volatiles from well-watered citrus seedlings infested by the psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), but not if the plants were drought-stressed (Martini and Stelinski 2017).

We were unsuccessful in previous (unpublished) experiments in recovering subterranean HIPVs using in situ methods such as Haysep-Q (Ali et al. 2010), solid-phase microextraction (SPME), or freeze-drying and crushing root samples using SPME (Rasmann et al. 2005). Nevertheless, direct tissue extraction by  $n$ -hexane revealed higher amounts of several volatile compounds in insect-damaged, drought-stressed roots, compared to

those of well-watered plants. Benzoic acid was reported to increase in plants under water stress, possibly to provide abiotic stress tolerance (Senaratna et al. 2003, Sampietro et al. 2006). The adverse effect of benzoic acid derivatives on nematodes was investigated by several researchers. Compounds such as methyl 4-hydroxybenzoate (Tada et al. 1988), 3,4-dihydroxybenzoic acid (Nguyen et al. 2016), and 4-hydroxybenzoic acid (Bogner et al. 2017) were lethal to the root-knot nematode, *Meloidogyne incognita*. Aoudia et al. (2012) found that 4-hydroxybenzoic acid and salicylic acid, a benzoic acid derived phenol, had a paralyzing effect on *M. incognita*. Thus, increased production of benzoic acid, 4-ethoxy-, ethyl ester may have reduced EPN migration to herbivore infested, drought-stressed, compared to well-watered potatoes in this study.

Weissteiner (2010) reported that when potato plants were subjected to belowground herbivory by the forest cockchafer larvae, *Melolontha hippocastani* Fabricius (Coleoptera, Scarabaeidae), or the wireworm larvae, *Agriotes* sp. (Coleoptera, Elateridae), roots and tubers released compounds such as decanal, nonanal, octanal, undecane, 6-methyl-5-hepten-2-one, 2-ethyl-1-hexanol and 1,2,4-trimethylbenzene, while undamaged potato plants released only 2-ethyl-1-hexanol. We found different compounds due to our extraction method. Moreover, different classes of HIPVs have different emission/abundance patterns following the onset of herbivory (Rodriguez-Saona and Frost 2010, Joo et al. 2018), generally, increasing with time (Brilli et al. 2009, Holopainen and Blande 2013, Copolovici et al. 2014). Perhaps we did not detect such compounds due to a relatively short duration of insect feeding. Larvae fed for 1 wk in the study by Weissteiner (2010), in contrast to 70 h in our experiments. Further study of this system is warranted because identification of the active semiochemicals could potentially be used to improve the efficacy of EPNs as biological control agents of potato pests.

Reports suggest that the land area affected by drought has doubled since 1970 (Jamieson et al. 2012), thus, understanding ecological consequences of drought on tritrophic interactions is becoming increasingly important. The results of this study suggest that drought might reduce the services provided by EPNs where tritrophic interactions are involved. Understanding such incremental change is especially important in agricultural systems where climate change could threaten sustainable food production (Grinnan et al. 2013).

## Acknowledgments

This work is a part of first author's PhD dissertation which has joint support by "Ferdowsi University of Mashhad" (p.3/39620) and Citrus Research and Education Center, University of Florida (UF). The authors thank members of the Dr. Killiny and Dr. Stelinski lab (UF) for their help.

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