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Biol. Lett. 2013 **9**, 20130341, published 24 July 2013

biology letters

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Research

Cite this article: McKenzie SW, Vanbergen AJ, Hails RS, Jones TH, Johnson SN. 2013 Reciprocal feeding facilitation between above- and below-ground herbivores. Biol Lett 9: 20130341.
http://dx.doi.org/10.1098/rsbl.2013.0341

Received: 15 April 2013
Accepted: 1 July 2013

Subject Areas:
ecology

Keywords:
interspecific competition, plant–insect interactions, *Otiorhynchus sulcatus*, *Amphorophora idaei*

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2013.0341 or via http://rsbl.royalsocietypublishing.org.

Community ecology

Reciprocal feeding facilitation between above- and below-ground herbivores

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Interspecific interactions between insect herbivores predominantly involve asymmetric competition. By contrast, facilitation, whereby herbivory by one insect benefits another via induced plant susceptibility, is uncommon. Positive reciprocal interactions between insect herbivores are even rarer. Here, we reveal a novel case of reciprocal feeding facilitation between above-ground aphids (*Amphorophora idaei*) and root-feeding vine weevil larvae (*Otiorhynchus sulcatus*), attacking red raspberry (*Rubus idaeus*). Using two raspberry cultivars with varying resistance to these herbivores, we further demonstrate that feeding facilitation occurred regardless of host plant resistance. This positive reciprocal interaction operates via an, as yet, unreported mechanism. Specifically, the aphid induces compensatory growth, possibly as a prelude to greater resistance/tolerance, whereas the root herbivore causes the plant to abandon this strategy. Both herbivores may ultimately benefit from this facilitative interaction.

1. Introduction

Plant-mediated interactions between insect herbivores feature prominently in most terrestrial ecosystems [1]. Above- and below-ground insect herbivores attacking the same plant can affect each other's performance [2,3] via induced changes in plant architecture [4], allocation of primary metabolites [5] or chemical defences [6]. Such plant-mediated herbivore interactions can also have consequences for higher trophic levels [7].

While competition dominates plant-mediated herbivore interactions [1,6], facilitation—whereby herbivory by one species benefits another—has been reported in only 11% of interspecific interactions between above-ground herbivores [1]. Such facilitative interactions, however, tend to be asymmetric, benefiting only a single species [8]. There is still less evidence that reciprocal facilitation between herbivore species occurs frequently [9]. This lack of evidence for reciprocal facilitation may have arisen because many above–below-ground studies focus on above-ground herbivore performance, whereas below-ground herbivore performance remains under-reported [10].

In a microcosm experiment, we tested the hypothesis that reciprocal facilitation, identified by increased insect abundance, would occur between an above-ground (large raspberry aphid *Amphorophora idaei*) and below-ground (vine weevil *Otiorhynchus sulcatus*) herbivore, interacting via a host plant (red raspberry *Rubus idaeus* L.) that varies susceptibility to herbivory. This experiment mimics the natural phenological succession of these herbivore species on the plant in field situations. Weevils over-winter on plants in all life-stages [11], whereas aphids over-winter as eggs and do not feed on the plant until the growing season is underway [12]; consequently, weevil herbivory generally precedes aphid herbivory.

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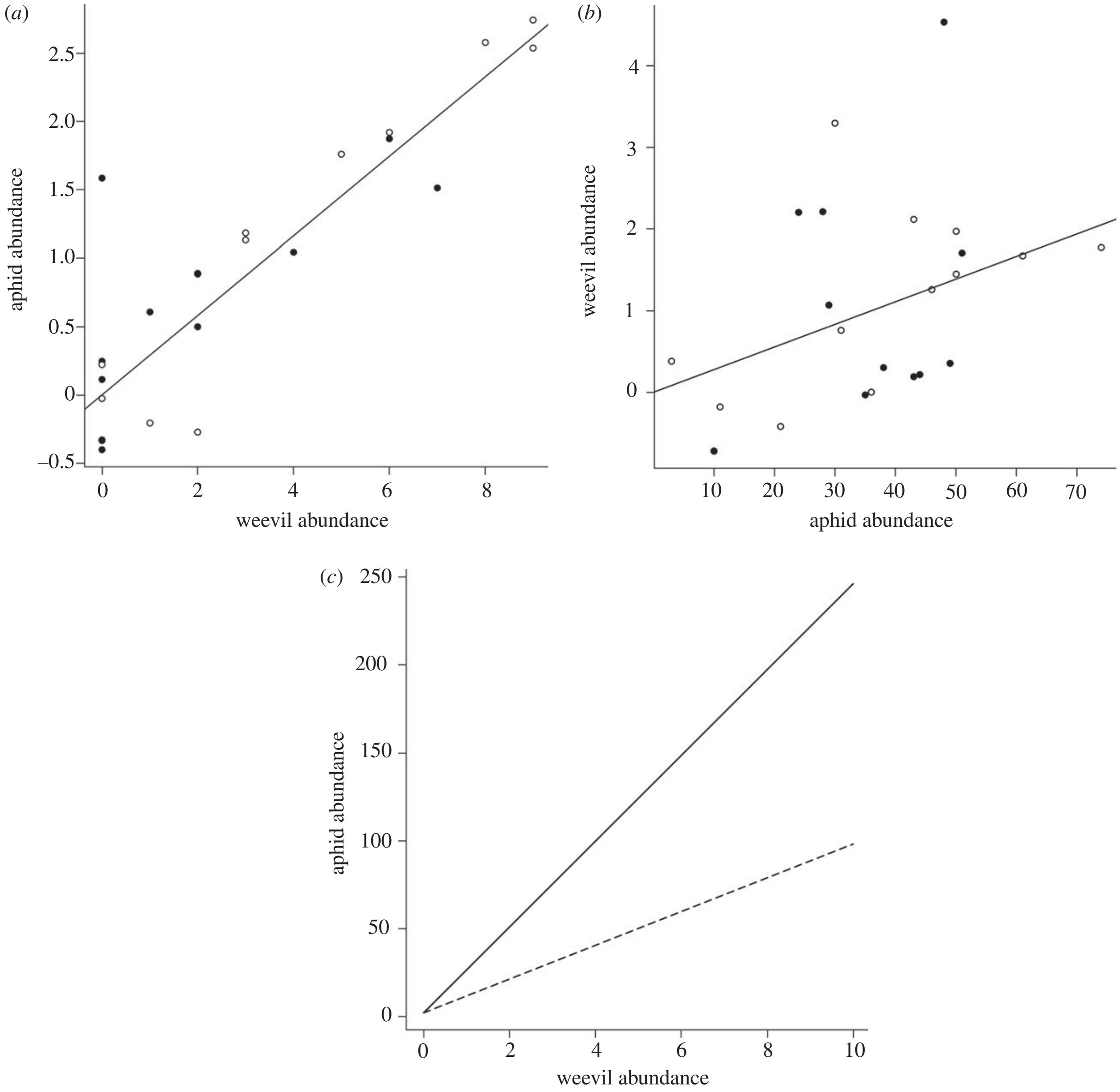

Figure 1. Partial residual plots on the linear predictor scale of the response of (*a*) aphid and (*b*) weevil abundance to the other herbivore concurrently feeding on a moderately (filled circle) or highly (open circle) susceptible cultivar in the 'combination' treatment only; (*c*) effect of weevil abundance on aphid abundance at modelled high (solid line) and low (dotted line) above-ground dry weight (predicted slopes (m) used when fixing above-ground dry weight at its highest and lowest value, respectively, with the modelled intercept (c) from the final model output. This equation was then applied to weevil abundance (x), giving a resultant aphid abundance value (y)).

2. Material and methods

Two raspberry cultivars varying in susceptibility to both experimental herbivores (Glen Ample: highly susceptible; Glen Clova: moderately susceptible) [11–13] were challenged with *A. idaei* and *O. sulcatus*. Each replicate plant (grown from rootstock at the James Hutton Institute (JHI), Dundee, UK) was established in a rhizotube (40 cm plastic cable trunking containing 2 : 1 compost: sand [13]) that allowed access to roots and weevils. Insects were obtained from cultures at the JHI [11–13].

The experiment ran in a climate- (day 20°C ± 2°C; night minimum 10°C ± 2°C) and photoperiod- (16 L : 8 D) controlled glasshouse for 10 weeks. Experimental treatments applied to 48 replicates of each raspberry cultivar comprised: a control (no insects), a single herbivore ('weevil' or 'aphid') or weevil and aphid together ('combination'; $n = 12$ each). Eight plants were randomly assigned to spatial blocks, each a full replicate of every insect treatment–cultivar combination (week 0). In week 4, replicates randomly assigned to 'weevil' and 'combination' treatments were inoculated with 20 weevil eggs, with eclosion occurring in week 6. Three adult aphids were added to each 'aphid' and 'combination' replicate in week 8. Plant height was recorded at week 0 and again at week 10. Insects were counted and plant biomass oven-dried (80°C for 24 h) and weighed in week 10. The phenology of herbivore arrival can influence the outcome of above–below-ground herbivore interactions [8]. Consequently, the effects of variation in the relative timing of above- and below-ground herbivory were checked in a separate experiment, with aphids preceding (the reverse of natural situations) and following weevil herbivory (see the electronic supplementary material).

Data were analysed using generalized linear mixed models (GLMM), with insect (aphid or weevil larvae) counts and plant biomass (above- or below-ground dry weight) modelled with Poisson and Gaussian error distributions, respectively (PROC GLIMMIX, SAS Institute). Parameter estimation used restricted maximum likelihood for plant biomass and pseudo-likelihood for insect counts. Replicate plant nested within spatial block was specified as a random effect and, for aphid counts, an

Table 1. GLMM results summary for herbivore and plant response parameters. Italicized entries indicate parameters retained in the final model. MPE, multiple parameter estimates.

response variable	explanatory variables	estimate	$F_{(\mathrm{ndf,ddf})}$	p
herbivore				
aphid abundance	cultivar	MPE	$1.78_{(1,16)}$	0.2021
random effect estimate = 0.1766	*weevil abundance*	*0.2910*	*$13.79_{(1,16)}$*	*0.0021*
	below-ground dry weight	−0.03500	$0.04_{(1,14)}$	0.8513
	above-ground dry weight	*0.5524*	*$13.79_{(1,30)}$*	*0.0019*
	above-ground dry weight × weevil abundance	*−0.1308*	*$7.25_{(1,15)}$*	*0.0163*
weevil larvae abundance	*cultivar*	MPE	*$10.53_{(1,20)}$*	*0.0041*
random effect estimate = 0.4054	*aphid abundance*	*0.02774*	*$5.68_{(1,14)}$*	*0.0316*
	below-ground dry weight	−0.1991	$1.62_{(1,19)}$	0.2181
	above-ground dry weight	−0.2310	$1.59_{(1,17)}$	0.2239
plant				
above-ground dry weight	cultivar	MPE	$0.21_{(1,20)}$	0.6502
random effect estimate = 0.5555	*weevil abundance*	*−0.1676*	*$4.70_{(1,21)}$*	*0.0417*
residual variance = 0.5176	*aphid abundance*	*0.03154*	*$5.21_{(1,21)}$*	*0.0330*
below-ground dry weight	cultivar	MPE	$5.01_{(1,45)}$	0.5165
random effect estimate = 1.6410	*weevil abundance*	*−0.1934*	*$8.31_{(1,46)}$*	*0.0060*
residual variance = 0.7008	aphid abundance	0.03526	$4.18_{(1,21)}$	0.0537

observation-level random component was included to account for overdispersion [14]. Cultivar (categorical) was fitted to all models. Models of weevil and aphid responses also included the abundance of the co-occurring herbivore ('combination' treatment only). Above- and below-ground plant biomass were always fitted to models of insect abundance. Models of the above- or below-ground plant biomass response did not, however, include the corresponding biomass measure as an explanatory term, because they were strongly positively correlated ($p < 0.0001$, $r = 0.89$). Plant height at week 0 was fitted to all models to account for initial between-replicate variation in growth. Models underwent forward stepwise selection until a minimum adequate model was obtained. Statistical significance of main effects are always reported, whereas two-way interactions are reported only where $p < 0.05$. Degrees of freedom were estimated using the Satterthwaite approximation [15].

Partial residual plots were constructed to show the influence of particular explanatory variables on response parameters accounting for other significant terms retained in the model. Gaussian and Poisson models used raw and standardized (residuals/fitted) values, respectively [16].

3. Results

Aphid and weevil abundance were positively correlated (figure 1*a*,*b* and ce 1). This relationship suggests reciprocal feeding facilitation, although weevil abundance exerted a larger positive effect on aphid densities than *vice versa* (figure 1*a*,*b* and table 1). This facilitative relationship persisted regardless of whether weevils preceded aphids or *vice versa* over the experimental timescale (see the electronic supplementary material, figure S1). Aphid abundance also increased with above-ground plant biomass, indicating an effect of the plant resources (table 1). While the positive effect of weevil abundance on aphid abundance occurred regardless of above-ground plant biomass, it was reduced when above-ground biomass was low (figure 1*c* and table 1: above-ground dry weight × weevil abundance). Cultivar did not affect aphid abundance, but these herbivores were less abundant on the moderately susceptible (Glen Clova) plants (table 1).

Above-ground and below-ground biomass did not vary between cultivars (table 1). The herbivore species affected above-ground plant biomass differently. Higher densities of aphids (figure 2*a* and table 1) and weevil larvae (figure 2*b* and table 1) increased and decreased above-ground biomass, respectively. Greater weevil abundance reduced root biomass, whereas aphids had no effect (table 1). Initial plant height did not affect the abundance of aphids ($F_{1,16} = 0.17$, $p = 0.6857$), weevils ($F_{1,15} = 0.00$, $p = 0.9456$) or final plant biomass (above-ground $F_{1,20} = 0.07$, $p = 0.8006$; below-ground $F_{1,45} = 0.04$, $p = 0.8512$).

4. Discussion

This paper provides compelling evidence for reciprocal feeding facilitation between root and shoot herbivores, a phenomenon that could be under-reported for above–below-ground interactions [8,10]. This positive relationship between the abundances of the two herbivore species persisted despite variation in above-ground plant biomass. Although facilitative, there remained a degree of asymmetry in the interaction, with weevils exerting a much greater effect on aphid abundance than *vice versa*. While feeding facilitation has been found above-ground [17], positive non-reciprocal effects of above-ground herbivores on below-ground herbivores are generally scarce (reviewed in [18]), and only one other study [5] has, to our knowledge, demonstrated reciprocal facilitation between above- and below-ground herbivores. In that case, the abundance of wireworms (*Agriotes* spp.) and the aphid *Rhopalosiphum padi*

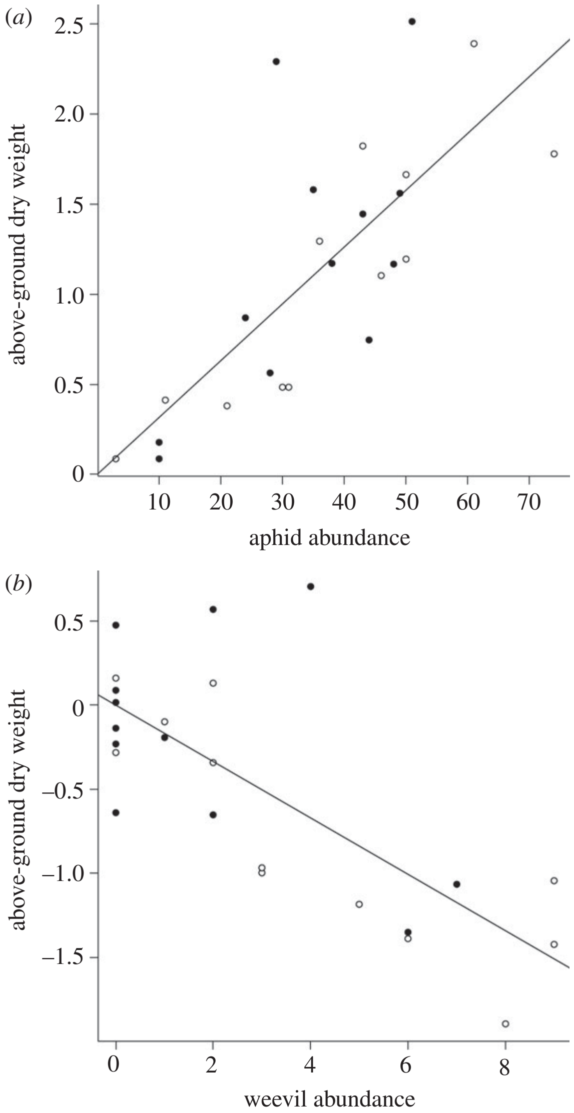

Figure 2. Partial residual plots on the linear predictor scale of the response of above-ground plant biomass to (*a*) aphid and (*b*) weevil abundance feeding on a moderately (filled circle) or highly (open circle) susceptible cultivar.

feeding on barley (*Hordeum vulgare*) increased by 30% and 25%, respectively [5]. Over a longer time-scale, facilitative relationships may give way to competitive interactions [1], and sustained herbivory in this system may still eventually lead to competition between the two herbivores. Even a short-term positive interaction may, however, affect greatly on the host plant, because *A. idaei* is the principal vector of raspberry viruses [12]. Therefore, the positive effects of root herbivory could have wider implications for plant pathogen transmission beyond the temporal conjunction of the herbivores.

Our study suggests that root-feeding weevils reduced overall plant biomass, whereas sap-sucking aphids stimulated compensatory plant growth above-ground, suggesting that functional adaptations are key to shaping plant–herbivore interactions. Vine weevils have a large and direct impact on plant biomass by chewing and severing primary roots, which compromises plant compensation by limiting water and nutrient uptake [3]. Stimulating plant compensatory growth is known to be an evolutionary strategy for tolerance or resistance to herbivory [11]. Our data suggest that aphid induction of plant compensation benefits the co-occurring, but spatially separated, vine weevil. This concurs with other studies showing that plant growth improved *O. sulcatus* performance [19,20].

Induced susceptibility to aphid colonization following root attack by beetles appears the most common above–below-ground herbivore interaction [8]. The potential for positive reciprocal interactions is, however, largely unknown, as few studies quantify both above- and below-ground herbivore performance simultaneously [8]. The sequence of herbivore arrival is often important in many above–below-ground herbivore interactions [10]. In this study, we simulated the sequence that reflects the natural phenology of herbivory (i.e. weevils before aphids), but even when weevil herbivory was manipulated to occur following aphid feeding, this did not alter the pattern of reciprocal feeding facilitation between the two herbivores (see the electronic supplementary material). Soler *et al.* [21] suggested that inter-guild herbivore interactions are more likely to result in positive outcomes than intra-guild interactions, because the former triggers different phytohormonal pathways, potentially leading to signal crosstalk [21]. For instance, root-feeding induces jasmonic acid, which reduces the salycilic acid defence response to aphid herbivory [21]. Other potential mechanisms are induction of ethylene or abscisic acid in leaves by root herbivory, which reduces plant resistance to aphids [21,22]. Above–below-ground interactions have a crucial role in multi-species interactions, and the reciprocal feeding facilitation between herbivores described here may be more prevalent than previously thought.

Acknowledgements. We thank Alison Dobson for plants; Adam Butler for statistical advice; Carolyn Mitchell, Will Hentley and Sheena Lamond for technical assistance.

Funding statement. NERC-CEH PhD Studentship (NEC04280) with CASE support from JHI. Data are available via the NERC Environmental Informatics Data Centre (EIDC).

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