

LETTER

Effects of a belowground mutualism on an aboveground mutualism

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Abstract

Studies of multitrophic interactions between below- and aboveground communities have generally focused on soil organisms and antagonists of plant shoots and leaves (herbivores). Despite the widespread occurrence of plant mutualists below- and aboveground which can occur on the same host plant, the potential for interactions between them has not been considered. Here we demonstrate that aboveground plant mutualists, insect pollinators, are strongly influenced by belowground plant mutualists, arbuscular mycorrhizal fungi. The presence of arbuscular mycorrhizal fungi in the roots of *Chamerion angustifolium* increased pollinator visitation and per cent seed set of this plant in the field by up to two times compared with non-mycorrhizal plants. We propose that interactions between belowground and aboveground mutualisms are widespread and may play important functional roles in populations and communities.

Keywords

Arbuscular mycorrhizal fungi, multitrophic interactions, mutualism, pollination.

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INTRODUCTION

Numerous studies have demonstrated plant-mediated linkages between aboveground and belowground biota (Wardle *et al.* 2004). For example, herbivores can remove or damage photosynthetic tissue of plants and can subsequently change allocation of photosynthate to roots and the rhizosphere, leading to changes in the structure and function of soil communities (Saikkonen *et al.* 1999; Gange *et al.* 2002; Bardgett & Wardle 2003). Conversely, soil organisms can change the morphology or chemical composition of plant tissues, altering the activity and fecundity of aboveground herbivores (Gange & West 1994; Gange *et al.* 1999; Goverde *et al.* 2000).

While most aboveground–belowground studies have focused on aboveground antagonists such as herbivores, aboveground biota that can have positive effects on plants could also indirectly interact with components of soil communities. Interactions between plants and mutualists, such as pollinators, may be affected by the abundance and composition of belowground mutualists. For example, arbuscular mycorrhizal (AM) fungi, root symbionts of most higher plant species that can often have positive effects on plants, can alter reproductive traits of flowering plants (Koide & Dickie 2002). Given that changes in reproductive traits can influence plant interactions with pollinators

(Thompson 2001; Elle & Carney 2003; Mitchell *et al.* 2004), AM fungal effects on host plants could change the dynamics of the interactions between the plant and its pollinators. As mutualisms are generally only considered between pairs of species or trophic levels (Stanton 2003), the relative importance of multi-species mutualisms, such as those between aboveground and belowground interactors, has not been considered (Strauss & Irwin 2004).

In this study, we tested the hypothesis that belowground mutualisms can alter aboveground mutualistic interactions. We tested this hypothesis by manipulating the presence and identity of AM fungi in the roots of fireweed, *Chamerion angustifolium* L. Holub (Onagraceae). *C. angustifolium* is a perennial plant that establishes in sites of primary and secondary succession throughout much of the northern hemisphere (Mosquin 1966). This species is dependent on animal-mediated pollination, mainly by bees, for successful seed set (Benham 1969). AM fungal colonization of this plant in the field is variable, ranging from 0 to *c.* 60% (Allen 1988; Chapin 1995), reflecting patchiness of fungal propagules and possibly genetic variation in the plants ability to associate with AM fungi. In previous work with *C. angustifolium*, colonization by AM fungi did not significantly influence floral traits that might influence pollinator interactions with this plant such as nectar production and nectar composition (% fructose, glucose, and sucrose), but

AM fungi did increase the size of the floral display by *c.* 30% (Komlos 1999). Given that visitation by pollinators to this species is largely dependent on the flower morphology and size of floral display (Galen & Plowright 1988; Schmid-Hempel & Speiser 1988; Husband & Schemske 2000; Routley & Husband 2003), *C. angustifolium* is an appropriate model system to test the stated hypothesis.

METHODS

The experiment consisted of 90 seedlings of *C. angustifolium*, each growing in an 8-in pot. Pots were divided among three treatments: presence of the AM fungal species *Glomus intraradices*, presence of the AM fungal species *Gigaspora gigantea*, or absence of AM fungi. Plants were generated from seed collected in population D2, a diploid, outbred population along the Beartooth Pass Highway, near Red Lodge, southern Montana (Flint 1980; Husband & Schemske 1998). Seedlings were grown in a greenhouse substrate free of AM fungal propagules comprised of Pro-Mix (Premier Horticulture Lt.'ee, Rivière-du-Loup, Canada), Turface (Aimcor, Deerfield, USA), and Perlite (VIL Vermiculite, Inc., Toronto, Canada) in a ratio of 4 : 1 : 1.

The AM fungi were isolated from the Long-Term Mycorrhiza Research Site (Klironomos 2003), University of Guelph Arboretum, and kept in culture in association with leek (*Allium porrum* L., cultivar Giant Musselburgh) roots. We used leek to culture the AM fungi because roots of this plant are highly colonized by AM fungi and can serve as a clean and efficient source of AM fungal inoculum grown on a common host for a range of ecological studies. We used these two species of fungi because they are from within the native range and habitat of *C. angustifolium* and the interactions of these fungi with other plant species have been well characterized (Van der Heijden *et al.* 1998; Klironomos 2003). AM fungi from this site show little host specificity (Klironomos 2000). The pots were filled with the growth substrate to *c.* 2.5 cm below the inner rim. A similar amount (*c.* 2 g) of leek roots from each of the AM fungal cultures was washed, cut into *c.* 1 cm pieces, spread in the middle region of the substrate and then covered with growth substrate to the top of the inner rim. The control plants received the same quantity of un-inoculated leek roots. The +AM fungi/–AM fungi treatments in this experiment are relevant to field conditions. *C. angustifolium* can range from being uncolonized by AM fungi to high levels of colonization. Therefore, natural variation in AM fungi colonization within this study system occurs on a similar spatial scale at which the treatments were performed, and on the same spatial scale at which pollinators make choices about which plants to visit within a population.

To measure pollinator visitation rates, we adopted a method described by Schmid-Hempel & Speiser (1988) for

natural populations of *C. angustifolium*. Plants were placed outside in an open field within the natural range of *C. angustifolium* (near Rivière-du-Loup, Quebec, Canada) that did not contain any other *C. angustifolium*. Plants were positioned 0.5 m apart, within a 9 × 10 grid, with treatments being randomized across grid locations. At 42, 62, and 82 days after transplant, the frequency of visits by pollinators was assessed. Ten plants from each treatment were randomly chosen for observation. Three plants were observed simultaneously (one plant per treatment) by three-field assistants. Plants were randomly assigned to each assistant, and they were not made aware of the treatment associated with the plants. For each plant, we measured the time elapsed until a pollinator visited the main inflorescence and the number of flowers visited within the inflorescence. Five minutes was the maximum amount of time spent waiting for a visit. This procedure was repeated five times for each plant and then averaged. The pollinator visitation rate for each unit was calculated as 1/(mean waiting time). If no pollinator had arrived after 5 min, the time for a pollinator to arrive was recorded as 5 min. The data was recorded in minutes, but was expressed on a per hour basis. For example, if it took 1.5 min (0.025 h) before the first pollinator visited, then arrivals per hour = 1 arrival/(0.025 h) = 40. In all cases only large bees (bumble bees and honey bees) were counted. Although a range of insects can visit the flowers of fireweed, only large bees have been confirmed to consistently transfer pollen. Furthermore, our observations (B. Kennedy, H. Sabara, and B.C. Husband, unpublished data) indicate that bees visit only during the day, thus we made all pollinator observations between 10:00 and 16:00 h. We did not identify the bees to species.

In addition to visits by pollinators we also measured herbivory on the plants. Percent leaf damage by arthropod herbivores was estimated on five random leaves from each plant prior to final harvest. Plants were also sampled for arthropods on the day following the pollinator observations. All arthropods were identified and counted. They were considered to be herbivores if they were observed feeding on the plants or were known herbivores based on the literature.

At 101 days after transplant, plants were harvested and biomass was determined by separating plants into the following components; main vegetative stem, main inflorescence, lateral vegetative stem, lateral inflorescence, leaves, and roots.

We decided to measure biomass because there is a strong correlation between shoot biomass, inflorescence height, and the number of flowers (Komlos 1999), and bees prefer to forage on individuals of *C. angustifolium* with a larger floral display, particularly those that are taller and contain more flowers (Galen & Plowright 1988; Schmid-Hempel & Speiser 1988; Husband & Schemske 2000; Routley & Husband 2003). At harvest, a small subsample of roots was

used to confirm the presence/absence of mycorrhizal colonization following the protocol of Brundrett *et al.* (1984). Finally, plant components were put into separate paper bags, dried at 80 °C for 72 h, and then weighed to determine biomass.

From the 30 plants that were observed for pollinator visitation, five randomly-chosen fruits were collected from the main inflorescence. For each fruit we counted the total number of ovules and the number of filled seed, under a dissecting microscope. Percent seed set per fruit was calculated as number of seeds/number of ovules. Percent seed set is a component of total seed production and a good reflection of the activity of pollinators at any one time because it controls for variation in ovule number. Also Husband (2000) estimated percent seed set and total seed production for a whole growing season, and they were highly correlated. To estimate percent germination, we collected 25 seed from each fruit, placed them on filter paper, kept moist with sterile distilled water, in Petri dishes (12-h light/dark, at 20 °C/20 °C, 50/50 humidity). Germination was assessed as the proportion of seeds with an emerging radicle after 2 weeks. For response variables that were measured once, mycorrhizal effects were evaluated using one-way analysis of variance (ANOVA), followed by Tukey *post-hoc* tests. For pollinator visitation data (multiple measures on individual plants) a repeated measures ANOVA was used.

RESULTS

All plants exposed to AM fungal inoculum were successfully colonized by AM fungi. We found the presence of some or all of the characteristic structures of AM fungi (inter- and intra-cellular hyphae, vesicles, arbuscules). Percentage colonization of plant roots ranged from 34 to 52% in the presence of *G. intraradices*, and 10–24% in the presence of *G. gigantea*. We did not detect any AM fungal structures in plants of the non-mycorrhizal treatment.

We found no significant effect of AM fungi on the abundance of herbivores on plants ($F_{2,81} = 0.15$, $P = 0.87$) or leaf damage by herbivores ($F_{2,81} = 0.19$, $P = 0.84$). However, plants that were infected by either of the AM fungi were more frequently visited by pollinators compared with non-mycorrhizal plants (Fig. 1). Differences in pollinator visitation were significant at three different times (42, 62, 82 days after transplant), although the differences were strongest at the latter two times. Overall, we detected a significant effect of time ($F_{2,81} = 66.9$, $P = 0.001$), mycorrhiza ($F_{2,81} = 77.7$, $P = 0.001$), and time \times mycorrhiza interaction ($F_{4,81} = 5.8$, $P = 0.001$). Across the mycorrhiza treatment, differences were only detected between mycorrhizal plants and non-mycorrhizal controls. There was no significant difference between plants colonized by the different isolates of AM fungus (Tukey,

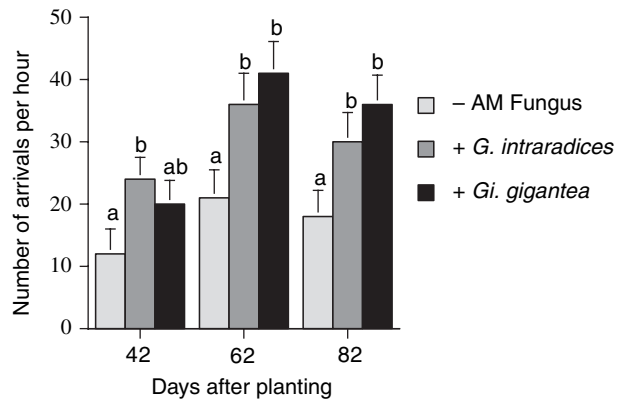


Figure 1 Effect of different arbuscular mycorrhizal (AM) fungal treatments on the pollinator visitation of *Chamerion angustifolium* at 42, 62, and 82 days after establishment of the AM fungal treatments. Bars represent ± 1 SEM. Different letters represent significant differences ($P < 0.05$) using ANOVA and Tukey *post-hoc* tests.

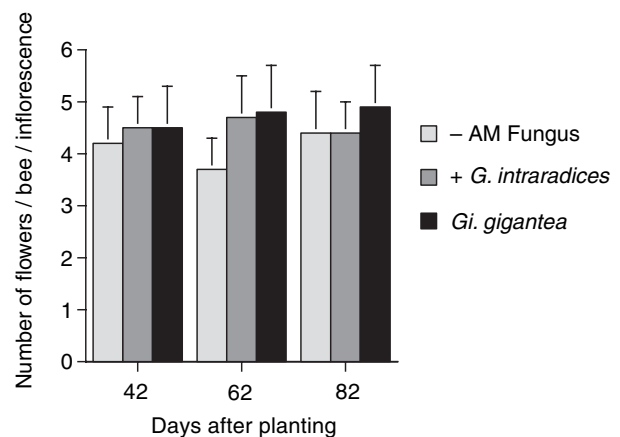


Figure 2 Effect of different arbuscular mycorrhizal (AM) fungal treatments on the number of flowers visited per bee per inflorescence of *Chamerion angustifolium* at 42, 62, and 82 days after establishment of the AM fungal treatments. Bars represent ± 1 SEM. Different letters represent significant differences ($P < 0.05$) using ANOVA and Tukey *post-hoc* tests.

$P = 0.96$). Also, when bees arrived at individual inflorescences we did not detect a difference in the number of flowers visited per inflorescence (time: $F_{2,81} = 0.13$, $P = 0.88$; mycorrhiza: $F_{2,81} = 0.17$, $P = 0.85$; time \times mycorrhiza: $F_{4,81} = 1.18$, $P = 0.33$) (Fig. 2). At harvest, mycorrhizal plants had higher per cent seed set ($F_{2,29} = 13.8$, $P = 0.001$), but seed germination did not differ among any treatments ($F_{2,29} = 1.19$, $P = 0.319$) (Fig. 3).

Plants grown in the presence of *G. intraradices* showed few differences in biomass compared with non-mycorrhizal plants (Table 1). We did not detect a difference in total

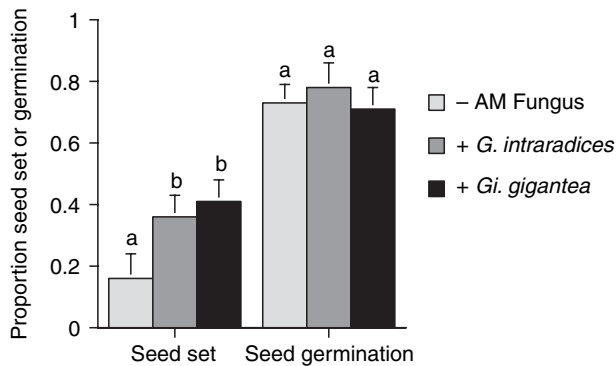


Figure 3 Effect of different arbuscular mycorrhizal (AM) fungal treatments on per cent seed set per fruit and seed germination of *Chamerion angustifolium* at 42, 62, and 82 days after establishment of the AM fungal treatments. Different letters represent significant differences ($P < 0.05$) using ANOVA and Tukey *post-hoc* tests.

biomass, nor a difference in the biomass of most other plant components. The only mycorrhizal effects detected were increases in the biomass of the main stem (vegetative and inflorescence). In contrast, plants exposed to the other AM fungus (*G. gigantea*) did show a significant but modest (15%) increase in total biomass. Significant increases were also detected in the biomass of most plant components (Table 1). Plants colonized by either of the AM fungi allocated more resources to reproductive vs. vegetative parts. We detected a significant effect ($F_{2,81} = 49.1$, $P = 0.001$) of mycorrhiza on the ratio of biomass in reproductive vs. vegetative stems (–AM fungi = 0.30; *G. intraradices* = 0.36; *G. gigantea* = 0.51). A similar effect ($F_{2,81} = 35.0$, $P = 0.001$) was detected in the ratio between reproductive stems and total non-reproductive plant biomass (–AM fungi = 0.18; *G. intraradices* = 0.22; *G. gigantea* = 0.31).

DISCUSSION

The results of this study illustrate that despite minimal effects on overall plant biomass, belowground mutualists

can induce changes in allocation of resources. Furthermore, changes in allocation favoured shoot growth, which resulted in an increase in pollinator visitation and percent seed set, at the cost of reduced allocation to roots. In this study, increased pollinator visitation in mycorrhizal treatments was probably associated with plants having larger inflorescences and hence being more conspicuous to bees. Mycorrhizal plants that had a higher shoot biomass were also taller, and taller individuals generally produce more flowers (Komlos 1999).

Other factors that can influence pollinator visitation that are linked to resource availability, such as changes in flower morphology (Ashman *et al.* 2000) or rewards for pollination (Zimmerman 1982), may also play a role in increased visitation to mycorrhizal plants, but previous work with this plant found limited effects of AM fungi on these floral traits (Komlos 1999), suggesting that inflorescence size was the main mechanism behind the observed effects. Studies with other plant systems are needed to assess if AM fungi can influence other floral traits, and subsequently alter plant interactions with pollinators. Experimental studies are also needed to more completely tease apart the mechanism driving the result in this study system.

Although increased seed set was observed in plants that also had higher levels of pollinator visitation, it is not possible to tease apart direct vs. indirect effects of AM fungal colonization on percent seed set in this experiment. For example, although AM fungal colonization did increase the number of pollinator visits per plant, the increases in percent seed set could have been indirectly caused by increased resource availability mediated by AM fungal uptake of nutrients from the soil, and not increased pollinator visitation. Although we cannot tease apart the direct vs. indirect effects of AM fungi on plant response, that does not detract from the main premise of our work, namely that below-ground mutualisms can affect above-ground mutualistic interactions between plants and pollinators. To tease apart the direct vs. indirect effects of AM fungal infection on plant response, future studies should

Table 1 Effect of different arbuscular mycorrhizal (AM) fungal treatments on mean dry weight (g) of vegetative and reproductive biomass of *Chamerion angustifolium*

Variable	–AM fungus	+ <i>G. intraradices</i>	+ <i>G. gigantea</i>
Main vegetative stem	0.74 (0.13)a	1.02 (0.09)b	0.91 (0.14)ab
Main inflorescence	0.83 (0.11)a	1.26 (0.13)b	1.79 (0.17)c
Lateral vegetative stem	3.21 (0.37)a	2.80 (0.27)a	3.14 (0.34)a
Lateral inflorescence	0.98 (0.09)a	0.81 (0.11)a	1.37 (0.15)b
Leaf	1.95 (0.21)a	1.90 (0.18)a	2.17 (0.31)b
Shoot	7.71 (0.41)a	7.79 (0.49)a	9.38 (0.37)b
Root	3.81 (0.20)a	3.54 (0.25)a	3.90 (0.30)a
Total	11.52 (0.65)a	11.32 (0.71)a	13.28 (0.55)b

Values in parentheses represent 1 SEM. Different letters represent significant differences ($P < 0.05$) using ANOVA and Tukey *post-hoc* tests.

combine AM fungal manipulation with hand-pollination treatments.

The above results pertain to just one plant species and its associated aboveground and belowground mutualists, but aboveground–belowground interactions between mutualisms may be mediated by many other plants species. Many of the plant species on which aboveground mutualistic interactions occur are colonized by some type of mycorrhizal fungi or nitrogen-fixing root symbionts, which can all serve as effective mutualists for plants. These belowground mutualists have the potential to affect resource allocation in host plants, which in turn can alter the outcome of aboveground mutualisms (Baylis & Pierce 1991; Heil *et al.* 2001). Development of other study systems with aboveground and belowground mutualists on a common host will allow us to understand the relative importance of these interactions for population and community dynamics.

While we only considered the effects of belowground mutualists on aboveground mutualists, aboveground mutualists also have the potential to alter the structure and function of belowground mutualists. When flowers of an individual plant are pollinated, photosynthate and stored resources that could otherwise be supplied to root mutualists are directed toward the production of reproductive biomass (Obeso 2002). Given that the amount of carbon supplied by the plant host can affect the abundance and composition of root mutualist communities (Zitler *et al.* 1996; Heinemeyer *et al.* 2004), variation in pollinator visitation and subsequent alterations in resource allocation within a plant could alter belowground interactions of a plant with belowground mutualists. This idea has yet to be tested.

The interplay between above- and belowground mutualisms can also have evolutionary implications, which have been largely unexplored. In general, all mutualists are continually under selection to maximize their fitnesses within a specific abiotic and biotic environment. However, if an organism participates in more than one mutualism and these interact, then the evolutionary responses to selection on any one mutualism may be facilitated or constrained by the other (Nuismer & Doebeli 2004). Ultimately, the joint evolution of fungal and pollinator mutualisms will depend on the availability of heritable variation within these respective complexes and genetic correlations between them (Nuismer & Doebeli 2004). While the quantitative genetics of floral traits affecting pollinators have received some attention (Elle 1998; Worley & Barrett 2000; Caruso 2004), to our knowledge the genetic basis of variation in colonizing ability of fungi or colonization-response in plants has not been quantified.

Generally, mutualistic interactions have been considered between sets of two species, and the potential for interactions between different sets of mutualists has rarely

been examined (but see Compton & Robertson 1988 for an example of interactions between two aboveground mutualisms). Given the importance of mutualisms for the organization and function of both aboveground and belowground communities (Kawanabe *et al.* 1993; Van der Heijden *et al.* 1998; Christian 2001), we propose that their interaction can have important consequences for the structure and functioning of communities. Future work examining the interactions between belowground and aboveground mutualisms should consider the relative importance of these interactions compared with the better-understood interactions between antagonists of plants.

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REFERENCES

- Allen, M.A. (1988). Re-establishment of VA-mycorrhizas following severe disturbance – comparative patch dynamics of a shrub desert and a subalpine volcano. *Trans. Br. Mycol. Soc.* 88, 413–417.
- Ashman, T.L., Swetz, J. & Shivittz, S. (2000). Understanding the basis of pollinator selectivity in sexually dimorphic *Fragaria virginiana*. *Oikos*, 90, 347–356.
- Bardgett, R.D. & Wardle, D.A. (2003). Herbivore-mediated linkages between aboveground and belowground communities. *Ecology*, 84, 2258–2268.
- Baylis, M. & Pierce, N.E. (1991). The effect of host-plant quality on the survival of larvae and oviposition by adults of an ant-tended Lycaenid butterfly, *Jalmenus evagoras*. *Ecol. Entomol.*, 16, 1–9.
- Benham, B.R. (1969). Insect visitors to *Chamaenerion angustifolium* and their behaviour in relation to pollination. *Entomologist*, 102, 221–228.
- Brundrett, M.C., Piche, Y. & Peterson, R.L. (1984). A new method for observing the morphology of vesicular-arbuscular mycorrhizae. *Can. J. Bot.*, 62, 2128–2134.
- Caruso, C.M. 2004. The quantitative genetics of floral trait variation in *Lobelia*: potential constraints on adaptive evolution. *Evolution*, 58, 732–740.
- Chapin, D.M. 1995. Physiological and morphological attributes of two colonizing plant species on Mount St. Helens. *Am. Midl. Nat.*, 133, 76–87.
- Christian, C.E. (2001). Consequences of a biological invasion reveal the importance of mutualism for plant communities. *Nature*, 413, 635–639.
- Compton, S.G. & Robertson, H.G. (1988). Complex interactions between mutualisms – ants tending homopterans protect fig seeds and pollinators. *Ecology*, 69, 1302–1305.

- Elle, E. 1998. The quantitative genetics of sex allocation in the andromonoecious perennial, *Solanum carolinense* (L.). *Heredity*, 80, 481–488.
- Elle, E. & Carney, R. (2003). Reproductive assurance varies with flower size in *Collinsia parviflora* (Scrophulariaceae). *Am. J. Bot.*, 90, 888–896.
- Flint, E. (1980). Ecology and distribution of diploid and tetraploid *Epilobium angustifolium* (fireweed) in the Beartooth Mountains of Wyoming and Montana. PhD Thesis, Duke University, Durham, NC.
- Galen, C. & Plowright, R.C. (1988). Contrasting movement patterns of nectar-collecting and pollen-collecting bumble bees (*Bombus terricola*) on fireweed (*Chamaenerion angustifolium*) inflorescences. *Ecol. Entomol.*, 10, 9–17.
- Gange, A.C., & West, H.M. (1994). Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata*. *New Phytol.*, 128, 79–87.
- Gange, A.C., Bower, E. & Brown, V.K. (1999). Positive effects of an arbuscular mycorrhizal fungus on aphid life history traits. *Oecologia*, 120, 123–131.
- Gange, A.C., Bower, E. & Brown, V.K. (2002). Differential effects of insect herbivory on arbuscular mycorrhizal colonization. *Oecologia*, 131, 103–112.
- Goverde, M., der Heijden, M.G.A., Wiemken, A., Sanders, I.R. & Erhardt, A. (2000). Arbuscular mycorrhizal fungi influence life history traits of a lepidopteran herbivore. *Oecologia*, 125, 362–369.
- Heil, M., Hilpert, A., Fiala, B. & Linsenmair, K.E. (2001). Nutrient availability and indirect (biotic) defence in a Malaysian ant-plant. *Oecologia*, 126, 404–408.
- Heinemeyer, A., Ridgway, K.P., Edwards, E.J., Benham, D.G., Young, J.P.W. & Fitter, A.H. (2004). Impact of soil warming and shading on colonization and community structure of arbuscular mycorrhizal fungi in roots of a native grassland community. *Global Change Biol.*, 10, 52–64.
- Husband, B.C. (2000). Constraints on polyploidy evolution: a test of the minority cytotype exclusion principle. *Proc. R. Soc. Lond. B* 267, 1–7.
- Husband, B.C. & Schemske, W.D. (1998). Cytotype distribution at a diploid-tetraploid contact zone in *Chamerion (Epilobium) angustifolium* (Onagraceae). *Am. J. Bot.*, 85, 1688–1694.
- Husband, B.C. & Schemske, W.D. (2000). Ecological mechanisms of reproductive isolation and coexistence of diploid and tetraploid *Chamerion angustifolium*. *J. Ecol.*, 88, 1–14.
- Kawanabe, H., Cohen, J.E. & Iwaski, K. (1993). *Mutualism and Community Organization*. Oxford University Press, Inc., New York.
- Klironomos, J.N. (2000). Host-specificity and functional diversity among arbuscular mycorrhizal fungi. In: *Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology* (eds. Bell, C.R., Brylinsky, M. & Johnson-Green, P.). Atlantic Canada Society for Microbial Ecology, Halifax, Canada. pp. 845–851.
- Klironomos, J.N. (2003). Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology*, 84, 2292–2301.
- Koide, R.T. & Dickie, I.A. (2002). Effects of mycorrhizal fungi on plant populations. *Plant Soil*, 244, 307–317.
- Komlos, D.A. (1999) Vegetative and sexual life-history attributes of *Chamerion angustifolium* (Fireweed) as influenced by arbuscular mycorrhizal fungi. MSc Dissertation, University of Guelph, Guelph, ON, Canada.
- Mitchell, R.J., Karron, J.D., Holmquist, K.G. & Bell, J.M. (2004). The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. *Funct. Ecol.*, 18, 116–124.
- Mosquin, T. (1966). A new taxonomy for *Epilobium angustifolium* L. (Onagraceae). *Brittonia*, 18, 167–188.
- Nuismer, S.L. & Doebeli, M. (2004). Genetic correlations and the coevolutionary dynamics of three-species systems. *Evolution* 58, 1165–1177.
- Obeso, J.R. (2002). The costs of reproduction in plants. *New Phytol.*, 155, 321–348.
- Routley, M. & Husband, B.C. (2003). The effect of protandry on siring success in *Chamerion angustifolium* (Onagraceae) with different inflorescence sizes. *Evolution*, 57, 240–248.
- Saikkonen, K., Ahonen-Jonnarth, U., Markkola, A.M., Helander, M., Tuomi, J., Roitto, M. *et al.* (1999). Defoliation and mycorrhizal symbiosis: a functional balance between carbon sources and below-ground sinks. *Ecol. Lett.*, 2, 19–26.
- Schmid-Hempel, P. & Speiser, B. (1988). Effects of inflorescence size on pollination in *Epilobium angustifolium*. *Oikos*, 53, 98–104.
- Stanton, M.L. (2003). Interacting guilds: moving beyond the pairwise perspective on mutualisms. *Am. Nat.*, 162, S10–S23.
- Strauss, S.Y. & Irwin, R.E. (2004) Ecological and evolutionary consequences of multispecies plant–animal interactions. *Annu. Rev. Ecol. Syst.*, 35, 435–466.
- Thompson, J.D. (2001). How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? *Oecologia*, 126, 386–394.
- Van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, P., Boller, T. *et al.* (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69–72.
- Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H. & Wall, D.H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, 304, 1629–1633.
- Worley, A.C. & Barrett, S.C.H. (2000). Evolution of floral display in *Eichhornia paniculata* (Pontederiaceae): direct and correlated responses to selection on flower number. *Evolution*, 54, 1533–1545.
- Zimmerman, M. (1982). The effect of nectar production on neighborhood size. *Oecologia*, 52, 104–108.
- Zitzer, S.F., Archer, S.R. & Boutton, T.W. (1996). Spatial variability in the potential for symbiotic N₂ fixation by woody plants in a subtropical savanna ecosystem. *J. Appl. Ecol.*, 33, 1125–1136.

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