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PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Belowground fungal associations and water interact to influence the compensatory response of *Ipomopsis aggregata*

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Abstract Although the concept that some plants benefit from being eaten is counterintuitive, there is now considerable evidence demonstrating enhanced fitness following herbivory. It has been assumed that plants growing in high resource conditions are the ones best able to compensate for herbivory. However, just the opposite has been found for dicotyledonous plants exhibiting patterns of overcompensation, with most occurring in resource-poor conditions. Long-term studies of the monocarpic biennial, scarlet gilia, *Ipomopsis aggregata* growing in resource-poor conditions have shown that ungulate herbivory by mule deer and elk can result in a threefold increase in plant fitness over uneaten controls. These observations led us to hypothesize that fungal associations would facilitate the compensatory response most commonly observed in this Arizona population of scarlet gilia; perhaps mutualistic associations with fungi, such as arbuscular mycorrhizal fungi, would explain the phenomenon of overcompensation altogether. Fungal removal experiments, using Captan®, a commercially available fungicide, showed that a reduction in fungal abundance altered the compensatory response following ungulate herbivory, particularly in years in which water was limited, increasing fitness compensation from equal compensation to overcompensation. A multifactorial experiment revealed that the interactive effects of water and fungicide maximized fruit production following herbivory. Our results are counter to the "modification of tolerance hypothesis" in which plants associating with mycorrhizal fungi will have higher tolerance to herbivory. It is likely that arbuscular mycorrhizal fungi and dark septate endophytes compete with plants for photosynthates following herbivory, thereby limiting the magnitude of compensation. Thus, fungi appear to be parasitic on scarlet gilia following ungulate herbivory.

Keywords Overcompensation · Ungulate herbivory · Fungi · Scarlet gilia

Introduction

Ecologists are gaining an increasing appreciation of how multispecies interactions, both above- and belowground, can alter the ecological and evolutionary outcomes of interactions in ways that differ from the outcomes of pairwise interactions alone (Garrido et al. 2010). Soil biota contributing to these interactions include arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE), both of which colonize the roots of a great diversity of plant species in associations that can be mutually beneficial to both plant and fungus (Jumpponen 2001; Smith and Read 2008; Newsham 2011). Association with arbuscular mycorrhizal fungi, for example, can increase plant access to scarce or immobile soil minerals, facilitating the uptake of up to 70 % of the phosphorus (P) and 30 % of the nitrogen (N) required by the plant, and provide other services, such as improved water relations; in turn, mycorrhizal fungi receive

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4–20 % of a plants' photosynthate production (Jakobsen et al. 2002; Smith and Read 2008). Similarly, a meta-analysis showed that inoculation of DSE can increase shoot N and P content, as well as root, shoot, and total biomass (Newsham 2011).

These plant–fungal interactions, however, are not always mutualistic and have been shown to span a continuum from mutualistic to parasitic (Johnson et al. 1997; Jumpponen 2001; Bennett et al. 2006). The outcome of these interactions may be contingent upon the particular species of fungi associating with the plant, the environmental conditions under which the interactions occur, and/or varying combinations of plant and fungal genotypes (Johnson et al. 1997). In addition, the benefit of a plant associating with a fungal symbiont depends not only on the presence of a particular fungal species, but also on its colonization density (Gange and Ayres 1999; Garrido et al. 2010).

An association with mycorrhizal and dark septate fungi may also change the outcome of biotic interactions between plants and their parasites, pathogens, and herbivores (Johnson et al. 1997; Borowicz 2001; Gange and Brown 2002; Gehring and Whitham 2002; Bennett et al. 2006). For example, mycorrhizal fungi have been shown to interfere with plant pathogen attack (reviewed in Borowicz 2001). Gange and West (1994) showed that fungal colonization reduced herbivore damage to *Plantago lanceolata* caused by a leaf-chewing lepidopteran, *Arctia caja*. Gehring et al. (1997) found a negative effect of scale insect herbivory on ectomycorrhizal colonization, and DSE have been noted to protect hosts from pathogens or herbivores through the production of inhibitory metabolites (Mandyam and Jumpponen 2005). These seemingly contrasting results suggest that mycorrhizal fungi may compete directly, or indirectly, with herbivores for limiting photosynthates (Borowicz 1997). Clearly the outcomes of such interactions are complex; if there are general trends in the effects of fungi on plant–herbivore interactions it may not be apparent until more fungus–plant–herbivore systems are examined over a wide range of conditions (Borowicz 2001).

Over the past three decades we have been studying fitness compensation by the scarlet gilia (*Ipomopsis aggregata*; Family Polemoniaceae) following ungulate herbivory by mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus*). Scarlet gilia is a monocarpic biennial/perennial herb growing in western montane regions of the USA (USDA PLANTS Database; http://plants.usda.gov/java/). Post-germination, a leafy rosette forms, which overwinters; in the following spring the majority of rosettes bolt, each forming a single paniculate-racemose inflorescence that flowers from early–mid July through to late September (Paige and Whitham 1985). During the period of stem elongation, mule deer and elk browse, on average, 80 % of scarlet gilia plants (Paige 1992a).

Although scarlet gilia are known to interact with a diverse suite of insect herbivores, including lepidopteran fruit predators [the owlet moth *Heliothis phloxiphaga* (Family Noctuidae) and the tortrix/leafroller moths *Olethreutes* sp. (Family Tortricidae)], a specialist seed fly [*Hylemya* sp. (Family Anthomyiidae)], a stem-boring moth [*Sparganothis belfrageana* (Family Noctuidae)], an unidentified green aphid, and an unidentified dipteran root borer (Juenger and Bergelson 1998; Anderson and Paige 2003), the high-elevation populations that we have been studying in Arizona have been relatively depauperate of insect herbivores. Only green aphids and a lepidopteran fruit predator, *Heliothis phloxiphaga*, have been observed, and at exceptionally low levels ($\ll$1 % of plants). Even in a year when *Heliothis* was abundant, there was no significant effect on plant fitness (see Anderson and Paige 2003). Thus, our studies have focused primarily on the effects of ungulate herbivory on scarlet gilia.

In earlier studies we have shown that when ungulate herbivores remove $\geq$95 % of the aboveground biomass of the monocarpic biennial scarlet gilia, the product of lifetime seed production, seed germination, and seedling survival was on average threefold higher than that of the uneaten controls (plant overcompensation; i.e., an increase in plant fitness; Paige 1992a, b; 1994; 1999; Paige and Whitham 1987; Anderson and Paige 2003). Although the notion that some plants benefit from being eaten is counterintuitive, there is now considerable evidence demonstrating enhanced fitness following herbivory (e.g., see Maschinski and Whitham 1989; Alward and Joern 1993; Lowenberg 1994; Nilsson et al. 1996; Lennartsson et al. 1997; Mauricio et al. 1997; Juenger et al. 2000; Weinig et al. 2003; Rautio et al. 2005; Scholes and Paige 2011, among others).

There are at least three mechanisms (not necessarily independent) that may lead to overcompensation, including (1) increased net photosynthetic rates following damage; (2) increased branching following the release of apical dominance; (3) pre-existing high levels of carbon stored in roots for allocation to aboveground growth and reproduction following damage (Strauss and Agrawal 1999). Two of these mechanisms appear to be involved in the compensatory response of scarlet gilia, namely, increased photosynthetic rates and increased branching following the removal of apical dominance (Paige and Whitham 1987; KN Paige, unpublished data). The third mechanism does not appear to be important in this system given that both above- and belowground biomass increases following the removal of apical dominance.

These energy consumptive processes would predict that overcompensation by plants requires a greater demand for nutrients and water following herbivory and has led to the assumption that plants growing in high-resource conditions are the ones best able to compensate for herbivory

(Bryant et al. 1983; Coley et al. 1985; Maschinski and Whitham 1989). However, just the opposite has been found for dicotyledonous plants exhibiting patterns of overcompensation, with most occurring in resource-poor conditions (Hawkes and Sullivan 2001; Wise and Abrahamson 2007). These studies, however, have ignored the potential effects of belowground interactions with other organisms, such as AMF, and other fungal associates, such as DSF, which could provide the necessary nutrients needed for a plant to overcompensate. This would be consistent with Bennett et al.'s (2006) hypothesis (referred to as the "modification of tolerance hypothesis") suggesting that plants associating with mycorrhizal fungi will have higher tolerance (or compensatory capability) following herbivory.

In the study reported here, we were interested in how fungal associations affect the compensatory response of scarlet gilia, *Ipomopsis aggregata*, following ungulate herbivory. To date, no one has studied the effects of fungal species on plants showing patterns of overcompensation. We used a fungicide treatment to significantly reduce fungal abundance and assess their effects on plant fitness following natural patterns of ungulate herbivory. In addition, given that mycorrhizal fungi in particular can improve water relations, we were also interested in the interactive role between annual variation in precipitation, fungi, ungulate herbivory, and plant compensation. The fungal community in scarlet gilia roots at this site consist of ascomycetous dark septate root endophytes (DSE) characterized by dark septate hyphae and sclerotia and arbuscular mycorrhizal fungi (AMF) of the Phylum Glomeromycota characterized by arbuscules (finely-branched tree-like structures) and coenocytic hyphae (lacking cross-walls). In addition, roots were colonized by a small group of non-AMF/DSE fungi, including pathogenic fungi identified via oospores (Allsup and Paige, this study). Pathogenic fungi produce enzymes that kill host tissue, which could inhibit plant growth or regrowth following aboveground herbivory (Latijnhouwers et al. 2003). However, as noted above, mycorrhizal fungi can provide protection from infection by pathogens. The outcome of these interactions are dependent upon the identities of the plant or fungal species involved (Borowicz 1997; Sikes et al. 2009).

Specifically, we addressed the following issues: (1) What is the effect of the fungal community on the compensatory outcome of scarlet gilia, following ungulate herbivory? Do they facilitate, inhibit, or have no effect on compensation; i.e., are they mutualistic, parasitic, or of no consequence and (2) Is the interaction between ungulate herbivory, mycorrhizal fungi, and plant compensation altered under varying climatic conditions (i.e., variation in precipitation)?

Methods and materials

Field site

Our studies on the interactive effects of scarlet gilia, ungulate herbivory, and fungi were conducted within White Horse Meadow in the San Francisco Peaks (approx. 2500 m a.s.l.) in Coconino County, Arizona, USA. This population comprises >30,000 flowering individuals of scarlet gilia located in a montane meadow surrounded by ponderosa pine and aspen. Arizona soils are primarily composed of sand and silt with small amounts of clay (USDA Soil Series Descriptions; https://soilseries.sc.egov.usda.gov/). Precipitation, including snowfall, has averaged 58.2 cm per year over a 50-year period [National Oceanic and Atmospheric Administration (NOAA) 2008]. In the 3-year span of this project, 2 of the 3 years (2007 and 2009) experienced lower than average precipitation during the growth of scarlet gilia [Electronic Supplementary Material (ESM) Fig. S1]. In 2007, the site experienced lower than average precipitation in late July and August during the flowering period of scarlet gilia, and in 2009 it received lower than average precipitation in May and June during the bolting stage of scarlet gilia [2007: 27.7 cm; 2009: 22.6 cm; both years experienced moderate drought conditions and were among the 5 driest years over a 59-year history (Hereford 2007)]. In 2008, the site approached average precipitation (53.3 cm). All precipitation data were collected each year from NOAA's National Weather Service station at Pulliam Airport (2129 m a.s.l.) in Flagstaff, Arizona. Over the 3 years of this study, 83–91 % of bolting scarlet gilia plants were browsed (CM Allsup, unpublished data).

Experimental design

To assess the interactive effects of belowground fungal associations and water availability on the compensatory capability of scarlet gilia, we designed and conducted a factorial experiment in each of the 3 years (2007–2009) following herbivory by mule deer and elk in late May. For this experiment, we randomly selected 68–160 bolting plants (depending on year) within a 100 × 50-m grid covering approximately two-thirds of the entire population. The grid was divided into ten columns spaced 10 m apart. Within each column, we randomly selected 6–16 plants (depending on year) spaced approximately 3 m apart; half were naturally browsed and half were unbrowsed. Plants were then assigned to one of four treatments: (1) unbrowsed controls, (2) naturally browsed controls, (3) unbrowsed fungicide-treated plants, and (4) naturally browsed fungicide-treated plants. All plants were caged with wedding veil-covered wire cages. Overall, sample sizes per treatment ranged from 46 to 62 (average of 57/treatment) in 2007, from 20 to 26 (average of 23/treatment) in 2008, and from 11 to 16 (average of 14/treatment) in 2009. The smaller sample sizes/treatment in 2008 and 2009 were due to the addition of a watering treatment (see below).

It is important to note that previous studies (Paige and Whitham 1987; Paige 1992a; 1994; 1999) had failed to show any significant differences in fitness between naturally browsed and experimentally clipped plants (whether chosen from the pool of uneaten individuals before or following natural herbivory). These results therefore argue that plant selectivity by herbivores has no effect on scarlet gilias' ability to compensate, or alternatively, that herbivores are not selective (Anderson and Paige 2003); both sides of the argument justify the use of naturally browsed plants in the experiments described herein.

Fungicide treatments were administered to the base of each plant throughout the growing season at 2-week intervals for a total of five treatments. Each treatment consisted of 0.5 g of the fungicide Captan® in 0.28 L of water (per manufacturer's recommendation). Captan® (CIS-*N*-trichloromethyl thio-4-cyclohexane-1,2-di-carboximide) acts to halt cellular respiration of fungal organisms and reduce fungal colonization in plant roots (Kough et al. 1987). We chose Captan® as an alternate fungicide over the commonly used fungicide benomyl because Dupont ceased production of benomyl in 2001 and it was no longer available during the study period. No fungicide eradicates mycorrhizae; they only decrease development for a short time after application. The duration of this effect depends on the length of time the chemical persists in the environment. For Captan®, persistence is on the order of 2 weeks (see, for example, Watson 1965); hence we repeated our treatment applications at 2-week intervals throughout the growing season. The remaining non-fungicidal controls received an equivalent amount of water (0.28 L/treatment × 5 treatments = 1.40 L) to mimic the liquid that treatment plants were given.

Following the moderate drought conditions observed in 2007, a water treatment was added to half (160) of all herbivory and fungicide combinations in both 2008 and 2009, given that the compensatory response is dependent upon water availability (Levine and Paige 2004). To assess the interactive effects of fungi and water on compensation by scarlet gilia, we applied a total of 1.0 L of water over a 10-week time span (200 mL at 2-week intervals for a total of 5 treatments) to mimic a 7.5-cm (1.0 L) increase in overall precipitation during the flowering period, totaling 60.8 cm of precipitation in 2008 (10.5 cm during the 2.5-month flowering period) and 30.1 cm in 2009 (9.9 cm during the 2.5-month flowering period).

We also assessed the effects of fungi on plant selection by ungulate herbivores. In 2008, we added fungicide to half ($N = 68$) of a sampling of rosettes and no fungicide to the other half ($N = 69$) during the course of the season, as in the experiments described above. Plants were allowed to overwinter and in the spring of 2009, during the period of stem elongation, we compared ungulate selection between these two treatments.

Fungicide efficacy

To test whether fungicide had a non-target impact on the microbial community that could alter soil nutrients, the non-mycorrhizal plant species Hoary Stock [*Matthiola incana* (Family Brassicaceae)] and Dianthus [*Dianthus chinensis* (Family Caryophyllaceae)] were grown in Arizona soils within our field site during the study period. Twenty plants of each species were treated with and without a fungicide (representative of the main experiment; see above). In general, plant species in the Brassicaceae and Caryophyllaceae families are considered to be non-host plants for AMF. However, there have been a few contradictory reports of AMF colonization in these two species (DeMars and Boerner 1995; Gaur and Adholeya 2005). Plants with and without a fungicide treatment were compared in terms of biomass at the end of the flowering season and assessed for fungal colonization.

Fungicide-treated and -untreated soils from the experimental plots of scarlet gilia plants were also compared to assess whether there were differences in soil nutrients. Soils were collected from plants following the fifth and final fungicide treatment. Ten randomly selected samples from each treatment were tested for pH, organic matter (OM), and available potassium (K), P, and N. All chemical analyses were conducted at the A & L Great Lakes Laboratory in Fort Wayne, Indiana (http://www.algreatlakes.com/).

Plant and fungal collections

In September of each year, whole plants from our multifactorial experiment were collected following senescence at the end of the growing season (approx. 4 months from bolting to senescence). Fine roots were washed free of soil and transported on ice for storage in a 15 °C cold room. The shoots and a subset of roots (see below) were air dried and assessed for above- and belowground biomass and number of fruits. Seed weight was estimated from a randomly drawn subset of ten seeds for each of ten plants collected from each of the treatments.

Roots of scarlet gilia were collected to detect the presence of belowground fungal structures through microscopic examination of cleared and stained roots. To assess fungal colonization of roots, we sampled 0.15 g of fibrous roots from a subset of ten roots per treatment. Fibrous roots were removed, placed in cassettes for clearing using hot 10 % potassium hydroxide (Gardner 1975), then acidified for a stain uptake in a 1 % HCl acid solution, and finally stained with a hot 0.05 % Direct Blue solution [International Collection of Vesicular-Arbuscular Mycorrhizal Fungi (INVAM); http://invam.caf.wvu.edu]. Root colonization was assessed using the "gridline intersection" method at 100 intersections for three separate categories: (1) overall AMF colonization (hyphae, arbuscules, vesicles, internal spores); (2) DSE hyphae and sclerotia; (3) a bin of other fungi, including pathenogenic oospores under a compound microscope (Giovannetti and Mosse 1980; McGonigle et al. 1990; Brundett et al. 1996) at 400× magnification.

Data analysis

To assess whether fungicide applications to non-mycorrhizal plant species had non-target impacts on soil nutrients (N, P, K, OM, and pH), a multivariate analysis of variance (MANOVA) was used to determine the difference between plants given a fungicide treatment and those that were not given a fungicide treatment. In addition, plant biomass of each of the non-mycorrhizal species, with and without a fungicide treatment, was compared with a t test to further assess whether there were non-target effects of the fungicide treatment that could enhance nutrient availability.

We also assessed fungal colonization using fungicide treatment as an independent variable on percentage fungal colonization of roots in each year. Separate ANOVAs were run assessing the impact of AMF, DSE, and other root fungal colonization for each year.

We analyzed the compensatory responses of scarlet gilia to the interactive effects of a fungicide treatment, herbivory, and water in analyses of covariance (ANCOVAs) for fruit number, seed mass, aboveground biomass, and belowground biomass over the 3 years of the study [2007 (contained no water treatment), 2008 and 2009 (included a water treatment)] followed by a comparison of all treatment combinations with a Tukey's pairwise comparison test. All data were normally distributed, with the exception of fruit data (see below). In the assessment of treatment effects, stem diameter was included as a covariate to control for initial size differences of plants. Stem diameter was measured immediately after browsing and matched for size with unbrowsed plants (we also know that stem scars do not change through time; thus, stem diameter does not respond to treatment; Paige 1994). We employed the MASS package in R for count data. A negative binomial distribution with a logarithmic link, typical of count date, was used to statistically test the total number of fruits per plant (Bolker et al. 2008). All other analyses were conducted in package stats in R (R-3.0.1; Development Core Team 2013).

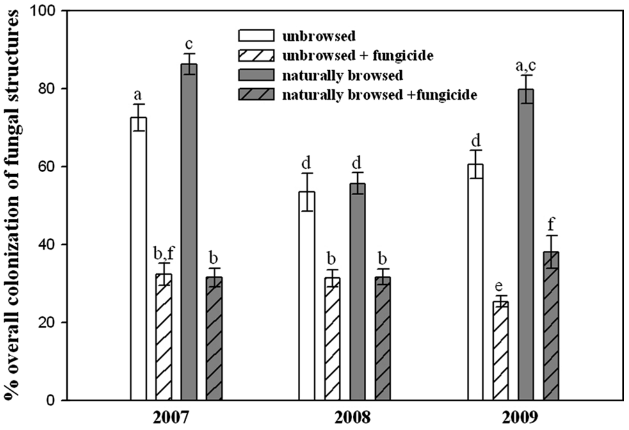

Fig. 1 Overall arbuscular mycorrhizal fungi (AMF) colonization in roots of browsed and unbrowsed scarlet gilia (*Ipomopsis aggregata*) plants with and without fungicide treatments in each of the 3 years (2007, 2008, and 2009) of the study. Data are presented as the means + 1 standard error (SE). Letters above bars represent significant differences among treatments

Results

Fungicide efficacy

There was no significant difference in N, P, K, pH, or OM content in soils with or without a fungicide treatment (MANOVA $p_{\text{fung}} = 0.86$; for N: $F = 1.36$, $df = 4{,}17$, $p = 0.29$; P: $F = 0.08$, $df = 4{,}17$, $p = 0.98$; K: $F = 0.72$, $df = 4{,}17$, $p = 0.59$, pH $F = 0.50$, $df = 4{,}17$, $p = 0.74$; OM: $F = 0.96$, $df = 4{,}17$, $p = 0.98$). Soils were particularly low in N (at approx. 2.5 ppm, N is much less than the 30 ppm that is typically needed) and intermediate in P (at approx. 31 ppm) (see ESM Table S1). The absence of fungicide-induced changes in soil nutrients (e.g., non-target impacts on the microbial community) was further substantiated by the observation that no significant difference in biomass was detected between non-mycorrhizal plants given a fungicide treatment and those that were not treated ($t = 1.6$, $df = 12$, $p_{\text{stock}} = 0.96$; $t = 4.2$, $df = 24$, $p_{\text{dianthus}} = 0.69$). Furthermore, no fungal colonization was observed in any plant roots of these two species at the end of the study when plants were collected.

Scarlet gilia plants that were administered a fungicide showed a reduction in fungal structures in all 3 years for browsed and unbrowsed plants, with only a trend in DSE in 2009 (ANOVAs 2007: $F = 233.5$, $df = 1{,}51$, $p_{\text{amf}} \leq 0.001$, $F = 17.3$, $df = 1{,}51$, $p_{\text{dse}} \leq 0.001$, $F = 6.1$, $df = 1{,}51$, $p_{\text{other}} = 0.02$; ANOVAs 2008: $F = 95.6$, $df = 1{,}53$, $p_{\text{amf}} \leq 0.001$, $F = 6.42$, $df = 1{,}53$, $p_{\text{dse}} = 0.014$, $F = 22.6$, $df = 1{,}53$, $p_{\text{other}} \leq 0.001$; 2009: $F = 38.9$, $df = 1{,}25$, $p_{\text{amf}} \leq 0.001$, $F = 2.6$, $df = 1{,}25$, $p_{\text{dse}} = 0.116$, $F = 9.5$, $df = 1{,}25$, $p_{\text{other}} = 0.005$; Fig. 1; ESM Tables S2, S3). In

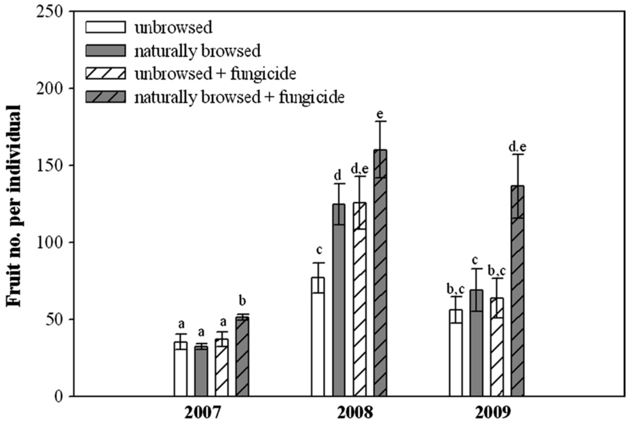

Fig. 2 Number of fruits produced by browsed and unbrowsed plants of *I. aggregata* with and without fungicide treatments in each of the 3 years (2007, 2008, and 2009) of the study. Data are presented as the mean ± 1 SE. Letters above bars represent significant differences among treatments

2007, a fungicide treatment reduced AMF by 57.5 %, DSE by 87.7 %, and all other fungi by 62.3 %. In 2008, fungicide reduced AMF by 43.3 %, DSE by 58.9 %, and all other fungi by 73.1 %, and in 2009, fungicide reduced AMF by 43.3 %, DSE by 58.9 %, and all other fungi by 50.6 %.

Effects of fungi on herbivore selection

There was no significant difference in herbivory between plants with and without a fungicide treatment, indicating no differential herbivory as a result of the presence or absence of fungi ($\chi^2 = 0.91$, $df = 1$, $p = 0.34$; 40/68 (58.8 %) fungicide-treated plants and 35/69 (50.7 %) untreated plants were browsed). All plants were browsed in a similar fashion, with 95 % of the aboveground biomass removed and browing down to approximately 1–2 cm in height.

Interactive effects of browsing and fungi on compensation

In 2007 and 2009 (under drought conditions), there was a significant interaction between browsing and fungicide treatments for fruit number and aboveground biomass [generalized linear model (GLM) ANCOVA, fruit number: $z = 1.96$, $df = 1{,}267$, $p_{2007} = 0.05$, $z = 2.2$, $df = 1{,}120$, $p_{2009} = 0.03$; ANCOVA, aboveground biomass: $F = 4.6$, $df = 1{,}227$, $p_{2007} = 0.03$, $F = 4.66$, $df = 1{,}101$, $p_{2009} = 0.03$; Fig. 2; ESM Fig. S2A; Tables 1, 2]. Without a fungicide treatment, plants equally compensated, in terms of the numbers of fruit produced and aboveground biomass (i.e., no significant difference between browsed and unbrowsed plants; GLM ANCOVA, fruit number: $z = 0.33$, $df = 1{,}105$, $p_{2007} = 0.78$, $z = -0.72$, $df = 1{,}56$, $p_{2009} = 0.45$; ANCOVA, aboveground biomass: $F = 0.22$, $df = 1{,}127$, $p_{2007} = 0.27$, $F = 0.28$, $df = 1{,}32$, $p_{2009} = 0.68$; Fig. 2; ESM Fig. S2A). However, with a fungicide treatment plants overcompensated; browsed individuals produced 55 % more fruit and 33 % greater aboveground biomass

Table 1 Effect of herbivory, fungicide, and water treatments (in 2008 and 2009) on fruit number in scarlet gila (*Ipomopsis aggregata*) plants

	2007			2008			2009		
	df	z	p	df	z	p	df	z	p
Herbivory	1	-3.55	**<0.001**	1	-2.66	**<0.008**	1	-5.03	**<0.001**
Fungicide	1	-2.78	**<0.01**	1	-1.66	0.25	1	-3.72	**<0.001**
Stem	1	4.75	**<0.001**	1	5.01	**<0.001**	1	5.43	**<0.001**
Water				1	2.3	**0.02**	1	0.96	**0.03**
H X F	1	1.96	**0.05**	1	-0.61	0.55	1	2.2	**0.03**
H X W				1	-0.32	0.75	1	0.86	0.39
F X W				1	-0.99	0.32	1	-0.06	0.95
H X F X W				1	0.37	0.71	1	0.5	0.98
Error df	267			187			120		

Data were analyzed using a negative binomial distribution with a logarithmic link. Stem diameter is used as the covariate to control for size. *Grey shading* indicates no watering treatment in 2007

Significant p-values are in bold

Table 2 Effect of herbivory, fungicide, and water treatments (2008 and 2009) on attributes of plant fitness [aboveground biomass (g), belowground biomass (g), and seed mass (mg)] in *I. aggregata* plants

	2007			2008			2009		
Aboveground biomass	df	F	p	df	F	p	df	F	p
Herbivory	1	17.06	**<0.01**	1	12.83	**<0.01**	1	0.84	0.36
Fungicide	1	4.47	**0.04**	1	3.39	0.07	1	0.03	0.86
Stem	1	49.54	**<0.01**	1	22.88	0.82	1	50.01	**<0.01**
Water				1	0.05	**<0.01**	1	2.08	0.15
H X F	1	4.6	**0.03**	1	0.07	0.79	1	4.66	**0.03**
H X W				1	1.33	0.25	1	2.68	0.10
F X W				1	4.6	**0.03**	1	1.06	0.31
H X F X W				1	4.79	**0.03**	1	2.62	0.11
Error df	227			184			101		
Belowground biomass	df	F	p	df	F	p	df	F	p
Herbivory	1	4.66	**0.03**	1	7.57	**<0.01**	1	0.12	0.73
Fungicide	1	1.8	0.18	1	2.6	0.11	1	0.22	0.64
Stem	1	59.77	**<0.01**	1	5.26	**0.02**	1	23.43	**<0.01**
Water				1	27.54	**<0.01**	1	<0.01	0.98
H X F	1	2.91	0.09	1	0.13	0.72	1	0.54	0.54
H X W				1	2.87	0.09	1	0.07	0.07
F X W				1	3.35	0.07	1	0.91	0.91
H X F X W				1	0.73	0.40	1	0.27	0.27
Error df	227			184			101		
Seed mass	df	F	p	df	F	p	df	F	p
Herbivory	1	4.38	**0.04**	1	0.02	0.90	1	0.55	0.46
Fungicide	1	4.6	**0.03**	1	0.02	0.88	1	2.62	0.11
Stem	1	0.25	0.62	1	0.04	0.84	1	0.03	0.86
Water				1	9.37	0.11	1	2.1	0.15
H X F	1	1.07	0.30	1	3.05	0.08	1	0.1	0.75
H X W				1	0.6	0.44	1	0.19	0.66
F X W				1	0.07	0.79	1	3.9	**0.05**
H X F X W				1	0.3	0.58	1	1.36	0.25
Error df	121			184			101		

Data were analyzed with an ANCOVA. Stem diameter was used as the covariate to control for plant size. *Grey shading* indicates no watering treatment in 2007

compared to unbrowsed individuals (GLM ANCOVA, fruit number: $z = -2.72$, $df = 1{,}122$, $p_{2007} = 0.01$, $z = -3.66$, $df = 1{,}56$, $p_{2009} = 0.01$; ANCOVA, aboveground biomass: $F = 47.6$, $df = 1{,}138$, $p_{2007} = 0.04$, $F = 13.8$, $df = 1{,}31$, $p_{2009} = 0.01$; Fig. 2; ESM Fig. S2A).

In contrast, in 2008 (under normal levels of precipitation) there was no significant interaction between browsing and fungicide on fruit production or aboveground biomass (GLM ANCOVA, fruit number: $z = -0.61$, $df = 1{,}91$, $p_{2008} = 0.55$; ANCOVA, aboveground biomass: $F = 0.07$, $df = 1{,}46$, $p_{2008} = 0.79$; Fig. 2; ESM Fig. S2A; Tables 1, 2). Without a fungicide treatment, plants overcompensated in response to herbivory in terms of fruit production and aboveground biomass (GLM ANCOVA, fruit number: $z = -3.7$, $df = 1{,}91$, $p_{2008} = 0.012$; ANCOVA, aboveground biomass: $F = 1.7$, $df = 1{,}46$, $p_{2008} < 0.01$). When fungicide was applied, there was a trend toward overcompensation [i.e., a trend toward higher fruit production in browsed vs. unbrowsed fungicide treated plants (see Fig. 2), albeit, non-significant; GLM ANCOVA, $z = -2.47$, $df = 1{,}45$, $p_{2008} = 0.24$]. It is important to note that both browsed and unbrowsed plants significantly increased fruit production in 2008 following a fungicide treatment when compared to their natural controls (GLM ANCOVA, $z = -1.06$, $df = 1{,}45$, $p_{2008} = 0.05$, $z = -1.89$, $df = 1{,}47$, $p_{2008} = 0.05$, respectively; see Fig. 2). Naturally browsed plants with a fungicide treatment produced 27 % more fruit than naturally browsed plants without a fungicide treatment, and unbrowsed plants with a fungicide treatment produced 57 % more fruit than unbrowsed plants without a fungicide treatment. Aboveground biomass significantly increased for unbrowsed fungicide-treated plants (ANCOVA, $F = 45.6$, $df = 1{,}47$, $p_{2008} < 0.02$) in 2008 when compared to their natural controls (ESM Fig. S2A), producing 82 % more biomass.

There was no significant interaction between browsing and fungicide treatments on belowground biomass or seed mass in any year (ANCOVA, belowground biomass: $F = 2.9$, $df = 1{,}227$, $p_{2007} = 0.09$, $F = 0.13$, $df = 1{,}184$, $p_{2008} = 0.72$, $F = 0.54$, $df = 1{,}101$, $p_{2009} = 0.54$; seed mass: $F = 1.07$, $df = 1{,}121$, $p_{2007} = 0.30$, $F = 3.05$, $df = 1{,}184$, $p_{2008} = 0.08$, $F = 0.1$, $df = 1{,}101$, $p_{2009} = 0.75$; Table 2; Fig. ESM S2b, c).

Interactive effects of browsing and water on compensation

No significant interactions between browsing and water were found in either 2008 or 2009 for any of the traits measured (number of fruits, above- or belowground biomass or seed mass) (GLM ANCOVA, fruit number: $z = -0.32$, $df = 1{,}184$, $p_{2008} = 0.75$, $z = 0.86$, $df = 1{,}120$, $p_{2009} = 0.39$; ANCOVA, aboveground biomass: $F = 1.33$, $df = 1{,}184$, $p_{2008} = 0.25$, $F = 2.68$, $df = 1{,}101$, $p_{2009} = 0.10$; belowground biomass: $F = 2.87$, $df = 1{,}184$, $p_{2008} = 0.09$, $F = 0.07$, $df = 1{,}101$, $p_{2009} = 0.07$; seed mass: $F = 0.06$, $df = 1{,}184$, $p_{2008} = 0.44$, $F = 0.19$, $df = 1{,}101$, $p_{2009} = 0.66$; Tables 1, 2). However, in 2008, overall water additions significantly increased fruit production in both unbrowsed and browsed plants (by 32 and 27 % over unbrowsed and browsed controls, respectively) (Table 1; Fig. 3a; GLM ANCOVA, $z = 2.3$, $df = 1{,}184$, $p = 0.02$) and in 2009, water additions significantly increased fruit production in unbrowsed plants (by 82 % over unbrowsed controls) (Fig. 3b; GLM ANCOVA, $z = 2.2$, $df = 1{,}51$, $p = 0.04$).

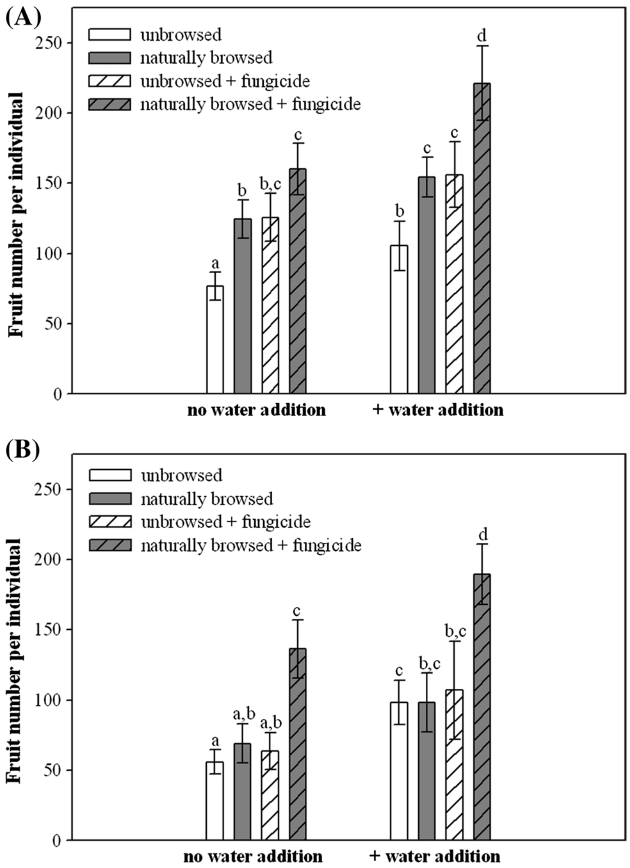

Fig. 3 **a** Number of fruits produced by browsed and unbrowsed plants of *I. aggregata* with and without all combinations of fungicide and water in 2008, **b** number of fruits produced by browsed and unbrowsed plants of *I. aggregata* with and without all combinations of fungicide and water in 2009. Data are presented as the mean ± 1 SE. Letters above bars represent significant differences among treatments

Interactive effects of browsing, fungicide, and water on compensation

In 2008, browsed plants given a water and fungicide treatment significantly increased fruit production over browsed plants with just a fungicide treatment (GLM ANCOVA, $z = 2.05$, $df = 1{,}45$, $p = 0.05$). Water- and fungicide-treated plants, both unbrowsed and browsed, significantly increased fruit production over unbrowsed and browsed plants receiving only a water treatment (Fig. 3a; GLM ANCOVA, $z = -1.89$, $df = 1{,}43$, $p = 0.04$ vs. $z = 4.43$, $df = 1{,}46$, $p = 0.05$, respectively) and unbrowsed and browsed plants without water or fungicide treatments (Fig. 3a; GLM ANCOVA, $z = 1.81$, $df = 1{,}47$, $p < 0.01$ vs. $z = -3.91$, $df = 1{,}49$, $p = 0.04$, respectively). Browsed plants given a fungicide and water treatment produced 43 % more fruit than browsed plants given only a water treatment and 52 % more fruit than browsed controls (no fungicide, no water). Unbrowsed plants given a fungicide and water treatment produced 48 % more fruit than unbrowsed plants given only a water treatment and 103 % more fruit than unbrowsed controls (no fungicide, no water).

There was also a significant two-way interaction between water and fungicide and a significant three-way interaction for herbivory, fungicide, and water on aboveground biomass (ANCOVA, $F = 4.6$, $df = 1{,}184$, $p = 0.03$ and $F = 4.79$, $df = 1{,}184$, $p = 0.03$ for two-way and three-way interactions, respectively; Table 2). A fungicide and water treatment increased aboveground biomass by approximately 34 % over control plants without fungicide and water. The interactive effects of herbivory, water, and fungicide increased aboveground biomass by approximately 65 % over control plants without herbivory, water or fungicide.

In 2009, browsed plants given both a fungicide and a water treatment produced significantly more fruit (21 %) than plants receiving a fungicide treatment only (GLM ANCOVA, $z = -1.07$, $df = 1{,}24$, $p = 0.03$) and significantly more fruit (66 %) than plants receiving only a water treatment (GLM ANCOVA, $z = -4.63$, $df = 1{,}25$, $p = 0.03$). No significant differences were found between unbrowsed plants receiving both the water and fungicide treatment and unbrowsed plants receiving either only the water treatment or only the fungicide treatment (GLM ANCOVA, $z = 2.02$, $df = 1{,}22$, $p = 0.68$ and $z = -0.84$, $df = 1{,}20$, $p = 0.23$, respectively). Both unbrowsed and browsed water- and fungicide-treated plants produced significantly more fruit (101 and 154 %, respectively) than untreated controls (Fig. 3b; GLM ANCOVA, $z = -3.13$, $df = 1{,}23$, $p < 0.01$ and $z = -5.13$, $df = 1{,}26$, $p < 0.01$, respectively).

Main effects of browsing, fungicide, and water on plant fitness

Overall, the main effects of herbivory led to a significant increase in fruit production in all 3 years (30, 45, and 59 % greater in 2007, 2008 and 2009, respectively; GLM ANCOVA, $z = -3.55$, $df = 1{,}267$, $p_{2007} < 0.001$, $z = -2.66$, $df = 1{,}187$, $p_{2008} = 0.008$, $z = -5.03$, $df = 1{,}120$, $p_{2009} < 0.001$; Table 1) and a significant increase in aboveground and belowground biomass in 2 of the 3 years (53 % greater aboveground biomass in both 2007 and 2008 and 16 and 44 % greater belowground biomass in 2007 and 2008, respectively) for browsed individuals versus unbrowsed individuals (ANCOVA, aboveground biomass: $F = 17.06$, $df = 1{,}227$, $p_{2007} < 0.01$, $F = 12.83$, $df = 1{,}84$, $p_{2008} < 0.01$; belowground biomass: $F = 4.66$, $df = 1{,}227$, $p_{2007} = 0.03$, $F = 7.57$, $df = 1{,}184$, $p_{2008} < 0.01$; Table 2). Overall, seed mass was significantly greater (by 20 %) for browsed plants in 2007, with no significant differences in 2008 or 2009 (ANCOVA, $F = 4.38$, $df = 1{,}121$, $p_{2007} = 0.04$, $F = 0.02$, $df = 1{,}184$, $p_{2008} = 0.90$, $F = 0.55$, $df = 1{,}101$, $p_{2009} = 0.46$; Table 2).

The main effect of fungicide (fungicide vs. no fungicide) led to significant overall increases in fruit production in 2007 and 2009 (30 and 51.5 %, respectively) with no significant effect in 2008 (GLM ANCOVA, $z = -2.78$, $df = 1{,}267$, $p_{2007} < 0.01$, $z = -2.66$, $df = 1{,}187$, $p_{2008} = 0.25$, $z = -3.72$, $df = 1{,}120$, $p_{2009} < 0.001$; Table 1). Fungicide treatments also led to a significant increase in aboveground biomass (22 %) and seed mass (by 23 %) in 2007 (ANCOVA, aboveground biomass: $F = 4.47$, $df = 1{,}227$, $p_{2007} = 0.04$; seed mass: $F = 4.6$, $df = 1{,}121$, $p_{2007} = 0.03$; Table 2).

The main effect of water additions (assessed only in 2008 and 2009) led to a significant overall increase in fruit production in both years (29 and 56 %, respectively; GLM ANCOVA, $z = 2.3$, $df = 1{,}187$, $p_{2008} = 0.02$, $z = 0.96$, $df = 1{,}120$, $p_{2009} = 0.034$, Table 1) and a significant increase in aboveground biomass (by 24 %) in 2008 (ANCOVA, $F = 0.05$, $df = 1{,}184$, $p < 0.01$, Table 2). In addition, water addition led to an overall significant decline in belowground biomass (by 34 %) in 2008 (ANCOVA, $F = 27.5$, $df = 1{,}184$, $p < 0.01$, Table 2).

Discussion

Overall, our results indicate that the fungal community strongly influences the compensatory response of scarlet gilia plants and that the magnitude of the response is dependent upon environmental conditions (in this case water). Experimental water additions alone did not affect the compensatory response, although water additions did increase general plant fitness. The reduction in fungi, however, did alter the compensatory response, particularly under water-limited conditions, increasing compensation from equal to over. In a year of normal precipitation, a fungicide treatment reduced the compensatory response from overcompensation to a trend toward overcompensation by simultaneously increasing the reproductive success of both browsed and unbrowsed plants. We were therefore able to show experimentally that the interactive effects of water and fungicide maximize fruit production following herbivory. Thus, fungi are likely parasitic on scarlet gilia following ungulate herbivory.

In this study we were primarily interested in how fungal associations affected the compensatory response of scarlet gilia following ungulate herbivory. We hypothesized that fungal associations would facilitate the compensatory response most commonly observed in this Arizona population of scarlet gilia and that mutualistic associations with fungi would explain the phenomenon of overcompensation altogether. However, the results of our fungal removal experiments showed an increase in the compensatory response, particularly under the combined effects of herbivory and drought. Fungicide-treated browsed plants produced 1.2- to 1.9-fold more fruits than browsed plants without a fungicide treatment over the 3-year period of this study and 1.7- to 1.9-fold more fruit under drought conditions. In addition, the interactive effects of herbivory, fungicide treatment, and water additions resulted in even greater compensatory capabilities in terms of fruit production whether under drought conditions or environmentally favorable conditions (normal levels of precipitation). Compared to unbrowsed plants without a fungicide treatment, fungicide-treated unbrowsed plants showed no significant increase in fruit production in either 2007 or 2009, but they did show significantly higher fruit production in 2008. Specifically, drought conditions appear to limit the capacity of unbrowsed plants to respond to the fungicide treatment. This is substantiated by the fact that when unbrowsed plants received both fungicide and water treatments in a drought year (2009), there was a significant increase in fruit production over unbrowsed control plants.

These results lead one to ask why are there differences between browsed and unbrowsed plants in terms of their fitness responses following a fungicide treatment. Recent studies on *Arabidopsis thaliana* in our laboratory have shown that differences in the degree of endopolyploidy (i.e., plasticity in cellular ploidy through endoreduplication) following the removal of apical dominance leads to enhanced reproductive success, i.e., overcompensation (Scholes and Paige 2011; Scholes et al. 2013; Scholes and Paige 2014). *Ipomopsis aggregata* also plastically endoreduplicates following the removal of apical dominance (KN Paige, unpublished data), thus we suspect that it responds similarly to *Arabidopsis* from a molecular genetic perspective. Increasing chromosome number, and thus gene copy number, may provide a means of increasing gene expression, likely by the upregulation of selected genes or gene families. We have recently shown significant upregulation of glucose-6-phosphate dehydrogenase 1, the key regulatory enzyme in the oxidative pentose-phosphate pathway that plays a central role in plant metabolism converting glucose to ribose-5-phosphate, following the removal of apical dominance in genotypes that overcompensate (Siddappaji et al. 2013). Furthermore, increasing chromosome number increases the total DNA content and hence cell size, leading to extensive cell growth/expansion through endoreduplication. Hence, greater demand by plants for nutrients and water following herbivory and greater nutrient and water transport as a consequence of endoreduplication may explain the differential responses of browsed and unbrowsed plants to the removal/reduction of fungi. We observed that root biomass also generally increased following browsing, initially acting as a sink for carbon from the newly regenerating tissues (e.g., see Paige and Whitham 1987). The increase in root biomass and spread in both the tap and fibrous portions of the root system may further facilitate the accumulation and transport of nutrients and water contributing to enhanced aboveground biomass and fruit production.

Our results are counter to the “modification of tolerance hypothesis” proposed by Bennett et al. (2006) that plants associating with mycorrhizal fungi will have higher tolerance to herbivory, given that scarlet gilia plants with fungi showed lower fitness following herbivory. It is likely that AMF and DSEs are competing with plants for carbon following herbivory (Johnson et al. 1997; Jones and Smith 2004; Hoeksema et al. 2010), driven in part by water limitations. Plants only equally compensated under the drought conditions of 2007 and 2009, but overcompensated following a fungicide treatment which reduced their fungal competitors. In a year of normal precipitation plants naturally overcompensated (browsed plants produced significantly more fruit than unbrowsed plants), and following a fungicide treatment both browsed and unbrowsed plants significantly increased fruit production with a nonsignificant trend toward overcompensation (see Fig. 2). Despite the low levels of pathogenic colonization (less than 1 % of all fungi), scarlet gilia plants may have been challenged by root-infecting pathogenic fungi (oospores) that could decrease plant growth. However, a comparison of plant fitness following herbivory with and without pathogenic fungi showed no effect of these fungi on fitness in any of the 3 years of study. Variation in the level of colonization could also play a role in constraining the compensatory response following herbivory. For example, Garrido et al. (2010) showed a negative correlation between plant tolerance to defoliation in *Datura stramonium* and AMF colonization levels.

Herbivory may also influence AMF species composition (Murray et al. 2010). For example, fungal species that colonize roots following browsing may differ from those that colonize plants which are not browsed, and individual species of AMF are known to differ in their effects on plant growth, ranging from mutualistic to antagonistic (Johnson et al. 1997; Klironomos 2003; Bennett et al. 2006). Thus, the outcome of any interaction may be contingent on the particular fungal species that colonize roots following browsing (Bennett and Bever 2007). Of course, additional studies will be necessary to address how different fungal species differentially affect the compensatory response.

Overall, this study represents one of few to consider the interactive effects of mammalian herbivory and fungal associations on plant fitness and represents the only study to date addressing the phenomenon of overcompensation. It also represents one of few studies which focuses on plant reproduction as opposed to biomass alone.

Aboveground biomass and fruit production were at best weakly correlated ($R^2 = 0.26$, $df = 1{,}433$, $p = 0.001$), possibly indicating that biomass may not give a clear picture of changes that are ultimately of evolutionary importance.

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