Arbuscular mycorrhizal fungi and belowground herbivore interact to determine plant productivity and subsequent conspecifics performance by changing plant metabolites

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Research Article

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Abstract

Insect herbivores and arbuscular mycorrhizal fungi (AMF) often occur simultaneously on a host plant, altering plant morphological and biochemical traits and thereby not only affecting each other’s performance, but also plant interactions with subsequent above- or belowground herbivores. Here, we investigate the combined effects of AMF and above- and belowground herbivory on plant productivity and performance of subsequent above- and belowground herbivores. We conducted a 3×2 full-factorial experiment with three factors of ‘Herbivory (no herbivory, leaf herbivory, and tuber herbivory) and two factors of ‘AMF inoculation’ (no AMF inoculation and AMF inoculation) in the tuber-plant, potato (*Solanum tuberosum*). We showed that both AMF and tuber herbivory increased tuber biomass and tuber primary metabolites (protein, starch). Tuber herbivory reduced the performance of subsequent conspecifics feeding on leaves potentially via increased leaf levels of phenolics, α-solanine and α-chaconine. By contrast, it increased the performance of subsequent conspecifics feeding on tubers potentially via increased protein, however, only in plants inoculated with AMF. This indicates that the belowground facilitation among conspecific insects was contingent upon the presence of AMF. Leaf herbivory did not affect subsequent above- or belowground insect performance. These feedings improve our understanding of the ecological consequences of antagonists and mutualists interactions mediated by phytochemistry, especially for agroecosystems.

Key Message

AMF and tuber herbivory increased potato tuber biomass, and concentrations of tuber primary metabolites.

Tuber herbivory reduced the performance of subsequent conspecifics feeding on leaves both in plants with and without AMF.

Tuber herbivory only enhanced the performance of subsequent conspecifics feeding on tubers in the presence of AMF, indicating that the presence of AMF was essential for this belowground facilitation.

Such facilitation and inhibition potentially depend on increased levels of tuber protein and leaf phenolics, α-solanine and α-chaconine.

Introduction

Plants interact with a multitude of above- and belowground beneficial and harmful organisms that often occur simultaneously or sequentially in the plant-associated food web (Bennett et al. 2006; Pozo and Azcon-Aguilar 2007; Huang et al. 2014; Biere and Goverse 2016). Through the morphological and biochemical changes that they induce in their shared host plant, above- and belowground herbivores can affect each other’s performance as well as the performance of later arriving herbivores, both within and across compartments, i.e., shoots and roots (Rasmann and Turlings 2007; Johnson et al. 2013; Erb et al.
Insect herbivores feeding on plants can induce plant defence, resulting in plant-mediated interactions between above- and belowground herbivorous insect (Bezemer and van Dam 2005; Erb et al. 2009; Sun et al. 2019). Plants also often associate with belowground microbial symbionts, e.g., arbuscular mycorrhizal fungi (AMF). Such root symbionts can affect plant-herbivore interactions by changing plant size, apparency, and induce changes in above- and belowground metabolite profiles that result in induced resistance or susceptibility, or by priming of plant defenses (Hartley and Gange 2009; Koricheva et al. 2009; Jung et al. 2012). AMF-induced changes in the response of plants to leaf- or root herbivores (Frew et al. 2020; Locke and Crawford 2022) can eventually result in altered population dynamics of later arriving above- and belowground feeders and subsequently determine community structure (Biere and Bennett 2013; Vannette et al. 2013; Tao et al. 2016).

In above- and belowground interactions with herbivorous insect, herbivores feeding on different plant compartments (i.e., leaf and root) can positively or negatively affect each other by inducing systemic changes in primary and secondary metabolites. For example, above- or belowground herbivory-induced changes in the composition or quantity of particular secondary metabolites (e.g., glucosinolates, terpenoids, phenols, tannins, and glycoalkaloids) can hinder the development of herbivores feeding in the other compartment (van Dam et al. 2004; Huang et al. 2013; Kumar et al. 2016). By contrast, systemic herbivore-induced changes in primary metabolites such as nitrogen and amino acids can increase their performance (Awmack and Leather 2002). Belowground herbivores can thus strongly affect aboveground feeders and vice versa, but these effects are often asymmetric. For example, belowground tuber-feeding by *Tecia solanivora* larvae reduced the performance of aboveground *S. exigua* and *Spodoptera frugiperda* by increasing chlorogenic acid and glycoalkaloid contents in potato plants, but *S. exigua* aboveground damage had no noticeable effect on belowground *T. solanivora* performance (Kumar et al. 2016). Such below-aboveground interactions not only occur among simultaneously feeding herbivores but also among sequentially feeding herbivores. Notably, herbivores often differ in their phenology, which establishes priority effects where plant defensive responses to previous attackers affect the opportunities for successful establishment of later arriving herbivores (Ohgushi 2005; Stam et al. 2018; Abdala-Roberts et al. 2019). A recent study showed that previous herbivory by the leaf-feeder *Apion godmani* reduced the performance of later arriving seed-eating beetles, *Zabrotes subfuscatus*, on lima bean plants (*Phaseolus lunatus*) (Hernández-Cumplido et al. 2016). However, plant-mediated effects between attackers may vary with for instance sequence of herbivory, plant species, and herbivore type (Johnson et al. 2012).

Not only previous herbivory, but also previous establishment of plant-associated microbes such as diseases or beneficial root symbionts can affect the success of later arriving herbivores. Arbuscular mycorrhizal fungi (AMF) are among the most common symbiotic microorganisms in soil. They can form symbiotic relationships with more than 80% of land plants. They easily acquire soil phosphorus, nitrogen, and micronutrients as well as water through their extraradical mycelial network and trade phosphorus and nitrogen for photosynthates in the form of sugars and lipids with their host plant (Smith and Read 2008; Smith and Smith 2011; Keymer and Gutjahr 2018). Under conditions where nutrients rather than carbon are limiting plant growth, root symbiosis with AMF therefore often results in enhanced plant growth and leaf concentrations of P and N (Leigh et al. 2009). In addition, root colonisation by AMF can
affect the resistance or tolerance of host plants to herbivory in above- or belowground plant tissues (De Deyn et al. 2009; Sharma et al. 2017; Eichholtzer et al. 2021; Rivero et al. 2021). Plant biochemicals associated with resistance against herbivorous insects such as phenolics, flavonoids, tannins, and alkaloids, can be systemically induced by AMF in leaves and roots (Ceccarelli et al. 2010; Hill et al. 2018; Frew et al. 2021). On the other hand, plants with AMF often have a higher nutrient status (Leigh et al. 2009), which could benefit herbivores. Thus the impacts of AMF on the performance of herbivorous insects has been shown to have positive, negative, and variable to no effects (Koricheva et al. 2009; Pineda et al. 2013). The magnitude and direction of these effects depends not only on improved nutrition or local changes within the roots and rhizosphere, but also on systemic changes including priming of jasmonate signaled defenses (Pozo et al. 2007; Jung et al. 2012). Moreover, the feeding mode and diet breadth of the herbivorous insect and the identity of fungi play an important role in the outcome of such interactions (Koricheva et al. 2009).

Interestingly, recent studies have shown that AMF may not only affect the performance of above- or belowground herbivores feeding on a shared host plant, but also modulate more complex interactions. In particular, AMF can modify the extent to which previous herbivory induces resistance or susceptibility to later arriving insect herbivores. For instance, Kempel et al. (2010) showed that in a number of plant species, previous herbivory only led to successfully induction of defense against ensuing herbivores if AMF were present, indicating interactive effects of AMF and prior herbivory on later arriving herbivores. By contrast, Wang et al. (2015) showed that AMF mitigated the induction of defense metabolites by prior herbivory as well as the reduction in relative growth rate of later arriving caterpillars in *Plantago lanceolata*. Clarifying such tripartite plant-insect-AMF interactions helps to better understand above- and belowground ecological consequence of interspecific interactions, but we lack a comprehensive insight in how AMF and prior belowground herbivory jointly affect subsequent aboveground herbivory and vice versa.

Here, we investigate how prior above- and belowground herbivory (i.e., leaf herbivory and tuber herbivory) by the potato tuberworm, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) and AMF symbiosis interactively affect the performance of subsequent above- and belowground conspecific larvae in a tuber-plant, potato (*Solanum tuberosum*). The potato tuberworm is a worldwide pest of Solanaceous crops, which causes serious losses to potato production by mining leaves and tubers (Rondon 2010; Horgan et al. 2013). Potato is the fourth largest food crop in the world and contains a series of primary metabolites (starch, protein and carbohydrate) and secondary metabolites (phenolics, α-solanine and α-chaconine) in leaves and tubers. The concentrations of these chemicals can be systemically altered by insect herbivores (Pacifico et al. 2019; Wang et al. 2020a; del Pulgar et al. 2021). Moreover, AMF can improve tuber nutrient and primary metabolite levels in potato (Lone et al. 2020). Considering that the occurrence of potato tuberworm is multigenerational (2–13 generations) every year (Rondon 2010), early-season herbivory may influence the performance of conspecific larvae that subsequently feed in above- and belowground plant compartment by systemically induced plant defences. Therefore, this is an ideal study system to investigate how herbivory (i.e., leaf herbivory and tuber herbivory) and AMF jointly affect plant productivity and subsequent above- and below-ground feeders and whether this could be mediated by
induced changes in plant primary and secondary metabolites. Based on previous studies (Kumar et al. 2016; Kumar et al. 2018), we hypothesize that tuber herbivory may reduce subsequent foliar herbivore performance by increasing leaf defensive responses and increase tuber production by inducing an overcompensation effect. Considering the asymmetry of interaction between above- and belowground herbivores, we also hypothesize that leaf herbivory does not necessarily affect or increase tuber-feeder performance. In addition, we hypothesize that AMF could modulate these above- and belowground interactions by interfering with induced changes in primary and secondary metabolites.

Specifically, we asked the following questions: how do above- or belowground herbivory and AMF, alone and in combination, affect (1) plant productivity (biomass), (2) subsequent conspecific larvae performance in above- and belowground compartments, and (3) the concentrations of primary (soluble sugar, soluble protein, starch, C, N, and C: N ratio) and secondary metabolites (phenolics, α-chaconine, and α-solanine) in leaves, roots, and tubers.

**Materials And Methods**

**Plant species**

We used the potato cultivar FAVORITA in our experiments, which is widely planted in northern China. Seed potatoes were obtained from the Zhengzhou Vegetable Research Institute, Henan, China. Before planting, seed potatoes were soaked in 10% H$_2$O$_2$ for 10 minutes to remove fungi on the surface of tubers, and then washed with deionized water. All seed potatoes were placed in a plastic container (80 cm×40 cm×20 cm) sterilized with 75% alcohol and covered with field soil sterilized by gamma radiation (25 kGy). After 2 weeks of germination, we cut the tubers with 1cm long buds into 25 g pieces using knives sterilized with 75% alcohol and 10% H$_2$O$_2$, each with an eye bud. All seed potatoes were planted 8cm deep into pots (height:28cm and diameter:36cm) with mixed soil (50% field soil and 50% sphagnum peat moss) sterilized by gamma radiation (25 kGy). All plants were covered with fabric (nylon netting: 10 m×5 m×2 m) in an open-sided greenhouse with natural temperature and light at Henan University, Kaifeng, China (34°49’ N, 114°18’ E).

**Insect rearing**

*Phthorimaea operculella* were obtained from a potato field in Qujing, Yunnan, China. We collected potato tubers and leaves with *P. operculella* larvae and reared them in nylon cages (40 cm×40 cm×40 cm) whose bottom was covered with 2cm deep sterilized sand for pupating. All nylon cages were placed in an insectary with 27 ± 2°C temperature, 70–80% R.H. (Relative Humidity) and a 12: 12 L: D (Light: Dark) photoperiod following published methods by Wang et al. (2020b). After 13–25 days, these mature larvae left the tuber or leaf and pupated in the sand in the cages. Around 10–15 days later, *P. operculella* adults began to emerge and 50 pairs of adults were collected and placed into plastic containers (height: 25 cm and diameter: 20 cm) with 10% honey water for mating and oviposition. After 5–10 days, we collected the filter paper with eggs on the top of the container and placed them into zip-lock bags. Larvae started to...
emerge 3–5 days later, and these newly hatched larvae were used in our experiments within 12 h after emergence.

**Experimental design: AMF inoculation and herbivory treatment**

We designed a 2×3 full-factorial experiment to investigate the effects of AMF and herbivory by *P. operculella* on plant morphological and biochemical traits (plant productivity and plant primary and defensive metabolites) and the performance of subsequently feeding conspecific larvae. Herbivory treatments included no herbivory, leaf herbivory, and tuber herbivory, while AMF treatments included AMF inoculation and No AMF inoculation. AMF was applied to potato plants one week after roots were formed.

The inoculated AMF spores were obtained by wet screening (Klironomos 2002, Qin and Yu 2019). We selected field soil that had been planted with potato crops to increase the probability that screened AMF spores could form a symbiotic relationship with potatoes. The soils were first screened through a 1cm-sieve to remove rocks and dead leaves. Then, 150 aliquots of 160 g (10% of the mass of potting soil per pot) were individually placed into beakers filled with 100ml sterile water. The resulting suspensions were successively passed through a 800µm-, 250µm- and 45µm-sieve. The sieves were washed with 10% sodium hypochlorite solution to remove residual bacteria. Finally, the obtained AMF spores were transferred to beakers with sterile water adjusted to a total volume of 200mL. Half of the filtrates (75 in total) were autoclaved at 15 lbs pressure and 120°C for 30 minutes with heating pressure steam sterilizer (LDZF-75KB, Shanghai, China) and inoculated into one-half of potato plants (No AMF inoculation). The other half was directly inoculated into the other half of potato plants (AMF inoculation).

We established three levels of herbivory (leaf herbivory, tuber herbivory, and no herbivory) in mycorrhizal and non-mycorrhizal plants with 25 replicates for each treatment after 8 weeks of plant growth. For leaf herbivory treatment we inoculated 10 newly hatched *P. operculella* larvae on the third and fourth leaf from the top with a small soft brush, and covered them with a small net (15 cm length and 10 cm width) to prevent the larvae from falling down or damaging other tissues (i.e. stems, tuber). For tuber herbivory treatment, to avoid damaging the tubers and fine root tissue, we carefully removed the potting soil with a big soft brush, until an intact tuber was completely exposed. Then, 10 newly hatched *P. operculella* larvae were placed onto this tuber with a small soft brush and covered the tuber with black cloth to reduce light-induced changes in tuber metabolism. After 2 hours, when all mining larvae had entered the tuber, tubers were covered with 2 cm thick soil. Control plants (no herbivory treatment) received a similar treatment (i.e., soil removal, covering the tuber with cloth, and returning into the soil), but without adding *P. operculella* larvae. All pots were placed at random positions at a distance of about 30cm from each other. Every two weeks all pots were randomly re-arranged to reduce any spatial or edge effects.

**Response variables**
After 3 weeks of infestation by *P. operculella*, all larvae were removed from leaves or tubers and we harvested all the plants. We then analyzed plant biomass, and primary and defensive metabolites using 15 replicates of each treatment. The remaining 10 replicates were used to assess subsequent insect performance (see below). We measured the biomass of shoots, roots and tubers to evaluate how plant productivity responded to AMF and herbivory treatments. The plant stem was cut off with scissors at the soil surface, and shoots, roots, and tubers were separated and washed. These plant tissues were placed into paper bags, dried at 60°C for five days, and then weighed. These dried leaves, roots, and tubers were ground into a powder with a ball mill (Heng'ao HMM-400A, Tianjin Heng'ao Technology Development Co., Ltd., Tianjin, China) for chemical analysis.

To determine colonisation of roots under the ‘AMF inoculation’ treatment and absence of colonisation of the roots under the ‘No AMF inoculation’ treatment we measured AMF colonisation of potato roots following established methods (Giovannetti et al. 1994). In brief, a subset of fresh root sample was randomly selected from all plants (45 no-AMF plants and 45 AMF plants) with different herbivory levels and was cleared with 10% KOH for 10 minutes. Fungal structures were stained with 0.05% Trypan blue for 24h at 4°C. Then for each sample, ten 1 cm root segments were neatly arranged on a slide and the presence of hyphae, vesicles and arbuscules were recorded at 300 grid-line intersections at 200×microscope magnification. Our results showed that AMF colonisation rate was 13.7% in plants with AMF inoculation, while no hyphae were detected in the roots of plants that had not been inoculated with AMF. Moreover, tuber herbivory increased AMF colonisation rate compared to no herbivory (Fig S1).

To assess the effect of AMF and prior herbivory on the weight gain of conspecific larvae that subsequently fed on leaves and tubers, we conducted bioassay experiments with detached leaves and tubers obtained from potato plants of the different treatment combinations. Detached leaves of similar size from different treatments were placed separately in Petri dishes (diameter: 9cm) with filter paper, 10 replicates per treatment. Distilled water was appropriately added every day to maintain leaf moisture. Similarly, 30 detached tubers of similar size from different treatments were placed separately in 200 ml beakers with wet filter paper at the bottom. These beakers were covered with a black cloth to mimic belowground light conditions. The laboratory conditions for bioassay were at 27 ± 2°C, 70–80% R.H. and a 12:12 h L: D photoperiod. Before the experiment, the weight of newly hatched larvae was measured. Since the weight of the newly hatched larvae was very small, we weighed larvae in batches of 100 individuals. The average initial weight of individual larvae was 0.064 mg. Three leaves and three tubers of each plant were individually inoculated with 1 newly hatched larva (within 12 h from hatching) with a soft brush. Each of the 12 treatment combinations (2 AMF×3 prior herbivory×2 plant tissues exposed to subsequent herbivory) had 10 replicates/plants, and 360 larvae were used throughout the bioassay. After 10 days, all larvae were isolated from the leaves and tubers and placed in a 1 mL centrifuge tube. These larvae were allowed to starve for 24 hours to excrete feces from the body before weighing. The larval weight gain was calculated as the final weight minus the average initial weight. Before data analysis, the weight of three larvae feeding on leaves or tubers on each plant was averaged to obtain a single value per plant tissue for each plant.
For plant primary metabolites, we measured the concentrations of carbon (C), nitrogen (N), soluble sugars, soluble protein, and starch in leaves, roots, and tubers. Plant C contents, N contents, and C:N ratio were determined by an elemental auto-analyzer (Vario MAX CN; Elementar, Hanau, Germany) (Schumacher et al. 2009). Plant soluble sugar and soluble protein contents were analyzed by ultraviolet spectrophotometer (Thermo Scientific GENESYS 10S, Waltham, MA, USA) at 630 nm and 595 nm wavelength, respectively, following published methods by Elleuch et al. (2007) and Bradford (1976). To analyze starch levels, the starch in tissue samples was broken down into soluble sugars with perchloric acid in boiling water conditions (100°C). The starch levels were then determined by ultraviolet spectrophotometer following the same methodology as used for the analysis of soluble sugars.

To measure plant defensive chemicals, the concentrations of phenolics, α-solanine and α-chaconine were determined in leaves, roots and tubers. Plant phenolics levels were analyzed following the methods of Ainsworth and Gillespie (2007), using gallic acid as a standard and measuring absorbance at 765 nm wavelength by ultraviolet spectrophotometer. The two glycoalkaloids α-solanine and α-chaconine were extracted following reported methods (Sotelo and Serrano 2000, Friedman et al. 2003) and their concentrations were determined with HPLC (high-performance liquid chromatography) (Agilent 1260, Palo Alto, CA, USA) at room temperature (25°C), using a reversed-phase ZORBAX SB-C18 (4.6×250 mm, 5 µm) column. Acetonitrile - water (35:65, v/v) containing 0.05 M monobasic ammonium phosphate was used as mobile phase and the flow rate was 1mL/min. The UV absorbance was measured at 210 nm and the injected sample size was 20 µL.

Data analysis

All analyses were performed using R version 3.4.2 (R Development Core Team 2017). All data were checked for deviations from normality and for homogeneity of variance before analysis. If necessary, the data were log(x + 1)-transformed. Two-way ANOVAs were used to assess the influence of ‘AMF’ (no AMF inoculation, AMF inoculation), ‘Herbivory’ (no herbivory, leaf herbivory, tuber herbivory) and their interactions on plant productivity, subsequent herbivore performance, plant primary metabolites and plant defensive metabolites, using the R package ‘car’ and post hoc Tukey’s HSD test from the R package ‘agricolae’. Additional orthogonal contrasts were added to the above two-way ANOVAs to evaluate whether ‘Herbivory’ effects were caused by ‘Leaf herbivory’ or by ‘Tuber herbivory’, or by both. The variables that were analysed were plant biomass (shoot biomass, root biomass, tuber biomass, and total biomass), larval weight gain on leaves and tubers, concentrations of C, N, soluble sugars, soluble proteins, starch, phenolics, α-solanine, and α-chaconine in leaves, roots, and tubers, and the C:N ratio. In addition, for each of the primary metabolites (protein, sugar, starch) we calculated the proportional investment of the metabolite in tubers relative to its investment in other tissues (“tuber allocation ratio”) by calculating the ratio of the absolute amount of the compound present in tubers to the absolute amount of that compound present in leaves plus roots (amount in tuber / amount in shoot + root).

Results
Effects of AMF and herbivory on plant productivity

Plant productivity was considerably impacted by AMF and *P. operculella* leaf and tuber herbivory (Table S1, S2; Fig. 1). Overall, herbivory decreased shoot and root biomass by 8% and 7%, respectively, but increased tuber biomass by 6%. The decrease in shoot biomass was mainly due to leaf herbivory (15% decrease, Fig. 1A), whereas the increase in tuber biomass was mainly due to tuber herbivory (17% increase, Fig. 1C). Moreover, AMF increased shoot, root and tuber biomass by 28, 26 and 26%, respectively (Fig. 1A, 1B, 1C). Although AMF-induced increases in shoot and total biomass were proportionally larger in plants under herbivory (33 and 29%, respectively) than in control plants (20 and 22%, respectively), there were no significant interactions between AMF and herbivory, indicating that effects of AMF on plant growth were independent of herbivory (Table S1, S2).

Effects of AMF and herbivory on subsequent conspecific larvae

*Leaf feeding larvae* - Prior tuber herbivory by *P. operculella* significantly decreased the weight gain of conspecific larvae that were subsequently feeding on leaves compared to the weight gain of larvae feeding on leaves from plants with no prior herbivory or prior leaf herbivory, whereas AMF had no significant impact on the weight gain of subsequent leaf-feeding larvae (Table S1, S2; Fig. 2A).

*Tuber feeding larvae* - By contrast, prior tuber herbivory did not affect the weight gain of conspecific larvae that subsequently fed on the tubers of these plants if these plants had been grown without AMF. However, prior tuber herbivory enhanced the weight gain of larvae subsequently feeding on these tubers if these plants had been inoculated with AMF (Fig. 2B). This indicates that AMF interacted with previous tuber herbivory (Table S1,S2, AMF×Tuber herbivory interaction, p < 0.05) to facilitate ensuing tuber feeders. AMF also overall enhanced larval weight gain on tubers (Table S1; Fig. 2B)

Effects of AMF and herbivory on primary metabolites

*Leaves* - AMF and herbivory had minor effects on leaf nitrogen and soluble proteins. Tuber and leaf herbivory slightly reduced leaf concentrations of nitrogen and soluble proteins, respectively, whereas AMF slightly enhanced leaf soluble protein levels (Table S3, S4; Fig. 3A, 3B). Leaf carbon contents and C: N ratio were not affected by AMF, herbivory or their interaction (Table S3). However, AMF strongly reduced leaf soluble sugar concentrations by 18.5%, whereas leaf herbivory reduced leaf soluble sugar concentrations (by 10.8%) but only in plants without AMF (Table S3, S4; Fig. 3C). Both tuber and leaf herbivory reduced leaf starch concentrations (Table S3, S4; Fig. 3D).

*Tubers* - Tuber primary metabolite concentrations were strongly altered by AMF and herbivory. Tuber carbon concentrations were significantly enhanced by independent effects of tuber herbivory and AMF (Table S3, S4; Fig. 3E), whereas AMF and herbivory did not affect tuber concentrations of nitrogen or their C: N ratio (Table S3). Tuber soluble protein and starch concentrations were strongly enhanced by AMF and tuber herbivory, but decreased by leaf herbivory (Table S3, S4; Fig. 3F, H). Tuber herbivory and AMF
significantly increased tuber starch concentrations. AMF tended to enhance the net effect of leaf and tuber herbivory on tuber starch levels (Table S3, S4), but the interactions were only marginally significant (AMF×Tuber herbivory, p = 0.098; AMF×Leaf herbivory, p = 0.056). Tuber soluble sugar concentrations were also significantly enhanced by AMF, but not affected by leaf herbivory and reduced by tuber herbivory (Fig. 3G).

Roots - Root primary metabolite concentrations were significantly affected by AMF but not by herbivory. AMF significantly increased the root concentrations of carbon and soluble proteins but decreased those of starch (Table S3; Fig. 3I, 3J, 3K), whereas it did not affect root concentrations of nitrogen and root soluble sugar or root C:N ratio.

Mycorrhizal plants exhibited a 22% higher protein tuber allocation ratio than plants that had not received AMF inoculation, and tuber herbivory dramatically increased this ratio by 52% compared with no herbivory. Leaf herbivory had only minor effects on the protein tuber allocation ratio; it tended to enhance it in the absence of AMF but decrease it in their presence, resulting in a significant interaction between AMF and leaf herbivory (Table S3, S4; Fig. 4A). The tuber allocation ratio for sugars was increased by 36% by AMF, but not affected by tuber herbivory. As observed for proteins, leaf herbivory tended to enhance the sugar tuber allocation ratio in the absence of AMF but to decrease it in their presence (Table S3, S4; Fig. 4B). The tuber allocation ratio for starch was increased by 12% by AMF, whereas tuber herbivory increased this ratio by 57% compared to plants without herbivory. Again leaf herbivory tended to enhance this ratio for starch in the absence of AMF but to reduce it in their presence (Table S3, S4; Fig. 4C).

Effects of AMF and herbivory on secondary defensive metabolites

Leaves - Leaf secondary metabolites were significantly affected by AMF and herbivory. AMF inoculation strongly enhanced leaf concentrations of phenolics (Table S5; Fig. 5A). Overall, herbivory also enhanced leaf phenolic concentrations, but these effects depended on AMF. Specifically, tuber herbivory enhanced leaf phenolic concentrations in the presence of AMF but not in their absence, resulting in a significant AMF×Herbivory interaction (Table S5, S6; Fig. 5A). Leaf concentrations of the two glycoalkaloids, α-solanine and α-chaconine were mainly affected by tuber herbivory. Both of them were significantly enhanced by tuber herbivory both in plants with and without AMF (Table S5, S6; Fig. 5B, C).

Tubers and roots - Effects of AMF and herbivory on secondary metabolites in roots and tubers were generally less strong than in the leaves. In tubers, both AMF and herbivory slightly reduced the concentration of phenolics (Table S5; Fig. 5D). Tuber concentrations of α-chaconine were increased by leaf herbivory, but only in the presence of AMF (Table S5, S6; Fig. 5E), whereas tuber concentrations of α-solanine were unaffected by AMF and herbivory (Table S5). Root total phenolic levels depended on the interactive effects of AMF and herbivory. Specifically, AMF reduced root phenolics in control plants without herbivory, but not in plants exposed to leaf or tuber herbivory (Table S5, S6; Fig. 5F). AMF did not
affect levels of root glycoalkaloids and herbivory only slightly enhanced levels of root α-chaconine (Table S5, S6; Fig. 5G).

Discussion

Our results show that both AMF colonisation and tuber herbivory increased potato productivity by increasing tuber biomass, suggesting that plants overcompensated tuber growth in response to herbivory. Furthermore, AMF and tuber herbivory independently improved tuber nutritional status by increasing tuber carbon, soluble protein and soluble starch concentrations. Our results also demonstrate that AMF increased developmental performance of *P. operculella* larvae subsequently feeding on tubers, potentially via the observed increase in tuber primary metabolite levels (i.e., soluble protein). Interestingly, tuber herbivory only enhanced the performance of later arriving tuber feeders in the presence of AMF, indicating that the presence of AMF was essential for this belowground facilitation. By contrast, in the leaves, tuber herbivory by *P. operculella* significantly increased α-solanine and α-chaconine concentrations, independent of AMF. These changes in leaf α-solanine and α-chaconine might be involved in the negative effect of tuber herbivory on subsequent conspecific larvae feeding on leaves (Fig. 6). Our findings improve our understanding of the importance of above- and below-ground antagonistic and mutualistic interactions when predicting plant responses to subsequent herbivore damage.

Overcompensatory tuber growth and altered tuber quality in response to tuber herbivory by *P. operculella*

Overcompensation occurs when herbivory results in a higher plant biomass or seed production in damaged plants than in undamaged plants (Strauss and Agrawal 1999). Occurrence and degree of overcompensation depends on a series of factors, including the feeding site of herbivores (Houle and Simard 1996), feeding intensity of herbivores (Stieha and Poveda 2015), abiotic conditions, and the presence or absence of other biotic factors such as mycorrhizal fungi (Allsup and Paige 2016). In this study, tuber herbivory exerted an overcompensatory effect by increasing tuber biomass, and this overcompensation effect was also observed for total biomass but only in the presence of AMF (Fig. 1). It is well documented that low-level herbivore infestation in tubers can enhance tuber yield. For instance, low level tuber herbivory doubled the yield of potato plants compared to that of plants without herbivory (Poveda et al. 2010; Poveda et al. 2018). In addition to the quantity of plant tissue, herbivory can also affect the quality of plant tissue. Kumar et al. (2018) reported that tuber infestation by *T. solanivora* larvae caused an 80% decrease in sucrose content in systemic tubers and this effect was attributed to an increased conversion of sucrose into starch. In our study, analyses of plant primary metabolites showed that both tuber herbivory and AMF, largely independently, increased the concentrations of starch, soluble proteins, and carbon in the tubers (Fig. 3). Their combined effects not only resulted in the highest tuber biomass and total biomass, but also in the highest tuber concentrations of carbon, soluble proteins, and starch. We speculate that tuber herbivory alters the source-sink relationship of primary metabolite transport from leaves (source tissue) to tubers (sink tissue) during tuberization (Baroja-Fernandez et al.
2009), and that this effect is enhanced by AMF. Moreover, our results also show a higher allocation of protein and starch to tuber tissue at the whole plant level in response to tuber herbivory (Fig. 4). Management strategies for control of tuber pests in farmland systems could benefit from capitalizing on such knowledge to enhance potato productivity and quality (Poveda et al. 2018).

**Induced plant chemicals potentially affect subsequent above- and below-ground feeders**

Increasing evidence shows that plants subjected to attack in one compartment show induced responses to subsequent attackers in the other compartment (Erb et al. 2009; Huang et al. 2013). According to our findings, tuber infestation by *P. operculella* enhanced the performance of conspecific larvae that subsequently fed on tubers of AMF-inoculated plants, but decreased the performance of subsequent conspecific larvae feeding on leaves. The latter result is in agreement with previous studies reporting that below-ground herbivory can reduce above-ground herbivore performance, and that plant-induced primary and secondary metabolites play an important role in regulating the plant-mediated interactions between herbivores feeding in different (aboveground and belowground) plant compartments (Johnson et al. 2009; Johnson et al. 2012; Huang et al. 2014; Biere and Goverse 2016). For instance, root herbivory by the nematode *Meloidogyne incognita* increased leaf trypsin protease inhibitor activity in tomato plants, and negatively affected *Tuta absoluta* pupation by increasing the duration of the pupal phase and the proportion of dead and deformed pupae (Arce et al. 2017). *T. solanivora* tuber feeding decreased the larval mass of leaf feeding herbivores by increasing the accumulation of chlorogenic acid and glycoalkaloids in potato plants (Kumar et al. 2016).

Phenolics are usually associated with plant resistance to herbivory, which can reduce the developmental performance of herbivores (Mithofer and Boland 2012; Agrawal and Weber 2015). Similarly, glycoalkaloids (α-solanine and α-chaconine) can confer enhanced resistance to herbivores in potato by affecting their palatability (Hlywka et al. 1994; Dinkins et al. 2008). Consistent with these results, we observed that leaf concentrations of phenolics, as well as leaf concentrations of α-solanine, and α-chaconine were higher in plants exposed to prior tuber herbivory. Interestingly, leaf concentrations of phenolics were only enhanced in response to tuber herbivory in the presence of AMF, indicating that these responses are primed by AMF. By contrast, leaf concentrations of α-solanine and α-chaconine were enhanced in response to tuber herbivory both in the presence and absence of AMF. Therefore, these glycoalkaloids may have contributed to the tuber herbivory-induced resistance to subsequent conspecific leaf feeders that we observed in both AMF and non-AMF plants.

We found positive effects of AMF on the performance of subsequent *P. operculella* larvae feeding on tubers. Multiple mechanisms have been proposed by which AMF may affect plant-herbivore interactions. The current paradigm is that AMF enhance plant defense against leaf chewing insect herbivores through priming of plants for jasmonic acid signaled defenses (Pozo and Azcon-Aguilar 2007; Jung et al. 2012; Song et al. 2013). However, AMF can also modulate a suite of other plant traits including plant primary metabolites that can positively or negatively affect herbivore performance (Bennett et al. 2006;
Schoenherr et al. 2019). Our results are consistent with prior studies showing that AMF can improve herbivore growth and survival (Tomczak et al. 2016; Malik et al. 2018). In our study, AMF enhanced the tuber concentrations of primary metabolites (carbon, soluble protein, and starch). Especially soluble protein can be efficiently used by herbivorous insects to acquire nourishment (Awmack and Leather 2002). In addition, AMF reduced the levels of tuber phenolics. These changes may have contributed to the positive effect of AMF on subsequent tuber feeders.

**AMF and previous herbivory interactively affect the performance of subsequent herbivores**

A growing number of studies shows that effects of previous herbivory on subsequent herbivory is contingent upon the present or absence of root symbiotic fungi such as AMF (e.g. Kempel et al. 2010; Wang et al. 2015; Frew et al. 2017; Locke et al. 2022). Most of these studies focused on the impact of AMF and previous herbivory on aboveground insects, and only a few studies focused on belowground herbivores. In this study, we comprehensively evaluated how above- and belowground insects and AMF jointly affect the performance of subsequent above- and belowground herbivores. An important finding was that whereas the impact of tuber feeding on aboveground insects was negative and not affected by AMF, the impact on belowground insects was significantly affected by AMF. More specifically, the effect of tuber feeding on subsequently arriving belowground insects was positive, but only in the presence of AMF. This indicates that the belowground conspecific facilitation that we observe in this system is dependent on the presence of AMF. A series of studies have shown that belowground herbivores can facilitate the development of co-occurring or later arriving insects. For example, belowground infestation by wireworms *Agriotes spp.* increased foliar-feeding aphid *Rhopalosiphum padi* numbers on barley *Hordeum vulgare* plants (Johnson et al. 2009). Root-feeding weevils *Otiorhynchus sulcatus* increased phloem-feeding aphid *Cryptomyzus galeopsisidis* abundance on blackcurrant plants (Johnson et al. 2013). However, according to our results, these facilitation effects may depend on AMF colonisation. Thus herbivorous insects and AMF interactively determine herbivore performance. This information can be useful for optimizing pest management and potato production in the future (Johnson et al. 2021). Moreover, our work extends the conventional approach of studying the joint effects of AMF and herbivory on subsequent herbivores in the same compartment to responses in both above- and belowground compartments and suggests that future work should fully embrace the complexity of plant-associated above- and belowground herbivore communities.

**Conclusions**

In summary, our study demonstrates the differential impact of above- and belowground herbivory and arbuscular mycorrhizal fungi on plant productivity and the performance of subsequent herbivores feeding above- and belowground on potato plant. While tuber herbivory results in induced resistance to aboveground feeding conspecifics, it leads to facilitation of tuber feeding conspecifics in interaction with fungal plant symbionts (i.e., AMF). In addition, such tripartite plant-herbivore-AMF interactions lead to systemic alterations in primary and secondary metabolites in above- and belowground plant tissues.
Whereas resistance to conspecific foliar feeders induced by tuber feeders could be associated with induction of one of the glycoalkaloid secondary metabolites, facilitation of conspecifics tuber feeders induced by tuber herbivory and AMF were more likely mediated by changes in primary metabolite profiles. These findings provide novel insights into the role and ecological consequences of plant-mediated interactions with antagonists and mutualists in shaping the interactions with later arriving above- and below-ground organisms within the plant-associated food web (Rodriguez-Rodriguez et al. 2017; Mejia-Alva et al. 2018; Van Goor et al. 2021).

Declarations

Author Contributions

J. D. and D.W. conceived the idea; J.D. and D.W. designed the experiments; D.W. performed the experiments; J.D., A.B. and D.W. analysed the data; all authors wrote the manuscript.

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Data availability

The data supporting the results of this study will be made available in the Dryad repository.

Conflict of Interest Statement

The authors declare no conflict of interest.

Ethical approval

This article does not contain any studies with vertebrates performed by the authors.

References


**Figures**
Figure 1

Effects of AMF (No AMF inoculation and AMF inoculation) on (A) shoot biomass, (B) root biomass, (C) tuber biomass and (D) total biomass in *Solanum tuberosum* with different herbivory treatments of *Phthorimaea operculella* (no herbivory, leaf herbivory and tuber herbivory). Differences between treatments were determined by two-way ANOVAs (* 0.01 ≤ P < 0.05, ** 0.001 ≤ P < 0.01, *** P < 0.001) followed by post hoc Tukey's HSD test (different letters indicate P < 0.05).
Figure 2

Effects of AMF (No AMF inoculation and AMF inoculation) and prior herbivory treatment of *Phthorimaea operculella* (no herbivory, leaf herbivory and tuber herbivory) on weight gain of conspecific larvae feeding on detached (A) leaves and (B) tubers of *Solanum tuberosum*. Data are means±SE. Differences between treatments were determined by two-way ANOVAs (**P**<0.001) followed by post hoc Tukey’s HSD test (different letters indicate **P**<0.05).
Figure 3

Effects of AMF (No AMF inoculation and AMF inoculation) on primary metabolites of leaf (A-D), tuber (E-H) and root (I-K) in *Solanum tuberosum* with different herbivory treatments of *Phthorimaea operculella* (no herbivory, leaf herbivory and tuber herbivory). Differences between treatments were determined by two-way ANOVAs (* 0.01 ≤ P < 0.05, ** 0.001 ≤ P < 0.01, *** P < 0.001) followed by post hoc Tukey's HSD test (different letters indicate P < 0.05).
Figure 4

Effects of AMF (No AMF inoculation and AMF inoculation) on (A) Tuber/(leaf+root) ratio for protein, (B) Tuber/(leaf+root) ratio for sugar and (C) Tuber/(leaf+root) ratio for starch in *Solanum tuberosum* with different herbivory treatments of *Phthorimaea operculella* (no herbivory, leaf herbivory and tuber herbivory). Differences between treatments were determined by two-way ANOVAs ( ** 0.001 ≤ P < 0.01, *** P < 0.001) followed by post hoc Tukey's HSD test (different letters indicate P < 0.05).
Figure 5

Effects of AMF (No AMF inoculation and AMF inoculation) on secondary metabolites of leaf (A-C), tuber (D, E) and root (F, G) in *Solanum tuberosum* with different herbivory treatments of *Phthorimaea operculella* (no herbivory, leaf herbivory and tuber herbivory). Differences between treatments were determined by two-way ANOVAs (* 0.01 ≤ P < 0.05, ** 0.001 ≤ P < 0.01, *** P < 0.001) followed by post hoc Tukey's HSD test (different letters indicate P < 0.05).
Figure 6

Diagram illustrating the impacts of AMF inoculation and tuber herbivory by Phthorimaea operculella on tuber productivity, tuber quality and performance of subsequent conspecific larvae feeding on leaves and tubers by changing plant metabolites. Positive or negative effects are indicated by ‘green arrow’ or ‘red arrow’ respectively. (A) plant treatment of tuber herbivory and AMF inoculation; (B) changes in plant primary metabolites, defensive metabolites and tuber quality; (C) responses of subsequent insect performance and tuber productivity.

Supplementary Files

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