Potato aphid Macrosiphum euphorbiae performance is determined by aphid genotype and not mycorrhizal fungi or water availability

Karley, Alison Jane; Emslie-Smith, Matthew; Bennett, Alison Elizabeth

Published in:
Insect Science

DOI:
10.1111/1744-7917.12445

Publication date:
2017

Document Version
Peer reviewed version

Correspondence: Alison Jane Karley, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK. Tel: +44 (0)1382 568820; email: Alison.Karley@hutton.ac.uk

ORIGINAL ARTICLE

Potato aphid *Macrosiphum euphorbiae* performance is determined by aphid genotype and not mycorrhizal fungi or water availability

Alison Jane Karley[1], Matthew Emslie-Smith[2], and Alison Elizabeth Bennett[1]

[1]The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK; [2]School of Biology, University of St. Andrews, St Andrews, KY16 9ST, UK

Abstract

Intra- and inter-specific variation in plant and insect traits can alter the strength and direction of insect-plant interactions, with outcomes modified by soil biotic and abiotic conditions. We used the potato aphid (*Macrosiphum euphorbiae* Thomas) feeding on cultivated *S. tuberosum* and wild *S. berthaulti* to study the impact of water availability and plant mutualistic arbuscular mycorrhizal (AM) fungi on aphid performance and susceptibility to a parasitoid wasp (*Aphidius ervi* Haliday). Plants were grown under glass with live or sterile AM fungal spores and supplied with sufficient or reduced water supply. Plants were infested with one of three genotypes of *M. euphorbiae* or maintained as aphid-free controls; aphid abundance was scored after one week, after which aphid susceptibility to *A. ervi* was assayed *ex planta*.

This is an Accepted Article that has been peer-reviewed and approved for publication in the Insect Science but has yet to undergo copy-editing and proof correction. Please cite this article as doi: 10.1111/1744-7917.12445.

Solanum tuberosum accumulated *c.* 20% more dry mass than *S. berthaultii*, and root mass of *S. berthaultii* was smallest under reduced water supply in the presence of AM fungi. Aphid abundance was lowest on *S. berthaultii* and highest for genotype'2' aphids; genotype '1' aphid density was particularly reduced on *S. berthaultii*. Aphid genotype '1' exhibited low susceptibility to parasitism and was attacked less frequently than the other two more susceptible aphid genotypes. Neither AM fungi nor water availability affected insect performance. Our study suggests a fitness trade-off in *M. euphorbiae* between parasitism resistance and aphid performance on poor quality *Solanum* hosts that warrants further exploration, and indicates the importance of accounting for genotype identity in determining the outcome of multi-trophic interactions.

Key words *Aphiduis ervi*; arbuscular mycorrhizal fungi; multi-trophic interaction; parasitism resistance; *Solanum* spp.

Introduction

Herbivorous insects interact directly and indirectly with multiple organisms during their life history, spanning host plants, natural enemies, and other insect herbivores and microbial communities above- and below-ground (e.g. Koricheva *et al.*, 2009; Johnson *et al*., 2012). The outcome of these interactions will depend on the species identity of each organism, which determines the nature of the interaction, but can also depend on the species genotype and the composition of the community of organisms (e.g. Kempel *et al*., 2010; Kempel *et al*., 2013; Bennett *et al*., 2016). Studies of multi-trophic interactions are, therefore, crucial to understand the behaviour and performance of individuals in a community of organisms, particularly those that take into account the impact of within-species variation at each trophic level (Gehring & Bennett, 2009; Hackett *et al*., 2013; Bennett *et al*., 2016).

Phloem-feeding aphids are a successful group of herbivores that frequently form an abundant component of insect communities in natural and agricultural vegetation. The survival and success of aphids is strongly influenced by plant quality and the activity of natural enemies (e.g. Karley *et al*., 2004). Plant suitability and nutritional quality for aphid feeding and growth can vary widely between, and even within, plant species. For example, wild and cultivated *Solanum* species differ dramatically in their suitability for potato aphid (*Macrosiphum euphorbiae* Harris) and peach-potato aphid (*Myzus persicae* Sulzer) (Fréchette *et al.*, 2010; Askarianzadeh *et al.*, 2013; Bennett *et al*., 2016), while the quality of cultivated *Solanum tuberosum* for aphids varies significantly between cultivars (Aldmen & Gerowitt, 2009) and during plant development (Karley *et al.*, 2002; Karley *et al.*, 2003). Similarly, within aphid populations, individuals with reduced susceptibility to natural enemies can be detected with varying frequency. For example, several aphid species are known to exhibit phenotypes with high levels of resistance to attack by entomopathogenic fungi and parasitism

by parasitoid wasps, which has been linked both to the presence of 'protective' facultative endosymbiont bacteria and to aphid genetic variation (e.g. Łukasik *et al*., 2013; Parker *et al*., 2013; Asplen *et al*., 2014; Martinez *et al*., 2014).

Plant quality for herbivores is also subject to the prevailing growing conditions. Soils harbour a diverse community of microbes that have the potential to influence the growth and performance of plants and plant-associated insects. In particular, mutualistic arbuscular mycorrhizal (AM) fungi, which colonise plant roots and facilitate soil exploration and resource capture, can enhance plant growth and nutrition (Smith & Read, 2008). Through this association, AM fungi have significant potential to alter plant responses to herbivore attack through direct effects on plant quality and palatability, for example by promoting the accumulation of plant defensive chemicals and priming plant defense signalling, leading to rapid and more effective induction of defense responses to herbivore attack (Bennett *et al.*, 2009; Jung *et al*., 2012). Aphids are highly likely to respond to plant colonisation by AM fungi: a meta-analysis has shown that most sucking insect herbivores tend to benefit from AM fungal colonisation of plant roots (Koricheva *et al*., 2009). The effects of AM fungi on aphid fitness can filter through to higher trophic levels by enhancing plant volatile emissions that attract natural enemies. For example, the aphid parasitoid *Aphidius ervi* Haliday was equally attracted to tomato plants colonised by *Glomus mosseae* and to uncolonised plants infested with *M. euphorbiae*, and both were more attractive to parasitoids than aphid-free and uncolonised control plants (Guerrieri *et al*., 2004). In no-choice studies, presence of AM fungi has been associated with increased parasitism of cereal aphids (Hempel *et al*., 2009) and *M. euphorbiae* (Bennett *et al*., 2016), indicating indirect effects on aphid quality for parasitism.

Such a broad range of potential outcomes of these trophic interactions raises the likelihood that the effect of AM fungi on plants and higher trophic levels is highly dependent on the species and genotype identity of the interacting organisms. Abiotic conditions can further influence the outcome of these trophic interactions. Phloem-feeding insects are strongly influenced by plant water status (Huberty & Denno, 2004), which can lead to changes in abundance and quality that influence the success of aphid parasitoids (Johnson *et al*., 2011; Aslam *et al.*, 2012), while AM fungi can confer plant tolerance to drought stress (Augé, 2004; Augé *et al.*, 2014). Although genotypic variation in traits forms the basis of selection in crop breeding and in natural systems (e.g. Strauss & Agrawal, 1999), it is rare for multi-trophic interaction studies to take into account the influence of genotype identity on the outcomes of these interactions, and even rarer for them to include abiotic stress.

This study aims to address this knowledge gap using multiple plant and aphid genotypes to disentangle the effect of soil microbial mutualists below-ground on insect herbivores above-ground, mediated by the host plant, in relation to soil water availability. We use an experimental system comprising two species of *Solanum*, cultivated *S. tuberosum* and a wild relative *S. berthaultii*; the latter species shows a different volatile emission profile and is less attractive to aphids (Avé *et al*., 1987; Gibson & Pickett, 1983) and provides a lower quality substrate for aphid growth (Bennett *et al*., 2016). These plants were exposed to three genotypes of *M. euphorbiae* known to vary in fitness and susceptibility to the parasitoid wasp *A. ervi* (Clarke *et al*. in press). To best replicate naturally-occurring associations between plants and AM fungi, we used a native community of AM fungi, extracted from live soil adjacent to potato cultivation (compared to a sterile control inocula), to investigate the effects of AM fungi on plant and insect performance in the presence of sufficient and low water availability treatments. The study was designed to test the following predictions: (i) *Solanum* species will influence aphid performance, with reduced aphid fitness on *S. berthaultii*; (ii)

root colonisation by AM fungi will promote plant growth, particularly under low water availability; and (iii) aphid susceptibility to parasitism will vary between aphid genotypes, with enhanced parasitism success in the presence of AM fungi.

Materials and methods

Study system

Seed of *Solanum tuberosum* (accession TBR5642) and *Solanum berthaultii* (accession BER7348) were obtained from the Commonwealth Potato Collection held at the James Hutton Institute, Dundee. Seeds were germinated in steam-sterilized coir; two weeks after sowing, seedlings were transplanted individually to 0.8 L pots containing a background soil consisting of 2 : 1 sand : loam (Keith Singleton Steam Sterilized Loam, Clydesdale Trading, Lanark, UK) mixture that had been autoclaved twice at 121°C (15 psi) for 4 hours, with an interim overnight cooling period.

For each experimental block of plants (see below), following seedling transfer, AM fungal spores were extracted from 7 L of soil (a total of 21 L of soil across all three blocks) collected from a site with a known AM fungal community at the James Hutton Institute, Dundee (Bennett *et al.*, 2016) by wet sieving and sucrose density centrifugation (Daniels & Skipper, 1982). This volume was chosen to allow the equivalent amount of spores from 100 mL of soil to be added to each pot as inocula. Once extracted, the total volume of the spore solution was reduced to 70 mL. A microbial wash was prepared from each set of spore extractions by vacuum filtering 3 ml of the AM fungal inoculum and extraneous liquid from the inocula through a Whatman filter paper to exclude fungal spores and hyphae. Half of the spore inocula and half of the microbial wash were sterilized by autoclaving. Each pot received 1

ml of either live or sterile AM fungal spore inocula and 1 mL of either live or sterile microbial wash. Live inoculum consisted of live AM fungal spore solution and sterile microbial wash, while the sterile inoculum comprised sterile AM fungal spore solution and live microbial wash to ensure the only difference between the treatments was the presence of AM fungal spores. Spore abundance and morphotype diversity was counted in three 1 ml samples of the live spore solution for each block. On average, inoculum applied to each pot in Block 1 contained an average (± std. error) of 28.67 (± 4.37) spores and 6.67 (± 0.88) morphotypes and a Shannon diversity of 1.97 (± 0.18) per pot, in Block 2, 24.67 (± 2.33) spores and 4.67 (± 0.67) morphotypes and a Shannon diversity of 1.41 (± 0.46) per pot, and in Block 3, 38 (± 3.61) spores and 7 (± 0) morphotypes and a Shannon diversity of 2.46 (± 0.11) per pot with a total of 11 morphotypes across all blocks and samples. Assessment of roots for colonisation by AM fungi showed that a significantly larger proportion of root length was colonised in the AM fungi treatment ($F_{1,154}$ =347.71, $P < 0.0001$), indicating successful establishment of the sterile and AM fungal treatments (data not shown).

Three clonal lineages of the potato aphid *Macrosiphum euphorbiae* originating from commercial potato crops and belonging to three distinct genotypes (genotype '1', '2' and '3'; Clarke *et al.*, in press) were cultured on excised potato leaves (cv. Desirée), with the excised petiole submerged in a water reservoir, at 21°C with 16h light: 8h dark. *Aphidius ervi* mummies were obtained from a commercial supplier (Syngenta, Essex, UK) and wasps were reared on pre-flowering *Vicia faba* plants infested with pea aphids (*Acyrthosiphon pisum*) at 21°C with 16h light: 8h dark for at least three generations before use in parasitism assays.

Experimental design

The experiment was conducted as a 2×2×2×4 factorial design (*Solanum* sp × AMF treatment × water treatment × aphid treatment) with six replicates per treatment, giving 192 plants in total. The experiment was divided into three spatial/temporal blocks, with two replicates of each treatment combination per block, to allow parasitism assays to be staggered temporally, with a period of one week between each block.

AM fungal inocula was added to the root zone at the time of seedling transfer. The plants were grown in well-watered substrate conditions for a further three weeks before administering the water treatments. Plants received either 240 mL water (ambient water supply) or 120 mL water (reduced water supply) weekly and soil moisture content was monitored using a soil moisture probe (AT WET-1 moisture meter, Delta-T Devices Ltd.); on average, soil moisture content (% vol) was 20.45% (±0.38) in the ambient water treatment and 10.21% (±0.38) in the reduced water supply treatment. Plants were fertilised weekly with 40 mL of a simplified Hoagland's solution (1 mmol/L KNO_3 and 0.5 mmol/L NH_4NO_3) from week six after seedling transfer. At eight weeks after seedling transfer, two apterous adults of *M. euphorbiae* were confined to the underside of a mid-stem terminal *Solanum* leaflet using mesh clip-on cages of 25 mm internal diameter. Empty cages were attached to aphid-free control plants. After a period of one week, aphids were removed from each cage and the number of nymphs was counted. Ten nymphs (where available) were selected at random from each cage for use in parasitism assays. Nymphs were transferred to a potato leaf (cv. Desirée) embedded in 1% agarose (w/v in water) with abaxial surface uppermost in a 100 mm Petri dish 'arena'. Aphids were allowed to settle for a period of up to 4 h, after which a single female *A. ervi* (2-6 d old, presumed mated) was introduced to the arena for a period of 30 minutes and the number of wasp attacks in the first ten minutes was recorded. At the end of the assay, the wasp was removed and nymphs were transferred to an excised potato leaf (cv. Desirée) and maintained in the conditions described above for insect cultures. The

number of mummified aphids and the number of successfully emerged wasps was recorded after a further 12-16 days. Plants were harvested 14 weeks following seedling transfer to pots. Shoots were separated into stem and leaf fractions. Belowground parts were washed free of soil and separated into roots, stolons and tubers. All plant fractions were dried at 70°C and weighed.

Statistical analysis

Type III ANOVA was applied to all data using the glm procedure of SAS 9.2 (SAS, Cary, NC, USA). Dependent variables included leaf, stem, root, stolon and tuber mass as well as aphid success (number of nymphs per plant) and independent variables included the main and interactive effects of Block and the treatments (water treatment, AMF treatment, *Solanum* species and aphid genotype). To determine differences between nymph production on different host plants we ran a post-hoc contrast within the *Solanum* species × aphid genotype interaction (titled "Aphid by *Solanum*" in Table 1). Due to poor performance of aphids on *S. berthaultii*, analysis of parasitism success (number of attacks in the first ten minutes of the 30 min assay, number of mummies and number of successfully emerged wasps) was conducted for aphids collected from *S. tuberosum* plants only. Values for all dependent variables were $\log_{10}$-transformed prior to analysis to ensure the data met the requirements of parametric analysis for normal distribution and limited heteroscedasticity.

Results

Total plant mass was significantly larger in *S. tuberosum* than *S. berthaultii* (Fig. 1A; Table 1). This difference was due to larger mass of tubers and roots in *S. tuberosum* (Fig. 1A; Table

1). Root mass varied with *Solanum* species depending on water treatment and AMF treatment. In the reduced water treatment, *S. berthaultii* root mass decreased in the presence of AM fungi relative to root mass in sterile soil (Fig. 1B; Table 1). Water and AM fungal treatments did not affect any other component of plant mass (not shown).

Aphid success (number of nymphs produced per plant) was significantly affected by *Solanum* species, with very few nymphs produced on *S. berthaultii* compared to *S. tuberosum* (Table 1; Fig. 2). Nymph production also varied significantly between aphid genotypes, with the highest number of nymphs produced by genotype 2 aphids and the fewest by genotype 3 aphids (Table 1; Fig. 2). Nymph production by genotype 1 aphids was depressed to a greater extent on *S. berthaultii* compared to genotype 2 and genotype 3 aphids (Aphid by *Solanum* contrast in Table 1, Fig. 2), resulting in a significant interaction between these two factors. No significant effects of AM fungi or water treatment on aphid performance were detected.

Due to the low number of aphids produced on *S. berthaultii* plants, oviposition behavior and parasitism success of *A. ervi* was analysed only for aphids reared on *S. tuberosum*. Parasitoid success varied significantly with aphid genotype (Fig. 3). The number of attacks in the first ten minutes of the assay was significantly lower for genotype 1 aphids compared to the other two aphid genotypes, and the number of mummies produced 12 d after the assay was significantly smaller for genotype 1 aphids (Table 2; Fig. 3). No significant effects of AM fungi or water treatment on parasitism were detected.

Discussion

The outcome of trophic interactions in communities of organisms is known to vary with the species identity at each trophic level (Bennett *et al*., 2016). The findings of the present study

reinforced that not only species identity, but also the genotype within each species, influences the strength and direction of plant-herbivore and herbivore-natural enemy interactions. A novel finding of particular interest was the fact that aphid genotypes exhibited differential responses to *Solanum* species, leading to differences between aphid genotypes in their fitness on each host plant species. Contrary to previous work, however, this study did not find strong evidence that soil AM fungi and soil water availability modified the outcome of multi-trophic interactions, although these factors had an interactive effect on plant growth.

The two *Solanum* species differed considerably in their growth and allocation to vegetative structures, with the cultivated *S. tuberosum* investing more mass in tubers while the wild relative *S. berthaultii* invested more mass in stolons. A similar pattern of resource allocation was observed in a previous study using these two species (Bennett *et al*., 2016) and likely reflects selection for a desirable trait (tuber bulking) in the cultivated *Solanum* species. While plant mass allocation alone is unlikely to have dictated suitability for insect herbivores, it was clear that the larger *S. tuberosum* plants provided a more suitable host for *M. euphorbiae*, and supported higher abundance of aphids than *S. berthaultii*, confirming our first prediction that aphid fitness would be reduced on *S. berthaultii*, in line with the findings of previous studies (Bennett *et al*., 2016; Gibson & Pickett, 1983). It is likely that the wild species *S. berthaultii* expresses a suite of traits that influence plant quality for insect herbivores, including production of volatiles and defensive chemicals that deter aphids from settling and prevent sustained feeding (Avé *et al*., 1987; Gibson & Pickett, 1983), as well as other unidentified factors that enhance resistance to aphids in *Solanum* (Rossi *et al*., 1998; Cooper & Goggin, 2005) and thus influence *Solanum* host plant range.

Fitness of *M. euphorbiae* varied significantly between the three aphid genotypes. Within the experimental period, genotype 1 and 2 aphids produced higher nymph densities per plant

than genotype 3 aphids, which might have resulted from the higher survival, faster development and higher fecundity shown for genotype 1 and 2 aphids in a previous study (Clarke *et al.*, in press). In addition, our third prediction was partially confirmed as *M. euphorbiae* susceptibility to parasitism varied significantly between aphid genotypes. The highest frequencies of aphid attacks exhibited by *A. ervi* wasps were observed in assays with genotype 2 and 3 aphids, and the highest levels of parasitism were also observed in these two aphid genotypes, both in terms of the number of mummies produced and the number of emerging wasps. Within-species variation in aphid susceptibility to parasitism has been studied extensively, particularly in the pea aphid (*Acyrthosiphon pisum* Harris), but also in a number of other aphid species. While resistance to parasitism can be conferred by aphid infection with one or more types of facultative bacterial endosymbionts (Vorburger, 2014), frequently referred to as 'protective' endosymbionts', there is increasing recognition that aphid-encoded resistance to parasitism can be detected in aphid populations (Martinez *et al*. 2014). To date, *M. euphorbiae* resistance to the parasitoid *A. ervi* has been detected only for genotype 1 aphids irrespective of the presence of bacterial endosymbionts (Clarke *et al.*, in press). However, given that the genotype 1 clonal line used in the present study also harboured the facultative endosymbiont *Hamiltonella defensa*, which provides protection against parasitism in other aphid species (Vorburger, 2014), we cannot entirely rule out a contribution from protective endosymbionts.

An unanticipated finding was that frequency of parasitoid attack was low in assays of *M. euphorbiae* genotype 1, suggesting that wasps were less capable of attacking these aphids. Parasitoid wasps might avoid ovipositing in unsuitable aphid hosts, either in response to indicators of host quality such as aphid development stage, morph and colour (Liu *et al*., 1984; Ives *et al*., 1999; Michaud & Mackauer, 1994), or due to aphid defensive behaviors such as rearing and kicking which physically deter wasp attack (Rehman & Powell, 2010). A

particularly interesting focus for future work would be to explore whether reduced wasp attack of genotype 1 aphids is associated with more aggressive aphid behaviour, as the opposite scenario has been demonstrated for *A. pisum* harbouring protective endosymbionts (i.e. parasitism-resistant pea aphids are less aggressive towards parasitoid wasps: Dion *et al*., 2011). Although parasitism assays were conducted *ex planta* in the present study, it is possible that parasitoid behavior *in planta* could be influenced further by differences between *Solanum* species in plant volatile emissions (Avé *et al*., 1987; Gibson & Pickett, 1983) as has been shown in other aphid-parasitoid systems (reviewed in Rehman & Powell, 2010).

Whatever the causal factor(s), genotypic variation in aphid resistance to parasitism, combined with genotypic differences in aphid responses to the two *Solanum* species, gave rise to a key novel finding: the differential negative effect of *Solanum berthaultii* on aphid abundance. While numbers of all three aphid genotypes were low on this *Solanum* species, nymph abundance was particularly depressed for aphid genotypes 1 and 2, and most pronounced for genotype 1, which barely survived on *S. berthaultii*. This raises the possibility that a trade-off exists between aphid fitness traits, with allocation of resources to parasitism resistance resulting in reduced investment in aphid growth/reproduction on less suitable host plants. While trade-offs between defence and growth are predictable in nature (e.g. Agrawal, 2011), and indeed have been demonstrated for parasitism resistance in relation to aspects of performance in some aphid species (Oliver *et al*., 2006; Foster *et al*., 2011; Vorburger & Gouskov, 2011; Vorburger, 2014), they have previously eluded detection in *M. euphorbiae* (Clarke *et al*., in press). Our experimental work to date on this aphid species, conducted on a commercial cultivar of *S. tuberosum*, has shown that the parasitism-resistant aphid genotype performs as well as the fittest susceptible genotypes, exhibiting rapid development, and high survival rates and fecundity (Clarke *et al.*, in press; Hackett *et al.*, 2013). However, it is possible that fitness trade-offs are observed only under certain conditions, for example on

poorer-quality hosts such as *S. berthaultii* that are less suitable for aphid infestation. If future work shows that parasitism resistance consistently incurs a reproduction cost to *M. euphorbiae* colonising poor quality plant hosts, it would imply a trade-off between natural enemy defence and host plant range that could influence population genetic structure and distribution of this aphid species in cultivated and natural vegetation.

Surprisingly, and contrary to our second and third predictions, this study did not detect a significant impact of soil AM fungi on plant or insect performance, irrespective of water availability. Although previous work has uncovered limited evidence for effects of AM fungi on *Solanum* growth (Bennett *et al*., 2016), based on findings from other research, we predicted that AM fungi would promote plant growth, particularly under reduced water supply (Augé *et al*., 2004, 2014), but this was not observed. However, a significant interactive effect of AM fungi and water availability on root mass was detected for *S. berthaultii*, which resulted in reduced root mass in AM fungal-colonised plants when water availability was limited. Previous studies have produced mixed results for the response of AM fungal-infected roots to drought (reviewed in Veresoglou *et al.*, 2012). However this variation may depend on other abiotic factors, such as nutrient availability (e.g. Valliere & Allen, 2016), that were not manipulated in this study. The reduced investment in *S. berthaultii* roots under reduced water supply in the presence of AM fungi might have arisen because AM fungal exploration of the soil can enhance water uptake (Smith & Read, 2008) allowing AM fungi to compensate for reduced plant water acquisition through the roots and allowing plants to invest limited resources elsewhere. *S. tubersosum* is highly susceptible to water deficit (Monneveux *et al.*, 2013), although wild relatives can be more tolerant (Coleman, 2008), thus the limited plant response and lack of herbivore response to water treatment suggests that water availability was not sufficiently limiting in the present study to elicit detectable effects for many plant variables and at higher trophic levels. Plants were

grown from seed and were therefore smaller than typical tuber-generated *Solanum* plants, suggesting that more severe water restriction might need to be imposed in future work with this study system. Further, previous work has shown that aphid attack by *A. ervi* and parasitism success was enhanced on these two *Solanum* species when roots were colonised by AM fungi (Bennett *et al*. 2016). The difference between the two studies might have arisen because the present study employed three genotypes of *M. euphorbiae* which varied in parasitism susceptibility while Bennett *et al*. (2016) examined four aphid clones belonging to a single genotype that was susceptible to parasitism (genotype 2). Consequently, the strength of the AM fungal effect might have been weakened in the present study by use of genotypes with different levels of parasitism susceptibility, and thus not detectable with the level of replication. This possibility highlights the importance of considering genotype identity in multi-trophic interaction studies, and also for confirming the differences between aphid genotypes using multiple representatives of each genotype.

In conclusion, this study confirmed our prediction that intraspecific variation, driven by genotype-specific differences in key fitness traits, can markedly alter the outcome of multi-trophic interactions, highlighting the importance of considering this aspect of organism identity in community ecology studies. Further, we report novel data revealing the existence of ecological trade-offs in aphid fitness traits depending on host plant species identity that has potential implications for persistence of different aphid genotypes in agroecosystems. When combined with previous work in this study system, we emphasise the importance of variation at both the plant and insect level for structuring the outcome of plant-microbe-insect interactions, and have identified some of the factors that limit predictability when interpreting complex multi-trophic interactions.

Acknowledgments

This research was funded by a British Mycological Society vacation bursary awarded to MES, with financial support to AEB and AJK provided by the Scottish Government through the RESAS strategic research programme Environmental Change (2011-16) and Productive and Sustainable Land Management and Rural Economies (2016-2021). We thank Sandra Caul and Carolyn Mitchell for assistance with experimental techniques, Emils Gedrovic for helping with experimental set up and parasitism assays, Tommer Wallace, Ellen Eyles, Lucy Abel, Sarah Mitchell, Anupol Chareesri, and Pil Rasmussen for assistance with the experimental harvest, and Hannah Clarke and Rebecca Cornwell for aphid molecular characterisation. Thanks also to Dr Steve Foster (Rothamsted Research, UK) for supplying the *M. euphorbiae* genotype 1 clonal line used here. The study was conceived by AEB and AJK and conducted by MES. Statistical analysis was conducted by AEB and MES and all authors contributed to writing the manuscript. The sponsors did not contribute to study design, data collection, analysis or reporting.

Disclosure

All the authors confirm that they have no financial or other involvement in activities or organisations that might bias the work reported here.

References

Aldmen, H. and Gerowitt, B. (2009) Influence of selected potato cultivars on the reproduction rate of the aphid species *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas). *Journal of Plant Diseases and Protection*, 116, 278–282.

Agrawal, A.A. (2011) Current trends in the evolutionary ecology of plant defence. *Functional Ecology*, 25, 420–432.

Askarianzadeh, A., Birch, A.N.E., Ramsay, G. and Minaeimoghadam, M. (2013) study of wild *solanum* species to identify sources of resistance against the green peach aphid, *Myzus Persicae* (Sulzer). *American Journal of Potato Research*, 90, 66–70.

Aslam, T.J., Johnson, S.N. and Karley, A.J. (2012) Plant-mediated effects of drought on aphid population structure and parasitoid attack. *Journal of Applied Entomology*, 137, 136–145.

Asplen, M.K., Bano, N., Brady, C.M., Desneux, N., Hopper, K.R., Malouines, C., Oliver, K.M., White, J.A. and Heimpel, G.E. (2014) Specialisation of bacterial endosymbionts that protect aphids from parasitoids: defensive symbiosis in the cowpea aphid. *Ecological Entomology*, 39, 736–739.

Augé, R.M. (2004) Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science*, 84, 373–381.

Augé, R.M., Toler, H.D. and Saxton, A.M. (2014) Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. *Mycorrhiza*, 25, 13–24.

Avé, D.A., Gregory, P. and Tingey, W.M. (1987) Aphid repellent sesquiterpenes in glandular trichomes of *Solanum berthaultii* and *S. tuberosum*. *Entomologia Experimentalis et Applicata*, 44, 131–138.

Bennett, A.E., Bever, J.D. and Deane Bowers, M. (2009) Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory. *Oecologia*, 160, 771–779.

Bennett, A.E., Millar, N., Gedrovics, E. and Karley, A.J. (2016) Plant and insect microbial symbionts alter the outcome of plant-herbivore-parasitoid interactions: implications for invaded, agricultural and natural systems. *Journal of Ecology*, 104, 1734–1744.

Clarke, H.V., Cullen D., Hubbard, S.F. and Karley, A.J. (2016) Susceptibility of *Macrosiphum euphorbiae* to the parasitoid *Aphidius ervi*: larval development depends on host aphid genotype. *Entomologia Experimentalis et Applicata* (in press).

Coleman, W.K. (2008) Evaluation of wild *Solanum* species for drought resistance: 1. *Solanum gandarillasii* Cardenas. *Environmental and Experimental Botany*, 62, 221–230

Cooper, W.R. and Goggin, F.L. (2005) Effects of jasmonate-induced defenses in tomato on the potato aphid, *Macrosiphum euphorbiae*. *Entomologia Experimentalis et Applicata*, 115, 107–115.

Daniels, B. and Skipper, H. (1982) Methods for the recovery and quantitative estimation of propagules from soil. *Methods and Principles of Mycorrhizal Research* (ed. N.C. Schenk), pp. 29–35. The American Phytopathological Society, Minnesota.

Dion, E., Polin, S.E., Simon, J.-C. and Outreman, Y. (2011) Symbiont infection affects aphid defensive behaviours. *Biology Letters*, 7, 743–746.

Foster, S.P., Denholm, I., Poppy, G.M., Thompson, R. and Powell, W. (2011) Fitness trade-off in peach-potato aphids (*Myzus persicae*) between insecticide resistance and vulnerability to parasitoid attack at several spatial scales. *Bulletin of Entomological Research*, 101, 659–666.

Fréchette, B., Bejan, M., Lucas, É., Giordanengo, P. and Vincent, C. (2010) Resistance of wild *Solanum* accessions to aphids and other potato pests in Quebec field conditions. *Journal of Insect Science*, 10, 161.

Gehring, C. and Bennett, A. (2009) Mycorrhizal fungal–plant–insect interactions: the importance of a community approach. *Environmental Entomology*, 38, 93–102.

Gibson, R.W. and Pickett, J.A. (1983) Wild potato repels aphids by release of aphid alarm pheromone. *Nature*, 302, 608–609.

Guerrieri, E., Lingua, G., Digilio, M.C., Massa, N. and Berta, G. (2004). Do interactions between plant roots and the rhizosphere affect parasitoid behaviour? *Ecological Entomology*, 29, 753–756.

Hackett, S.C., Karley, A.J. and Bennett, A.E. (2013) Unpredicted impacts of insect endosymbionts on interactions between soil organisms, plants and aphids. *Proceedings of the Royal Society B*, 280 (1768), 1471–2954.

Hempel, S., Stein, C., Unsicker, S. B., Renker, C., Auge, H., Weisser, W.W. and Buscot, F. (2009) Specific bottom–up effects of arbuscular mycorrhizal fungi across a plant–herbivore–parasitoid system. *Oecologia*, 160, 267–277.

Huberty, A.F. and Denno, R.F. (2004) Plant water stress and its consequences for herbivorous Insects: a new synthesis. *Ecology*, 85, 1383–1398.

Ives, A.R., Schooler, S.S., Jagar, V.J., Knuteson, S.E., Grbic, M. and Settle, W.H. (1999) Variability and parasitoid foraging efficiency: a case study of pea aphids and *Aphidius ervi*. *The American Naturalist*, 154, 652–673.

Johnson, S.N., Clark, K.E., Hartley, S.E., Jones, T.H., McKenzie, S.W. and Koricheva, J. (2012) Aboveground-belowground herbivore interactions: a meta-analysis. *Ecology*, 93, 2208–2215.

Johnson, S.N., Staley, J.T., McLeod, F.A.L. and Hartley, S.E. (2011) Plant-mediated effects of soil invertebrates and summer drought on above-ground multitrophic interactions. *Journal of Ecology*, 99, 57–65.

Jung, S.C., Martinez-Medina, A., Lopez-Raez, J.A. and Pozo, M.J. (2012) Mycorrhiza-induced resistance and priming of plant defenses. *Journal of Chemical Ecology*, 38, 651–664.

Karley, A.J., Douglas, A.E. and Parker, W.E. (2002) Amino acid composition and nutritional quality of potato leaf phloem sap for aphids. *Journal of Experimental Biology*, 205, 3009–3018.

Karley, A.J., Pitchford, J.P., Douglas, A.E., Parker, W.E. and Howard, J.J. (2003) The causes and processes of the mid-summer crash in potato aphids Macrosiphum euphorbiae and *Myzus persicae* (Hemiptera: Aphididae). *Bulletin of Entomological Research*, 93, 425–437.

Karley, A.J., Douglas, A.E., Parker, W.E. and Pitchford, J.P. (2004) The mid-summer aphid population crash: how and why does it occur? *Ecological Entomology*, 29, 383–388.

Kempel, A., Schmidt, A.K., Brandl, R. and Schädler, M. (2010) Support from the underground: induced plant resistance depends on arbuscular mycorrhizal fungi. *Functional Ecology*, 24, 293–300.

Kempel, A., Nater, P., Fischer, M. and van Kleunen, M. (2013) Plant-microbe-herbivore interactions in invasive and non-invasive alien plant species. *Functional Ecology*, 27, 498–508.

Koricheva, J., Gange, A.C. and Jones, T. (2009) Effects of mycorrhizal fungi on insect herbivores: a meta-analysis. *Ecology*, 90, 2088–2097.

Liu, S.S., Morton, R. and Hughes, R.D. (1984) Oviposition preferences of a hymenopterous

parasite for certain instars of its aphid host. *Entomologia Experimentalis et Applicata*, 35, 249–254.

Łukasik, P., van Asch, M., Guo, H., Ferrari, J. and Godfray, H.C.J. (2013) Unrelated facultative endosymbionts protect aphids against a fungal pathogen. *Ecology Letters*, 16, 214–218.

Martinez, A.J., Ritter, S.G., Doremus, M.R., Russell, J.A. and Oliver, K.M. (2014) Aphid-encoded variability in susceptibility to a parasitoid. *BMC Evolutionary Biology*, 14, 127.

Michaud, J.P. and Mackauer, M. (1994) The use of visual cues in host evaluation by aphidiid wasps: I. Comparison between three *Aphidius* parasitoids of the pea aphid. *Entomologia Experimentalis et Applicata*, 70, 273–283.

Monneveux, P., Ramírez, D.A. and Pino, M.T. (2013) Drought tolerance in potato (*S. tuberosum* L.) Can we learn from drought tolerance research in cereals? *Plant Science*, 205–206, 76–86.

Oliver, K.M., Moran, N.A. and Hunter, M.S. (2006) Costs and benefits of a superinfection of facultative symbionts in aphids. *Proceedings of the Royal Society of London B*, 273, 1273–1280.

Parker, B.J., Spragg, C.J., Altincicek, B. and Gerardo, N.M. (2013) Symbiont-mediated protection against fungal pathogens in pea aphids: a role for pathogen specificity? *Applied and Environmental Microbiology*, 79, 2455–2458.

Rehman, A. and Powell, W. (2010) Host selection behaviour of aphid parasitoids (Aphidiidae: Hymenoptera). *Journal of Plant Breeding and Crop Science*, 2, 299–311.

Rossi, M., Goggin, F.L., Milligan, S.B., Kaloshian, I., Ullman, D.E. and Williamson, V.M. (1998) The nematode resistance gene Mi of tomato confers resistance against the potato aphid. *Proceedings of the National Academy of Sciences*, 95, 9750–9754.

Smith, S.E. and Read, D.J. (2008) *Mycorrhizal Symbiosis*. Academic Press, New York.

Strauss, S.Y. and Agrawal, A.A. (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution*, 14, 179–185.

Valliere, J.M. and Allen, E.B. (2016) Interactive effects of nitrogen deposition and drought-stress on plant-soil feedbacks of *Artemisia californica* seedlings. *Plant and Soil*, 403, 277–290.

Veresoglou, S.D., Menexes, G. and M.C. Rillig, M.C. (2012) Do arbuscular mycorrhizal fungi affect the allometric partition of host plant biomass to shoots and roots? A meta-analysis of studies from 1990 to 2010. *Mycorrhiza*, 22, 227–235.

Vorburger, C. (2014) The evolutionary ecology of symbiont-conferred resistance to parasitoids in aphids. *Insect Science*, 21, 251–264.

Vorburger, C. and Gouskov, A. (2011) Only helpful when required: a longevity cost of harbouring defensive symbionts. *Journal of Evolutionary Biology*, 24, 1611–1617.

Wu, Q.S. and Xia, R.X. (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology*, 163, 417–425.

Manuscript received October 31, 2016

Final version received January 20, 2017

Accepted February 13, 2017

Table 1 Statistical output from a Type III ANOVA in the glm procedure of SAS for the log of total plant dry mass, root dry mass and number of aphid nymphs per plant as dependent variables. The post-hoc contrast *Aphid by Solanum* (represented by an indentation and italic type) within the *Solanum* species-by-Aphid genotype interaction was run to test the influence of *S. berthaultii* on the production of nymphs by genotype 1 aphids versus the other two aphid genotypes. Due to missing data the error degrees of freedom for the different analyses differed for each variable and are listed at the bottom of the *F* column. Significant *P* values are in bold.

		Total dry mass		Root dry mass		No. nymphs	
		F	*P*	*F*	*P*	*F*	*P*
Block	2	9.92	**<0.0001**	1.09	0.3394	0.06	0.9450
Solanum species	1	4.14	**0.0437**	2.95	0.0877	40.29	**<0.0001**
Water treatment	1	0.13	0.7156	0.15	0.6981	0	0.9761
AMF treatment	1	0.88	0.3510	1.54	0.2172	0.12	0.7334
Aphid genotype	3	0.33	0.8018	0.29	0.8343	33.19	**<0.0001**
Water×AMF	1	0.91	0.3423	1.01	0.3170	0.29	0.5897
Solanum×Water	1	0.02	0.8757	0	0.9961	0.05	0.8262
Solanum×AMF	1	0.27	0.6056	0.14	0.7071	0.19	0.6661
Water×Aphid	3	0.13	0.9442	0.05	0.9839	0.49	0.6921
AMF×Aphid	3	0.21	0.8925	1.19	0.3143	0.14	0.9381
Solanum×Aphid	3	1.03	0.3790	1.12	0.3440	11.18	**<0.0001**
Aphid by Solanum	1					5.64	**0.0188**
Solanum×Water×AMF	1	1.5	0.2222	4.99	**0.0269**	1.84	0.1769
Solanum×Water×Aphid	3	0.54	0.6539	0.98	0.4040	0.07	0.9747
Water×AMF×Aphid	3	0.59	0.6246	0.69	0.5589	1.05	0.3730
Solanum×AMF×Aphid	3	0.63	0.5947	0.55	0.6461	0.41	0.7454
Solanum×Water×AMF×Aphid	3	1.78	0.1538	1.12	0.3448	0.41	0.7475
Error df		151		153		155	

Table 2 Statistical output from a Type III ANOVA in the glm procedure of SAS for the log of number of attacks by the wasp in the first ten minutes of the 30 minute assay, number of emerged wasps, and number of mummies as dependent variables for aphids that fed on only *S. tuberosum* plants. Significant *P* values are in bold.

		No. wasp attacks		No. emerged wasps		No. mummies	
		F	*P*	*F*	*P*	*F*	*P*
Block	2	1.14	0.3357	3.73	0.0388	3.35	0.0520
Water treatment	1	0.05	0.8203	0.07	0.7889	0.09	0.7625
AMF treatment	1	0.69	0.4159	0.02	0.8958	0.02	0.8959
Aphid genotype	2	5.27	**0.0127**	18.45	**<0.0001**	19.45	**<0.0001**
Water×AMF	1	1.17	0.2902	0.48	0.4963	0.43	0.5182
Water× Aphid	2	0.14	0.8699	0.36	0.7007	0.37	0.6951
AMF × Aphid	2	0.32	0.7324	1	0.3822	1.04	0.3684
Water× AMF × Aphid	2	2.4	0.1122	0.66	0.5261	1.39	0.2684
Error	24						

Figure legends

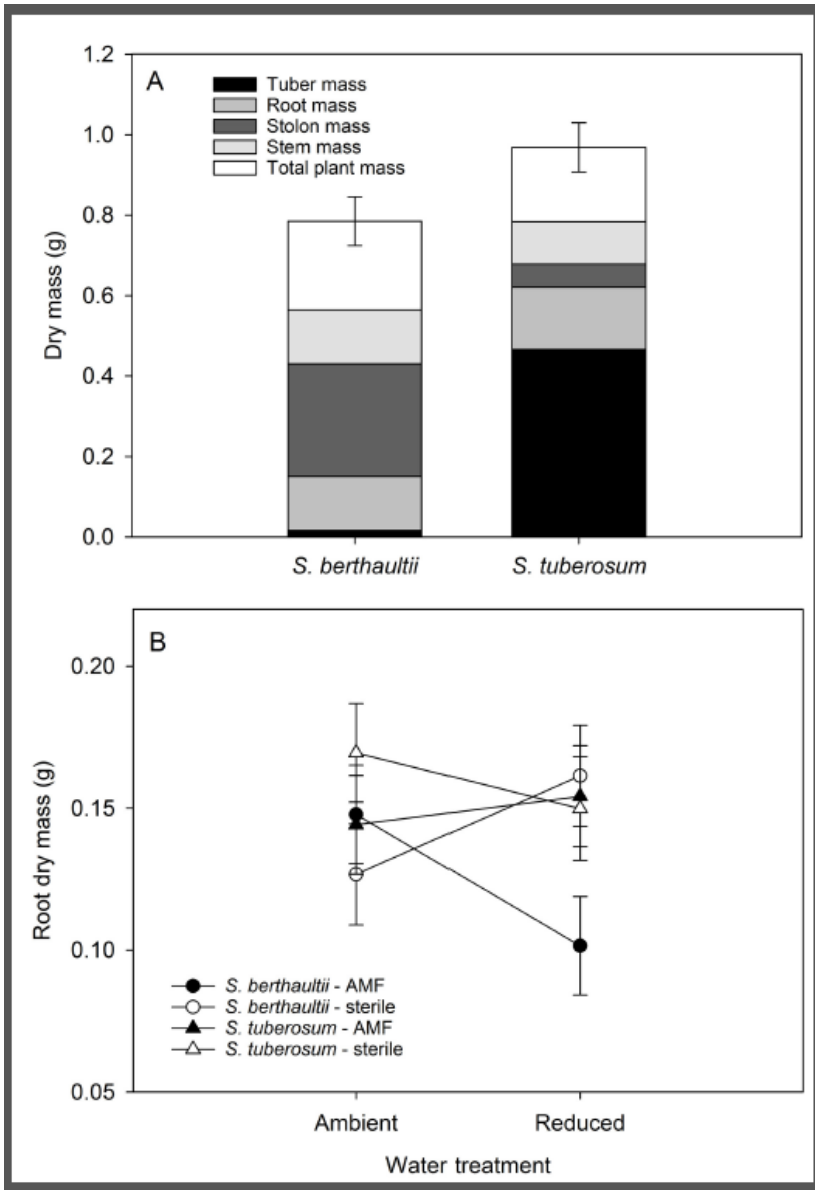

Fig. 1 (A) Total plant dry mass and allocation to stems, stolons, tubers and roots in the two *Solanum* species. Values are LSmeans (± std. error) of n = 96 plants. (B) Root mass in the two *Solanum* species in response to water treatment and AM fungal presence. Values are LSmeans (±std. error) of n = 24 plants.

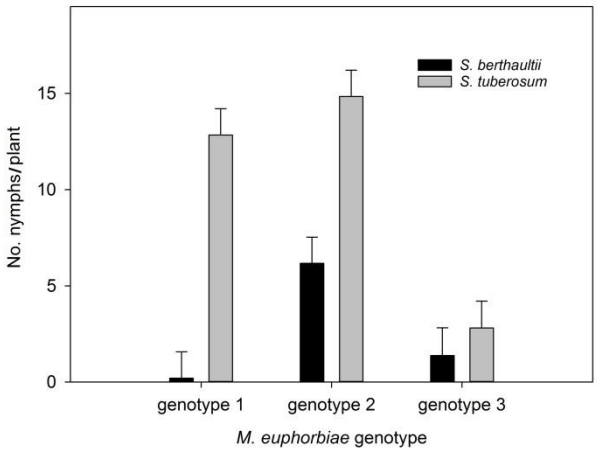

Fig. 2 Number of aphid nymphs of three *M. euphorbiae* genotypes supported by each *Solanum* species. Values are LSmeans (± std. error) of $n = 24$ plants.

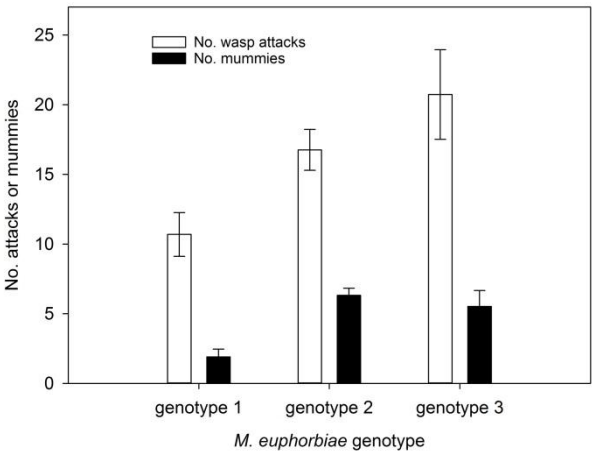

Fig. 3 Success of aphid parasitism by *A. ervi* with nymphs of three *M. euphorbiae* genotypes collected from *S. tuberosum* plants, measured as number of wasp attacks in the first ten minutes of the 30 minute assay and number of mummies formed after 12 d. Values are LSmeans (± std. error) of $n = 16$ (genotype 1), $n = 18$ (genotype 2) and $n = 4$ (genotype 3) assays.