Ecology, 89(7), 2008, pp. 1791–1801
© 2008 by the Ecological Society of America

DISRUPTION OF A BELOWGROUND MUTUALISM ALTERS INTERACTIONS BETWEEN PLANTS AND THEIR FLORAL VISITORS

James F. Cahill, Jr.,[1,3] Elizabeth Elle,[2] Glen R. Smith,[1] and Bryon H. Shore[1]

[1]*Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9 Canada*
[2]*Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6 Canada*

Abstract. Plants engage in diverse and intimate interactions with unrelated taxa. For example, aboveground floral visitors provide pollination services, while belowground arbuscular mycorrhizal fungi (AMF) enhance nutrient capture. Traditionally in ecology, these processes were studied in isolation, reinforcing the prevailing assumption that these above- and belowground processes were also functionally distinct. More recently, there has been a growing realization that the soil surface is not a barrier to many ecological interactions, particularly those involving plants (who live simultaneously above and below ground). Because of the potentially large impact that mycorrhizae and floral visitors can have on plant performance and community dynamics, we designed an experiment to test whether these multi-species mutualisms were interdependent under field conditions. Using benomyl, a widely used fungicide, we suppressed AMF in a native grassland, measuring plant, fungal, and floral-visitor responses after three years of fungal suppression. AMF suppression caused a shift in the community of floral visitors from large-bodied bees to small-bodied bees and flies, and reduced the total number of floral visits per flowering stem 67% across the 23 flowering species found in the plots. Fungal suppression has species-specific effects on floral visits for the six most common flowering plants in this experiment. Exploratory analyses suggest these results were due to changes in floral-visitor behavior due to altered patch-level floral display, rather than through direct effects of AMF suppression on floral morphology. Our findings indicate that AMF are an important, and overlooked, driver of floral-visitor community structure with the potential to affect pollination services. These results support the growing body of research indicating that interactions among ecological interactions can be of meaningful effect size under natural field conditions and may influence individual performance, population dynamics, and community structure.

Key words: Aspen Parkland, Kinsella, Alberta, Canada; community ecology; grassland; indirect interactions; interactions at multiple trophic levels; mutualisms; mycorrhizae; plant–soil interactions; pollination biology.

Introduction

Ecological interactions do not occur in isolation, but instead seemingly spatially discrete ecological processes can be functionally linked. For example, by increasing predation on larval dragonflies, which as adults eat a variety of pollinating insects, fish can enhance pollination services delivered in the surrounding terrestrial environment (Knight et al. 2005*a*). Even within terrestrial communities, there has historically been a tendency to view aboveground and belowground processes as distinct. However, in recent years there has been a significant paradigm shift associated with the realization that above and below ground processes can be of meaningful-effect sizes (Wardle 2002). For example, through direct effects on plant community composition (via consumption) and indirect effects on bee nest sites (via soil compaction), cattle grazing can increase bee abundance (Vulliamy et al. 2006). Interactions within communities can also be trait-based, such as when plant competition for soil resources reduces shoot growth, which in turns impacts a plant's ability to compete for light (Cahill 1999), or when reductions in flower size due to foliar herbivory leads to reduced floral visitation (Strauss et al. 1996). Although trait-mediated changes due to one type of interaction may in turn affect interactions with other guilds (Agrawal 2001, Palmer et al. 2003, Strauss and Irwin 2004), our understanding of the ecological importance of interactions between multiple interacting guilds is extremely limited. This lack of knowledge of the effect of interactions among ecological interactions is particularly acute when the interactions of interest are mutualisms rather than trophic cascades.

Two widespread mutualisms involving plants are plant–pollinator and plant–mycorrhizal fungal interactions. Although these appear independent due to our perception of the soil surface as a barrier, individual plants grow on both sides of this "barrier" and form a link between two seemingly independent guilds: pollinators and fungi. Feedbacks between plant and soil

Manuscript received 2 May 2007; revised 21 November 2007; accepted 29 November 2007. Corresponding Editor: C. Galen.

[3] E-mail: jc.cahill@ualberta.ca

PLATE 1. The native fescue grassland in the Aspen Parkland ecoregion contains a diverse assemblage of native flowering plants and pollinators. Shown here is (left) a large bee (*Bombus* sp.) visiting *Astragalus* sp. and (right) a large number of plant species in flower, including *Gaillardia aristata* and *Oxytropis campestris* (available in color in Appendix A). Photo credit: J. F. Cahill, Jr.

communities can alter plant and microbial community structure and ecological functioning (e.g., Wardle 2002, Bezemer et al. 2006). The extent to which changes to the plant–fungal mutualism can have a cascading effect on the plant–pollinator mutualism in natural communities is unknown. Whether shifts in plant–fungal mutualisms could lead to shifts in the community structure and activity of pollinating insects will depend not only on whether functionally relevant connections exist, but also on the absolute effect size of these connections.

Arbuscular-mycorrhizal fungi (AMF) form associations with the roots of most vascular plant species, consuming photosynthate and often providing enhanced nutrition or other benefits (Allen et al. 1991). Because of the intimacy of the plant–AMF interaction, mycorrhizal suppression can have widespread direct and indirect effects on plant communities, including shifts in species diversity (Hartnett and Wilson 1999), altered competitive outcomes (Smith et al. 1999), and increased growth of non-mycorrhizal or weakly mycorrhizal species (van der Heijden et al. 1998). As mycorrhizae can affect both floral trait expression of individuals (Gange and Smith 2005, Wolfe et al. 2005) and plant community composition (Hartnett and Wilson 1999), there is reason to expect mycorrhizae to influence plant–pollinator interactions.

Two recent studies of potted plants found the addition of mycorrhizae to sterile soil increased flower-visitation rates (Gange and Smith 2005, Wolfe et al. 2005) due to species-specific increases in flower production and/or rewards (Gange and Smith 2005, Wolfe et al. 2005). In a complex natural community, however, floral visitors respond not only to shifts in floral display of potential host species, but also to the collective display and reward properties of the co-flowering plant community (e.g., Johnson et al. 2003, Moeller 2004, Knight et al. 2005*b*, Larson et al. 2006). There is substantial evidence indicating that AMF can have impacts on plants that range from parasitic to mutualistic (Klironomos 2003), suggesting that floral displays may be enhanced by AMF in some species, and reduced by AMF in others. As a result AMF may alter community-level floral displays through direct changes to floral traits of individual species, altered growth of mycorrhizal-dependent species, and changes in competitive interactions involving release of non-mycorrhizal or weakly mycorrhizal species. Such complex interactions are best examined in natural conditions in intact communities.

Animal-mediated pollen movement is essential for plant fitness in many species, and disruption of this service can impact plant population dynamics and community composition (Ashman et al. 2004, Knight et al. 2005*b*, Biesmeijer et al. 2006). The benefits of pollinator services to a plant are influenced by visitation rate (Ashman et al. 2004), which can be affected by plant species richness and evenness (Ghazoul 2006), floral density (Moeller 2004, Hegland and Boeke 2006), and floral display and reward sizes (Thompson 2001, Elle and Carney 2003). In addition to influencing the behavior of pollinators that are already present, floral traits can also influence which floral visitors will be present at a given location (Potts et al. 2003, Fenster et al. 2004). As a result, changes in the flowering-plant community can lead to shifts in the visiting community of insects (Larson et al. 2006). These shifts can also go in the opposite direction, with evidence that a decline in bee populations is associated with a decline in outcrossing bee-pollinated plant species (Biesmeijer et al. 2006).

It is clear that both aboveground and belowground mutualisms can independently impact both the fitness of individual plants and the composition of natural communities. As a first step toward determining whether the plant–AMF mutualism has the potential for additional impacts on the plant community by influencing plant–pollinator interactions, we suppressed AMF in a native grassland using the fungicide benomyl. We then measured insect-visitation rates to the flowering-plant community in treated and control plots, as well as AMF colonization and floral display for the six most abundant plant species (Table 1). We hypothesized that suppressing AMF would lead to a cascade of effects through the plant community to impact the interacting community of floral visitors. Experiments performed on potted plants suggest that suppressing AMF should reduce the floral display and so reduce pollination services. In an intact community, however, more complex interactions are expected, so that a reduction in performance of some plant species will be coupled with increased performance of others. We therefore expect plant-species-specific changes in the interactions with the community of floral visitors.

METHODS

Field site and experimental layout

Field work was conducted in a native rough fescue grassland (*Festuca hallii*) at the University of Alberta Research Station in Kinsella, Alberta, Canada (Appendix A). The field site is in the Aspen Parkland, which is a savanna-type habitat consisting of interdispersed patches of grassland and aspen stands (see Plate 1). The site has never been seeded or tilled, and was not grazed for the two years prior to or during the study. Over 70% of the biomass is in grasses, while over 70% of the species diversity is in the forbs (J. F. Cahill, *personal observation*). Competitive interactions for resources between plants are primarily belowground (Lamb et al. 2007). It is unknown whether the plants in this system compete above ground for access to floral visitors.

We established 20, 2 × 4.5 m blocks distributed haphazardly across 6 ha. All blocks were located in grassland regions of the savanna, and consisted of two 2 × 2 m plots, separated by 0.5 m. The plots were established in 2003 and 2004, and all measures for this study took place only in 2005. There were no effects of plot age in the initial analyses, and this term was excluded from the final statistical models.

Mycorrhizal suppression

One plot in each block received additions every two weeks of the fungicide benomyl (methyl 1-[(butylamino)carbonyl]-1H-benzimidazol-2-ylcarbamate) in a soil drench (2.5 g Benlate per 1.75 L water per square meter (Benlate: 50% active ingredient [DuPont, Wilmington, Delaware, USA]), while the control plots received an equivalent amount of water. By the end of this study, plots had received either two or three years of benomyl application. Benomyl is effective in reducing colonization of arbuscular-mycorrhizal fungi (AMF) (Fitter and Nichols 1988, Smith et al. 2000) and has been widely used in ecological studies (e.g., Newsham et al. 1994, Hartnett and Wilson 1999, 2002, O'Connor et al. 2002, Callaway et al. 2003). However, benomyl also causes a variety of unintended effects, including suppression of parasitic fungi (Newsham et al. 1994) and nematodes (Schmidt et al. 2000).

We recognize that understanding the potential confounding effects of experimental treatments is critical to the proper interpretation of data. For example, nematodes can alter plant growth and interactions between plants and aboveground herbivores (Bezemer et al. 2005), and thus benomyl-induced changes to nematode densities could cause a variety of impacts independent of AMF-mediated pathways. We also recognize that an alternative mesocosm design in which one uses sterile soil, to which a subset of even-aged plant and fungal species are added, is not representative of natural communities, and thus the ability to extrapolate results from such a study to real-word conditions is questionable. We believe the greatest advances will come when both of these approaches are used in a diversity of studies. Many of the specific concerns regarding the use of benomyl in ecological studies have been discussed extensively in the literature by Smith et al. (2000:944) who conclude that "the principal effect of benomyl in tall-grass prairie soils is suppression of mycorrhizal fungi, and that benomyl applications remain the most useful tool for experimentally manipulating mycorrhizal symbiosis in the field."

One potential direct confounding effect not tested by Smith et al. (2000) was whether benomyl itself directly impacted the behavior of floral visitors. To test for such an effect, five blocks were set up every two weeks during the main study (with each block used only once). Blocks contained two 50 × 50 cm plots, one of which received benomyl application. Attraction of insects that are common floral visitors was measured by placing three plastic pan traps (one each of white, blue, and yellow) half filled with soapy water into each plot immediately following benomyl application. Insects in the traps were counted eight hours later. There were no significant differences in numbers of trapped insects (mostly bees and flies) between treatments (t test, $t_{34} = 0.133$, $P = 0.895$) indicating that benomyl itself does not attract or repel flying insects.

Fungal colonization

We measured root colonization by AMF in the roots of six focal species. In August fine roots of 1–5 individuals per focal species per plot (depending upon abundance) were taken from all plots in which they were found, and stained (Giovannetti and Mosse 1980).

TABLE 1. Characteristics for the six focal plant species visited in at least five plots of each treatment, collected from various floras and observations in the field.

Species	Family	Peak flowering date (2006)	General floral characters
Achillea millefolium	Asteraceae	8–12 July	white; numerous small heads in flattened dome-shaped inflorescences
Aster laevis	Asteraceae	22–28 August	head with yellow disk and purple ray florets
Campanula rotundifolia	Campanulaceae	24–28 July	purple; nodding radially symmetric
Cerastium arvense	Caryophyllaceae	28–30 May	white; upright radially symmetric
Erigeron philadelphicus	Asteraceae	24–28 July	head with yellow disk and white-pink ray florets
Solidago missouriensis	Asteraceae	24–28 July	yellow; rounded, branched flower cluster, narrow at top

Note: Morphological variation necessitated measuring flower/inflorescence and display sizes differently for each species.

Colonization was calculated using a modified line–intersect method at 400× magnification (McGonigle et al. 1990). This magnification is higher than generally used for determining root-length colonization (e.g., Newsham et al. 1995, Hartnett and Wilson 1999, Hodge 2003), and reduces ambiguity in fungal structure identification. We recorded the proportions of intersections that contained arbuscules, vesicles, or hyphae visibly connected to either, within the field of view. We also recorded the colonization by hyphae unlikely to be AMF, including those on the root surface, septate hyphae, or those with clamp connections within hyphae.

Flowering-stem counts and observation of floral visitor

Approximately every two weeks for the entire flowering season (May–August 2005), flowering stems (having at least one open flower) in each plot were counted, and floral visitors were observed for 15 min. Frequency of interactions between plants and floral visitors is considered a good predictor of the importance of the interaction (Vazquez et al. 2005, Sahli and Conner 2006). Floral visits are required for seed production (female fitness) in obligately outcrossing species such as our focal species with self-incompatibility mechanisms (Table 1). "Flower" in this paper refers to showy flowers that are likely entomophilous. In total, 39 entomophilous plant species were recorded in the plots of this study.

The order of observations of floral visitors was randomly assigned among blocks. Observations occurred only on sunny days between 10:00 and 16:00 hours, so each round of observations to all plots would take ~3 days. A "visit" was classified as a single insect contacting the sexual parts of a flower. A single insect contacting multiple flowers on a single flowering stem was counted as one visit. This was necessary because many species in our study area had numerous small flowers in an inflorescence (Table 1), and accurate observation at the scale of the individual flower probed was not possible. Each floral visitor was identified on the wing to "morphospecies" using morphological traits such as color pattern and location of scopae, as well as behavior. We also established a reference collection of insects using pan traps and netting, from which we identified to at least genus the identity of the morphospecies; several morphospecies groups actually include several genera. For analysis, we combined morphospecies into logical groupings based on size, as body size can be correlated with pollen movement (e.g., Kremen et al. 2002). Within the bees, our groupings included large-bodied bumble bees (*Bombus* spp); mid-sized bees, including Andrenidae (mining bees), Halictidae (sweat bees), and Megachilidae (mason bees); and small bees, primarily *Dialictus* spp. (Halictidae) but also including other halictids and Colletids (Table 2). The Dipterans were either floral specialists such as Syrphidae (hoverflies) and beeflies (*Bombylius* spp.), or other generalist flies such as muscids and sawflies that tended to land on flowers. A final "other" category included wasps and butterflies.

Floral display

Aspects of the floral display were measured on six focal plant species (Table 1). All six species are perennials, with evidence for clonal growth. These species were chosen because they were the only ones visited by insects in at least five plots of each treatment, an arbitrarily chosen minimum necessary level of replication to ensure confidence in the results. We measured three aspects of floral phenotype during the peak of flowering for each species, to estimate the total display of flowers or inflorescences by species in a plot: (1) size of flower or inflorescence, (2) display size, normally the average number of flowers or inflorescences, including buds for some species, and (3) the number of flowering stems (see *Flowering-stem counts*, above). Species-specific details of floral display measurement are provided in Table 1; flower or inflorescence size, in particular, needed to be measured differently for the different focal species as they had very different morphologies. Our attempts to measure nectar volume were unsuccessful, due to both the small amounts produced by these taxa and the dry conditions in which they were growing. Note that all of our focal species were self-incompatible, weakly self-incompatible, or in a genus with many self-incompatible species in cases where the breeding system of the focal species has not been investigated (Table 1). This suggests that for our

TABLE 1. Extended.

Mating system	Flower or inflorescence size	Display size
self-incompatible	surface area of largest inflorescence (elliptical shape)	no. inflorescences per flowering stem
self-incompatible	width of the largest flower per stem	no. flowering heads and buds per stem
protandrous, weakly self-incompatible	width of the largest flower per stem	no. flowering heads and buds per stem
self-incompatible	width of largest flower per stem	no. flowering heads and buds per stem
unknown; genus is largely self-incompatible	width of largest flower per stem	no. flowering heads per stem
unknown; genus includes self-incompatible and self-compatible species	surface area of inflorescence (cylindrical shape)	not applicable (one inflorescence per flowering stem)

focal species at least, floral visitors are required for seed production, and visit rate may be a surrogate for fitness.

Statistical analyses

For all analyses we summed data across the growing season, rather than conducting repeated-measures analyses, because observations within any one period tended to be low. Some flowering stems were present in more than one observation period, and thus we will have overestimated flowering stem numbers. However, this was consistent across treatments, and thus will not alter interpretation of the results. Additionally, modeling with simulated data indicated that our results were robust regardless of whether we used total flowering-stem number (the most liberal measure of display over time) or maximum stem number observed in any time period (the most conservative measure of flower display).

Visitation rates, floral display, and AMF colonization.—Visitation rates were analyzed both as (1) total visits to a plot, representing a community level analysis, and (2) standardized by the total number of flowering stems in a plot (per-stem analysis). These analyses were conducted both at the whole-community level, and on a subset of the data that included only the six focal species. Unless otherwise noted, all analyses were conducted using the Proc Glimmix procedure (an extension of Proc Mixed; SAS Institute 2003). A generalized, linear, mixed model (GLMM) was used to determine the effect of treatment on the total number of visits per plot. In the whole-community model, treatment served as a fixed effect and block as a random effect. Total visit number was log transformed prior to analysis to normalize the data, and a Gaussian error distribution was modeled in Proc Glimmix. A second GLMM was conducted on only the data from the six focal species, including species and treatment as fixed effects and block as a random effect. Because multiple measures were taken within each plot (visits to each species), we also included plot, nested within the block × treatment interaction, as a second random effect to avoid pseudoreplication. Species-specific responses to benomyl application would be indicated by a significant species × treatment interaction. Due to the large number of zeros in the focal-species data set, we used a Poisson error distribution.

Both of the analyses of floral visits were also conducted on a per stem basis by standardizing the total number of visits that occurred in a plot by the total number of flowering stems that were counted within the plot. In both the whole-community and focal-species per stem analyses, the log(number of visits per flowering stem +1) served as the response variable, with a Gaussian error distribution modeled in Proc Glimmix. In the community model, treatment served as a fixed effect and block as a random effect. In the focal-species model, treatment and species served as fixed effects and block and plot, nested in the block × treatment interaction, served as random effects.

The effects of benomyl application on floral display, AMF colonization (required a pseudo-binomial distribution for a reasonable model fit), and flowering-stem counts were determined for each of the six focal species using a series of GLMMs. We used the average value of each response variable for a given plot, and thus the statistical model included block as a random effect and benomyl application as a fixed effect. Except for AMF colonization for which we again used Proc Glimmix, these analyses were conducted using the Mixed Model function in SPSS (SPSS 2004), which assumes a Gaussian error distribution.

Floral visitor community composition.—To test whether the composition of the floral-visitor community

TABLE 2. Groupings of floral visitors used for analysis.

Group	Size (cm)
Bumble bees	>2
Mid-sized bees (include species of *Andrena*, *Halictus*, *Lasioglossum, Osmia*, and *Megachile*)	1–2
Small bees (primarily *Dialictus* spp., but also *Duforea*, *Halictus*, *Heriades*, *Hylaeus*, and *Sphecodes*)	0.5
Hoverflies and beeflies	0.5–1
Other Diptera	variable
Other: wasps and Lepidoptera	variable

Notes: These are based largely on size, though they fall out partially along the lines of bee families. Flies are grouped into the Syrphidae plus beeflies, which are flower specialists but only occasionally land on flowers, and other flies (i.e., muscids) that are opportunistic floral visitors and normally land. Bee genera are based on identifications made from insects caught via netting or pan-trapping, as genera cannot be reliably distinguished on the wing.

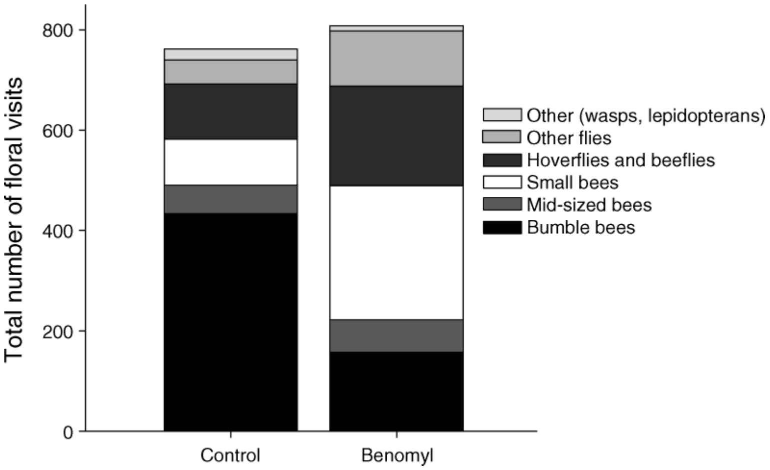

FIG. 1. Visiting-insect community composition at the study site in a native rough fescue grassland in Kinsella, Alberta, Canada. Total number of floral visits by each of the major groups of visiting insects, as a function of benomyl treatment. We used benomyl, a common fungicide, to suppress AMF (arbuscular mycorrhizal fungi), measuring floral-visitor responses after three years of fungal suppression. Post hoc tests indicated significant effects of benomyl application on the abundance of all groups of floral visitors (at $P < 0.008$) except medium-bodied visitors ($P = 0.334$) and other visitors ($P = 0.130$).

differed among benomyl treatments we conducted an additional GLMM. In this analysis, the total number of floral visitors recorded in a plot (regardless of the plant species they were seen visiting), served as the Poisson-distributed response variable (using Proc Glimmix). Block again served as a random effect, with treatment and floral-visitor identity serving as two fixed effects. As noted above, insect morphospecies were grouped for analysis based on body size and coarse taxonomic affiliation (Table 2).

RESULTS

AMF (arbuscular-mycorrhizal fungi) root colonization varied among focal species (mean % (SE) colonization by arbuscules, vesicles, or AMF hyphae: *Campanula*, 4.8% ± 2.3%; *Cerastium*, 5.6% ± 1.5%; *Achillea*, 13.2% ± 2.7%; *Aster*, 15.4% ± 3.2%; *Erigeron*, 16.8% ± 2.9%; *Solidago*, 31.3% ± 2.8% [means ± SE]). Benomyl application reduced AMF colonization rates ~33% ($F_{1,125} = 7.27$, $P = 0.008$), and this was consistent among species (species × treatment interaction: $F_{5,135} = 0.65$, $P = 0.660$). There was no shift in non-AMF fungal colonization as a function of benomyl application (control, 67.3% ± 2.2%; benomyl, 69.9% ± 2.0%; $F_{1,135} = 2.22$, $P = 0.139$). The vast majority of these non-AMF fungi were hyphae external to the root surface.

Plant and insect community-level effects

Benomyl application caused a shift in the relative frequencies of the body-size-based floral-visitor groups (significant treatment × floral visitor group interaction; Appendix B; Fig. 1). In general, benomyl application resulted in a reduction in visits by large-bodied bumble bees and an increase in visits by small-bodied bees and flies. At the whole-community level, AMF suppression did not impact the total number of visits to all flowering stems within a plot ($F_{1,19} = 0.1.43$, $P = 0.246$, Fig. 2A), but did result in a 67% reduction in visits per flowering stem ($F_{1,19} = 15.50$, $P = 0.0009$, Fig. 2C)

Focal-plant effects

Visitation rates to the six focal species responded to benomyl application in a species-specific manner (Appendix C), regardless of whether data were analyzed as total visits or on a per stem basis. A posteriori least-squares means contrasts indicated that there were significant treatment effects on total visits for *Aster, Cerastium,* and *Solidago* (Fig. 2A). In contrast, benomyl application had a significant effect on per stem visitation rates for *Aster* (Fig. 2C). It is important to note that these species are not random subsets of the larger community, but instead were chosen based upon their abundance (present in at least five plots of each treatment).

Benomyl application only influenced floral morphology for one species (Table 3), and the effects observed were counter to expectation. When mycorrhizae were suppressed, flower size was larger and there were more flowers per stem for *Cerastium*. No other effects of benomyl application on floral traits were significant for any other species (Table 3). Mycorrhizal suppression had species-specific effects on flowering-stem production (Fig. 2B; species × treatment interaction: $F_{5,140.9} = 3.715$, $P = 0.003$), increasing flowering-stem numbers per plot for *Achillea* from 7.41 ± 1.38 stems to 20.70 ± 5.99 stems, *Campanula* from 8.31 ± 3.26 stems to 18.38 ± 5.20 stems, and *Cerastium* from 72.13 ± 20.59 stems to 394.58 ± 54.24 stems, (means ± SE) while having no significant effect on flowering-stem numbers for *Aster,*

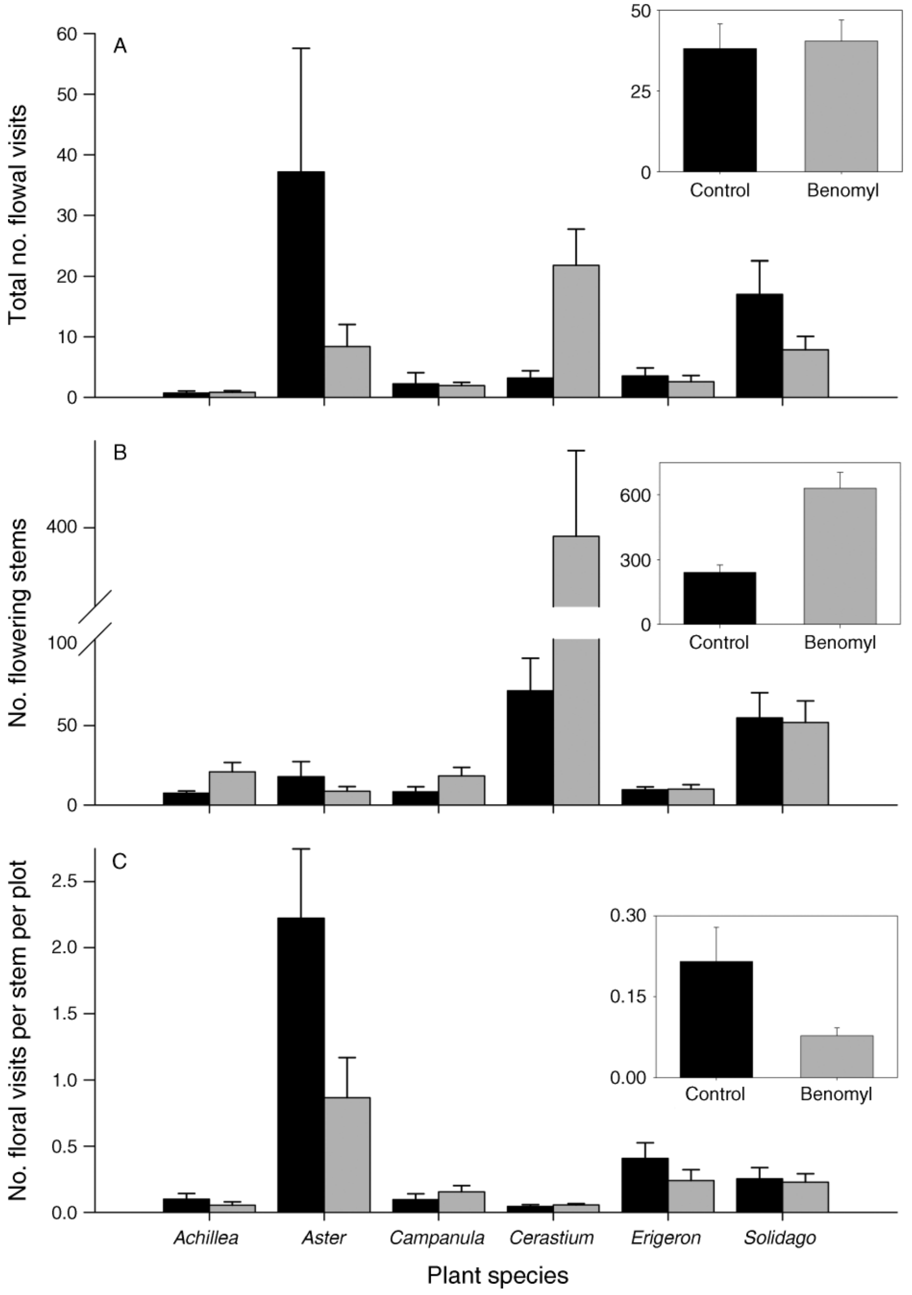

FIG. 2. Total number of insect floral visits, numbers of flowering stems, and insect visits per stem, for control plots and benomyl-treated plots. Data are means + SE. In all panels, black corresponds to controls, and grey to benomyl application. (A) The total number of floral visits per plot observed for each of the six focal plant species that were visited by insects in at least five plots of each treatment. Inset: the mean number of visits observed for the entire flowering community in plots of each treatment. (B) Flowering stem production per plot for each of the six focal species. Inset: the mean number of flowering stems in plots of each treatment. (C) The average number of visits per stem for each of the six focal plant species. Inset: the mean visits per stem observed for the entire flowering community, by treatment.

Erigeron, or *Solidago* (Table 2, Fig. 2B). Interestingly, the three species whose flowering increased with benomyl application were also the three species with the lowest amount of AMF infection in the control plots, suggesting that indirect interactions, rather than the direct impact of benomyl on AMF, were important. Mycorrhizal suppression did not affect flowering-plant species richness ($F_{1,19} = 2.879$, $P = 0.106$), but did reduce flowering plant evenness by 50% ($F_{1,19} = 16.736$, $P = 0.001$). This effect was largely driven by the large increase in the number of *Cerastium* flowering stems with benomyl application.

TABLE 3. Impact of benomyl application on the floral morphology and flowering stem production of six focal species.

	Flower or inflorescence size†			No. flowers or inflorescences per stem‡			Total no. flowering stems		
Species	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Achillea millefolium	0.473	1, 14.97	0.502	0.089	1, 8.34	0.773	4.950	1, 17.04	**0.040**
Aster laevis	0.193	1, 4.94	0.679	0.640	1, 18	0.434	0.194	1, 5.78	0.675
Campanula rotundifolia	0.724	1, 17	0.407	0.047	1, 17	0.831	21.915	1, 11.42	**0.001**
Cerastium arvense	7.149	1, 17.94	**0.016**	6.599	1, 19.22	**0.019**	46.711	1, 9.92	**<0.001**
Erigeron philadelphicus	0.669	1, 9.34	0.434	0.008	1, 25	0.932	0.001	1, 27	0.994
Solidago missouriensis	0.642	1, 28	0.430	§	§	§	0.079	1, 12.06	0.784

Note: Significant results at an alpha level of 0.05 are shown in boldface.

† Size refers to the width of the largest flower or inflorescence on a stem for *Aster*, *Campanula*, *Cerastium*, and *Erigeron*; the surface area calculated for an ellipse for *Achillea*; and the outer surface area calculated as a cylinder for *Solidago*.

‡ Analyses of flower/inflorescence size and number were done on per-plot means.

§ Analysis of the number of flowers or inflorescences per stem was not performed for *Solidago*, as there were never multiple infloresences per flowering stem for this species in these plots.

DISCUSSION

Indirect effects among species and ecological processes are of great importance in communities, but poorly studied in comparison to our understanding of direct effects (Lortie et al. 2004). Here we show that disrupting belowground interactions between plants and mycorrhizae has important consequences aboveground, changing both the relative composition of the visiting-insect community and the number of floral visits received. These changes do not appear to be caused by changes in floral morphology of the most common species (Table 3).

There are two main paths through which we might expect disruption of mycorrhizae to affect interactions between plants and floral visitors. Direct effects may occur if arbuscular-mycorrhizal fungi (AMF) disruption reduces plant attractiveness because the benefit of photosynthate or water delivery is removed, such as when potted plants lacking AMF produced fewer or smaller flowers or less nectar than plants with AMF (Gange and Smith 2005, Wolfe et al. 2005). This could, in turn, lead floral visitors to select against plants with suppressed AMF because of their reduced attractiveness. A second path is indirect; disruption of AMF could change competitive interactions among members of the plant community if they differ in their reliance on AMF or the benefits they gain from the mutualism (van der Heijden et al. 1998, Hartnett and Wilson 1999, 2002, Smith et al. 1999). Changes in the network of competitive interactions could lead to changes in the display of relatively non-mycorrhizal plants, size of individual plants, and/or their relative apparency to floral visitors, leading to a spatial change in visitation that is related to the cascading effects in the plant community rather than direct changes on plant traits. It is these latter, indirect effects that we believe were principally responsible for the results found here, though future experiments are needed to identify the specific mechanisms involved. We acknowledge that unintended consequences of benomyl application, such as those described in *Methods: Mycorrhizal suppression*, above, could also have contributed to our results. Differentiating among all possible soil influences (mycorrhizae, bacterial groups, other fungal groups, nematodes, other soil biota, chemical changes, and so forth) is simply not possible within the constraints of an experiment conducted in a natural community.

Our most striking result was a shift in the relative frequency of visits by different floral- visitor groups. We observed a dramatic reduction in visits by bumble bees, and an increase in visits by small bees and flies, with AMF suppression (Fig. 1). These shifts may be a consequence of the increase in flower size, flower numbers, and the number of flowering stems of *Cerastium* (Table 3) with benomyl application. Note that this could be considered an indirect effect, as *Cerastium* is relatively non-mycorrhizal, and the direction of change (larger flowers when AMF are suppressed) is opposite of that predicted by pot studies. *Cerastium* was highly attractive to smaller insects (100% of the 51 total visits to *Cerastium* in control plots and 99.2% of the 415 visits in benomyl plots were by sweat bees and flies). However, this does not explain the decrease in total bumble bee visits with mycorrhizal suppression. *Aster* and *Solidago* were consistently visited by bumble bees (80.2% and 50.2% of flowering stems visited by bumble bees were to these species in control and benomyl plots, respectively), and the total number of visits by bumble bees to these species was reduced by >2.5 times with mycorrhizal suppression (from 478 to 187 visits in control and benomyl plots, respectively). Neither plant species exhibited a shift in the number of flowering stems or floral morphology among treatments (Table 3), but both *Solidago* and *Aster* showed a dramatic drop in visits on a per plot basis, as well as on a per stem basis for *Aster* (Fig. 2). Clearly the reduction in bumble bee visits with mycorrhizal suppression was mediated through shifts external to the individual plant. We suggest that the dramatic increase in *Cerastium* flowering-stem production with benomyl application may have altered the visual or chemical cues normally used by bumble bees to locate their preferred species (*Aster* and *Solidago*). That is, the floral-visitor community responded to the change in the relative composition of the flowering-plant community that was altered through the suppression of AMF.

Competitive effects on pollination due to shifts in the co-flowering plant community have been documented in other studies focussed on specific plant species. Bumble bees visited *Collinsia parviflora* only in a plant community lacking *Plectritis congesta* (Elle and Carney 2003). Sweat bees visited native plants less often, and less pollen was deposited, when the alien plant *Euphorbia esula* invaded plant communities (Larson et al. 2006). Pollen deposition by hummingbirds on *Ipomopsis aggregata* decreased when *Castilleja linariaefolia* was present in experimental plots (Caruso 1999). These results highlight the importance of considering the larger community in studies of plant–pollinator interactions, but our results further suggest that shifts may occur in the community of floral visitors due to other mechanisms than interplant competition for floral visitors. Our results are more akin to disruption of the mutualism, where the abundance of an unattractive plant species (*Cerastium*) changes the spatial patterns of insect foraging.

Our finding that flowering-stem number and floral display increased with AMF suppression (for some species) is contrary to expectations that mycorrhizal suppression should have negative consequences for plants, as shown by previous pot studies (Gange and Smith 2005, Wolfe et al. 2005). For two of our focal species, *Achillea* and *Campanula*, increases in stem number did not translate into a change in visitation, possibly because the number of visits observed to these species was relatively low (29 visits to *Achillea* and 56 to *Campanula* out of 1570 total visits observed); in contrast, the change in flowering-stem number for *Cerastium* had a major impact on visitation rates. It is important to note that even in the control plots these three species have the lowest rates of infection among all six focal species, and infection rates for *Campanula* and *Cerastium* were <5%. Although it is difficult to relate infection rates directly to physiological importance, these findings do suggest the main effect of mycorrhizal suppression was not due to changes in infection rates within the plants, but instead due to changes in competitive interactions with neighboring plants. In this system competition for soil resources is intense (Cahill 2003), and mycorrhizal grasses represent the majority of the potential resource competitors for our focal species. If mycorrhizal suppression reduced foraging efficiency or competitive ability of grasses, one would expect to see competitive release for entomophilous species similar to what we have shown here for flowering-stem production. Unfortunately, measures of competitive interactions were not a part of this study, and thus the information needed to test this hypothesis is not available.

The decline in the number of visits per stem with AMF suppression may have followed directly from the increase in total stem number. Visits per stem are generally considered an indication of the potential fitness benefits of the mutualism (Vazquez et al. 2005), as increased insect visitation is associated with increased pollen deposition, increased seed production, and improved opportunities for mate choice (Knight et al. 2005*b*). However, when plants are clonal, as our focal species are (Table 1), genetic individuals consist of multiple flowering stems within a plot. In such a case, total visits to a species in a plot may better reflect the fitness potential of genetic individuals, and the potential for a species-specific impact of mycorrhizal suppression on population dynamics and future plant community composition. At the plot level, we saw no change in total visits per plot with AMF suppression, but visits to particular plant species changed in a species-specific manner (Fig. 2). These species-level changes are likely also the best indicator of potential community-level impacts of AMF mediated through a shift in plant–pollinator interactions. Species-specific changes in visitation rates have the potential to alter seed production and population dynamics, which itself could alter future plant community composition (Ashman et al. 2004, Knight et al. 2005*b*). To fully explore this question, we would need to investigate the effectiveness of different floral visitors as pollinators, and determine whether recruitment into the plant community is seed limited.

We observed no change in flowering-plant species richness with mycorrhizal suppression, but did observe a change in evenness that affected visitation in a species-specific manner. The importance of plant species richness as a predictor of bee species richness or abundance has been shown in several studies (Potts et al. 2003, Hegland and Boeke 2006, Vulliamy et al. 2006), but we are unaware of any study demonstrating the importance of evenness of the flowering-plant community for variation in insect-community visitation patterns. Visual inspection of the data suggests that the evenness of number of flowering stems of other species is positively related to the total number of visits received by a particular species. We suggest more research into whether AMF influence visitation rates primarily through a shift in floral evenness is warranted.

One of the realities of ecological research, particularly at the community level, is that experimental designs necessary to capture the contingent nature of species interactions are often overwhelmingly complex (Lawton 1999). As a result, there has been a tendency to focus experiments on single "interactions" such as mycorrhizal associations, plant–pollinator interactions, or plant–herbivore interactions. Our work can be added to a growing body of literature that demonstrates that ecological interactions are not independent (Haag et al. 2004, Lortie et al. 2004, Knight et al. 2005*a*, Vulliamy et al. 2006). The 67% reduction in per stem visitation rates that we found here is of such a magnitude as to suggest that by simplifying experiments to focus on single interactions, we unfortunately fail to include what are potentially very important factors, and thus end up with a biased and incomplete understanding of ecological processes. Specifically, we show that two community subsets generally assumed to be independent of one another, AMF and floral visitors, are not. The interaction between these factors can alter species composition (of floral visitors), with the potential to affect the delivery of pollination services. The ecological and evolutionary consequences of these altered services remain to be discovered, but such a drastic decline in visitation rates could lead to pollen limitation with implications for the stability of populations (Knight et al. 2005*b*). We suggest that ecologists need to pay more attention to the downstream impacts of treatments, such as interactions at multiple trophic levels. In addition, we support the growing diversity of community interactions being considered by pollination biologists in their attempt to refine models of plant reproduction.

Acknowledgments

J. F. Cahill and E. Elle contributed equally to this work. We thank members of the Cahill lab, the Elle lab, B. Irwin, J. Biernaskie, and B. Casper for comments on drafts of the manuscript. Initial ideas were developed during the multitrophic brainstorming session organized by A. Agrawal and J. Thaler. S. Good-Avila kindly provided compatibility information for Asteraceae. R. Currah assisted with identification of fungal structures. Financial support was provided by NSERC discovery grants awarded to J. F. Cahill and E. Elle, an NSERC undergraduate award to G. R. Smith, and an ACA Biodiversity grant awarded to J. F. Cahill and B. H. Shore.

Literature Cited

Agrawal, A. A. 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294:321–326.

Allen, M. F., H. J. B. Birks, and J. A. Weins. 1991. The ecology of mycorrhizae. Cambridge University Press, Cambridge, UK.

Ashman, T. L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. Ecology 85:2408–2421.

Bezemer, T. M., G. B. De Deyn, T. M. Bossinga, N. M. van Dam, J. A. Harvey, and W. H. Van der Putten. 2005. Soil community composition drives aboveground plant–herbivore–parasitoid interactions. Ecology Letters 8:652–661.

Bezemer, T. M., J. A. Harvey, G. A. Kowalchuk, H. Korpershoek, and W. H. Van der Putten. 2006. Interplay between *Senecio jacobaea* and plant, soil, and aboveground insect community composition. Ecology 87:2002–2013.

Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemuller, M. Edwards, T. Peeters, A. P. Schaffers, S. G. Potts, R. Kleukers, C. D. Thomas, J. Settele, and W. E. Kunin. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and The Netherlands. Science 313:351–354.

Cahill, J. F., Jr. 1999 Fertilization effects on interactions between above- and belowground competition in an old field. Ecology 80:466–480.

Cahill, J. F., Jr. 2003 Lack of relationship between belowground competition and allocation to roots in 10 grassland species. Journal of Ecology 91:532–540.

Callaway, R. M., B. E. Mahall, C. Wicks, J. Pankey, and C. Zabinski. 2003. Soil fungi and the effects of an invasive forb on grasses: neighbor identity matters. Ecology 84:129–135.

Caruso, C. M. 1999. Pollination of *Ipomopsis aggregata* (Polemoniaceae): effects of intra- vs. interspecific competition. American Journal of Botany 86:663–668.

Elle, E., and R. Carney. 2003. Reproductive assurance varies with flower size in *Collinsia parviflora* (Scrophulariaceae). American Journal of Botany 90:888–896.

Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. Annual Review of Ecology, Evolution, and Systematics 35:375–403.

Fitter, A. H., and R. Nichols. 1988. The use of benomyl to control infection by vesicular arbuscular mycorrhizal fungi. New Phytologist 110:201–206.

Gange, A. C., and A. K. Smith. 2005. Arbuscular mycorrhizal fungi influence visitation rates of pollinating insects. Ecological Entomology 30:600–606.

Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. Journal of Ecology 94:295–304.

Giovannetti, M., and B. Mosse. 1980. Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84:489–500.

Haag, J. J., M. D. Coupe, and J. F. Cahill, Jr. 2004. Antagonistic interactions between competition and insect herbivory on plant growth. Journal of Ecology 92:156–167.

Hartnett, D. C., and G. W. T. Wilson. 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. Ecology 80:1187–1195.

Hartnett, D. C., and G. W. T. Wilson. 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. Plant and Soil 244:319–331.

Hegland, S. J., and L. Boeke. 2006. Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. Ecological Entomology 31:532–538.

Hodge, A. 2003. Plant nitrogen capture from organic matter as affected by spatial dispersion, interspecific competition and mycorrhizal colonization. New Phytologist 157:303–314.

Johnson, S. D., C. I. Peter, L. A. Nilsson, and J. Agren. 2003. Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. Ecology 84:2919–2927.

Klironomos, J. N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301.

Knight, T. M., M. W. McCoy, J. M. Chase, K. A. McCoy, and R. D. Holt. 2005*a*. Trophic cascades across ecosystems. Nature 437:880–883.

Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T. L. Ashman. 2005*b*. Pollen limitation of plant reproduction: pattern and process. Annual Review of Ecology and Systematics 36:467–497.

Kremen, C., N. M. Williams, and R. W. Thorp. 2002. Crop pollination from native bees at risk from agricultural intensification. Proceedings of the National Academy of Sciences (USA) 99:16812–16816.

Lamb, E. G., B. H. Shore, and J. F. Cahill, Jr. 2007. Water and nitrogen addition differentially impact plant competition in a native rough fescue grassland. Plant Ecology 192:21–33.

Larson, D. L., R. A. Royer, and M. R. Royer. 2006. Insect visitation and pollen deposition in an invaded prairie plant community. Biological Conservation 130:148–159.

Lawton, J. H. 1999. Are there general laws in ecology? Oikos 84:177–192.

Lortie, C. J., R. W. Brooker, P. Choler, Z. Kikvidze, R. Michalet, F. I. Pugnaire, and R. M. Callaway. 2004. Rethinking plant community theory. Oikos 10:433–438.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115:495–501.

Moeller, D. A. 2004. Facilitative interactions among plants via shared pollinators. Ecology 85:3289–3301.

Newsham, K. K., A. H. Fitter, and A. R. Watkinson. 1994. Root pathogenic and arbuscular mycorrhizal fungi determine fecundity of asymptomatic plants in the field. Journal of Ecology 82:805–814.

Newsham, K. K., A. R. Watkinson, H. M. West, and A. H. Fitter. 1995. Symbiotic fungi determine plant community structure: changes in a lichen community induced by fungicide application. Functional Ecology 9:442–447.

O'Connor, P. J., S. E. Smith, and E. A. Smith. 2002. Arbuscular mycorrhizas influence plant diversity and community structure in a semiarid herbland. New Phytologist 154:209–218.

Palmer, T. M., M. L. Stanton, and T. P. Young. 2003. Competition and coexistence: exploring the mechanisms that restrict and maintain diversity within mutualist guilds. American Naturalist 162:S63–S79.

Potts, S. G., B. Vulliamy, A. Dafni, G. Ne'eman, and P. Willmer. 2003. Linking bees and flowers: How do floral communities structure pollinator communities? Ecology 84: 2628–2642.

Sahli, H. F., and J. K. Donner. 2006. Characterizing ecological generalization in plant-pollination systems. Oecologia 148: 365–372.

SAS Institute. 2003. SAS system for Windows. Version 9.1. SAS Institute, Cary, North Carolina, USA.

Schmidt, I. K., L. Ruess, E. Baath, A. Michelsen, F. Ekelund, and S. Jonasson. 2000. Long-term manipulation of the microbes and microfauna of two subarctic heaths by addition of fungicide, bactericide, carbon and fertilizer. Soil Biology and Biochemistry 32:707–720.

Smith, M. D., D. C. Hartnett, and C. W. Rice. 2000. Effects of long-term fungicide applications on microbial properties in tallgrass prairie soil. Soil Biology and Biochemistry 32:935–946.

Smith, M. D., D. C. Hartnett, and G. W. T. Wilson. 1999. Interacting influence of mycorrhizal symbiosis and competition on plant diversity in tallgrass prairie. Oecologia 121:574–582.

SPSS. 2004. SPSS for Windows. Version 13.0. SPSS, Chicago, Illinois, USA

Strauss, S. Y., J. K. Conner, and S. L. Rush. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. American Naturalist 147:1098–1107.

Strauss, S. Y., and R. E. Irwin. 2004. Ecological and evolutionary consequences of multispecies plant–animal interactions. Annual Review of Ecology, Evolution and Systematics 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., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72.

Vazquez, D. P., W. F. Morris, and P. Jordano. 2005. Interaction frequency as a surrogate for the total effect of animal mutualists on plants. Ecology Letters 8:1088–1094.

Vulliamy, A., S. G. Potts, and P. G. Willmer. 2006. The effects of cattle grazing on plant-pollinator communities in a fragmented Mediterranean landscape. Oikos 114:529–543.

Wardle, D. A. 2002. Communities and ecosystems: linking the aboveground and belowground components. Princeton University Press, Princeton, New Jersey, USA.

Wolfe, B. E., B. C. Husband, and J. N. Klironomos. 2005. Effects of a belowground mutualism on an aboveground mutualism. Ecology Letters 8:218–223.

APPENDIX A

Images of the field site and study plots in Kinsella, Alberta, Canada (*Ecological Archives* E089-107-A1).

APPENDIX B

GLMM results from analyses of total floral visits as a function of treatment and floral visitor groupings (*Ecological Archives* E089-107-A2).

APPENDIX C

GLMM results from analyses of floral visits to the six focal species (*Ecological Archives* E089-107-A3).