

A whole-plant perspective reveals unexpected impacts of above- and belowground herbivores on plant growth and defense

FABIANE M. MUNDIM,^{1,4} HANS T. ALBORN,² ERNANE H. M. VIEIRA-NETO,¹ AND EMILIO M. BRUNA^{1,3}

¹Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, Florida 32611-0430 USA

²Center for Medical, Agricultural and Veterinary Entomology, Agricultural Research Service,
U.S. Department of Agriculture, Gainesville, Florida 32608 USA

³Center for Latin American Studies, University of Florida Gainesville, Gainesville, Florida 32611-5530 USA

Abstract. Trade-offs between plant growth and defense are central to theoretical frameworks used to study the ecology and evolution of plant defense against herbivores. However, these frameworks, as well as the experiments designed to test them, rarely include belowground herbivores. We experimentally challenged seedlings of the tropical shrub *Solanum lycocarpum* (Solanaceae) with either aboveground foliar herbivores (*Spodoptera* caterpillars) or belowground root herbivores (the nematode *Meloidogyne incognita*) and measured the resulting changes in plant growth rates, biomass allocation, and the concentration of defensive terpenoids in roots and leaves. We found that plants that suffered aboveground herbivory responded with aboveground growth but belowground defense. Similarly, belowground herbivory resulted in root growth but elevated defenses of leaves. These results underscore the importance of belowground plant–herbivore interactions, and suggest that, in contrast to theoretical predictions, plants can simultaneously invest in both growth and defense. Finally, they emphasize the need for a “whole-plant” perspective in theoretical and empirical evaluations of plant–herbivore interactions.

Key words: aboveground–belowground interactions; allocation; chemical defenses; growth-defense trade-off; herbivory; neotropical savanna; plant responses; plant–herbivore interactions; *Solanum lycocarpum*.

INTRODUCTION

Herbivory is a selective force that can fundamentally change plant morphology and physiology (Karban and Baldwin 1997, Strauss and Agrawal 1999, Ohgushi 2005) and has resulted in a vast diversity of plant defenses (Ehrlich and Raven 1964, Thompson 1988). Most theoretical frameworks used to study plant–herbivore interactions emphasize the optimal allocation of these resources or potential trade-offs between growth and defense because the resources allocated to the regrowth of tissues consumed by herbivores and the defense of remaining ones are costly (McKey 1974, Coley et al. 1985). In addition, the foci in almost all experimental tests of these frameworks are aboveground herbivory and the subsequent aboveground (AG) plant responses (see van Dam et al. 1996, Karban and Baldwin 1997, Strauss and Agrawal 1999, Fine et al. 2006, Mundim and Bruna 2016), which ignores the effects of belowground (BG) herbivores and plant defenses of belowground plant structures such as roots and rhizomes (e.g., Bardgett et al. 1999, Bais et al. 2006).

Root-feeding herbivores can enhance the production of root exudates (Denton et al. 1999, Bais et al. 2006), reduce plant growth (Bardgett et al. 1999, Bais et al. 2006)

and elevate defenses against other belowground herbivores (Bais et al. 2006). It has therefore been suggested that conclusions regarding plant–herbivore theory derived from experimental tests conducted with aboveground herbivores can be readily extended to BG subsystems (van Dam 2009). For instance, it has been hypothesized that severing of roots by BG herbivores should drive plants to invest in root growth to compensate for the loss of absorptive surface, in much the same way foliar herbivory results in the rapid production of new leaves necessary for photosynthesis (van Dam 2009). Recent work, however, suggests it is premature to assume that belowground responses should parallel those aboveground. Under abiotic stress, for example, more energy should be allocated to protection of roots given that plant performance can be severely reduced in limiting soil conditions (Erb and Lu 2013).

Despite the fact that the above and belowground plant–herbivore subsystems are often independently conceptualized and evaluated, they are not physiologically independent. For instance, defenses could be optimally allocated across the above and belowground plant compartments (Huang et al. 2014) such that a belowground herbivore could induce aboveground defenses and vice-versa (Huang et al. 2013, 2014). Such “asymmetrically induced systemic responses” (sensu Huang et al. 2012) could alter the outcome of interactions with other herbivores (Bais et al. 2006, Barber et al. 2015) and plant performance (Bardgett and Wardle 2010, Barber et al. 2011,

Manuscript received 3 May 2016; revised 28 September 2016; accepted 30 September 2016. Corresponding Editor: Evan L. Preisser.

⁴E-mail: fabianemmundim@gmail.com

Zvereva and Kozlov 2012). Accounting for such asymmetries, however, requires a whole-plant perspective, experimentally manipulating herbivory in one plant compartment and measuring plant responses in the other. Studies to have taken this whole-plant approach are rare (e.g., Soler et al. 2005, van Dam et al. 2005, Erb et al. 2009). Even fewer have measured responses in both plant compartments, and those to have done so have only measured the defensive chemistry of plants following herbivory (see Huang et al. 2013, 2014). Without measuring both growth and defense, and doing so both above- and belowground, it is impossible to determine if plant responses are limited to within one compartment or extend across the entire plant. Such information is essential to development of more precise and comprehensive plant defense theory, especially in light of ongoing global change (Mundim and Bruna 2016).

We experimentally manipulated AG and BG herbivory of seedlings of the tropical shrub *Solanum lycocarpum* (Solanaceae) to address the following questions: (1) Does AG herbivory result in the expression of BG defenses? (2) Does BG herbivory result in the expression of AG defenses? (3) Are patterns of root growth and root defense following belowground herbivory similar to those in leaves following herbivory aboveground? (4) Do above and belowground herbivory result in similar patterns of plant growth and biomass allocation? Our results indicate that evaluating both above and belowground responses to herbivory is necessary to accurately interpret patterns of growth, defense, and their implications for theoretical predictions.

MATERIALS AND METHODS

Solanum lycocarpum (Solanaceae) is a perennial, deciduous, pioneer shrub common in the more open physiognomies and disturbed areas of South America's savanna woodlands, known as the *cerrado* (Lorenzi 2002). This species is commonly known as *lobeira* or wolf's apple because its large fruits are central to the diet of the maned wolf, *Chrysocyon brachyurus* (Dietz 1984). The leaves of *S. lycocarpum* are protected by trichomes and thorns (Lorenzi 2002), while roots, leaves and fruits are protected by terpenoids, alkaloids, and other chemical compounds (e.g., Dall'Agnol and von Poser 2000, Sá et al. 2000).

As in many other *Solanum* species, the aboveground parts of *S. lycocarpum* are frequently attacked by generalist herbivores such as Armyworm larvae (*Spodoptera* spp.). The roots of *S. lycocarpum* are damaged by the root-knot nematode *Meloidogyne incognita*, an endogenous root-feeder that infects species from a broad spectrum of plant families (e.g., *S. lycopersicum* (Solanaceae), *Gossypium* spp. (Malvaceae), *Daucus carota* (Apiaceae); Shurtleff and Averre 2005). While the infective stage in many phytophagous nematodes are adults (e.g., *Pratylenchus*, *Radopholus*), in *M. incognita* they are the second-stage juveniles (J2; Shurtleff and Averre 2005).

They migrate through the soil and burrow into roots in which they feed, develop, and reproduce; adult females form large galls on roots (Shurtleff and Averre 2005).

In August 2013, we collected fruits from 10 *Solanum lycocarpum* shrubs and removed all pulp from seeds under running water with a strainer. The seeds were then placed in a shadehouse in germination trays filled with a 1:3 ratio of sand and soil; the soil was collected in an area of *cerrado* at the Universidade Federal de Uberlândia's (UFU) Panga Ecological Station (19°10' S, 48°24' W). In November 2013, $n = 45$ seedlings with fully expanded cotyledons (mean total leaf area \pm SE: 2.27 ± 0.069 cm²) were transplanted into 12 cm diameter \times 20 cm tall transparent bags containing the same ratio of sand and soil from Panga Station. We randomly assigned $n = 15$ plants to each of three treatments—AG herbivory, BG herbivory, or no-herbivory controls—then arranged them at random in the shadehouse. Several plants died in the three months between this assignment and application of experimental herbivory (final sample sizes, $n = 10$ AG, $n = 9$ BG, $n = 14$ control); there was no significant difference between treatments in the leaf area of plants at the time experimental herbivory was initiated ($F_{2,32} = 0.014$; $P = 0.986$).

On 20 February 2014, the plants in the BG herbivory treatment were inoculated with 10 mL of water in which we diluted $\sim 5,000$ *M. incognita* eggs; this solution was delivered to three points around the base of the plant with a pipette (see Bonetti and Ferraz 1981). The eggs were obtained from a colony maintained at the UFU's Department of Agronomy Nematology Research Lab. We estimate the number of eggs with which we inoculated plants was $\sim 50\%$ of the amount present in the soil surrounding naturally occurring *Solanum lycocarpum* seedlings (1.6 *Meloidogyne* adult males and juveniles per cm³ of soil; F. Mundim, *unpublished manuscript*).

It takes approximately 10 d for nematodes to emerge from eggs, develop into J2 stage juveniles, migrate, and infect roots (Shurtleff and Averre 2005). We therefore began the AG herbivory treatment on 2 March 2014 so that herbivory on plants in the AG and BG treatments began on approximately the same date. The AG herbivory treatment was applied by placing one second-instar or later *Spodoptera* larva on a leaf until it consumed 10% of total leaf area, which typically took about two hours. This value of leaf area consumed was selected because pilot studies of plant growth and *Spodoptera* consumption rates suggested it would ensure plants had sufficient tissue at the end of the experiment for chemical analyses. Induced defensive responses in leaves of *Solanum lycopersicum* can last at least 20 d (Underwood 2012). We therefore challenged plants with *Spodoptera* every 10 d to ensure a continuous response comparable to one resulting from the continuous BG infection. Plants in the control treatment were not exposed to any leaf or root herbivores. All plants received ~ 200 mL of water every 2 d and were rotated to random positions in the shadehouse weekly. They continued to grow throughout the duration of the experiment (see *Results*), indicating

the observed results are not due to inter-treatment differences in resource utilization or plants becoming pot bound. After approximately 125 d of exposure to herbivores all surviving plants were removed from the soil and washed. For each plant, we measured stem length and diameter, the final number of leaves, leaf area, total root length, and root complexity. We then separated leaves, stems, and roots, dried them at 60°C for 2 d, weighed them, and stored them separately in paper bags for subsequent chemical analyses.

Measuring root length and complexity immediately prior to challenging plants with herbivores would require uprooting them; this could damage them, induce plant defenses, or result in plant mortality. We therefore measured total root length, root complexity, and initial dry mass of leaves and roots in $n = 15$ plants set aside at the time experimental seedlings were transplanted. These values were then used as the initial ones for all experimental plants. We used the software package ImageJ (Rasband 1997) to calculate leaf areas and total root lengths. We used the centripetal link based ordering system to quantify the topological complexity of the root system (see Fitter 1987, Berntson 1997).

We calculated the relative growth rates (RGR) of total leaf area and total root length as

$$\text{RGR} = \frac{(\ln L_2 - \ln L_1)}{t_2 - t_1}$$

where L_1 is the initial total leaf area or total root length, L_2 is the final total leaf area or root length, and $t_2 - t_1$ is the length of the experiment in days. We also used the dry biomass of leaves, and roots at the end of the experiment to calculate the relative allocation of biomass below and aboveground (i.e., the root:shoot ratio).

Solanum species are known to use a broad profile of terpenoids for defense (War et al. 2012), and our preliminary analyses with gas chromatography/mass spectrometry (GC/MS) found most treatment-dependent variation was in an area of the chromatogram consisting primarily of sesquiterpenes. We therefore used the concentration of root and leaf sesquiterpenes as a metric of plant chemical responses to AG and BG herbivory. The roots and leaves from each experimental seedling were weighed, frozen with liquid nitrogen, and ground. The pulverized tissue was then diluted in a 1:2 volume of dichloromethane (CH_2Cl_2) and incubated in an ultrasonic bath for 30 min. The tubes were centrifuged and 100 μL of supernatants from the final extracted volume were pipetted for analyses. We used an Agilent 7890A gas chromatogram (GC) (Agilent Technologies, Santa Clara, California, USA) combined with a G7001B mass spectrometry (MS) in electron impact and single quad mode for the GC/MS analyses. We injected a 1- μL sample, using cool on column, on a 30 m \times 0.25 mm ID Agilent DB5 column with 0.25 μL film thickness with helium carrier at a constant flow of 35 cm/min. The column was initially held at 30°C for 1 min and then temperature programmed to increase 10°C/min to 280°C and held at that temperature for 4 min (total time = 30 min).

We focused on peaks in the 15–21 min retention time (RT). To estimate sesquiterpenes quantities based on peak area, known amounts of e-b- caryophyllene were injected to give an ng/au factor. For each sample, all peaks within the retention time range were integrated to give their area after values similar to the blank solvent and values from background compounds were eliminated. We then eliminated values caused by instrument background fluctuations (i.e., “noise,” here those <55,000 μmol). We eliminated solitary RT values and those appearing in fewer than three samples per treatment to avoid any biases related to the sample size of seedlings per treatment, then normalized the values for each RT peak by dividing the area by the dry mass (dm) of the plant sample. Finally, we calculated total sesquiterpenes concentration by summing all the normalized peak values for each plant sample. Note that it is possible, albeit unlikely, that some of the peaks in the 15–21 min retention time are not sesquiterpenes, so in the strictest terms, the concentrations we report here should be considered putative. Nevertheless, we found no evidence that particular peaks were overrepresented in individual treatments, so we are confident that any potential misidentifications would lead us to overestimate the magnitude but not the direction of the effects.

To determine if the different types of herbivory influenced growth and terpenoid concentrations we used linear mixed models. The response variables were relative growth rate (RGR), terpenoid concentration, and biomass. The main effects were herbivore treatment (i.e., AG herbivory, BG herbivory, control), plant part (i.e., leaves, roots), and their interaction. The random effects attributable to variation among individual seedlings were included by treating each plant as a split-plot, with herbivory type as the whole-plot treatment and the location of measurements (i.e., roots, leaves) as the within-plot treatment (B. Bolker, *personal communication*). To test for the effects of herbivore type on root:shoot ratio, total biomass, total terpenoid concentration, final stem length, final stem diameter, final number of leaves, final length of the primary root, and root complexity we used ANOVA after confirming the residuals met the assumptions of the test with graphs and the Lilliefors test. To determine if there was a difference among treatments we computed post hoc Tukey HSD tests. We conducted all analyses using the R statistical programming language (v3.2.4; R Core Team 2016) with package lme4 (Bates et al. 2015), nlme (Pinheiro et al. 2016), and the glht function in package multcomp (Bretz et al. 2010). Data are available from the Dryad Digital Repository.

RESULTS

No plants died as a result of the experimental treatments. Across treatments, final stem length ranged from 5.5 to 31.5 cm and final diameter ranged from 1.3 to 3.8 mm (Table 1), with no significant effect of herbivore

TABLE 1. Measurements of the aboveground (AG) and belowground (BG) plant parameters.

	Overall		Experimental treatments (mean \pm SE)		
	Range	Mean \pm SE	AG herbivory	BG herbivory	Control
<i>AG parameters</i>					
Stem length (cm)	5.5–31.5	14.63 \pm 1.25	11.15 \pm 2.47	14.944 \pm 1.544	16.93 \pm 2.02
Stem diameter (mm)	1.3–3.8	2.412 \pm 0.115	2.19 \pm 0.252	2.60 \pm 0.113	2.45 \pm 0.187
Final leaf area (cm ²)	8.85–71.38	37.325 \pm 3.17	52.852 \pm 4.268	27.687 \pm 4.65	32.43 \pm 4.58
Dry biomass (g)	0.095–0.788	0.288 \pm 0.031	0.212 \pm 0.049	0.273 \pm 0.034	0.352 \pm 0.057
Number of leaves	2–11	5.36 \pm 0.334	7.3 \pm 0.578	4.444 \pm 0.377	4.57 \pm 0.358
Leaf terpenoids (μ mol/g dry mass)	0–13,230.97	3,596.07 \pm 643.3	2,782.1 \pm 570.68	6,660.896 \pm 1594	2,207.25 \pm 706.363
<i>BG parameters</i>					
Final root length (cm)	61.2–583.1	262.436 \pm 19.29	231.44 \pm 35.874	308.222 \pm 47.39	255.143 \pm 21.75
Root complexity [†]	3.5–99	34.53 \pm 3.82	18.1 \pm 4.11	49.333 \pm 9.225	36.75 \pm 4.085
Dry biomass (g)	0.039–1.44	0.512 \pm 0.065	0.239 \pm 0.088	0.609 \pm 0.108	0.645 \pm 0.101
Root terpenoids (μ mol/g dry mass)	0–46,514.24	7,183.47 \pm 2124.77	14,686.54 \pm 6152.52	6,115.79 \pm 2,276.33	2,510.49 \pm 772.56

[†] For root complexity, we used the centripetal link-based ordering system (see Fitter 1987, Berntson 1997). In this system, each root segment is assigned an order of one. The complexity of the root system is equal to the number of segments in the system.

type on either (length $F_{2,31} = 1.851$, $P = 0.174$; diameter $F_{2,31} = 0.953$, $P = 0.396$; Table 1). However, there was a significant effect of herbivore type on both final leaf number and the relative growth rate of leaves. The final number of leaves on plants exposed to AG herbivores was 1.6 times greater than that of plants in the other treatments ($F_{2,31} = 13.4$, $P < 0.001$; Table 1), while the RGR of leaves in the AG herbivory treatment was 1.3 times greater (Fig. 1A; Appendix S1: Table S1).

All plants in the BG herbivory treatment were infected with nematodes (Appendix S1: Table S2). Across treatments the final length of the primary root ranged from 4 to 58.5 cm and there was no inter-treatment difference ($F_{2,31} = 2.301$, $P = 0.117$). Roots of plants exposed to BG herbivory had a growth rate 1.2 times greater than roots from control plants (Appendix S1: Table S1; Fig. 1B), however, and root complexity was 2.7 times greater in plants exposed to BG herbivory than in those challenged with AG herbivory ($F_{2,31} = 6.504$, $P = 0.004$; Table 1).

Although there was a trend toward higher total terpenoid concentrations in plants exposed to herbivory, none of the differences between treatments were significant (Appendix S1: Fig. S1; $F_{2,34} = 2.91$, $P = 0.05$; Table 1). Instead, the responses to herbivory appear to be in the subsystem opposite to the location of herbivore activity. For instance, the leaves of plants exposed to AG herbivory had terpenoid concentrations similar to those observed in control plants. However, the concentration of terpenoids in their roots was 2.4 times greater than in the roots of both control plants and those exposed to belowground herbivores (Appendix S1: Table S1; Fig. 1D). Similarly, plants inoculated with nematodes had similar root terpenoid concentrations to control plants, but their leaves had 2.4 times more terpenoids than both control plants and those exposed to AG herbivores (Appendix S1: Table S1; Fig. 1C).

At the end of the experiment control plants had greater total biomass than plants exposed to herbivores, although only the difference in biomass between control and AG plants was significant (Fig. 2A; $F_{2,30} = 3.958$, $P = 0.03$). There were differences in the allocation of this biomass, however. Across all treatments, the proportional investment by *Solanum lycocarpum* seedlings in roots is 1.75 times greater than in aboveground parts ($F_{1,66} = 8.441$, $P = 0.004$). There was no significant difference when comparing the root:shoot ratios of plants in either the AG or BG herbivory treatments with those of plant in the control group. However, plants exposed to AG herbivory had root:shoot ratios significantly lower than plants exposed to BG herbivory (Table 1; Fig. 2B).

DISCUSSION

Although plants are always exposed to both above and belowground herbivores, to date, the experiments used to study plant–herbivore interactions have mostly focused on aboveground herbivory and plant responses (e.g., Karban and Baldwin 1997, Fine et al. 2006). Furthermore, despite extensive study on belowground herbivory in agricultural systems (reviewed in Blackshaw and Kerry 2008), only recently have ecologists begun to incorporate it in theoretical frameworks (Masters et al. 1993) and empirically elucidate its breadth in natural systems (e.g., Bezemer et al. 2004, Erb et al. 2011, Barber et al. 2015). Our results underscore the importance of experimentally investigating the potential impact of belowground plant–herbivore interactions, despite their complexity and the challenges to doing so (Bezemer and van Dam 2005, Johnson et al. 2012). As predicted, plants responded to aboveground herbivory with higher leaf growth rates and belowground herbivory with elevated rates of root growth (Fig. 1A, B). More importantly, we show that

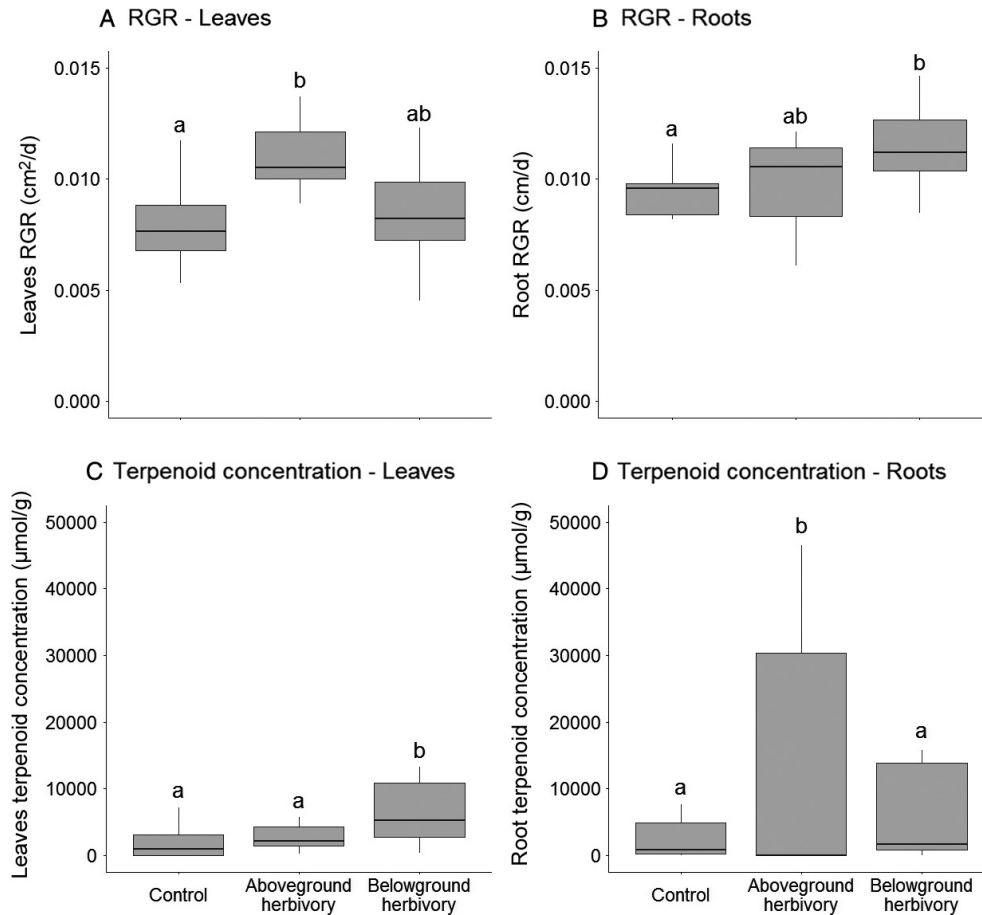


FIG. 1. (A) Relative growth rate (RGR) of leaf area, (B) RGR of total root length, (C) terpenoid concentration in leaves, and (D) terpenoid concentration in roots for plants exposed to aboveground herbivory (AG), belowground herbivory (BG), and no-herbivory controls. AG herbivory was performed by a generalist caterpillar (*Spodoptera* sp.) and BG herbivory was performed by a generalist endoparasitic nematode (*Meloidogyne incognita*). The boxes represent the interquartile range, the horizontal black lines indicate the medians, whiskers extend to 25% and 75% quartiles, and outliers are indicated by dots. Different letters indicate significant differences among herbivory treatments ($P < 0.05$) based on post hoc Tukey's tests.

belowground herbivory strongly influences a plant's aboveground defensive responses and vice-versa. This novel result highlights a need for a whole-plant perspective in the theoretical frameworks used to study herbivory; it also adds to an emerging body of literature (see Johnson et al. 2009, Huang et al. 2013, 2014) arguing for a need to both reevaluate the results of prior experiments and ensure future ones test for a broader diversity of responses.

There is growing evidence of the potential feedbacks between above- and belowground subsystems and how herbivory in one could influence the other (see Soler et al. 2007, Erb et al. 2008, 2011, Rasmann et al. 2009, Barber et al. 2015). For instance, because the main function of roots is nutrient uptake, changes in plant physiology and allocation in response to root herbivory could ultimately have negative effects on aboveground herbivores (Erb et al. 2011, Rasmann et al. 2011). These potential changes have been often attributed to distributive source-sink defensive allocation: plants respond

to herbivory by allocating defenses produced elsewhere than the part being attacked (van Dam and Heil 2011). However, we found the opposite to be true as, on average, plants experimentally infected by nematodes had significantly greater terpenoid concentrations in leaves, not roots (Fig. 1). This cross-subsystem response was even more dramatic in plants where herbivory was aboveground, which resulted in root terpenoid concentrations that were 2.4-fold higher than in the other treatments. This suggests BG herbivory leads plants to protect the tissue needed for photosynthesis while the attacked parts recover, a conclusion consistent with the lower growth and greater defense of their leaves. Similarly, AG herbivory likely leads plants to allocate primary metabolites to regrowth while preemptively protecting the undamaged roots or sequestering terpenoids in roots for reallocation to regrown leaves. While it has been suggested that defensive responses could be organ specific and compartmentalized (Rasmann and Agrawal 2008), our study demonstrates that AG and

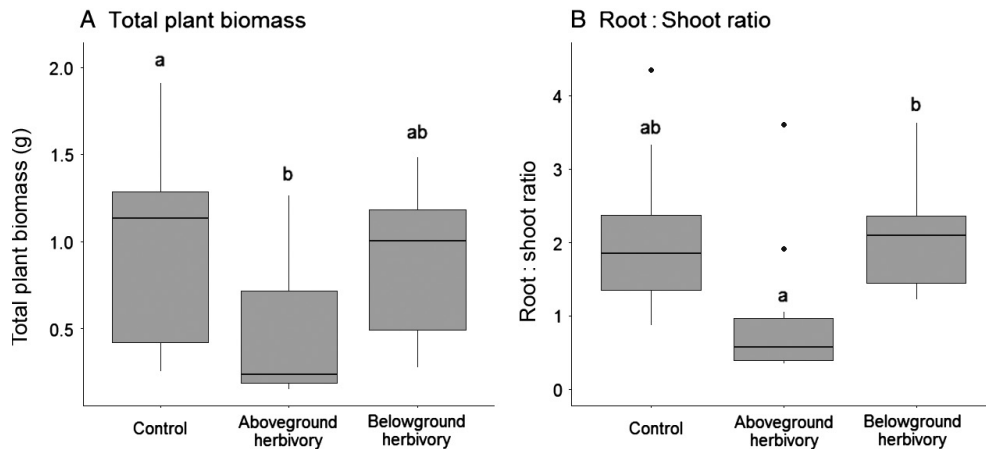


FIG. 2. (A) Total plant biomass and (B) root:shoot ratio of *Solanum lycocarpum* in the three different herbivory treatments. Aboveground herbivory was performed by *Spodoptera* sp. and belowground herbivory was performed by the nematode *Meloidogyne incognita*. The boxes represent the interquartile range, the horizontal black lines indicate the medians, whiskers extend to 25% and 75% quartiles, and outliers are indicated by dots. Different letters indicate significant differences among herbivory treatments ($P < 0.05$) based on post hoc Tukey's tests.

BG herbivory can simultaneously trigger regrowth, biomass allocation, and allocation to defense, and in a different subsystem than that attacked by herbivores (Figs. 1, 2).

A fundamental concept underlying plant responses to herbivory is that because resources cannot be allocated simultaneously to defense and growth, plants invest in defending tissues with the greatest fitness value (McKey 1974). The basis for this trade-off has been shown to depend on such factors as the duration or severity of herbivore and pathogen attacks, the availability of resources, and plant species identity (e.g., Bryant et al. 1983, Coley et al. 1985, Fine et al. 2006). While our study was designed to test for growth–defense trade-offs, the results do suggest properly interpreting patterns of plant–defense trade-offs likely requires a more comprehensive evaluation of where herbivores are feeding and how plants respond to them. Simultaneously evaluating growth and defense responses above- and belowground also suggests that, in contrast to predictions from theory, plants facing herbivore pressure can concurrently grow and defend: plants in the nematode inoculation treatment both defended leaves and produced longer and more complex roots (Fig. 2, Table 1). This observation was only evident with the benefit of a whole-plant perspective, however, as measuring plant responses solely above or belowground would have led to different conclusions about growth–defense trade-offs.

Our results also have implications for another important framework used to study plant–herbivore interactions. Plants are often identified as either resistant or tolerant to herbivores (van der Meijden et al. 1988, Agrawal and Fishbein 2006), with the former having traits that decrease the feeding of herbivores and the latter responding to herbivory with rapid regrowth (van der Meijden et al. 1988, Karban and

Baldwin 1997, Agrawal and Fishbein 2006). These alternative adaptive responses have been primarily assessed with AG herbivores (e.g., Bryant et al. 1983, Fine et al. 2006), so it is unclear if they extend to BG plant structures and herbivores. While the finding that *S. lycocarpum*'s AG subsystem is tolerant of foliar herbivory is not unexpected based on prior work with other *Solanum* species (Wise and Cummins 2006), it is reassuring to see that this can be extended to the BG subsystem as well. Most notable, however, is the intriguing possibility that plants can actually shift between resistance and tolerance as a function of where herbivory is occurring and the subsequent responses of above and belowground subsystems. If so this suggests the “tolerant vs. resistant” dichotomy may also be overly simplistic, and that – as with growth–defense trade-offs – a more holistic perspective would also advance this body of theory and empiricism.

Our study is the first to isolate simultaneous above and belowground responses to both above and belowground herbivores. Although it was conducted with a single plant species, we are nevertheless cautiously optimistic the results will be broadly applicable across plant taxa. First, our results are consistent with those showing plants under attack by AG herbivores can allocate chemical defenses to their roots (e.g., Bezemer et al. 2004, Huang et al. 2014). Second, both herbivores with which we challenged plants are widespread generalists, so the observed changes in plant chemistry and growth are not simply the result of how *S. lycocarpum* responds to specialized behavior or feeding physiology (Karban and Agrawal 2002). Finally, while the type and quantity of defensive secondary metabolites varies with plant and herbivore identity (see Adler and Wink 2001, Bezemer et al. 2004, Chen et al. 2004, Walls et al. 2005), terpenoids are the largest class of secondary defensive compounds,

are found in all plants, and both directly and indirectly deter herbivory by a wide range of pests (Mithöfer and Boland 2012).

Our results suggest four directions for future empirical and theoretical studies of plant–herbivore interactions. First, heterogeneity in water, soil nutrients, and other resources can have a major influence on plant growth, plant defense, and herbivore activity. Experimental manipulations of resources superimposed on manipulations of the location of herbivory are therefore an important next step in gaining a more comprehensive understanding of asymmetries in plant responses to herbivores. Second, future studies should consider how the whole plant responds when challenged with sequential or simultaneous above and belowground herbivory, since there can be important priority and additive effects of herbivores on plants (Erb et al. 2011). Third, better elucidating the physiological mechanisms underpinning the patterns we observed, such as the hormonal pathways responsible for defenses or environmental conditions limiting root or leaf growth, can help determine under what circumstances asymmetric responses are more or less likely to be observed. Fourth, plants also defend themselves against herbivores with biological defenses (e.g., mutualistic predatory ants), physical defenses (e.g., trichomes, spines), and many other types of secondary compounds. Experiments quantifying the responses of other plant defenses to above- and belowground herbivory are needed to both assess the generality of our results and suggest alternative hypotheses. Finally, the outcome of interactions between plants and herbivores can alter their respective population and community dynamics, other interspecific interactions, and even ecosystem processes (Huntly 1991). A whole-plant perspective can provide new insights into the nature of these cascading effects, for instance on above or belowground herbivore communities (e.g., Huang et al. 2014) and how they are influenced by heterogeneity in environmental conditions (Mundim and Bruna 2016).

ACKNOWLEDGMENTS

We thank M. A. Santos and the Nematology Research Lab at UFU for providing logistical support and nematode eggs, B. Bolker for providing statistical advice, and N. Underwood, E. Pringle, and anonymous reviewers for comments on the manuscript. F. Mundim and E. Vieira-Neto were supported by assistantships from the UF College of Agricultural and Life Sciences and Fellowships from Brazil's Science without Borders Program (Projects 237960/2012-5 and 202012/2012-3). E. Bruna was supported by a Special Visiting Researcher Fellowship from the Science without Borders Program (Project 061/2012).

LITERATURE CITED

- Adler, L. S., and M. Wink. 2001. Transfer of quinolizidine alkaloids from hosts to hemiparasites in two *Castilleja-Lupinus* associations: analysis of floral and vegetative tissues. *Biochemical Systematics and Ecology* 29:551–561.
- Agrawal, A. A., and M. Fishbein. 2006. Plant defense syndromes. *Ecology* 87:S132–S149.
- Bais, H. P., T. L. Weir, L. G. Perry, S. Gilroy, and J. M. Vivanco. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology* 57:233–266.
- Barber, N. A., L. S. Adler, and H. L. Bernardo. 2011. Effects of above- and belowground herbivory on growth, pollination, and reproduction in cucumber. *Oecologia* 165:377–386.
- Barber, N. A., N. J. Milano, E. T. Kiers, N. Theis, V. Bartolo, R. V. Hazzard, and L. S. Adler. 2015. Root herbivory indirectly affects above- and below-ground community members and directly reduces plant performance. *Journal of Ecology* 103:1509–1518.
- Bardgett, R. D., and D. A. Wardle. 2010. Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change. Oxford University Press, New York, New York, USA.
- Bardgett, R. D., C. S. Denton, and R. Cook. 1999. Belowground herbivory promotes soil nutrient transfer and root growth in grassland. *Ecology Letters* 2:357–360.
- Bates, D., M. Maechler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using *lme4*. *Journal of Statistical Software* 67:1–48.
- Berntson, G. M. 1997. Topological scaling and plant root system architecture: developmental and functional hierarchies. *New Phytologist* 135:621–634.
- Bezemer, T. M., and N. M. van Dam. 2005. Linking aboveground and belowground interactions via induced plant defenses. *Trends in Ecology and Evolution* 20:617–624.
- Bezemer, T. M., R. Wagenaar, N. M. van Dam, W. H. van der Putten, and F. L. Wackers. 2004. Above- and below-ground terpenoid aldehyde induction in cotton, *Gossypium herbaceum*, following root and leaf injury. *Journal of Chemical Ecology* 30:53–67.
- Blackshaw, R. P., and B. R. Kerry. 2008. Root herbivory in agricultural ecosystems. Pages 35–53 in S. N. Johnson, and P. J. Murray, editors. *Root feeders: an ecosystem perspective*. Cromwell Press, CABI, Oxfordshire, UK.
- Bonetti, J. I. S., and S. Ferraz. 1981. Modificações do método de Hussey & Barker para extração de ovos de *Meloidogyne exigua* em raízes de cafeeiro. *Fitopatologia Brasileira* 6:553.
- Bretz, F., T. Hothorn, and P. Westfall. 2010. *Multiple comparisons using R*. Chapman & Hall/CRC Press, Boca Raton, Florida, USA.
- Bryant, J. P., F. S. Chapin, and D. R. Klein. 1983. Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40:357–368.
- Chen, F., D. K. Ro, J. Petri, J. Gershenzon, J. Bohlmann, E. Pichersky, and D. Tholl. 2004. Characterization of a root-specific *Arabidopsis* terpene synthase responsible for the formation of the volatile monoterpene 1,8-cineole. *Plant Physiology* 135:1956–1966.
- Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. *Science* 230:895–899.
- Dall'Agnol, R., and G. L. von Poser. 2000. The use of complex polysaccharides in the management of metabolic diseases: the case of *Solanum lycocarpum* fruits. *Journal of Ethnopharmacology* 71:337–341.
- Denton, C. S., R. D. Bardgett, R. Cook, and P. J. Hobbs. 1999. Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. *Soil Biology and Biochemistry* 31:155–165.
- Dietz, J. M.. 1984. *Ecology and social organization of the maned wolf (Chrysocyon brachyurus)*. Smithsonian Institution Press, Washington, D.C., USA.
- Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants—a study in coevolution. *Evolution* 18:586–608.

- Erb, M., and J. Lu. 2013. Soil abiotic factors influence interactions between belowground herbivores and plant roots. *Journal of Experimental Botany* 64:1295–1303.
- Erb, M., J. Ton, J. Degenhardt, and T. C. J. Turlings. 2008. Interactions between arthropod-induced aboveground and belowground defenses in plants. *Plant Physiology* 146:867–874.
- Erb, M., C. Lenk, J. Degenhardt, and T. C. J. Turlings. 2009. The underestimated role of roots in defense against leaf attackers. *Trends in Plant Science* 14:653–659.
- Erb, M., C. A. M. Robert, B. E. Hibbard, and T. C. J. Turlings. 2011. Sequence of arrival determines plant-mediated interactions between herbivores. *Journal of Ecology* 99:7–15.
- Fine, P. V. A., Z. J. Miller, I. Mesones, S. Irazuzta, H. M. Appel, M. H. H. Stevens, I. Saaksjarvi, L. C. Schultz, and P. D. Coley. 2006. The growth–defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology* 87: S150–S162.
- Fitter, A. 1987. An architectural approach to the comparative ecology of plant-root systems. *New Phytologist* 106: 61–77.
- Huang, W., J. Carrillo, J. Ding, and E. Siemann. 2012. Invader partitions ecological and evolutionary responses to above- and belowground herbivory. *Ecology* 93: 2343–2352.
- Huang, W., E. Siemann, X. Yang, G. S. Wheeler, and J. Ding. 2013. Facilitation and inhibition: changes in plant nitrogen and secondary metabolites mediate interactions between above-ground and below-ground herbivores. *Proceedings of the Royal Society B* 280:20131318.
- Huang, W., E. Siemann, L. Xiao, X. Yang, and J. Ding. 2014. Species-specific defence responses facilitate conspecifics and inhibit heterospecifics in above-belowground herbivore interactions. *Nature Communications* 5:4851.
- Huntly, N. 1991. Herbivores and the dynamics of communities and ecosystems. *Annual Review of Ecology and Systematics* 22:477–503.
- Johnson, S. N., C. Hawes, and A. J. Karley. 2009. Reappraising the role of plant nutrients as mediators of interactions between root- and foliar-feeding insects. *Functional Ecology* 23:699–706.
- Johnson, S. N., K. E. Clark, S. E. Hartley, T. H. Jones, S. W. McKenzie, and J. Koricheva. 2012. Aboveground-belowground herbivore interactions: a meta-analysis. *Ecology* 93:2208–2215.
- Karban, R., and A. A. Agrawal. 2002. Herbivore offense. *Annual Review of Ecology and Systematics* 33:641–664.
- Karban, R., and I. T. Baldwin. 1997. *Induced responses to herbivory*. The University of Chicago Press, Chicago, Illinois, USA.
- Lorenzi, H.. 2002. *Brazilian trees: a guide to the identification and cultivation of Brazilian native trees*. Fourth edition. Instituto Plantarum de Estudos da Flora, Nova Odessa, São Paulo, Brazil.
- Masters, G. J., V. K. Brown, and A. C. Gange. 1993. Plant mediated interactions between aboveground and belowground insect herbivores. *Oikos* 66:148–151.
- McKey, D. 1974. Adaptive patterns in alkaloid physiology. *American Naturalist* 108:305–320.
- Mithöfer, A., and W. Boland. 2012. Plant defense against herbivores: chemical aspects. *Annual Review of Plant Biology* 63:431–450.
- Mundim, F., and E. M. Bruna. 2016. Is there a temperate bias in our understanding of how climate change will alter plant-herbivore interactions? A meta-analysis of experimental studies. *American Naturalist* 188:S74–S89.
- Ohgushi, T. 2005. Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annual Review of Ecology Evolution and Systematics* 36:81–105.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2016. nlme: linear and nonlinear mixed effects models version: 3.1-127. R-Core Team. <https://cran.r-project.org/web/packages/nlme/index.html>
- R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org
- Rasband, W. S. 1997. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA.
- Rasmann, S., and A. A. Agrawal. 2008. In defense of roots: a research agenda for studying plant resistance to belowground herbivory. *Plant Physiology* 146:875–880.
- Rasmann, S., A. A. Agrawal, S. C. Cook, and A. C. Erwin. 2009. Cardenolides, induced responses, and interactions between above- and belowground herbivores of milkweed (*Asclepias* spp.). *Ecology* 90:2393–2404.
- Rasmann, S., A. C. Erwin, R. Halitschke, and A. A. Agrawal. 2011. Direct and indirect root defences of milkweed (*Asclepias syriaca*): trophic cascades, trade-offs and novel methods for studying subterranean herbivory. *Journal of Ecology* 99: 16–25.
- Sá, R. C. S., A. A. Vireque, J. E. P. Reis, and M. O. Guerra. 2000. Evaluation of the toxicity of *Solanum lycocarpum* in the reproductive system of male mice and rats. *Journal of Ethnopharmacology* 73:283–287.
- Shurtleff, M. C., and C. W. Averre. 2005. Diagnosing plant diseases caused by nematodes. The American Phytopathological Society, St. Paul, Minnesota, USA.
- Soler, R., T. M. Bezemer, W. H. van der Putten, L. E. M. Vet, and J. A. Harvey. 2005. Root herbivore effects on above-ground herbivore, parasitoid and hyperparasitoid performance via changes in plant quality. *Journal of Animal Ecology* 74:1121–1130.
- Soler, R., T. M. Bezemer, A. M. Cortesero, W. H. van der Putten, L. E. M. Vet, and J. A. Harvey. 2007. Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid. *Oecologia* 152:257–264.
- Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution* 14:179–185.
- Thompson, J. N. 1988. Coevolution and alternative hypotheses on insect plant interactions. *Ecology* 69:893–895.
- Underwood, N. 2012. When herbivores come back: effects of repeated damage on induced resistance. *Functional Ecology* 26:1441–1449.
- van Dam, N. M. 2009. Belowground herbivory and plant defenses. *Annual Review of Ecology Evolution and Systematics* 40:373–391.
- van Dam, N. M., and M. Heil. 2011. Multitrophic interactions below and above ground: en route to the next level. *Journal of Ecology* 99:77–88.
- van Dam, N. M., T. J. DeJong, Y. Iwasa, and T. Kubo. 1996. Optimal distribution of defences: Are plants smart investors? *Functional Ecology* 10:128–136.
- van Dam, N. M., C. E. Raaijmakers, and W. H. van der Putten. 2005. Root herbivory reduces growth and survival of the shoot feeding specialist *Pieris rapae* on *Brassica nigra*. *Entomologia Experimentalis et Applicata* 115: 161–170.
- van der Meijden, E., M. Wijn, and H. Verkaar. 1988. Defense and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos* 51:355–363.
- Walls, R., H. Appel, M. Cipollini, and J. Schultz. 2005. Fertility, root reserves and the cost of inducible defenses in the

- perennial plant *Solanum carolinense*. *Journal of Chemical Ecology* 31:2263–2288.
- War, A. R., M. G. Paulraj, T. Ahmad, A. A. Buhroo, B. Hussain, S. Ignacimuthu, and H. C. Sharma. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signaling and Behavior* 7:1306–1320.
- Wise, M. J., and J. J. Cummins. 2006. Strategies of *Solanum carolinense* for regulating maternal investment in response to foliar and floral herbivory. *Journal of Ecology* 94:629–636.
- Zvereva, E. L., and M. V. Kozlov. 2012. Sources of variation in plant responses to belowground insect herbivory: a meta-analysis. *Oecologia* 169:441–452.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.1619/supinfo>