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Plant and insect microbial symbionts alter the outcome of plant–herbivore–parasitoid interactions: implications for invaded, agricultural and natural systems

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Summary

1. Understanding how soil microbial communities influence plant interactions with other organisms, and how this varies with characteristics of the interacting organisms, is important for multiple systems. *Solanum* spp. are a suitable model for trophic interactions in studies of agricultural and natural systems and can also provide useful corollaries in invaded systems. This study examined the influence of soil mutualist arbuscular mycorrhizal (AM) fungi on growth of different *Solanum* types fed on by the potato aphid, *Macrosiphum euphorbiae*, in relation to the presence of the aphid facultative endosymbiont *Hamiltonella defensa*.

2. Four *Solanum* types comprising two wild species, *S. berthaultii* and *S. polyadenum*, and two accessions of *S. tuberosum*, were grown with or without AM fungi and infested with one of four clonal lines of a single *M. euphorbiae* genotype (two with and two without *H. defensa*). Two experiments were conducted to (i) characterize plant responses to AM fungi and aphids and (ii) assess whether soil AM fungi could influence the success of the parasitoid wasp *Aphidius ervi* when attacking aphids reared on each *Solanum* type.

3. In both experiments, similar patterns of plant biomass were observed in relation to AM fungal and aphid treatments. *Solanum* biomass depended on plant type and aphid infection with *H. defensa*. Plants exposed to aphids harbouring *H. defensa* had smaller root biomass, and therefore total plant biomass, compared to plants infested with *H. defensa*-free aphids. *M. euphorbiae* performance varied with aphid clonal line, *Solanum* type and the presence of AM fungi.

4. Parasitoid success, measured as the proportion of aphids from which a wasp emerged, was highest from aphids that had fed on plants colonized by AM fungi, although this result also varied with *Solanum* type and aphid clonal line.

5. *Synthesis*. The presence of soil AM fungi, combined with within-species plant and insect variation in key traits, can have subtle – but significant – effects on plant fitness and insect success. This study highlights the importance of exploring genotypic variation in plant and pest responses to soil microbiota to identify suitable biocontrol options.

Key-words: aphid, *Aphidius ervi*, arbuscular mycorrhizal fungi, genotype-by-genotype interaction, *Hamiltonella defensa*, invasion ecology, *Macrosiphum euphorbiae*, multitrophic interaction, plant–soil (below-ground) interactions, *Solanum* spp.

Introduction

Soil microbial communities can influence the survival and persistence of native, naturalized and newly invasive plants (van der Putten, Klironomos & Wardle 2007; Bever *et al.* 2010; Philippot *et al.* 2013) and can alter the composition and dynamics of above-ground communities (Van Dam & Heil 2011). Arbuscular mycorrhizal (AM) fungi are particularly well known for their impact on plant fitness. AM fungi form symbiotic relationships with 80% of all known land plants and provide an enhanced supply of phosphorus, nitrogen, essential minerals and water in exchange for photosynthetic carbon from plants (Smith & Read 2008). As a consequence of the symbiosis function, AM fungi can change host plant quality for insect herbivores through their impact on plant nutritional quality and/or by priming effects that lead

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to enhanced inducible and constitutive plant defences (Gehring & Bennett 2009; Jung *et al.* 2012). Plant quality effects on herbivores could cascade to higher trophic levels aboveground (Gange, Brown & Aplin 2003; Bezemer *et al.* 2005; Hempel *et al.* 2009; Wooley & Paine 2011), altering the success of natural enemy control of insect pests, and thereby influencing plant survival and success. Understanding the interactions of a newly invasive plant species with AM fungi and other trophic groups is, therefore, crucial in gauging the potential impact of invasive species on ecosystem processes and properties (e.g. Wardle *et al.* 2004).

Phloem-feeding aphids are a successful group of insect herbivores in natural and managed vegetation, and populations are regulated in part by generalist and specialist natural enemies (Dixon 1998; Karley *et al.* 2004; Wajnberg, Bernstein & van Alphen 2008). AM fungi can alter the performance of aphids, although phloem-feeding herbivores tend to be less affected than chewing insects by AM fungal priming of plant defence, possibly due to the lower concentration of defence compounds found in phloem sap (Koricheva, Gange & Jones 2009). While previous research has shown that AM fungi can influence the production of plant volatiles that attract natural enemies to herbivore-infested plants (Guerrieri *et al.* 2004; Leitner *et al.* 2010; Schausberger *et al.* 2012; Babikova *et al.* 2013), no work has focused on the potential of AM fungi to alter the quality of aphids as hosts for their natural enemies despite the evidence that AM fungi likely improve aphid fitness through enhanced plant nutritional quality (Koricheva, Gange & Jones 2009). For example, many parasitoids show higher attack rates on larger insect hosts, due to increased resource availability for larval development (Godfray 1994; Ode & Hardy 2008), although the success of parasitism can be compromised by nymph availability for parasitism if changes in plant quality alter aphid population structure (Aslam, Johnson & Karley 2013) and by more costly handling of larger hosts (Harvey, Poelman & Tanaka 2013). Both cereal aphid (*Rhopalosiphum padi)* performance on *Agrostis capillaris* and *Anthoxanthum odoratum* and parasitoid fitness were reduced on plants inoculated with manipulated soil micro-organism and nematode communities, indicating that soil community, including AM fungi, can have a significant effect on multitrophic interactions (Bezemer *et al.* 2005). Thus, we expect that AM fungi may influence the interaction between herbivores and their natural enemies, and specifically parasitoid wasps, by altering the quality of the herbivore as a host for parasitism.

Aphids, however, harbour their own microbial symbionts that can alter the outcome of interactions with their food plant and natural enemies. In addition to the obligate nutritional endosymbiont *Buchnera aphidicola*, aphids can harbour one-to-several types of facultative bacterial endosymbiont, some of which provide varying degrees of protection to aphids from parasitism and other types of natural enemy attack (Schmid *et al.* 2012; Oliver, Smith & Russell 2014; Vorburger 2014). In particular, protection provided by the facultative endosymbiont *H. defensa* to some aphid species (although not all aphid species, e.g. von Burg *et al.* 2008) is thought to arise from toxin-encoding lysogenic bacteriophage (known as APSE) associated with the symbiont that halts the development of the parasitoid larva (Oliver & Moran 2009). The level of protection provided can depend both on the specific isolate of *H. defensa* as well as on parasitoid genotype (Oliver, Moran & Hunter 2005; Cayetano & Vorburger 2013). The frequency of *H. defensa* infection in aphid populations can vary significantly, both geographically and between host plant species, which could compromise parasitoid success on particular plant species (Ferrari *et al.* 2012; Brady & White 2013; Russell *et al.* 2013; Vorburger 2014). Moreover, in the absence of parasitoids, the frequency of *H. defensa* infection in the aphid population declines over time (Oliver *et al.* 2008; Vorburger & Gouskov 2011), suggesting a fitness cost associated with harbouring *H. defensa*, possibly related to the microbial maintenance of APSE bacteriophage (Vorburger 2014). Thus, the combined aphid and facultative endosymbiont genomes (termed 'holobiont': Mandrioli & Manicardi 2013) can contribute to intraspecific genetic and phenotypic variation in aphid susceptibility to parasitoid wasps. Little is known, however, about the role of facultative endosymbionts in shaping trophic interactions between parasitoids, aphids and plants (Frago, Dicke & Godfray 2012), and how insect endosymbionts might interact with AM fungi to alter outcomes of multitrophic interactions, particularly in invaded plant communities.

Overall, the interactions between AM fungal–plant mutualisms and aphid–endosymbiont–parasitoid interactions create a complex multitrophic network. Within this network, the fitness effects of each component can have cascading bottom-up or top-down effects on other members of the system, thus influencing the stability and function of the whole community (Gehring & Bennett 2009; Biere & Bennett 2013). This suggests that the success of plant invasion could depend to an extent on the presence and function of facultative microbial symbionts associated with the soil and insect herbivores. Yet, most studies on plant–herbivore interactions have treated herbivores and plants as individual entities, neglecting the fact that both of these organisms can harbour obligate or facultative endosymbiont communities that can have profound effects on fitness (Smith & Read 2008; Ferrari & Vavre 2011; Oliver, Smith & Russell 2014; Vorburger 2014). The outcomes of bitrophic plant–herbivore interactions observed in isolation may not, therefore, provide a realistic representation of plant–herbivore interactions in a community context. Additionally, the impact of AM fungal–plant symbiosis on multitrophic interactions has been shown to vary with plant and herbivore genotype (Gols *et al.* 2008; Vannette, Hunter & Rasmann 2013), thus focusing on a single plant or aphid type might not reveal the importance of genotype-specific interactions meaning that unusual outcomes can have undue influence on our understanding of trophic interactions. Agroecological models offer a useful proxy for understanding the multiple factors that could influence invasive and natural plant success: first, like many invasive species, crop plants are typically grown in monoculture in fertilized disturbed (ploughed) soil with less diverse soil microbial communities,

similar to many invaded systems, and are highly competitive relative to the native flora of the agroecosystem under these conditions. Secondly, invasive plants are often hypothesized to have undergone a bottleneck effect that will have limited their genetic diversity, and, because they often grow in monocultures, any genotypic variation within invasive plant types will be a significant influencing factor compared to diverse plant communities. Agroecological systems also typically consist of monocultures with low genetic diversity, but in agroecological systems, it is logistically easier to identify and manipulate genotypes and compare them to wild (or precursor) relatives. Thirdly, both agroecological and invasive systems frequently use insect biocontrol (either to promote or to suppress plant growth) which interact with a native or augmented community of natural enemies.

Here, we focus on *Solanum* spp. infested with the potato aphid *Macrosiphum euphorbiae*, which is attacked by the generalist aphid parasitoid *Aphidius ervi*. Using this study system, recent work has shown that the aphid facultative endosymbiont *H. defensa* can enhance an aphid-induced decrease in plant resource partitioning to roots in cultivated *Solanum tuberosum* (Hackett, Karley & Bennett 2013). In the present study, we explore this phenomenon in a wider range of *Solanum* types, encompassing wild and cultivated species; further, we explore the impact of AM fungi and aphid holobiont type on the outcome of plant–herbivore–parasitoid interactions.

The primary aim of this study was to investigate the influence of plant type, herbivore holobiont and root AM fungal infection on changes in root allocation and parasitism success in order to better understand how microbial symbionts might influence plant success in natural, invaded and agricultural systems. Globally, the genus *Solanum* includes wild, cultivated and invasive species, and thus, we expect the study findings to have ecological relevance for this wide range of systems.

Materials and methods

STUDY SYSTEM

For our study, two accessions of *S. tuberosum* (diploid accession STN4709 and tetraploid 'Chilean' accession TBR5642) and the two diploid wild potato species *S. berthaultii* and *S. polyadenum* (accessions BER7748 and PLD7778, respectively) were selected based on differences in susceptibility to aphid herbivory (Gibson & Pickett 1983; A.J. Karley and A.E. Bennett, unpublished data). Throughout the rest of the manuscript, we will refer to these as Solanum 'types' to encompass both the within- and between-species variation. *Solanum berthaultii* and *S. polyadenum* show higher constitutive resistance to aphids compared to *S. tuberosum* genotypes, thought to be in part due to the high production of plant defence compounds such as *E*-β-farnesene, a defensive compound that acts as an aphid alarm pheromone (Gibson & Pickett 1983). Seeds of each *Solanum* type were provided by the Commonwealth Potato Collection maintained at the James Hutton Institute, Dundee, Scotland.

The background soil was collected from an uncultivated site adjacent to potato cultivation near the source of the spore inocula, homogenized (well mixed to ensure the same biota, chemistry and physics throughout) and mixed with sand at a 1:1 ratio by volume. The soil–sand mixture was steam sterilized in an autoclave at 121 °C (15psi) for 2 h, allowed to cool for 24 h and then steam sterilized again at 121 °C (15psi) for a further 2 h.

While it is not possible to replicate the exact AM fungal community potato plants would be exposed to in the field, we attempted to replicate that community as closely as possible by extracting spores from the same volume of bulk soil we would typically add to a pot as inocula (ten per cent of total pot volume). This approach allows us to assess the influence of a realistic indigenous AM fungal community (and at realistic spore abundances) that *Solanum* is likely to be exposed to in a field environment. To avoid introducing potato pathogens into our system, AM fungal spores were isolated from a field verge adjacent to potato cultivation (GPS coordinates: 56°27′27.0″N 3°04′01.5″W) at the James Hutton Institute, Dundee, Scotland. Experimental pots were 1 L in size, 10% of the pot volume was therefore 100 mL, there were 160 pots per experiment, and so spores were isolated from 16.5 L of this soil (to allow extra for sampling to assess spore density and diversity and creation of a microbial wash). As a result, each plant received approximately the same spore numbers and diversity it would have been exposed to in the field. AM fungal spores were extracted using wet sieving and sucrose centrifugation (Daniels & Skipper 1982), collected in water, and extraneous spore extraction solution (without spores) was removed to produce a total spore solution of 200 mL. In both experiments, three replicate subsamples of this inoculum solution had similar average densities of spores (20.45 ± 1.56 mL^{-1} in 2013, 26.33 ± 1.20 mL^{-1} in 2014), equal maximum levels of species richness (6 morphospecies mL^{-1}) with an average richness in 2103 of 4.93 ± 0.48 and an average richness of 4.67 ± 0.33 in 2014. The same morphospecies appeared in the samples in each experiment, and two of the morphospecies could confidently be identified as *Funneliformis mosseae* and *Rhizophagus irregularis*. The remaining morphospecies will be identified using molecular techniques in a future publication. Extraneous spore extraction solution (not containing spores) and 10 mL of the spore solution (containing spores) were used to make a microbial wash by vacuum filtration through a Grade 1 11 μm Whatman filter paper (125 mm, Buckinghamshire, England) to create an equal volume of the microbial wash. Before addition to the pots, half of the AM fungal inoculum and the microbial wash (90 mL of each) were steam-sterilized for 20 min at 121 °C.

Clonal lines of the potato aphid, *M. euphorbiae*, collected in 2013 from *S. tuberosum* at cultivated and garden sites in Tayside and Perthshire, were maintained on excised *S. tuberosum* leaves (cv. Desirée) in ventilated cups at 20 °C with 16:8-h light:dark. Clonal lines belonged to a single aphid genotype (Clarke 2013) and the presence or absence of the facultative endosymbiont *H. defensa* was confirmed using the methodology of Clarke (2013) and Hackett, Karley & Bennett (2013). Clonal lines used in Experiment 1 included AK13/05 and AK13/18 (which hosted *H. defensa*), and AK13/19 and AK13/28 (which hosted no known facultative endosymbionts). Clonal lines used in Experiment 2 included AK13/18 and AK13/30 (which hosted *H. defensa*), and AK13/08 and AK13/22 (which hosted no known facultative endosymbionts).

Mummies of the generalist parasitoid *Aphidius ervi* were purchased from Syngenta Bioline (Essex, UK) and reared for at least one generation on a clonal line of *Acyrthosiphon pisum* that harbours no known facultative endosymbionts. Emerging wasps were removed daily to produce cohorts of known age; adult wasps were maintained in clear vented acrylic boxes at 20 °C with 16:8-h light:dark and supplied with 50% (v/v) honey solution using a soaked cotton ball.

EXPERIMENTAL DESIGN

Two experiments were conducted using a randomized 2 × 4 × 4 factorial design comprising two soil treatments (inoculated with sterile or live AM fungal spores), four *Solanum* types and four *M. euphorbiae* clones (two with and two without *H. defensa*). Each soil–plant–aphid treatment combination was replicated five times, giving a total of 160 plants, which were placed in random positions within two blocks. The first experiment (2013) focused on interactions between *Solanum* type, AM fungal presence and aphid identity, while the second experiment (in 2014) focused on parasitoid responses to our experimental treatments.

In both experiments, seeds were germinated in sterilized coir. After 3 weeks, the seedlings were transplanted into 160 one-litre pots filled with the sterilized background soil. Half of the pots were inoculated with 1 mL of the live AM fungal inoculum (containing spores extracted from 100 mL of soil) and 1 mL of the sterile microbial wash, while the other half of the pots were inoculated with 1 mL of the sterile AM fungal inoculum and 1 mL of the live microbial wash. Solutions were injected into the root ball of each plant using a pipette. Adding live microbial wash to the sterile treatment controlled for any effects of other microbes in the AM fungal inoculum on the measured variables. Pots containing seedlings were placed in a glasshouse at 18 °C: 14 °C (day/night) temperature with supplemental light (16:8 h light:dark) to replicate natural conditions. In Experiment 2 (2014), 40 mL of a simplified Hoagland's solution (1 mM KNO_3 and 0.5 mM NH_4NO_3) was applied weekly to each plant, beginning in week 3. In this experiment, after week 3, the glasshouse was invaded by the common pest Western flower thrips (*Frankliniella occidentalis*), and sticky traps were placed to control them. To account for any experimental variation altered by this unintentional treatment, thrips damage to plants was recorded on weeks 4, 6, 8 and 9 using a five-point scale (1 – no damage to 5–75% of leaves damaged) and added as a covariate in the statistical analyses. In the first experiment (2013), there was no evidence of thrips infestation.

EXPERIMENT 1: PLANT BIOMASS, APHID GROWTH AND AM FUNGAL COLONIZATION

In 2013, aphids were introduced to the plants eight weeks after transplanting. Each plant received two nymphs from one of the four aphid lines: AK13/19 and AK13/28, and *H. defensa* hosting lines AK13/05 and AK13/18. The plants were then covered with perforated plastic bags and connected to an automated watering apparatus. The aphids were left for three weeks to feed and reproduce. The plants were harvested at the end of this period. During the harvest, the heights of the plants were measured, aphids were removed and frozen, above- and below-ground structures were removed and separated into leaves, stems, stolons, roots and tubers, dried at 70 °C for one week, and mass of each structure in each sample recorded. Samples of the fine roots were then removed and rehydrated to assess AM fungal colonization.

To prepare samples for AM fungal colonization assessment, roots were boiled in 3% KOH for 10 min, rinsed and soaked in a 2% (v/v) HCl solution for 30 min. Roots were then boiled in a staining solution of 1:1:1 lactic acid:water:glycerol, with 0.05% (w/v) Trypan blue for 20 min. Samples were placed on microscope slides and assessed using the gridline intersect method (McGonigle *et al.* 1990). Briefly, ≥ 100 root fragments were examined per sample under a compound microscope (x40 magnification) for the occurrence of AM hyphae, arbuscules, vesicles, and spores, and non-AM fungi.

EXPERIMENT 2: APHID GROWTH AND PARASITISM BY *APHIDIUS ERVI*

In 2014, the aphids were also introduced to the plants after 8 weeks of plant growth. Each plant received two apterous adult aphids from all four clonal lines: AK13/08 and AK13/22, and *H. defensa* hosting lines AK13/18 and AK13/30. The aphids were left to feed for one week prior to harvest. To avoid the influence of host plant (and host plant volatiles) and focus on parasitoid choice solely based on aphid quality, ten aphid nymphs (*c.* 2nd–3rd instar) were removed from each plant and were transferred into Petri dish (100 mm wide × 15 mm high) containing an excised leaflet of *S. tuberosum* (cv. Desiree) set abaxial surface uppermost into 1% (w/v) agarose gel. A single female wasp, aged 2–5 days and presumed mated, was introduced to the arena for a period of 30 min, which was chosen based on our experience with this system (Clarke 2013); wasp behaviour was observed for the first 10 min to ensure that the wasp was active, and this observation period was used to collect additional information about the initial number of attacks (when the wasp inserted its ovipositor into the aphid). After the assay, aphids were transferred to excised leaflets of *S. tuberosum* (cv. Desirée) in ventilated cups and maintained at 20 °C with 16:8-h light:dark cycle. Aphids were checked daily, and after 10 days, the number of mummies and emerged wasps was recorded. Replication of parasitism assays was low for some plants, particularly *S. berthaultii* (8 replicates), due to poor aphid performance. For the rest of *Solanum* types, the number of assays conducted was 20 (*S. tuberosum* STN), 28 (*S. polyadenum*) and 31 (*S. tuberosum* Chilean).

The above-ground portions of the plants were harvested after aphids were removed. Shoots were removed and dried at 70 °C for one week, and mass recorded. Four core samples (12 mm wide × 100 mm deep) of the root system of each plant were taken, and the root samples were washed and stained as described for Experiment 1 (above) to measure AM fungal colonization. Measurement of AM fungal colonization in this experiment confirmed the success of the treatments, but due to uneven sampling of the root system was not used to assess response of AM fungal colonization to applied treatments.

STATISTICAL ANALYSES

Both experiments were analysed in a similar manner using an ANOVA in the general linear models procedure of SAS 9.2 (SAS, Cary, NC, USA). Different dependent variables were analysed in each experiment using the same model including the independent variables block, *Solanum* type, AM fungal treatment, aphid clonal line and interactions between these variables. Post hoc contrasts were included to test differences due to the use of different species and genotypes. In particular, two contrasts within the main effect *Solanum* type tested whether (i) there were differences in behaviour that could be attributed to variation between *Solanum* species (*S. berthualtii*, *S. polyadenum* and *S. tuberosum*) (labelled 'Solanum species') and (ii) whether the two genotypes of *S. tuberosum* differed in behaviour from the species *S. berthaultii* and *S. polyadenum* (labelled '*tuberosum* vs'). A contrast within the main effect 'aphid clonal line' labelled '*H. defensa* vs' tested whether the presence of *H. defensa* explained the influence of aphid clonal line in our system.

In Experiment 1, we analysed the dependent variables total plant weight, root weight, tuber weight, aphid number and proportion of root length colonized by AM fungi. Root weight was included as a covariate in the analysis of root length colonized by AM fungi in order to control for any influence of root size on root length colonized by AM fungi.

In Experiment 2, we analysed the dependent variables total aphid number, total parasitism (sum of emerged and unhatched mummies), total attacks by a parasitoid in the 10-min observation period (including multiple attacks on a single aphid), and successful attacks [measured as percentage of offspring emergence from all the attacked aphids in a replicate: 100*(No. wasp offspring/No. aphids attacked)], and root length colonized by AM fungi. Thrips score was initially included as covariate in the analysis of Experiment 2, but was removed from the final model because it explained very little variation.

Biomass and aphid variables were log-transformed, and fungal colonization variables were arcsin square-root-transformed to meet the normality assumptions of the statistical model.

Results

EXPERIMENT 1: PLANT RESOURCE ALLOCATION AND APHID PERFORMANCE

Plant mass and resource allocation varied with *Solanum* type: *S. tuberosum* Chilean had the largest total mass, while mass was smallest for *S. tuberosum* STN (Table 1). *Solanum tuberosum* Chilean was the only *Solanum* type to produce tubers consistently during the experimental period and, therefore, exhibited the largest tuber mass ($F_{3,127} = 186.03$, $P < 0.0001$; Table 1). Above-ground ($F_{1,127} = 13.17$, $P = 0.0004$), root ($F_{1,127} = 5.63$, $P = 0.0192$) and tuber ($F_{1,127} = 169.77$, $P < 0.0001$) masses were influenced by differences between *Solanum* species. When plants were infested by aphids infected with *H. defensa*, total plant mass ($F_{1,127} = 2.28$, $P = 0.0132$) and root mass ($F_{1,127} = 4.45$, $P = 0.0348$) were significantly smaller than for plants infested with *H. defensa*-free aphids (Fig. 1). Aphid herbivory reduced shoot mass, but there was only a trend for the influence of *H. defensa* on shoot mass ($F_{1,127} = 3.88$, $P = 0.0509$). The AM fungal (AMF) treatment had little influence on most of the measured plant mass or resource allocation variables. AMF presence did, however, decrease root mass (Table 1), and there was a significant interaction between *Solanum* type and AMF treatment (Table 1), wherein the presence of AMF reduced the root mass of *S. berthaultii* and *S. polyadenum,* but neither of the *S. tuberosum* types. There was also a significant three-way interaction between *Solanum* type, aphid line and AM fungal treatment for tuber mass ($F_{9,127} = 2.28$, $P = 0.0211$; Table 1), but there were no consistent patterns in this variation.

The sterile treatment successfully eliminated AM fungal colonization of the roots (Experiment 1: $F_{1,123} = 1356.06$, $P < 0.0001$; Fig. 2). There was a significant interaction between *Solanum* type and AMF treatment ($F_{3,123} = 10.13$, $P < 0.0001$; Table 1) due to higher levels of AMF colonization on *S. tuberosum* STN plants in the live treatment (Fig. 2). The interaction between *Solanum* type and aphid line on AM fungal colonization was also significant ($F_{9,123} = 2.85$, $P = 0.0042$; Table 1) due to differential effects of each aphid line on root AM fungal colonization in each *Solanum* type, but no consistent patterns could be discerned (data not shown). Non-AM fungi were also influenced by *Solanum* type ($F_{3,122} = 13.09$, $P < 0.0001$; Table 1) and AMF treatment ($F_{1,122} = 49.08$, $P < 0.0001$; Table 1). Variation in non-AMF colonization within the AMF treatment was partially due to an interaction with *Solanum* type ($F_{3,122} = 7.45$, $P = 0.0001$; Table 1), because some *Solanum* types showed no difference in colonization between soil treatments while others did (Fig. 2).

Solanum type significantly affected the abundance of aphids on each plant (Table 1). Lowest levels of aphid abundance were associated with *S. polyadenum,* while highest aphid abundance was associated with *S. tuberosum* STN (Fig. 3).

EXPERIMENT 2: PARASITOID BEHAVIOUR AND SUCCESS

Similar variation in plant mass between *Solanum* types was observed compared to the first experiment (Table 2), and the sterile treatment successfully eliminated AM fungal colonization in the roots ($F_{1,127} = 550.35$, $P < 0.0001$; Table 2).

Solanum type also significantly affected aphid abundance (Table 2), although there were some differences compared with Experiment 1. Unlike Experiment 1, the lowest levels of aphid abundance were associated with *S. berthaultii* (rather than *S. polyadenum*) and highest abundances with *S. tuberosum* Chilean (rather than *S. tuberosum* STN). This might have been related to the shorter period of aphid infestation in Experiment 2, which focused on quantifying parasitism success rather than assessing treatment effects on aphids.

The number of aphid attacks by the parasitoid *Aphidius ervi*, the number of mummies formed and the percentage of mummies from which adult wasps emerged were all unaffected by *Solanum* type and aphid line (Table 2). However, the presence of AM fungi significantly increased the percentage of mummies with successful emergence of *A. ervi*, although this AMF effect varied with *Solanum* type ($F_{3,55} = 4.03$, $P = 0.0116$). The number of wasp attacks ($F_{3,55} = 3.81$, $P = 0.0149$), the number of parasitized aphids ($F_{3,55} = 3.13$, $P = 0.0330$) and the percentage of successful wasp emergence all showed the same patterns of interaction between AMF treatment and *Solanum* type (Fig. 4; Table 2). For *S. berthaultii, S. tuberosum* STN and especially *S. polyadenum*, these parasitism measures were highest for aphids that fed on plants in the AM fungal treatment compared to the sterile treatment. Conversely, on *S. tuberosum* Chilean, parasitism measures were highest for aphids feeding on plants in the sterile treatment (Fig. 4).

Discussion

This study highlights the potential for plant type to alter the outcome of plant–herbivore–natural enemy interactions and indicates the multiple factors that can influence plant success and herbivore biocontrol in invaded, agricultural and natural systems. The study findings reveal the importance of characterizing genotype-by-genotype interactions in relation to environmental factors as a central feature in our understanding of trophic interactions, although such interactions are frequently overlooked in ecological studies, particularly of invaded

Table 1. Experiment 1 statistical output from a Type III ANOVA in the glm procedure of SAS for the log of total plant weight, root weight, tuber weight, aphid number, and the arcsin square-root-transformation of the proportion of root length colonized by AM fungi (AMF) and non-AMF (or 'Other' fungi) as dependent variables. The root weight was used as a covariate in the analysis of the proportion of root length colonized by fungi. Results of three post hoc contrasts are included in italics: *Solanum* species within the main effect *Solanum* type tests whether there were differences among the three species of *Solanum; tuberosum vs* within the main effect *Solanum* type tests whether the two genotypes of *S. tuberosum* behaved differently from the other two species; and *H. defensa vs* within the main effect Aphid clonal line tests whether the two aphid clonal lines hosting *H. defensa* behaved differently from the two clonal lines that did not host *H. defensa*. The error degrees of freedom for the proportion of root length colonized by fungi differed from the other analyses and are listed at the bottom of the F column for AM fungi. Significant *P* values are in bold

		Total plant weight		Root weight		Above-ground weight		Tuber weight		Aphid number		AMF		'Other' fungi	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Block	1	0.39	0.5313	4.62	**0.0335**	0.03	0.8546	4.67	**0.0327**	0.14	0.7131	0.82	0.3677	0.08	0.7842
Solanum type	3	32.92	**< 0.0001**	12.92	**< 0.0001**	4.09	**0.0082**	186.03	**< 0.0001**	36.59	**< 0.0001**	2.11	0.1028	13.09	**< 0.0001**
Solanum species	1	0.06	0.8070	13.17	**0.0004**	5.63	**0.0192**	169.77	**< 0.0001**	53.45	**< 0.0001**	2.54	0.1139	25.9	**< 0.0001**
tuberosum vs	1	0.83	0.3649	5.79	**0.0175**	9.04	**0.0032**	172.7	**< 0.0001**	54.44	**< 0.0001**	1.40	0.2389	27.99	**< 0.0001**
AMF	1	1.19	0.2770	4.35	**0.0391**	1.09	0.2994	0.98	0.3252	1.81	0.1813	1356.06	**< 0.0001**	49.08	**< 0.0001**
Aphid clonal line	3	2.26	0.0847	1.62	0.1877	3.04	**0.0313**	1.31	0.2746	1.51	0.2162	0.36	0.7812	0.16	0.9228
H. defensa vs	1	6.32	**0.0132**	4.55	**0.0348**	3.88	0.0509	0.61	0.4361	0.49	0.4852	0.04	0.8353	0.06	0.8049
Solanum*AMF	3	1.27	0.2871	2.95	**0.0353**	0.79	0.4992	0.42	0.7367	0.26	0.8561	10.13	**< 0.0001**	7.45	**0.0001**
Solanum*Aphid	9	0.64	0.7578	0.7	0.7046	0.91	0.5152	1.43	0.1801	1.9	0.0582	2.85	**0.0044**	1.54	0.1413
AMF*Aphid	3	0.31	0.8184	0.16	0.9223	0.17	0.9158	1.14	0.3338	1.1	0.3508	0.32	0.8078	0.81	0.4897
Solanum*AMF*Aphid	9	1.1	0.3705	1.52	0.1474	1.39	0.2008	2.28	**0.0211**	1.41	0.1903	1.89	0.0595	0.44	0.9131
Root weight	1											0.15	0.6960	3.71	0.0563
Error	127											122			

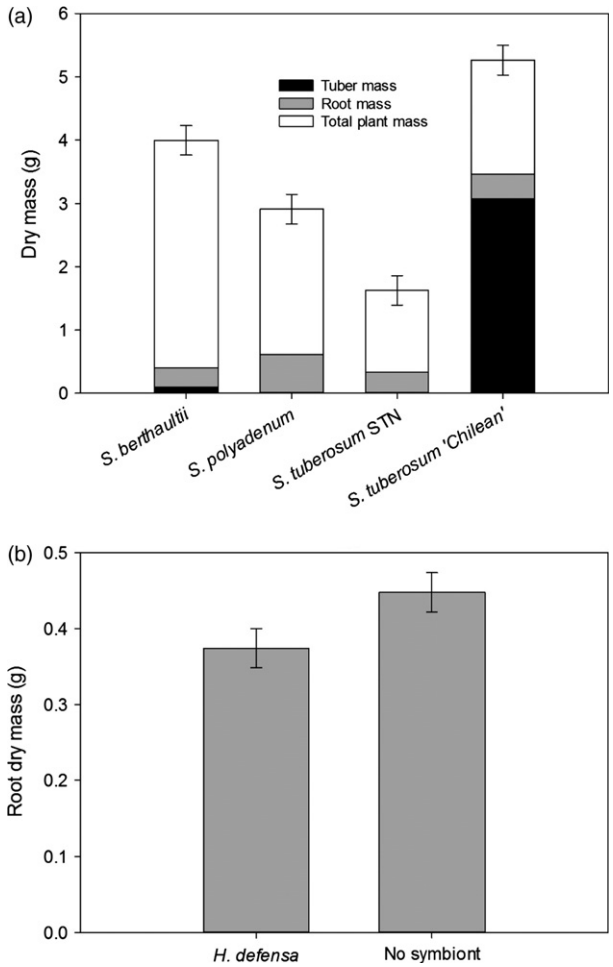

Fig. 1. (a) Total plant dry mass and allocation to tubers and roots in four *Solanum* types and (b) root dry mass in response to plant infestation with *M. euphorbiae* harbouring the facultative endosymbiont *H. defensa*. Values are lsmeans (±SEM) of $n = 40$ plants.

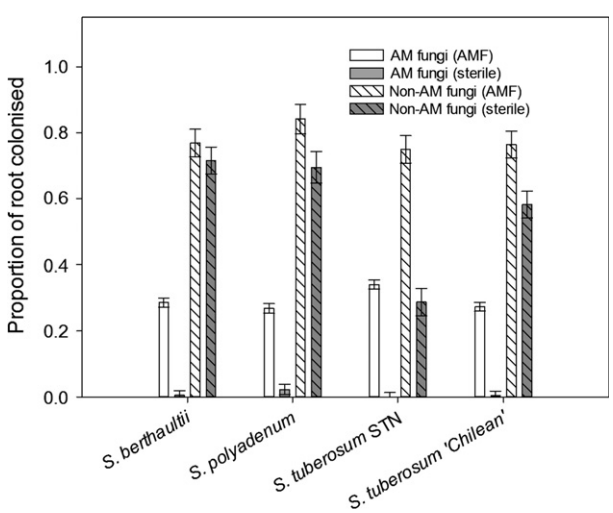

Fig. 2. Proportion of root length colonized by AM fungi and non-AM fungi on four *Solanum* types in the live AMF and sterile soil treatments. Values are lsmeans (±SEM) of $n = 20$ plants.

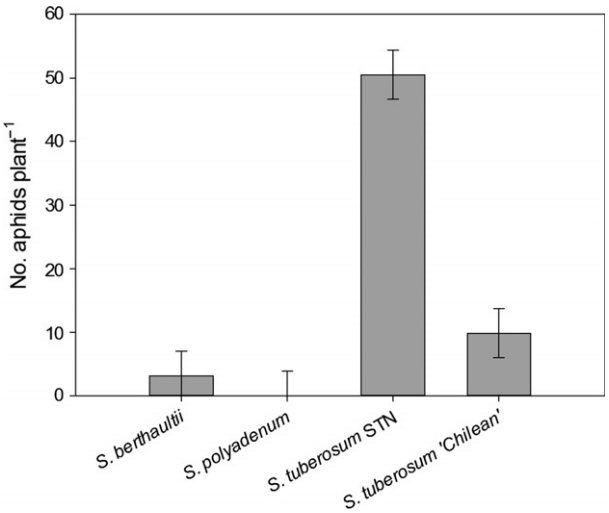

Fig. 3. Number of aphids supported by the four *Solanum* types. Values are lsmeans (±SEM) of $n = 40$ plants.

systems. In our study, plant productivity, and aphid survival and abundance, were highly dependent on *Solanum* type. A key novel finding was the indirect effect of biotic factors on plant and insect performance mediated by microbial symbionts. This was illustrated clearly in two of the measured responses: (i) the differential bottom-up effects of root AM fungal colonization on parasitoid success when attacking aphids infesting different *Solanum* types and (ii) the top-down influence of an aphid facultative endosymbiont on below-ground plant resource allocation.

Aphid population growth varied significantly amongst *Solanum* types in both experiments. Highest abundance of *M. euphorbiae* was associated with the cultivated *S. tuberosum* genotypes (*S. tuberosum* STN and *S. tuberosum* Chilean), while the wild *Solanum* types, *S. berthaultii* and *S. polyadenum*, supported only low levels of aphid infestation, which correlates with previous reports of reduced aphid susceptibility of these genotypes (Gibson & Pickett 1983; A.J. Karley and A.E. Bennett, unpublished data). Although positive (Gange, Bower & Brown 1999; Koricheva, Gange & Jones 2009) and negative (Hempel *et al.* 2009) effects of root colonization by AM fungi on aphid performance have been reported, there was no evidence for an effect of AM fungi on aphid fitness on the four *Solanum* types tested in the present study. Instead, the presence of AM fungi affected aphid fitness indirectly by influencing the outcome of aphid interactions with the parasitoid wasp *A. ervi*.

Arbuscular mycorrhizal fungi promoted aphid parasitism success in our system. In general, aphids feeding on plants colonized by AM fungi experienced elevated levels of attack by *A. ervi* and higher levels of mummification, and a higher proportion of successful wasp emergence, indicating consistent effects of AM fungi on several components of parasitoid fitness – searching behaviour, aphid mortality and offspring production. These effects varied with *Solanum* type and were particularly strong in *S. tuberosum* and *S. polyadenum*; by contrast, *S. tuberosum* Chilean was the only type where parasitoid success

Table 2. Experiment 2 statistical output from a Type III ANOVA in the glm procedure of SAS for the log of total aphid number, total parasitism (number of mummies), total attacks (number of attacks on all the aphids by the parasitoid), successful attacks (proportion of wasps which emerged from mummies), and the arcsin square-root-transformation of the proportion of root length colonized by AM fungi (AMF) as dependent variables. Results of two post hoc contrasts are included in italics: *tuberosum vs* within the main effect Solanum type tests whether the two genotypes of *S. tuberosum* behaved differently from the other two species and *H. defensa vs* within the main effect Aphid clonal line tests whether the two aphid clonal lines hosting *H. defensa* behaved differently from the two clonal lines that did not host *H. defensa*. The error degrees of freedom differed based on analysis and are listed at the bottom of the F column for each variable. Significant *P* values are in bold

	Total aphid number			Total parasitism		Total attacks		Successful attacks		AMF		
	df	*F*	*P*	df	*F*	*P*	*F*	*P*	*F*	*P*	df	*F*	*P*
Block	1	0.09	0.7645	1	1.18	0.2816	1.14	0.2897	0.3	0.5859	1	0.18	0.6706
Solanum type	3	16.48	**< 0.0001**	3	0.35	0.7899	2.36	0.0815	0.99	0.4055	3	1.47	0.2265
tuberosum vs	1	40.47	**< 0.0001**	1							1	2.06	0.1540
AMF	1	0.23	0.6323	1	0.96	0.3305	3.07	0.0852	5.19	**0.0266**	1	550.35	**< 0.0001**
Aphid clonal line	3	1.26	0.2906	3	0.89	0.4538	1.14	0.3422	2.28	0.0891	3	1.93	0.1275
H. defensa vs	1	0.02	0.8845	1							1	0.03	0.8547
Solanum*AMF	3	0.44	0.7233	3	3.13	**0.0330**	3.81	**0.0149**	4.03	**0.0116**	3	1.47	0.2265
Solanum*Aphid	9	0.29	0.9769	9	0.57	0.8196	0.58	0.8077	1.35	0.2357	9	0.93	0.5026
AMF*Aphid	3	1	0.3937	3	0.22	0.8807	0.9	0.4477	0.83	0.4851	3	1.88	0.1363
Solanum*AMF*Aphid	9	1.65	0.1077	8	0.86	0.5576	0.35	0.9435	2.49	**0.0221**	9	0.93	0.5026
Error	127			55							127		

on aphids was higher in the absence of AM fungi. Our study provides an explanation for the conflicting literature on AM fungal–plant mutualism effects on aphids and their natural enemies, in which AM fungi have had positive (Gange, Bower & Brown 1999; Koricheva, Gange & Jones 2009) and negative (Hempel *et al.* 2009) indirect effects on aphid resistance to parasitism. Here, we show that variation in AM fungal effects on aphids and *A. ervi* parasitism depends on *Solanum* type. The importance of species and genotype identity on trophic interactions has been demonstrated between insects and plants (Oliver, Moran & Hunter 2005; Bilodeau *et al.* 2012; Cayetano & Vorburger 2013; Hackett, Karley & Bennett 2013) and AM fungi and their host plants (Vandenkoornhuyse *et al.* 2003; Gange, Brown & Aplin 2005; Bennett, Alers-Garcia & Bever 2006; Jansa, Smith & Smith 2008; Hempel *et al.* 2009), and yet virtually no research has acknowledged the extent to which this factor could alter the outcome of multitrophic interactions. However, our study highlights the importance of considering different species and genotypes when studying multitrophic interactions.

In our study, we showed for the first time an indirect effect of the AM fungal–plant mutualism on parasitism of aphids by *A. ervi* that was not mediated by plant cues. Parasitism in our study occurred in the absence of plant-derived cues (e.g. constitutive or inducible plant volatiles) because parasitism assays were conducted *ex situ (*using leaves from a non-experimental cultivar of *S. tuberosum* Désirée). While plant-inducible defences are known to have direct and indirect effects on parasitoid behaviour (Guerrieri *et al.* 2004; Babikova *et al.* 2013, 2014), our findings suggest that root colonization by AM fungi influenced *A. ervi* parasitism of *M. euphorbiae* by alternative mechanisms. Parasitism success can depend on aphid development and size (Heimpel & Casas 2008; Henry, Ma & Roitberg 2009), and strength of the aphid immune response (Turlings & Benrey 1998; Bukovinszky *et al.* 2009; Bilodeau *et al.* 2012), all of which might have been affected by differences in plant nutritional quality for *M. euphorbiae* (Karley, Douglas & Parker 2002). Further work would be needed to identify precisely the causal factor(s) underlying the effect of AM fungal presence and *Solanum* type on increased aphid susceptibility to parasitism, and whether it was associated with larger aphid size, enhanced nutritional quality or compromised aphid physiological resistance. In contrast with other studies and other aphid species, aphid abundance and parasitism success did not vary significantly with insect clonal line or the presence of the facultative endosymbiont *H. defensa* (Oliver & Moran 2009; Vorburger *et al.* 2009; Martinez, Weldon & Oliver 2014). As a result, AM fungi have the potential to promote the parasitism of aphid herbivores, with negative consequences for control of an invasive plant species, although this might be countered by increased aphid size and fitness on AM fungal plants and would depend on plant species or genotype. Thus, we encourage future research incorporating these potential limitations to biocontrol.

Our findings confirmed that the aphid endosymbiont *H. defensa* has surprising impacts on plant biomass and resource allocation. Root biomass was reduced across all *Solanum* types when fed on by aphids hosting *H. defensa*, and biomass loss in roots was not compensated for in any other structures, leading to an overall decrease in plant mass. This *H. defensa*-associated reduction of root mass has been observed previously in *S. tuberosum* (Hackett, Karley & Bennett 2013) and suggests that the endosymbiont somehow influences plant resource allocation. The mechanism for this is unknown, but there are several hypotheses for how the endosymbiont might influence plant allocation. First, the aphid might transfer the endosymbiont to the plant during feeding, allowing the endosymbiont to interact directly with the plant. Although transmission of *H. defensa* to plants by phloem-feeding

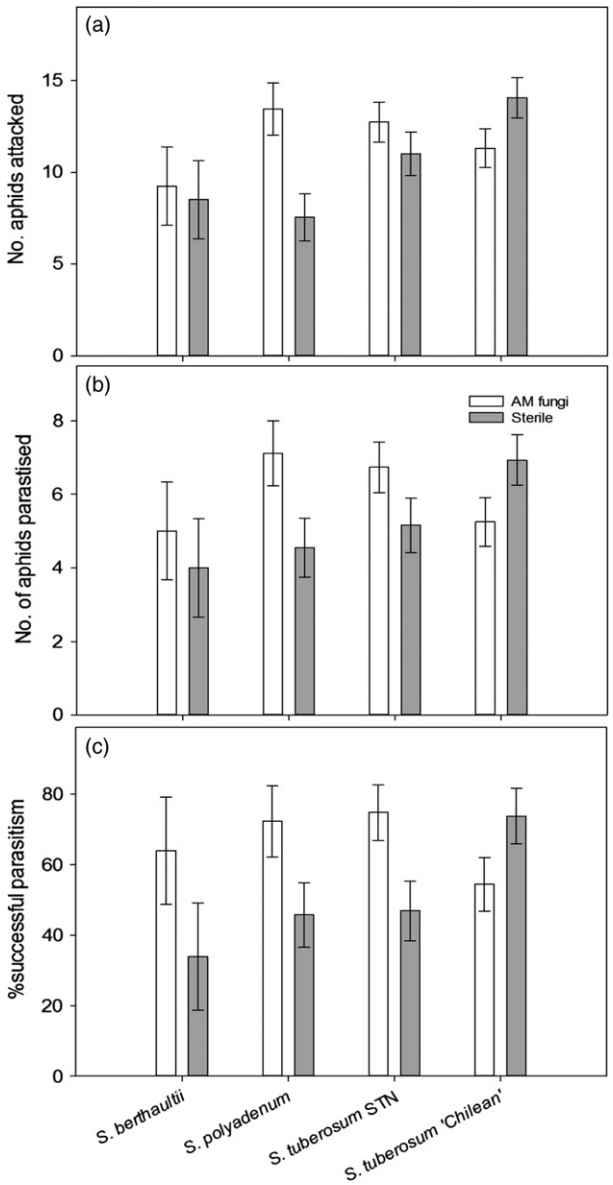

Fig. 4. Variables describing *A. ervi* parasitism by Solanum type and AM fungal treatment. (a) The number of times an *A. ervi* wasp attacked an aphid (including multiple attacks on a single aphid) during the initial 10 min of the parasitism assay, (b) the number of aphids that were parasitized following the parasitism assay and (c) the percentage of aphids that were successfully parasitized (i.e. parasitoids emerged from the aphid). Open bars represent aphids that fed on plants colonized by AM fungi, whereas grey bars represent aphids that fed on plants that were not colonized by AM fungi. Values are lsmeans (±SEM) of $n = 8$ (*S. berthaultii*), $n = 20$ (*S. tuberosum* STN), $n = 28$ (*S. polyadenum*) and $n = 31$ (*S. tuberosum* Chilean).

insects has not yet been demonstrated, other bacterial endosymbionts are capable of transfer via this infection route (Caspi-Fluger *et al.* 2012) and can function both as insect symbionts and plant pathogens (e.g. citrus disease caused by *Ca.* Liberibacter asiaticus transmitted by psyllids; Zhou *et al.* 2011). Also, members of the genus *Arsenophonus* include those that confer protection to the insect host and those that behave as plant pathogens (Bressan, Terlizzi & Credi 2012). Facultative endosymbionts share many features with invasive pathogens (Moran, McCutcheon & Nakabachi 2008), and *H. defensa* is known to have pathogenic ancestors (Degnan *et al.* 2009). Thus, there is potential for *H. defensa* to be introduced into a host plant and interact directly with host metabolic pathways. Secondly, aphids harbouring *H. defensa* might show modified saliva composition or production of specific aphid effectors that alter the plant response to aphid feeding (Bos *et al.* 2010; Chaudhary *et al.* 2014). *Hamiltonella defensa* has been associated with suppression of whitefly-induced plant defences in tomato (Su *et al.* 2015) which provides evidence of potential effectors generated by or modified by *Hamiltonella defensa*. The inhibition of root growth has been identified as part of the 'third danger signal' in plant responses to herbivory (Guiguet *et al.* 2016), but it is unknown whether this type of response can be triggered specifically by an insect endosymbiont. The influence of *H. defensa* on root mass represents a new function of insect endosymbionts and as a result demands future research to elucidate the mechanism.

This study highlights the importance of quantifying variation within- and between-species and its impact on the outcome of multitrophic interactions and is relevant to a range of systems. In our study, there were two levels of variation: species and genotypic variation within the host plant (*Solanum* spp.) and genotypic variation within the aphid holobiont (the presence or absence of *H. defensa*). Invasive species often form monocultures in disturbed soils and are expected to have lower genetic variability in their invaded environments than their native environments due to the bottleneck created by the invasion process. Thus, plant genotype becomes a significant factor structuring plant-trophic interactions within an invaded system. Our study, using both wild species and agricultural species typically grown in monoculture in disturbed agricultural soils, allowed us to manipulate variation in both the plant and herbivore and therefore provides a useful proxy for understanding the factors influencing success of agroecological, invasive and wild plant species. The degree of inter- and intraspecific variation observed in the present study suggests that plant species and genotype identity are likely important factors in structuring the wider scheme of interactions in both natural and invaded environments and thus could play a stronger role than previously considered in successful control of invasive plants. However, plant type was not the only factor influencing plant fitness and success of its insect herbivores. While root colonization by AM fungi did not appear to influence aphids feeding on host plants directly, AM fungi did influence the susceptibility of aphids to parasitism, creating a surprising impact on top-down control of aphids. As a result, our study indicates that soil biotic conditions could have unpredictable effects on the success of aphid biocontrol.

Conclusions

Here, we addressed the potential of the players (AM fungi, plants, aphid herbivores and their endosymbionts, and aphid parasitoids) within a multitrophic system to influence plant fitness (*Solanum* spp.) and insect herbivore performance (*M.*

euphorbiae). Genotypic variation (in both plants and aphid holobionts) altered the outcome of multitrophic interactions, indicating the importance of taking genotypic variation at all trophic levels into account in invaded, agricultural and natural systems. Our results demonstrate that AM fungi can have surprising impacts on higher trophic levels even if no impacts are recorded at lower trophic levels, aphid endosymbionts can have unpredicted impacts on plant resource allocation, and genotypic variation can alter the direction of the impacts of multitrophic interactions. These results have important implications for the success of invasive species and biocontrol of agricultural pests and might underlie variation in success of previously designed biocontrol strategies.

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Data accessibility

The raw data are available through the Dryad Digital Repository http://dx.doi.org/ 10.5061/dryad.5vv5v (Bennett *et al.* 2016).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. The number of aphids per aphid clonal line.