Multi-trophic consequences of plant genetic variation in sex and growth

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Abstract. There is growing evidence for the influence of plant intraspecific variation on associated multi-trophic communities, but the traits driving such effects are largely unknown. We conducted a field experiment with selected genetic lines of the dioecious shrub *Baccharis salicifolia* to investigate the effects of plant growth rate (two-fold variation) and gender (males vs. females of the same growth rate) on above- and belowground insect and fungal associates. We documented variation in associate density to test for effects occurring through plant-based habitat quality (controlling for effects of plant size) as well as variation in associate abundance to test for effects occurring through both habitat quality and abundance (including effects of plant size). Whereas the dietary specialist aphid *Uroleucon macolai* was unaffected by plant sex and growth rate, the generalist aphid *Aphis gossypii* and its tending ants (*Linepithema humile*) had higher abundances and densities on male (vs. female) plants, suggesting males provide greater habitat quality. In contrast, *Aphis* and ant abundance and density were unaffected by plant growth rate, while *Aphis* parasitoids were unaffected by either plant sex or growth rate. Arbuscular mycorrhizal fungi had higher abundance and density (both marginally significant) on females (vs. males), suggesting females provide greater habitat quality, but lower abundances (marginally significant) and higher densities on slow- (vs. fast-) growing genotypes, suggesting slow-growing genotypes provided lower resource abundance but greater habitat quality. Overall, plant sex and growth rate effects on associates acted independently (i.e., no interactive effects), and these effects were of a greater magnitude than those coming from other axes of plant genetic variation. These findings thus demonstrate that plant genetic effects on associated communities may be driven by a small number of trait-specific mechanisms.

Key words: *arbuscular mycorrhizal fungi; genetic variation; growth rate; herbivory; plant quality; plant sexual dimorphism; trophic interactions.*

Introduction

There is mounting evidence for the influence of plant intraspecific genetic variation on associated multi-trophic communities (reviewed by Bailey et al. 2009, Mooney and Singer 2012). Studies have found that plant genotypes vary in arthropod community structure (Dungey et al. 2000, Johnson and Agrawal 2005, reviewed by Bailey et al. 2009), and that plant genetic variation influences antagonistic (Fritz 1995, Hare 2002, Abdala-Roberts and Mooney 2013) and mutualistic (Mooney and Agrawal 2008, Abdala-Roberts et al. 2012) interactions associated with plant canopies. Likewise, equivalent effects of plant genetics on belowground communities have also been reported and appear to be similarly widespread (reviewed by Schweitzer et al. 2012).

Past studies testing for plant intraspecific genetic variation on associated communities have sampled genotypes randomly and without regard to any particular trait of importance to consumers (Hughes et al. 2008). While post hoc correlations between plant traits and community composition are useful, if many plant traits are assessed, this approach has relatively low statistical power and a high risk of implying spurious associations. Furthermore, plant genetic effects are likely mediated by multiple plant traits, yet little is known of the relative importance of variation in different traits or whether the effects of such traits on associated communities act additively or synergistically.

Plant genetic variation in growth rate may be an important driver of effects on associated communities

(Carmona et al. 2011). Growth rate is frequently related to food quality for herbivores; fast-growing plants produce more biomass and may be less defended against herbivores (and thus of higher quality) if growth and defenses trade off (Coley et al. 1985, Fine et al. 2004, Mooney et al. 2010). Alternatively, fast-growing plants may have greater nutrient content and in this way be of higher quality to herbivores independently of growth–defense trade-offs. In an experiment with 16 milkweed species, Mooney et al. (2010) demonstrated that aphid densities increase linearly with plant species growth rate. Similarly, Bridgeland et al. (2010) found that faster growth rates in cottonwood (*Populus*) hybrids were associated with greater herbivore loads and stronger bird predation. Moreover, plant growth rate may also have multi-trophic effects on herbivore habitat quality by mediating the effects of predators and parasitoids (Mooney and Singer 2012) and mutualists (Mooney and Agrawal 2008). Despite these predictions for the influence of intraspecific variation in plant growth rate on herbivore habitat quality, such effects have not been evaluated.

Plant sex is also an important axis of variation in ecologically important traits that is often genetically based (Ming et al. 2011), and dioecy occurs in 6–10% of all angiosperm species (Geber et al. 1999, Cornelissen and Stiling 2005, Renner 2014) including ecologically dominant grasses (Poaceae, Zosteraceae) and trees (e.g., most Moraceae and Salicaceae). Sexual dimorphism (e.g., males vs. females, females vs. hermaphrodites) in traits is proposed to originate from trade-offs with differing levels of reproductive investment between the sexes. There is evidence for plant sexual dimorphism in herbivore resistance (Ågren et al. 1999, Cornelissen and Stiling 2005) and interactions with above- (Ashman and King 2005, Cornelissen and Stiling 2005, Mooney et al. 2012*a*), and belowground (Varga and Kytöviita 2010, Vega-Frutis et al. 2013) consumers. However, tests for the traits and mechanisms mediating such effects remain scarce (but see Petry et al. 2013).

Plant sexual dimorphism in growth rate is an underlying mechanism by which plant sexes are proposed to vary in herbivore resistance. Specifically, females are proposed to invest more in reproduction than males or hermaphrodites (Lloyd and Webb 1977, Delph 1999), grow more slowly (Ågren et al. 1999, Cornelissen and Stiling 2005), and, as already described, slower female growth in turn leads to greater investment in herbivore resistance (Coley et al. 1985, Fine et al. 2004, Mooney et al. 2010). While females (vs. males or hermaphrodites) are frequently shown to have slow growth and high herbivore resistance (Ågren et al. 1999, Cornelissen and Stiling 2005), studies separating growth-dependent and growth-independent effects of plant sex are lacking.

In this study, we established genetic lines of fast- and slow-growing male and female plants of the dioecious shrub *Baccharis salicifolia* (Ruiz and Pav.)

Pers. (Asteraceae) to investigate the effects of these traits on associated above- and belowground communities. We documented variation in associate density (number of consumers per unit of plant biomass) to test for effects occurring through plant-based habitat quality (controlling for effects of plant size) and for variation in associate abundance to test for effects occurring through both habitat quality and abundance (including effects of plant size). Specifically, we addressed the following questions: (1) Are there individual and interactive effects of plant sex and growth rate on above- and belowground plant associates, namely a dietary specialist and dietary generalist aphid, aphid-tending ants, aphid parasitoids, and root-colonizing arbuscular mycorrhizal fungi? (2) What is the relative importance of plant sex and growth rate effects both within and among higher trophic levels? In addressing these first two questions, we thus provide a novel test for plant sexual dimorphism in interactions with insects and mycorrhizae while experimentally controlling for genetic variation in growth rate. (3) To what extent do these traits affect associated communities through changes in resource abundance (e.g., plant biomass) vs. changes in habitat quality independent of resource abundance? (4) How does the influence of genetic variation in growth rate and sex compare to other forms of genetic variation? And finally, (5) do plant genetic effects on herbivores differ between the dietary specialist and generalist aphids and, if so, does herbivore diet breadth mediate the cascading effects of plant genetics on other trophic levels? In addressing the above, this study builds towards understanding how two important axes of plant genetic variation concurrently shape plant-centered communities.

MATERIALS AND METHODS

Natural history

Baccharis salicifolia is a perennial, woody, dioecious shrub that is native to the southwestern United States and northern Mexico. It is commonly found near freshwater sources such as stream banks and wetlands. Flowering and most growth occur from March to May, and it is during this period that insect numbers are highest. At our field sites, within and adjacent to the University of California San Joaquin Marsh Reserve (33°39′47″ N, 117°51′7″ W; CA, USA), the two most abundant aboveground herbivores of this species are the generalist aphid *Aphis gossypii* (Glover) and the specialist aphid *Uroleucon macolai* Blanchard (Mooney et al. 2012*b*), both of which feed on young stems and leaves. *Aphis gossypii* (Glover) (*Aphis* hereafter) is a generalist herbivore feeding on numerous host plant species, including a number of important crops (Blackman and Eastop 2006), whereas *U. macolai* (*Uroleucon* hereafter) has a narrow diet breadth, feeding

only on *B. salicifolia* and one other *Baccharis* species (Blackman and Eastop 2006). In addition, at our study sites, *Aphis* is frequently tended by the nonnative argentine ant *Linepithema humile* Mayr, which feeds on the aphid's honeydew and in exchange protects aphids from predators and parasitoids (Mooney et al. 2012b, Moreira and Mooney 2013); *Uroleucon* is not tended by ants. The most common natural enemies of both aphid species are parasitic wasps (Hymenoptera: Braconidae; Mooney et al. 2012b).

Genotype selection

All plants used in this experiment were collected from a "source" common garden of genotypes originating from a naturally occurring population of *B. salicifolia* in the San Joaquin Marsh Reserve (33.65°N, 117.85°E; California, USA). The source common garden and the present experiment were adjacent to each other and to the Marsh Reserve from which wild-grown plants were randomly selected over a 35-ha area (with the most distant plants being approximately 900 m apart). Accordingly, the spatial scale of these experiments and of the distribution of the wild-grown genotypes was roughly equivalent, thus providing a realistic assessment of plant genetic effects on consumers (Tack et al. 2012).

In February 2008, shoot cuttings from 20 male and 20 female wild-grown plants were collected and dipped in a 20% solution of Dip 'N Grow Root Inducing Concentrate (Dip 'N Grow, Clackamas, Oregon, USA) and planted in horticultural perlite for 6 weeks. The cuttings from all but one male genotype (which failed to grow) were then transplanted to individual 1-L pots containing a soil mixture of equal parts silica sand, redwood compost, peat moss, and pumice, and grown in a greenhouse for 2 months. In May 2008, these cuttings were randomly planted out into a plot with 17 rows and 27 columns of plants, with 1.0-m separation among plants. Sample size ranged from 8 to 13 cuttings per genotype (mean 11.5 ± 0.2; mode 12),

with a total of 459 plants. Plants were irrigated as needed from an overhead sprinkler system until December 2008 when all plants were assessed for size by measuring the cumulative length of all shoots longer than 10 cm in length. Because all plants were initiated at the same size and time, any subsequent measurements of plant size represented an estimate of plant growth rate.

To test for genetic variation in plant growth rate, plant length (log-transformed to achieve normally distributed residuals) was modeled as dependent upon sex and genotype within sex, as well as common garden row and column (to account for spatial heterogeneity within the plot) using PROC GLM, SAS version 9.2 (SAS Institute 2008). Genotype was treated as a fixed effect because these models were used to generate least-square means and errors for each genotype's growth rate (accounting for plot row and column effects, which were both significant). Across all genotypes, there was significant variation in growth (total cumulative plant length) after 10 months ($\chi^2 = 186.4$, $P < 0.0001$), with the fastest genotype growing 3.7-fold more than the slowest (Appendix S1: Fig. S1), but there was no effect of plant sex ($F_{1,37} = 0.87$, $P = 0.35$; Fig. S1).

The three fastest and four slowest growing male and female genotypes ($N = 14$ genotypes total) were identified based on the above results and cloned for use in the present study. A greater number of slow- than of fast-growing genotypes (four vs. three for each sex, respectively) were used because of lower propagation success of the former. In the source common garden, the average growth of the six fast male and female genotypes was two-fold greater than the average growth of the eight slow male and female genotypes ($F_{1,10} = 33.75$, $P = 0.0002$), although there was no significant difference between males and females ($F_{1,10} = 0.03$, $P = 0.88$) or a sex × growth rate interaction ($F_{1,10} = 0.22$, $P = 0.65$).

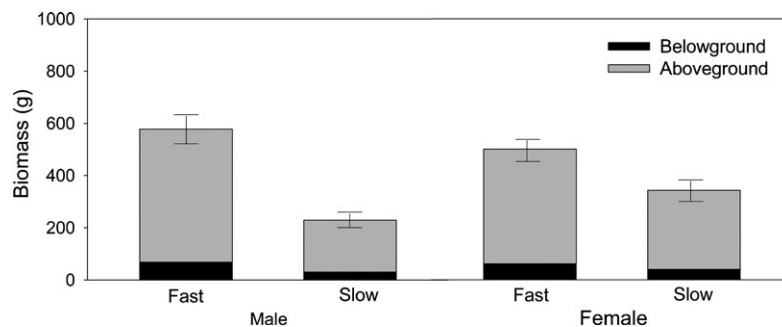In April 2009, three cuttings were collected from each of the approximately 12 copies of each plant within

FIG. 1. Least-square means (±SE) for total biomass (g dry mass) of fast- and slow-growing *Baccharis salicifolia* male and female genotypes. Total biomass is divided into above- and belowground biomass, and error bars show SE for total biomass. Least-square means account for the effects of plot and plant genotype nested within sex and growth rate.

the common garden (~36 cuttings per original genotype). These cuttings were in turn treated as described, growing in perlite until early June when they were transplanted into individual pots, and then grown in a greenhouse until November 2009 when they were planted into the field experiment. For this experiment, replicate copies of each of these 14 genotypes came from unique source plants in the common garden, thus eliminating non-genetic (maternal) effects.

Experimental design

In early November 2009, we planted *B. salicifolia* individuals as part of an experimental design that included elements beyond those relevant to the questions addressed here. The experiment consisted of 10 separate 2 × 2 m plots, each covered by 2.4 × 2.4 m cages made of PVC pipe frames encased with 70% transparent lumite fabric, and 1.4 m spacing between plots. For five plots, these cages were open on one side, although aphids, their natural enemies (e.g., parasitoids, coccinellids), and aphid-tending ants gained access and were common in all plots. Because preliminary analyses (not shown) found no main or interactive effects of cage type (open vs. not open) on plant growth or plant associates, we removed this aspect of the design from subsequent analyses.

Each plot was in turn divided into nine planting locations, each receiving a pair of plants, resulting in 18 plants per plot. The planting locations were on a three-by-three grid, with 0.67-m spacing among locations and with edge planting locations being 20 cm from the cage wall. The plants within each plot were approximately evenly divided between the four genetic classes of fast-growing males, fast-growing females, slow-growing males and slow-growing females, with four or five plants per genetic class. In total, this design resulted in 180 plants, with 45 plants each of the four genetic classes. With respect to the individual genotypes representing each genetic class, sample sizes ranged from 10 to 20 plants per genotype (median of 13), with the exception of one slow-growing female genotype for which only five replicates were used.

All plots were irrigated daily for the first 6 weeks and as needed throughout the summer and early fall of 2010 (May–November) and were weeded every other month from January through June of 2010 and 2011. All plants were inoculated with approximately 10 adult individuals of each aphid species in January 2010 and again in January 2011.

Insect sampling and plant biomass

We sampled insects on all plants twice, once in March 2010 and again in March 2011. During each survey we recorded the abundance of *Uroleucon*, *Aphis*, ants, and *Aphis* parasitoids by exhaustively examining the stems and leaves of each plant. Parasitoid abundance was estimated based upon the number of visibly parasitized aphids per plant, including the cuticle of killed aphids from which wasps had previously emerged. There was no visible parasitism of *Uroleucon*. All plants were harvested in May 2011, 18 months after planting. Plant material was divided into below-ground (i.e., root) and aboveground (woody tissues and leaves) biomass and dried until constant weight. With these data we calculated both insect abundance (counts) as well as density, measured as the number of insects per kilogram of aboveground dry biomass.

Mycorrhizal colonization

After weighing dry root biomass, fine roots were removed to assess colonization by arbuscular mycorrhizae. Although propagating plants from cuttings may influence rates of mycorrhizal colonization, the long duration of this experiment, and the close proximity (<25 m) of the experiment to wild-grown plants likely reduced such effects. Fine roots (about 0.5–1.0 g) were soaked in 10% KOH for 4–5 days and then transferred to a 1% HCl solution for 1 minute. Roots were stained by placing them in a 0.01% acid fuschin in 14:1:1 lactic acid:glycerol:diH$_2$O for 1 day. To destain, samples were transferred to 14:1:1 lactic acid:glycerol:diH$_2$O solution for 1 day. Percent root colonization by arbuscular mycorrhizal fungi hyphae was calculated by examining 100 random intersect points of a slide with stained fine roots. Five to six roots were placed horizontally on a slide with approximately eight drops of destain solution. Transect lines ran vertically through the slide; an observer then completed enough transect lines until 100 intersection points were recorded. An intersection point was defined as any time an observer encountered one of the root samples. At every intersection point, the presence or absence of mycorrhizal hyphae colonization was recorded, thus estimating the density of mycorrhizal colonization as percent root colonization (or the proportion of intersection points colonized). Mycorrhizal abundance (root mass [g] of *B. salicifolia* colonized) was then quantified by multiplying the proportion of root colonization by the total root dry biomass (g). This metric is based upon both fine and course root biomass and, as only the former is colonized, overestimates the mass of colonized roots, but nevertheless provides a relative estimate for comparison among plant genetic classes.

Statistical analyses

Our analyses of plant genetic effects on both associate density and abundance (hereafter "density models" and "abundance models," respectively) allow us to infer the mechanisms by which plant genetic effects occur. The density models control for variation in resource abundance and thus test for plant genetic effects occurring

only through habitat quality. Such variation in habitat quality might occur through a combination of plant genetic effects on plant quality (defenses or nutrients), mutualist services, the strength of competition and predation, plant mediation of the abiotic environment, and potentially other factors as well. In contrast, the abundance models test for effects occurring due to plant genetic variation in both habitat quality and resource abundance. By inference then, differences in findings between density and abundance models provides insight into the mechanisms at work. If there are significant plant genetic effects on abundance but not density, this would suggest such effects are mediated by differences in resource abundance alone. Conversely, if plant genetic effects on abundance are approximately equal to those on density, this would suggest that plant genetic effects are mediated by differences in habitat quality but not resource abundance.

We performed general linear mixed models testing for the fixed effects of plant sex (male vs. female), growth rate (slow vs. fast), their interaction, and the random effect of plant genotype (nested within sex and growth) on plant growth, mycorrhizae, *Aphis*, *Uroleucon*, parasitoids, and ants. Because initial analyses found no evidence between-year variation in the effects of plant sex or growth rate on insects (i.e., no interaction between plant sex or growth rate and year; Appendix S1: Table S1) we based subsequent analyses upon means across years. We tested for plant sex and growth rate effects on both the abundance (number of insects or hyphae biomass) and density (number of insects/kg dry aboveground mass or percent hyphae colonization) of insects and mycorrhizae. For all models, we also included plot (treated as random) to account for spatial heterogeneity in the common garden. The significance of the genotype effect (nested within sex and growth) on each associate (both abundance and density) was assessed using the likelihood ratio test, where the difference in two times the log-likelihood of models including and excluding the genotype effect is distributed as one-tailed χ^2 test with one degree of freedom (Littell et al. 2006). These tests account for the effects of plant sex and growth rate and thus evaluate residual variation among the 14 genotypes. To quantitatively describe this residual genetic variation, we ran the density models but excluded genotype to obtain the residuals (mean and SE) for each genotype.

Plant biomass, abundance of *Uroleucon, Aphis*, and parasitoids were log-transformed to achieve normality of residuals, whereas the ant abundance model used a Poisson distribution (with log-link function) as the data could not be transformed to produce normally distributed residuals. The density of *Uroleucon, Aphis*, parasitoid, and ants were all arcsine-square-root transformed to achieve normality of residuals. Mycorrhizal density and abundance were normally distributed and did not require transformation. We report least-square means and standard errors from original (untransformed) data as descriptive statistics. In all cases, results are based upon Type III sums of squares. The analysis of all variables except ant abundance was carried out in PROC MIXED, SAS version 9.2 (SAS Institute 2008). For ant abundance, tests for sex and growth effects were carried out in PROC GLIMMIX, but the effect of genotype on ant abundance was tested for using PROC MIXED and log-transformed data because the validity of likelihood ratio tests for random effects is questionable for generalized linear models (Bolker et al. 2009).

Results

Effects of plant sex and growth rate on biomass

Total biomass.—We found a significant effect of growth rate on total plant biomass (Table 1), with fast-growing genotypes (547.39 ± 32.42 g [mean ± SE]) having 1.9× more biomass than slow-growing genotypes (287.60 ± 31.75 g; Fig. 1), a result that was consistent with the differences observed in our source common garden (Fig. S1). Males and females did not differ in total biomass (412.05 ± 32.22 g and 422.95 ± 31.96 g, respectively; Table 1; Fig. 1). We found a significant sex × growth rate interaction (Table 1), where fast males (599.05 ± 42.77 g) had more biomass than fast females (495.74 ± 40.21 g), but slow females (350.15 ± 41.37 g) had more biomass than slow males (225.05 ± 39.68 g; Fig. 1); as a result, the difference between fast and slow genotypes was greater for males (2.7×) than for females (1.4×), i.e., for the particular genotypes selected for this experiment there was more variation in growth rate for males than females. Similar results were observed for shoot biomass and root biomass, where fast-growing genotypes exhibited greater mean values relative to slow-growing ones, and sexes did not differ (Table 1; Fig. 1)

Effects of plant sex and growth rate on associate abundance

Aphids.—We found a significant effect of plant sex, but not growth rate, on *Aphis* abundance, with males (29.86 ± 5.86 aphids) having 1.7× more aphids than females (18.10 ± 5.92 aphids). Fast-growing genotypes (28.97 ± 5.93 aphids) had 1.5× more aphids than slow-growing genotypes (18.99 ± 5.84 aphids), but this difference was not significant (Table 1; Fig. 2). In addition, we found a marginally significant sex × growth rate interaction on *Aphis* abundance, as fast males had 1.8× greater abundance than slow males, but fast females had 1.3× greater abundance than slow females (Table 1; Fig. 2), mirroring patterns of genetic variation in plant biomass (males varying more in biomass than females; Fig. S1). In contrast to *Aphis*, there were no effects of sex, growth rate, or their interaction on *Uroleucon* abundance (Table 1; Fig. 2).

TABLE 1. Results from linear mixed models testing for individual and interactive effects of *Baccharis salicifolia* genotype sex and growth rate on the (A) plant biomass, (B) abundance of plant associates, and (C) density of plant associates.

Response Variable	df	Sex	Growth	Sex × Growth
(A) Plant biomass				
Total	1, 14	2.25 (0.156)	**55.37 (0.0001)**	**8.28 (0.012)**
Aboveground ("Shoots")	1, 17	2.87 (0.108)	**54.59 (0.0001)**	**7.96 (0.011)**
Belowground ("Roots")	1, 14	0.08 (0.703)	**17.99 (0.0008)**	1.18 (0.295)
(B) Associate abundance				
Aphis	1, 17	**8.64 (0.009)**	2.62 (0.123)	*3.04 (0.099)*
Uroleucon	1, 18	1.91 (0.185)	0.18 (0.679)	0.77 (0.392)
Ants	1, 17	*3.53 (0.077)*	*4.00 (0.061)*	0.01 (0.990)
Parasitoids	1, 17	3.01 (0.100)	0.74 (0.402)	0.02 (0.892)
Mycorrhizae	1, 15	*3.68 (0.074)*	*4.38 (0.053)*	2.84 (0.112)
(C) Associate density				
Aphis	1, 17	**8.56 (0.009)**	0.64 (0.436)	0.01 (0.904)
Uroleucon	1, 18	0.56 (0.463)	0.29 (0.598)	0.03 (0.857)
Ants	1, 17	**8.20 (0.010)**	0.09 (0.767)	0.18 (0.680)
Parasitoids	1, 17	2.60 (0.125)	0.01 (0.929)	0.01 (0.920)
Mycorrhizae	1, 15	*3.31 (0.088)*	**5.61 (0.031)**	1.97 (0.180)

Notes: Associates are the dietary generalist herbivore *Aphis gossypii*, the dietary specialist herbivore *Uroleucon macolai*, parasitoids (Braconidae) of *A. gossypii*, *Linepithema humile* ants, and arbuscular mycorrhizal fungi. F and P values (in parenthesis) are shown. Significant ($P < 0.05$) results are shown in boldface type, marginally significant ($0.10 > P > 0.05$) results are shown in italic typeface. Models included the effect of genotype (random) nested within sex and growth, and the effect of plot (random).
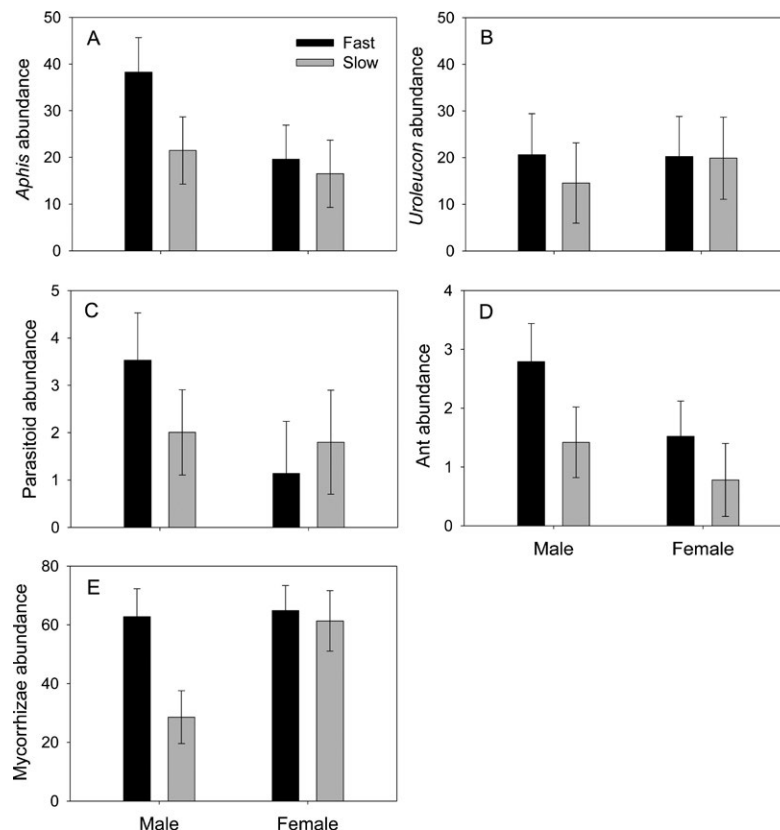

FIG. 2. Least-square means (±SE) of abundance of (A) the generalist aphid *Aphis gossypii*, (B) the specialist aphid *Uroleucon macolai*, (C) parasitoids of *A. gossypii* (Braconidae), and (D) ants (*Linepithema humile*), as well as (E) the abundance of arbuscular mycorrhizal fungi (calculated as the proportion root transect points colonized × gram of dry root biomass) on fast- and slow-growing male and female genotypes of *Baccharis salicifolia*. Least-square means account for the effects of plot and plant genotype nested within sex and growth rate.

Parasitoids and ants.—There were no effects of sex, growth rate, or their interaction on parasitoid abundance (Table 1; Fig. 2). However, males (2.76 ± 1.05 parasitoids) tended to have (1.9×) more parasitoids than females (1.47 ± 0.95 parasitoids; Fig. 2). On the other hand, there were marginally significant effects of growth rate and sex on ant abundance (Table 1), with fast-growing genotypes (2.16 ± 0.55 ants) having 2× more ants than slow-growing genotypes (1.10 ± 0.54 ants), and males (2.11 ± 0.54 ants) having 1.9× more ants than female plants (1.15 ± 0.55 ants; Fig. 2). There was no effect of the sex × growth rate interaction on ant abundance, though fast males tended to have a higher ant abundance than slow males but fast and slow females showed similar ant abundances (Table 1; Fig. 2).

Mycorrhizae.—We found marginally significant effects of growth rate and sex on mycorrhizae abundance (Table 1). Specifically, fast-growing genotypes had a 1.4× greater abundance of mycorrhizae (63.82 ± 6.76 g) relative to slow-growing genotypes (44.94 ± 7.21 g), and females (63.10 ± 7.10 g) showed 1.4× greater abundance than males (45.66 ± 6.93 g; Fig. 2). The sex × growth rate interaction was not significant (Table 1), though fast males had a greater (2.2×) abundance than slow males, but fast and slow females did not differ (Table 1; Fig. 2).

Effects of plant sex and growth rate on associate density

Aphids.—We found a significant effect of plant sex, but not of growth rate, on *Aphis* density (Table 1), with males (113.33 ± 24.84 aphids/kg) having a 1.9× greater density of this generalist aphid than females (60.75 ± 25.10 aphids/kg; Fig. 3). We found no effect of the sex × growth rate interaction on *Aphis* density (Table 1). In contrast, we found no effect of plant sex, growth rate, or their interaction on *Uroleucon* density (Table 1; Fig. 3).

Parasitoids and ants.—We found no effect of plant sex, growth rate, or their interaction on parasitoid density (Table 1; Fig. 3). However, males (10.95 ± 4.24 parasitoids/kg) tended to have greater mean value than females (5.01 ± 4.26 parasitoids/kg; Fig. 3). In addition, there was a significant effect of plant sex on ant density (Table 1), with males having 3× more ants than females (8.28 ± 1.57 and 2.80 ± 2.01 ants/kg, respectively; Fig. 3). There were no effects of growth rate or a sex × growth rate interaction on ant density (Table 1; Fig. 3), suggesting that the difference in ant abundance between fast- and slow-growing genotypes (see above; Fig. 3) was due to variation in resource abundance rather than habitat quality.
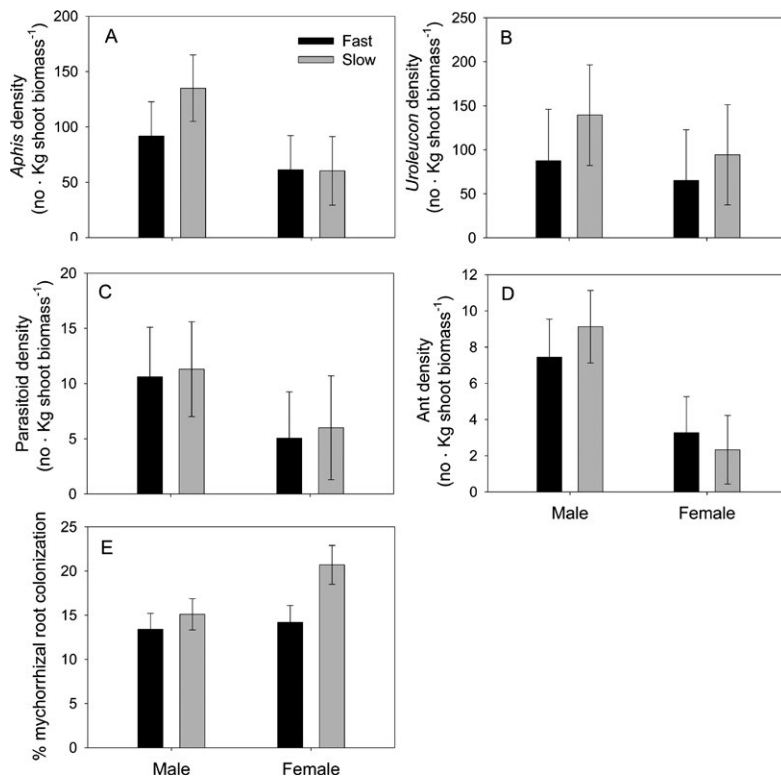
FIG. 3. Least-square means (±SE) of density (number of insects per kilogram of dry aboveground biomass) of (A) the generalist aphid *Aphis gossypii*, (B) the specialist aphid *Uroleucon macolai*, (C) *A. gossypii* parasitoids, and (D) ants (*Linepithema humile*), as well as (E) percentage of root colonization by arbuscular mycorrhizal fungi for fast- and slow-growing male and female genotypes of *Baccharis salicifolia*. Least-square means account for the effects of plot and plant genotype nested within sex and growth rate.

Mycorrhizae.—We found significant and marginally significant effects of growth rate and sex, respectively, on mycorrhizae density (i.e., percentage of colonized transect points; Table 1). Specifically, slow-growing genotypes had a 1.3× greater percent of mycorrhizal root colonization (17.95% ± 1.44%) relative to fast-growing genotypes (13.82% ± 1.37%), and females (17.49 ± 1.43%) showed 1.2× greater percent colonization than males (14.28% ± 1.40%) (Fig. 3). There was no evidence of a sex × growth rate interaction (Table 1), though slow-growing females tended to have greater colonization than fast-growing females, whereas fast and slow males did not differ (Fig. 3).

Residual plant genetic effects on associates

We compared the effects of plant sex and growth with other forms of (residual) genetic variation. We assessed residual plant genetic variation on the abundance and density of associates by testing for the effect of plant genotype from models accounting for sex and growth. Residual plant genetic effects were nonsignificant for aphids, parasitoids, and mycorrhizae, but there were significant and marginally significant genetic effects on ant abundance (up to 1.6-fold variation among genotypes) and density (up to 1.8-fold variation), respectively (Table S2; Fig. S2).

DISCUSSION

We show that genetic variation in *B. salicifolia* growth rate and sex acted independently and via different mechanisms on above- and belowground associates, and such effects superseded those coming from other (unrelated, residual) sources of plant genetic variation. Plant sex affected insects and arbuscular mycorrhizal fungi through variation in habitat quality (i.e., plant nutrients and defenses, natural enemies, mutualists, competitors) whereas plant growth rate exerted effects through variation in resource abundance (but not habitat quality). The magnitude and direction of plant genetic effects in turn varied among associates. For the dietary generalist herbivore *Aphis*, males provided superior habitat quality, fast-growing genotypes tended to provide greater resource abundance (though the growth effect on *Aphis* abundance was not significant), and these effects in turn cascaded up to influence ants (but not parasitoids). In contrast, there were no detectable genetic effects of plant sex or growth rate effects on the dietary specialist herbivore *Uroleucon*. For mycorrhizae, females and slow-growing genotypes had higher levels of root colonization, showing that plant genetic variation had opposing effects on below- vs. aboveground associates. Across all associates, the effects of plant sex and growth rate acted independently (with the single exception of a marginally significant interaction on *Aphis* abundance). Finally, having accounted for genetic variation in plant sex and growth

rate, weak residual genetic variation was only found for the abundance and density of ants. Overall, by manipulating genetic variation in plant sex and growth, this study builds toward a predictive understanding of the concurrent influence of multiple sources of genetically based plant trait variation on consumer communities.

Plant genetic effects on above- and belowground associates

Contrary to general patterns within dioecious species (Ågren et al. 1999, Cornelissen and Stiling 2005), male and female plants did not differ in growth rate (Table 1; Fig. 1). Although we found a significant sex × growth rate interaction for plant biomass, this occurred because variation between fast- and slow-growing genotypes was greater for males (2.7×) than for females (1.4×), while mean male and female growth was similar (Fig. 1). Importantly, the source garden of 39 randomly sampled genotypes showed no effect of plant sex on growth after 10 months (Fig. S1). Our conclusion of minimal sexual dimorphism for *B. salicifolia* growth is consistent with previous work on other *Baccharis* species (e.g., Espirito-Santo et al. 2012, see also Krischik and Denno 1990, Carneiro et al. 2006), possibly due to relatively equal investments by males and females in growth in this lineage.

Variation in plant growth rate in turn affected the abundance but not density of the dietary generalist *Aphis* (Table 1, Figs. 2, 3), demonstrating effects of growth rate through resource abundance but not habitat quality for this herbivore. Specifically, the greater variation in growth rate for males (vs. females; Fig. 1) translated into a trend for *Aphis* abundance to vary more between fast- and slow-growing males (1.8x) than females (1.3×; Table 1; Fig. 2). Plant genetic variation in growth rate thus cascaded up to influence higher trophic levels in a manner parallel to that occurring through ecological variation in nutrients and other resources affecting productivity (Mooney et al. 2010). Yet the fact that there was no effect of plant growth on *Aphis* density (Table 1, Fig. 3) demonstrates that habitat quality did not vary with plant growth. This finding runs counter to predictions for growth-resistance trade-offs that have been observed to occur among species (Coley et al. 1985, Fine et al. 2004, Mooney et al. 2010), and suggests that such trade-offs may be dependent on the scale of genetic variation (i.e., among vs. within species) at which they are investigated (Agrawal et al. 2010).

Male *B. salicifolia* (vs. female plants) had both a higher abundance and density of *Aphis* (Table 1, Figs. 2, 3), indicating that they conferred greater habitat quality to consumers. The specific plant traits (e.g., nutrients, defenses) or mechanisms (e.g., plant effects on herbivore mutualists or antagonists) mediating the effects of plant sex on *Aphis* are unknown. Nevertheless, this finding

is consistent with past studies of this plant (Mooney et al. 2012b) and with general patterns of lower resistance and defense in males (Ågren et al. 1999, Cornelissen and Stiling 2005). Furthermore, the fact that this sexual dimorphism in habitat quality was not associated with sexual dimorphism in growth (Table 1, Fig. 1) is suggestive that there are no growth-associated trade-offs with resistance in this species. More generally, this finding calls into question whether the broadly observed covariance between growth rate and resistance and defense in dioecious species (Ågren et al. 1999, Cornelissen and Stiling 2005) is underlain by growth–defense trade-offs, suggesting instead that each axes of sexual dimorphism may arise independently.

Sexual dimorphism in *Aphis* resource abundance and habitat quality in turn cascaded up to higher trophic levels. The abundance and density of ants associated with *Aphis* were also higher on males than females, and there was a nonsignificant pattern of parasitoids responding similarly (Table 1, Figs. 1, 2). Fast-growing *B. salicifolia* genotypes also tended to have higher ant abundance, but this effect was mediated by differences in plant biomass rather than habitat quality as there were no effects of growth rate on ant density. Petry et al. (2013) similarly found sexual dimorphism in *Valeriana edulis* recruitment of aphids, which in turn resulted in a greater abundance of aphid-tending ants, predators, and parasitoids on females. It is also possible that plant traits such as volatile emissions (Heil 2008) or floral resources (Petry et al. 2013) directly mediated the effects of plant sex on higher trophic levels.

Contrary to *Aphis*, neither plant sex nor growth rate affected the dietary specialist aphid *Uroleucon macolai* (Table 1, Figs. 1, 2). These findings are consistent with past studies showing dietary generalist to be more susceptible than specialists to both plant resistance (Bernays 1998, Cornell and Hawkins 2003, Coley et al. 2006) and plant-mediated variation in natural enemies (Singer et al. 2012, 2014). In addition, these results also agree with a past greenhouse study with *B. salicifolia* showing *Aphis* performed better on males (vs. females), whereas *Uroleucon* was unaffected by plant sex (Mooney et al. 2012b). Overall, these findings suggest that diet breadth may mediate the bottom-up effects of plants on higher trophic levels (Singer et al. 2014), although drawing such conclusions from the present study alone is speculative given that we only compare two herbivore species.

The effects of plant sex and growth rate on arbuscular mycorrhizal fungi contrasted with those on aboveground associations with insects. Female plants (vs. males) tended to have higher abundance and density of root colonization by arbuscular mycorrhizal fungi (Table 1, Figs. 2, 3). This agrees with results from Vega-Frutis et al. (2013) showing higher mycorrhizal colonization on females than males of the dioecious *Antennaria dioica*. Relatedly, Varga and Kytöviita (2010) showed that female plants of the gynodioecious *Geranium sylvaticum* had higher levels of nutrient acquisition from mycorrhizae than hermaphrodites. Our findings, together with these studies, suggest a broad pattern of sexual dimorphism in this association. At the same time, fast-growing genotypes had more root biomass and in turn higher mycorrhizal abundance, but slow-growing genotypes had higher mycorrhizae density (i.e., levels colonization). This suggests that slow-growing genotypes compensate for reduced growth by recruiting more mycorrhizal fungi to increase resource acquisition or, alternatively, that there is a trade-off between growing more and investing in this mutualistic association. Collectively, these findings indicate that sex- and growth-related traits represent important axes of plant genetic variation influencing interactions with mycorrhizal fungi, and calls for further work addressing the mechanisms underlying these effects (Johnson et al. 2012).

Differences in mycorrhizal colonization associated with plant sex or growth rate could have in turn affected observed patterns of biomass allocation as well as other plant traits influencing aboveground associates. For instance, higher mycorrhizal colonization in females may have reduced or eliminated differences in growth between male and female plants, weakening growth–defense trade-offs. Likewise, higher root colonization on female plants could have led to lower plant quality via induced defenses and reduced insect densities; previous studies have shown that arbuscular mycorrhizal fungi trigger the production of phenolic compounds and terpenoids (Jung et al. 2012), and the latter class of compounds are particularly abundant in *B. salicifolia*. Further work is necessary to tease a part the effects of mycorrhizae on plant traits (e.g., defenses, nutrients) and evaluate how such plant trait modifications influence other associates.

In comparison to plant genetic variation of sex and growth rate, there were no detectable residual genetic effects on aphids, parasitoids, or mycorrhizae, while genetic variation in ant abundance and density (Table S2, Fig. S2) was relatively small in magnitude. There is a substantial literature on the influence of plant intraspecific genetic variation on associated multitrophic communities (reviewed by Bailey et al. 2009, Mooney and Singer 2012) and few studies have failed most studies have found large, ecologically significant effects. Accordingly, our finding of weak residual genetic effects is suggestive that plant sex and growth rate are key axes of genetic variation driving interactions with associates.

Conclusion

Manipulating different sources of plant genetic variation for specific traits can significantly advance our

understanding of the mechanisms driving effects on associated communities. Focusing on specific traits or combinations of traits (vs. genotype identity per se) provides a means of moving beyond the descriptive realm to instead provide a predictive understanding of which traits or axes of trait variation drive these dynamics. This approach is especially valuable when applied to plant functional traits with both strong effects on consumers and for which there is a good understanding of the ecological and evolutionary factors that shape their variation. With the present study, both empirical studies and theory provide a framework for predicting within- and among-population variation in plant growth rate (e.g., Fine et al. 2004, Pratt and Mooney 2013) and plant sex (e.g., Wasser 1984, Mitton and Grant 1996). Accordingly, our understanding of the processes driving variation in sex- and growth-related plant traits may in turn allow prediction of concomitant patterns of variation in plant-centered communities.

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SUPPORTING INFORMATION