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Specificity of plant–plant communication for *Baccharis salicifolia* sexes but not genotypes

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Abstract. Plants are able to adjust their anti-herbivore defenses in response to the volatile organic compounds (VOCs) emitted by herbivore-damaged neighbors, and some of these changes increase resistance against subsequent herbivory. This phenomenon of plant–plant communication is thought to be widespread, but recent investigations have cautioned that it can be context dependent, including variation in the strength of communication based on the identity of plants and their associated herbivores. Here, we performed three greenhouse experiments using multiple male and female genotypes of the dioecious woody shrub *Baccharis salicifolia* and its specialist aphid *Uroleucon macolai* to test for specificity of plant–plant communication with respect to plant sex and genotype. Moreover, we evaluated plant sexual dimorphism and genotypic variation in VOC emissions (i.e., the "speaking" side of the interaction) and response of plants to VOC exposure (i.e., the "listening" side of the interaction) in order to identify the chemical mechanisms underlying such specificity. We did not find genotypic specificity of communication; emitter plants damaged by *U. macolai* significantly reduced subsequent *U. macolai* performance on receivers, but these effects were indistinguishable for communication within vs. among genotypes. In contrast, we found sex specificity of communication; male emitter plants reduced subsequent *U. macolai* performance on male and female receiver plants equally, while female emitter plants only did so for female receivers. We found sexual (but not genotypic) dimorphism in speaking but not listening; of the seven compounds induced by *U. macolai* feeding (speaking), pinocarvone was approximately fivefold greater in female than in male plants, while exposure of plants to pinocarvone emissions (listening) reduced *U. macolai* performance equally in both male and female plants. Together, our study demonstrates novel evidence for sexually dimorphic specificity of plant–plant communication and the chemical mechanism underlying this effect.

Key words: Baccharis salicifolia; *emitters; herbivory; plant dioecy; receivers;* Uroleucon macolai; *volatile organic compounds.*

Introduction

Three decades of work have demonstrated that plants send, receive, and respond to a rich suite of signals from their conspecifics (Karban 2015). In particular, plants are able to adjust their defenses in response to the volatile organic compounds (VOCs) emitted by herbivore-damaged neighbors, and some of these changes make them more resistant to subsequent herbivory (Karban 2015). This phenomenon of plant–plant communication has been documented in over 35 plant species spanning 16 families (reviewed by Heil and Karban 2010, Karban et al. 2014a). However, recent investigations have cautioned that such phenomena can be context dependent (Moreira and Abdala-Roberts 2019), including variation in the strength of communication depending on plant genotypic (Karban and Shiojiri 2009, Karban et al. 2014b, Moreira et al. 2016) and herbivore (Moreira et al. 2018) identity.

A wide range of herbivore-induced VOCs have been implicated in plant–plant communication, but little is known about the mechanistic basis for specificity in these interactions. The VOCs potentially underlying plant–plant communication include methylated forms of plant hormones, green leaf volatiles, and terpenoids (Rowen and Kaplan 2016, Aartsma et al. 2017), and such compounds have been shown to prime neighboring undamaged plants for enhanced responses upon subsequent insect attack (Erb et al. 2015, Moreira et al. 2018). The composition of herbivore-induced VOC blends is known to be highly specific to plant genotype (e.g., Degen et al. 2004, Wason and Hunter 2014), plant sex (e.g., Ashman 2009), and herbivore identity (Clavijo McCormick et al. 2012, Moreira et al. 2018).

Specificity of plant–plant communication occurs when one or more VOC compounds (or combinations of compounds) are uniquely emitted by damaged plants (specificity of "speaking") and when receiver plants also respond uniquely to these same compounds (specificity of "listening"). For example, Moreira et al. (2018) demonstrated *Baccharis salicifolia* to have herbivore-specific responses to two aphid species (*Uroleucon macolai* and *Aphis gossypii*), such that resistance in the receiver was specific to the identity of the herbivore damaging the emitter. In addition, the composition of herbivore-induced VOCs differed in response to damage by each herbivore, demonstrating specificity of speaking. While the experimental exposure of plants to the VOCs induced by *U. macolai* induced resistance to that same herbivore, this study did not investigate whether these same VOCs also induced resistance to *A. gossypii*, nor

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whether the VOCs induced by *A. gossypii* induced resistance to *U. macolai*. Neither this study nor any other study to our knowledge has confirmed the mechanisms underlying specificity of plant–plant communication on both the speaking and listening sides of the interaction.

Genotypic specificity of plant–plant communication has been demonstrated for two plant species (*Artemisia tridentata* [Karban and Shiojiri 2009, Karban et al. 2013, 2014b] and *Phaseolus lunatus* [Heil and Silva Bueno 2007, Moreira et al. 2016]), indicating stronger signaling and perceiving among genetically similar than distinct individuals. Three explanations have been offered to explain why genotypic specificity of plant–plant communication might occur. First, communication between non-related individuals should be costly for the emitter plants as they would be altruistically increasing competitors' fitness at the expense of their own (Heil and Ton 2008). Second, signaling among genetically similar individuals may arise as a by-product of plants using volatiles for rapid intra-plant signaling in cases where signaling via vascular connectivity is limited (Frost et al. 2007, Heil and Silva Bueno 2007). Finally, because plant susceptibility to herbivores and pathogens has a strong genetic component (e.g., Johnson and Agrawal 2005), responding to damage of genetically related individuals should be beneficial due to shared susceptibilities. Because few studies have tested for genotypic specificity of plant–plant communication, more information is needed in order to understand the prevalence, causes, and consequences of this phenomenon.

Sexual dimorphism is an important axis of plant genetic variation generally (Ashman 2009), and for herbivore defense in particular (Cornelissen and Stiling 2005), but no past studies have tested for its consequences for plant–plant communication. Male and female plants of dioecious species largely differ in how well defended they are against herbivores in terms of both direct (Agren et al. 1999, Cornelissen and Stiling 2005, Cepeda-Cornejo and Dirzo 2010) and indirect defense (Mooney et al. 2012a, Petry et al. 2013). Female plants typically invest more resources in reproduction than males, grow more slowly and have higher levels of defenses against herbivores (Agren et al. 1999, Cornelissen and Stiling 2005), and this may extend to volatile-induced defense. If this is the case, it implies that plant communication in response to herbivore damage should be stronger within than between sexes.

In this study, we investigated genotypic and sex specificity of plant–plant communication and the underlying chemical mechanisms. We carried out three greenhouse experiments using multiple male and female genotypes of the dioecious woody shrub *B. salicifolia* (Ruiz & Pav.) Pers. (Asteraceae) and the aphid *U. macolai* Blanchard (Hemiptera: Aphididae), a dietary specialist upon *Baccharis*. In separate experiments, we address three questions: (1) Is there genotypic and sex specificity of plant–plant communication? (2) Is there specificity in the VOCs emitted by herbivore damage, i.e., the "speaking" side of the interaction? And (3) is there specificity in the response of plants to these VOCs, i.e., the "listening" side of the interaction? In so doing, we build upon past studies to provide a novel test for the genetic basis and underlying mechanisms of specificity of plant–plant communication.

Material and Methods

Natural history

Baccharis salicifolia is a long-lived, dioecious, woody shrub that grows mostly in riparian or slightly wetter habitats throughout southwestern United States and northern Mexico. Our study was based on a natural population of *B. salicifolia* occurring in 80 ha of habitat within the University of California San Joaquin Marsh Reserve (33.66° N, 117.85° E; Orange County, California, USA; Mooney et al. 2012b, Moreira and Mooney 2013). Both plant sexes and genotypes vary in arthropod community composition (Abdala-Roberts et al. 2016, Nell et al. 2018). In particular, previous studies have demonstrated that male plants exhibit higher herbivore abundance and density than female plants (Abdala-Roberts et al. 2016, Nell et al. 2018). In this area, *B. salicifolia* is commonly colonized by the specialist aphid *Uroleucon macolai* that feeds on non-woody terminal stems (Mooney et al. 2012b, Abdala-Roberts et al. 2016). This aphid species has an exceptionally narrow diet breadth, feeding only upon *B. salicifolia* and *B. polifolia* Griseb (Blackman and Eastop 2006).

Experiment 1: Sex and genotype specificity of plant–plant communication

To investigate sex and genotype specificity of plant–plant communication, in January 2014 we cloned eight male and eight female *B. salicifolia* genetic lines ("genotypes" hereafter) from our study site. Clonal copies of parental genotypes originated from 10 cm long stem cuttings of mature plants. To obtain distinct genotypes, we collected cuttings from 16 plants, the most distant of which were separated by 500 m. In the greenhouse, we placed fresh cuttings in perlite medium under a misting bench for four weeks and then transplanted them to 2-L pots with a soil mixture composed of equal parts peat moss, redwood compost, silica sand, and pumice mixed with slow-release fertilizer at a concentration of 0.5 g/L of soil. At the same time, we collected aphids from a single stem and reared them on potted *B. salicifolia* in a separate greenhouse. We watered all plants every 2–3 d to field capacity and maintained them at 22–25°C. We grew plants under ambient light in a semiopaque greenhouse, thus providing natural light cycles and light levels less than full sun, but likely similar to those experienced by plants growing within the shaded environment of dense stands. We provided cooling by evaporative coolers, and we positioned experimental plants away from direct exposure to fans. On April 2014, when plants reached approximately 30 cm in height (main stem), we assigned three *B. salicifolia* plants to a mesh fabric cage (69 × 69 × 122 cm) in order to prevent aphid dispersal and colonization by aphid natural enemies. One plant acted as the emitter and was placed at the center of the cage, whereas the other two served as the receivers. Within each cage, one of the receiver plants was from the same sex as the emitter plant, while the other one was from a different sex. Within emitter–receiver plants from the same sex, in one-half of the cages, the receivers were from the same genotype (i.e., clones) than the emitter plant while, in the other one-half of the cages, the receivers were from a different genotype. We assigned emitter

plants to two treatments: (1) subjected to *U. macolai* feeding (herbivore-induced plants), and (2) control (untreated plants). In total, there were 96 cages (48 control and 48 herbivore-induced) for a total of 192 receiver plants and 96 emitter plants. We separated emitter and receiver plants inside the cages by a minimum of 20 cm. Adjacent cages were spaced by 2 m to prevent cross-communication among replicates: 60 cm was determined to be the likely distance for VOC transmission between plants in natural settings (Karban et al. 2006, Heil and Adame-Álvarez 2010).

In the herbivore-induced treatment, we added 15 unwinged, mature (reproductive) *U. macolai* individuals to a single growing tip of each emitter plant using a fine paintbrush. Aphids fed and reproduced on the emitter plants for 15 d, after which emitter plants were removed, while receivers remained inside the cages. During this period of exposure, aphid densities on emitters remained sufficiently low to avoid the induction of winged morphs. Upon removal of the emitter plants, each receiver plant was inspected to guarantee it was aphid free (all were) and then inoculated with two unwinged adult aphids using a fine paint brush. Aphids were placed on the growing tips of a branch and the length of this branch was recorded. After the aphids reproduced (between 24 and 48 h), the two inoculate adults and all but two nymphs were removed. We then monitored these two remaining nymphs for reproductive rate daily (number of nymphs per day and aphid) until the fifth day of reproductive maturity (Mooney et al. 2012b, Moreira et al. 2018).

We analyzed reproductive rate (nymphs produced) on the fifth reproductive day as a metric of aphid performance on receiver plants with linear mixed models using PROC MIXED in SAS (SAS 9.4; SAS, Cary, North Carolina, USA). We treated the main effects of emitter induction treatment (two levels: emitters as control and *U. macolai* feeding), plant sex heterogeneity treatment (receiver sex different vs. same as the emitter), plant genotype heterogeneity treatment (receiver genotype different vs. same as the emitter), and the emitter induction × sex heterogeneity treatment and emitter induction × genotype heterogeneity treatment interactions as fixed factors. We treated the effect of cage as a random factor to account for the non-independence of the two receivers paired with each emitter. Because larger branches can support larger aphid colonies and affect in turn aphid reproductive rate, we included the total length of each aphid-bearing branch as a covariate. As we found a significant emitter induction × sex heterogeneity treatment interaction on aphid reproductive rate (see *Results* section), we further assessed sex-specific responses underlying this interaction by analyzing emitter males and females separately.

Experiment 2: Plant sex and genetic variation in the emission of herbivore-induced VOCs (speaking side of interaction)

To address this goal, we used a subset of data presented in Moreira et al. (2018). In this previous paper, we tested for the specific VOCs emitted after feeding by two aphid species, the generalist *Aphid gossypii* and the specialist *U. macolai* (Moreira et al. 2018). In the current paper, we tested for sex and genetic variation in the emission of *U. macolai*-induced VOCs (speaking side of interaction). In January 2014, we cloned three male and three female *B. salicifolia* genotypes from the same population and using the same propagation methods from Experiment 1. After three months, we randomly assigned plants of each genotype to one of two induction treatments: control (untreated) and subjected to *U. macolai* feeding (15 individual aphids per plant). We assigned 18 plants to the control treatment and 20 plants to the *U. macolai* treatment, with plant genotypes represented approximately equally among and within the treatments (i.e., three clones of each genotype per treatment). We individually enclosed each plant in a mesh fabric bag. Two weeks after initiating induction treatments, we collected aboveground VOCs emitted from each plant following Rasmann et al. (2011). Briefly, we bagged plants with a 2-L bag and adsorbed VOCs on a charcoal filter trap (Orbo-32; Supelco, Bellefonte, Pennsylvania, USA) for 6 h at a rate of 0.25 L/min. We eluted traps with 150 μL dichloromethane (Merck, Dietikon, Switzerland) to which we had previously added the internal standard (tetraline [Sigma-Aldrich, St. Louis, Missouri, USA], CAS number: 119-64-2, 198 ng in 10 μL dichloromethane). We subsequently injected 5 μL of each sample onto a GC-MS (Agilent 6890 Gas Chromatograph [GC] coupled with a 5973N Mass Selective Detector; Agilent, Santa Clara, California, USA) fitted with a 30 m × 0.25 mm × 0.25 μm film thickness HP-5MS fused silica column (Agilent). We operated the GC in splitless mode with helium as the carrier gas (flow rate 1 mL/min). The GC oven temperature program was 1 min hold at 50°C, 10°C/min ramp to 130°C, 5°C/min ramp to 180°C, 20°C/min ramp to 230°C, and 1 min hold at 300°C. We identified volatile terpenes using Kovats retention index from published work (Loayza et al. 1995, Zunino et al. 1997) and by comparison with commercial standards when available. We measured total emission of individual VOCs as a proportion to the internal standard (Moreira et al. 2018).

We tested for the influence of plant sex (males vs. females) and genotype on the induced emission of volatile organic compounds. We restricted the analyses to those VOCs that were significantly induced by *U. macolai* feeding (Moreira et al. 2018): limonene, methyl salicylate, nonatriene, (E)-β-ocimene, ethanone, β-myrcene, and pinocarvone. Pinocarvone was previously referred to as an unknown compound with Kovats retention index = 11.59 (Moreira et al. 2018: Table 3). For each VOC, we calculated an effect size and 95% confidence interval (Hedges et al. 1999) for induction by aphids ("induction effect" hereafter) as the natural log of the ratio of emissions from plants fed upon by *U. macolai* to those from undamaged control plants. Because some induced compounds were not detected in the control treatment, a constant (the lowest emission detected) was added to each compound prior to calculating the response ratio. The VOCs' induction effects can be inferred to differ significantly between male and female plants or between plant genotypes when their 95% confidence intervals did not overlap, although this approach is liberal with respect to type I error if adjustments are not made for multiple comparisons (Garcia 2004).

Experiment 3: Plant sex variation in the response to herbivore-induced VOCs (listening side of interaction)

To understand whether there was sex variation in the response of plants to herbivore-induced VOCs (listening), in April 2018 we conducted a third greenhouse experiment in

which we exposed 32 individuals of *B. salicifolia* belonging to nine genotypes (four male and five female genotypes) to three volatile treatments: (1) exposure of plants to pinocarvone (Sigma-Aldrich, CAS number: 6485-40-1), (2) exposure of plants to a blend of six VOCs, namely pinocarvone, methyl salicylate (Sigma-Aldrich, CAS number: 119-36-8), ethanone (Sigma-Aldrich, CAS number: 122-00-9), limonene (Sigma-Aldrich, CAS number: 5989-27-5), myrcene (Sigma-Aldrich, CAS number: 123-35-3), and ocimene (Sigma-Aldrich, CAS number: 13877-91-3), or (3) a control. We chose pinocarvone because its emission significantly increased after *U. macolai* feeding (control, 8625.20 ± 4629.06 ng/h; *U. macolai* feeding, 24444.00 ± 3498.25 ng/h; Moreira et al. 2018) and significantly varied between plant sexes (see *Results*). We also chose the treatment containing a blend of six VOCs because all these compounds were highly inducible after *U. macolai* feeding (Moreira et al. 2018) and this treatment also provided a means to test whether the activity of pinocarvone required the presence of these other compounds. We grew experimental plants from cuttings as described above (Experiments 1 and 2). Within each treatment, we used 16 individual plants from nine different genotypes.

Volatile exposure treatments consisted of exposing plants to artificial emitters containing pinocarvone or empty artificial emitters for control plants following the methods of Moreira et al. (2018). We constructed artificial emitters with 2-mL glass chromatographic vials topped with screw thread caps with a Teflon septa through which we passed a 12.5-cm capillary tube (100-μL ring caps). Each vial contained a small piece of cotton inoculated with 100 μL of the pure compound (pinocarvone) or not (control). For the treatment containing a blend of six VOCs, we attached six artificial emitters to each plant, with 100 μL of a single compound in each vial. We then secured these emitters to the sides of pots of experimental plants within mesh cages for an exposure period of 7 d. We placed artificial emitters within 10 cm of *B. salicifolia* plants. During the exposure period, we randomly distributed cages at a minimum of 2.5-m separation. After exposure, we removed the emitter vials and conducted an aphid performance bioassay with *U. macolai* using the same methodology as described above (Experiment 1).

Table 1. Linear mixed model results showing the effects of the emitter induction treatment (herbivory by *Uroleucon macolai* vs. control), plant sex heterogeneity (different vs. same sex of emitter plant), plant genotype heterogeneity (different vs. same genotype of emitter plant), and their interactions on the performance of the specialist aphid *U. macolai* on *Baccharis salicifolia* receiver plants.

Variable	*F*	df	*P*
Treatment	47.34	1,74	**<0.001**
Sex	2.66	1,74	0.107
Genotype	6.12	1,74	**0.016**
Treatment × Sex	4.88	1,74	**0.030**
Treatment × Genotype	0.16	1,74	0.693
Branch length	2.33	1,74	0.131

Notes: Aphid performance was measured as the reproductive rate on the fifth reproductive day. Branch length was used as a covariate. Significant *P* values ($P < 0.05$) are shown in boldface type.

We analyzed reproductive rate (nymphs produced) on the fifth reproductive day with linear models using PROC GLM in SAS 9.4. We conducted two separate analyses, one comparing control to pinocarvone alone and one comparing control to all compounds in combination. In each analysis, we treated the main effect of exposure treatment, plant sex, and their interaction as fixed factors. To account for size differences among plants, we also included plant length as a covariate. We did not include the effect of plant genotype due to insufficient replication. A significant exposure treatment × plant sex interaction indicates plant sex variation in the response to exposure treatments.

Results

Experiment 1: Sex and genotype specificity of plant–plant communication

The emitter induction treatment significantly affected aphid reproductive rate on neighboring receiver plants

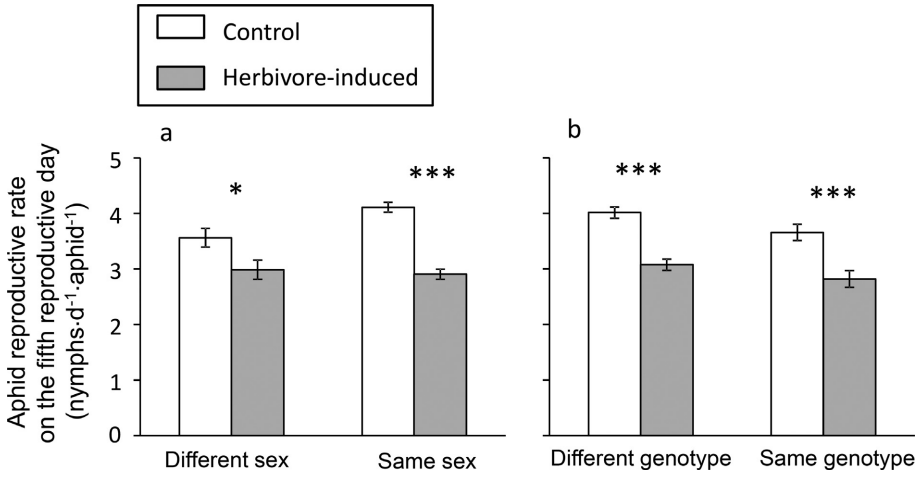

Fig. 1. Effect of herbivore-induced treatments in the emitter plants (control vs. herbivory by *Uroleucon macolai* feeding, white and gray bars, respectively) on the performance (reproductive rate on the fifth reproductive day) of the specialist aphid *U. macolai* on *Baccharis salicifolia* receiver plants belonging to (a) different vs. same plant sex and (b) different vs. same plant genotype. Values are least-square means ± SE ($N = 24$). Asterisks indicate significant differences within herbivore-induced treatments at $*P < 0.05$ and $***P < 0.001$.

(Table 1). Specifically, *Uroleucon macolai* reproductive rate on the fifth reproductive day on receiver plants that were adjacent to emitters damaged by *U. macolai* was 30% lower compared with the *U. macolai* reproductive rate on the fifth reproductive day on receiver plants that were adjacent to undamaged emitters (Fig. 1). However, this overall effect of plant–plant communication depended largely on emitter and receiver sex (significant emitter induction treatment × sex heterogeneity interaction in Table 1); decreases in aphid reproductive rate on receiver plants exposed to herbivore-damaged neighbors (compared to undamaged neighbors) were greater when emitter and receiver plants were from the same sex (Fig. 1a). In contrast, plant–plant communication did not depend on emitter and receiver genotype (no significant emitter induction treatment × genotype heterogeneity interaction in Table 1); decreases in aphid reproductive rate on receiver plants exposed to herbivore-damaged neighbors were similar whether or not the emitter and receiver plants were from the same genotype (Fig. 1b).

To further assess sex-specific responses underlying the above interaction, we analyzed data from male and female emitters separately. Aphid feeding on male emitters (vs. control) reduced aphid reproductive rate on the fifth reproductive day by 30% regardless of receiver sex (no emitter induction treatment × receiver sex interaction; Appendix S1: Table S1, Fig. 2a). In contrast, aphid feeding on female emitters (vs. control) reduced aphid reproductive rate on the fifth reproductive day by 30% on female receivers but had no effect on male receivers (significant emitter induction treatment × receiver sex interaction; Appendix S1: Table S2, Fig. 2b). Framed from the perspective of the receiver, females responded to herbivore-induced VOCs from both male and female emitters, whereas males only responded to male emitters.

Experiment 2: Plant sex and genetic variation in the emission of herbivore-induced VOCs (speaking side of interaction)

We found plant sex (but not genotypic) variation in the emission of herbivore-induced VOCs (speaking). In particular, we found that induction of pinocarvone significantly differed between plant sexes, being approximately fivefold greater in female than in male plants (Fig. 3a). Contrarily, we found no genotypic variation in total and individual herbivore-induced VOCs (Fig. 3b).

Experiment 3: Plant sex variation in the response to herbivore-induced VOCs (listening side of interaction)

Pinocarvone exposure significantly reduced aphid reproductive rate on the fifth reproductive day both alone and in combination with other compounds; in both cases, these effects were not contingent on plant sex (no sex-by-treatment interaction in Table 2, i.e., no sexual dimorphism in listening). Overall, aphid reproductive rate on the fifth reproductive day was 35% lower in plants exposed to pinocarvone than in control plants (Fig. 4). Similarly, aphid reproductive rate on the fifth reproductive day was 33% lower in plants exposed to a blend of six VOCs than in control plants (Fig. 4).

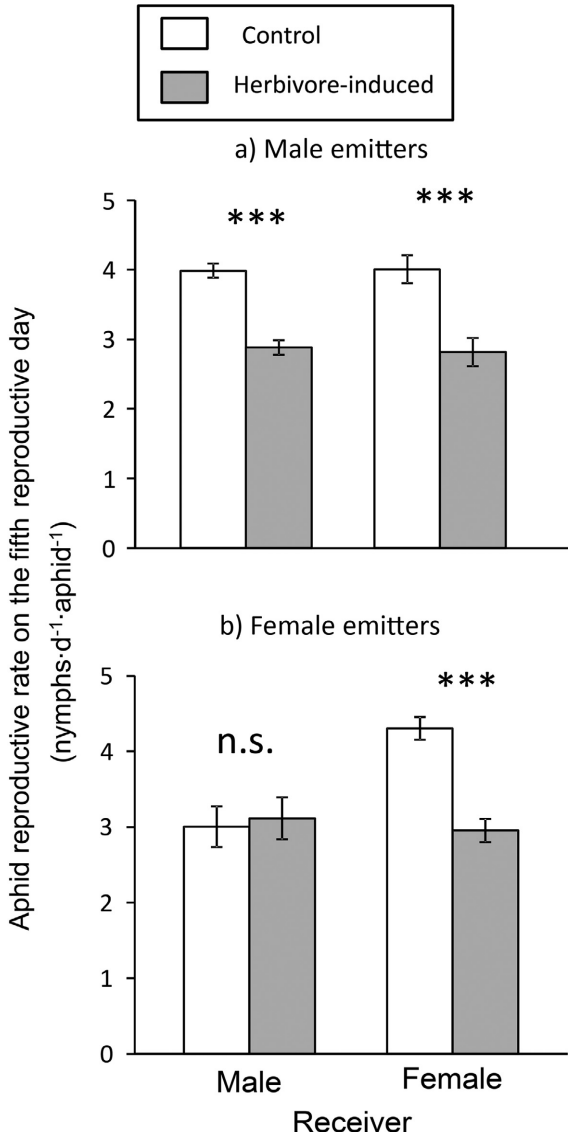

FIG. 2. Effect of herbivore-induced treatments in (a) male and (b) female emitter plants (control vs. herbivory by *Uroleucon macolai* feeding, white and gray bars, respectively) on the performance (reproductive rate on the fifth reproductive day) of the specialist aphid *U. macolai* on male and female *Baccharis salicifolia* receiver plants. Values are least-square means ± SE ($N = 24$). Asterisks indicate significant differences within herbivore-induced treatments at ***$P < 0.001$; n.s., not significant.

DISCUSSION

Our findings confirm the existence of communication between *Baccharis salicifolia* plants in response to *U. macolai* feeding (Moreira et al. 2018) and demonstrate specificity of communication with respect to plant sex but not genotype. In particular, female emitter plants only induced resistance on female receivers, whereas male emitter plants induced resistance on both male and female receivers. As a potential mechanism for these findings, we found sexually dimorphic induction of VOCs by *U. macolai* feeding, with especially strong induction of pinocarvone in female plants. However, this sex-based specificity of speaking was not

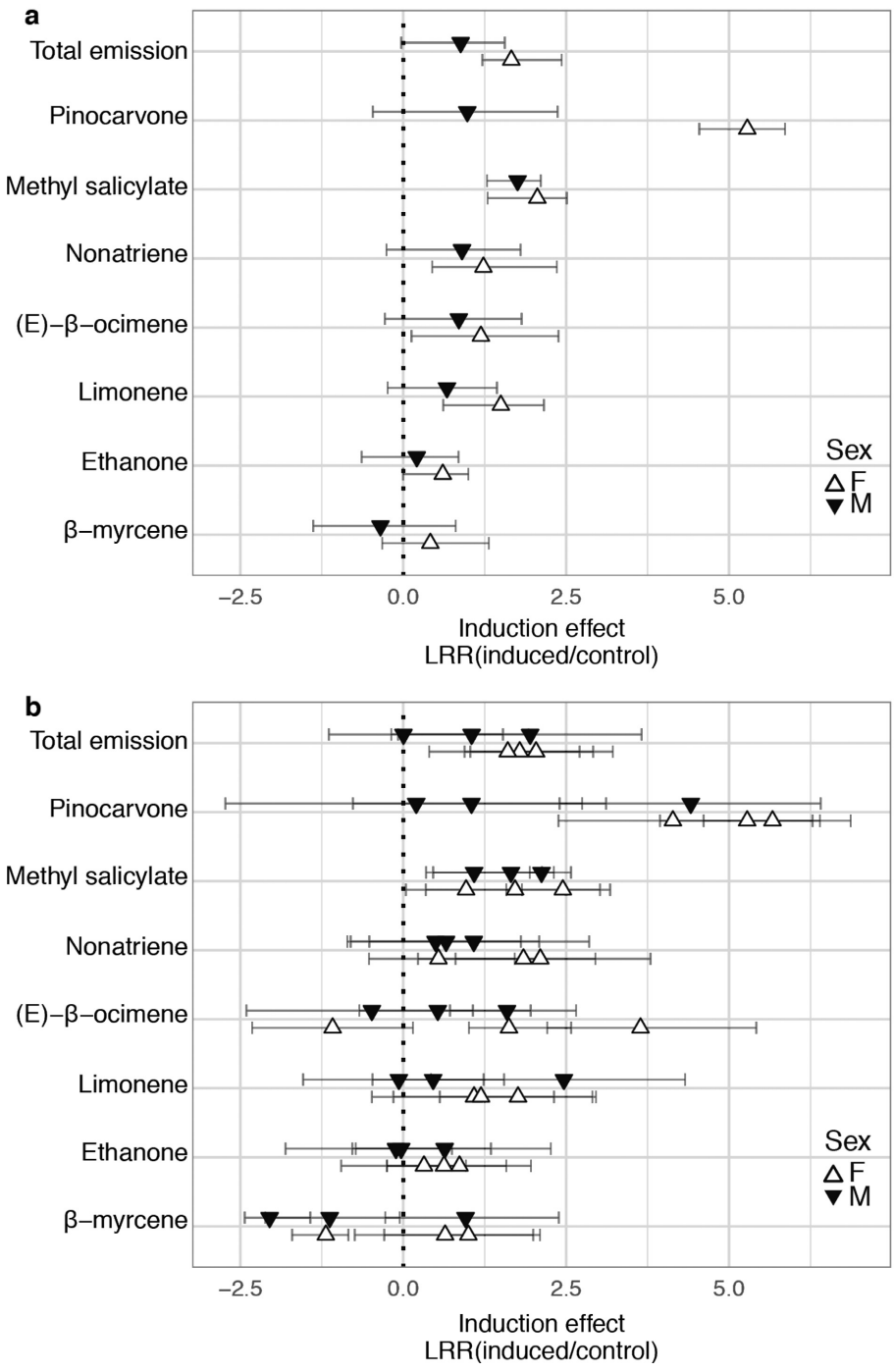

FIG. 3. Effect sizes for the influence of *Baccharis salicifolia* (a) sex (males vs. females) and (b) genotype (three males and three females) on the induced emission of volatile organic compounds (VOCs). For each VOC, we calculated an effect size as the log response ratio (LRRs) of the emissions induced by *Uroleucon macolai* feeding to control plants (induction effect) and its 95% confidence interval. VOCs induction effect significantly differed between male and female plants or between plant genotype within sexes when their 95% confidence intervals did not overlap. Open triangles represent female plants and solid triangles represent male plants. F, female; M, male.

complemented by parallel sex specificity of listening as this compound induced equal resistance in both sexes. In contrast to sex specificity, communication was not dependent upon emitter and receiver genotypes, and accordingly we found no genotypic variation in herbivore-induced VOCs. Together, our study finds novel evidence for sexually dimorphic (but not genotypic) specificity of plant–plant communication, as well as some indication of the chemical mechanisms underlying this effect.

Our study provides incomplete evidence for the particular VOCs underlying sex specificity of plant communication. Specificity of communication should only arise when the same VOCs are uniquely emitted from damaged plants (specificity of speaking) and uniquely detected by receivers

(specificity of listening). Consistent with past studies (Ashman 2009), we found sexually dimorphic induction of VOCs by *U. macolai* feeding (specificity of speaking), with the most notable difference being that females emitted fivefold more pinocarvone than males. While artificial exposure of undamaged plants to pinocarvone drastically reduced *U. macolai* performance, these effects were similar for both male and female plants, thus failing to demonstrate the requisite specificity of response (specificity of listening). Furthermore, this result was consistent independently of whether plants were exposed to pinocarvone alone or in combination with a blend of six aphid-induced VOCs. Our contrary findings may be due to unrealistic aspects of our experimental design; we speculate that both sexes possess receptors for pinocarvone, but that male receptors may be relatively insensitive compared with females. Under this scenario, the natural emissions of pinocarvone from damaged female plants may be sufficient to induce resistance in females but not males. In contrast, our artificial emitters likely provide unrealistically high doses of pinocarvone and, in so doing, may induce both sexes equally. Testing this hypothesis would require exposing males and females to an ecologically realistic gradient of pinocarvone concentrations.

Table 2. Linear mixed model results showing the effects of exposure treatments (control vs. pinocarvone and control vs. a blend of six VOCs [pinocarvone, methyl salicylate, ethanone, myrcene, limonene, and ocimene]), plant sex (males vs. females), and their interaction on the performance of the specialist aphid *Uroleucon macolai*.

Treatment and variable	df	*F*	*P*
Control vs. pinocarvone			
Exposure treatment (T)	1,24	17.68	**<0.001**
Sex (S)	1,24	3.66	0.068
T × S	1,24	0.00	0.976
Plant length	1,24	0.17	0.682
Control vs. a blend of six VOCs			
Exposure treatment (T)	1,22	7.37	**0.013**
Sex (S)	1,22	0.58	0.455
T × S	1,22	0.94	0.342
Plant length	1,22	2.08	0.163

Notes: Aphid performance was measured as the reproductive rate on the fifth reproductive day. Plant length was used as a covariate. Significant *P* values ($P < 0.05$) are shown in boldface type.

Our observation of sex specificity of *B. salicifolia* communication can be considered within the context of parallel sexual dimorphism in herbivore resistance. Two decades of work have demonstrated that male and female plants of dioecious species largely differ in how well defended they are against herbivores (Agren et al. 1999, Cornelissen and Stiling 2005), including induced defenses (Mooney et al. 2012a). In the case of *B. salicifolia*, our previous work has demonstrated sexual dimorphism in herbivore and predator abundances, with male plants exhibiting higher herbivore abundance (Abdala-Roberts et al. 2016, 2017), density (Nell et al. 2018), and performance (Mooney et al. 2012b) and lower predator density (Nell et al. 2018). Although we did not observe sexual dimorphism in aphid fecundity in the present experiment (Fig. 2), we attribute this to the relatively short-term nature of the bioassay. Sexual dimorphism in herbivore resistance thus parallels our observation of sexual dimorphism in response to neighbor cues; females are relatively sensitive to VOCs (respond to both sexes), whereas males are relatively insensitive to VOCs (respond only to other males). Accordingly, sexual dimorphism in plant–plant communication can be viewed as one component of a larger syndrome of sexual dimorphism in plant defense.

Differences in herbivore-induced VOCs and communication with plant sex may reflect divergent defensive strategies between males and females. Induced defensive strategies are hypothesized to be less costly for a plant to produce than constitutive defenses (Agrawal 2011, Karban 2011) and thus may be more beneficial to female plants that carry greater costs of reproduction. Herbivore-induced volatiles also play an important role in the attraction of predators for indirect plant defenses. Previous work in this same system found predator densities to be 50% greater on female plants compared to male plants (Nell et al. 2018), and this could be driven in part by increased VOC emission in female plants, although this has also been shown to be associated with higher female floral rewards (Nell et al. 2018). Together, this suggests that female plants of *B. salicifolia* may rely more on induced defenses than their male counterparts.

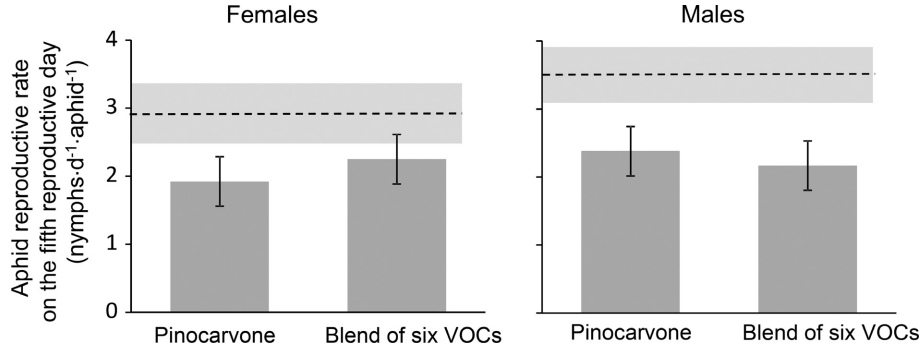

Fig. 4. Effect of VOC exposure treatment (control, pinocarvone, and a blend of six VOCs [pinocarvone, methyl salicylate, ethanone, limonene, myrcene, and ocimene]) on the performance (reproductive rate on the fifth reproductive day) of the specialist aphid *Uroleucon macolai* on female (left panel) and male (right panel) *Baccharis salicifolia* plants. Values are least-square means ± SE ($N = 16$). The dashed line represents the mean value for control plants, and the shaded area represents the standard error around that mean. The effect of each VOC exposure treatment (vs. control) is significant if their error bars do not overlap the shaded area.

Our results also showed that there was no genotypic specificity of *B. salicifolia* communication and that genotypes did not differ in their quantitative and/or qualitative emission of herbivore-induced VOCs. Plant–plant communication has been proposed to primarily evolve as a form of within-self communication in sectorial plants, with eavesdropping by non-self neighbors being either adaptive or a non-adaptive consequence of exogenous VOCs stimulating receptors intended for within-self communication (Heil and Silva Bueno 2007). In this sense, it has been commonly proposed that plants can discriminate between volatile cues released by genetically close relatives and respond positively toward their related individuals (e.g., Karban and Shiojiri 2009, Karban et al. 2013, 2014b, 2016, Moreira et al. 2016). For example, a recent study by Karban et al. (2016) found that cues emitted by sagebrush plants (*Artemisia tridentata*) vary geographically, resulting in more effective communication within than among populations in a reciprocal transplant experiment. Similarly, Moreira et al. (2016) found that lima bean (*Phaseolus lunatus*) plants exhibit population-specific "dialects" such that only receivers from the same source population as the damaged emitters suffered less leaf damage upon exposure VOCs. However, most previous studies have tested genotypic specificity of plant communication by using plant genotypes from different populations (in contrast to within a population as here, but see Karban et al. [2014b]), which would increase the likelihood of detecting genetic variation in VOC emission and reception.

In summary, the language that *B. salicifolia* plants use to communicate about risk of herbivory has been found to be highly specific of emitter and receiver plant sex. This sex specificity of plant communication seems to be underlain by the existence of sexual dimorphism in the emission of herbivore-induced VOCs. Further studies should address not only the chemical mechanisms underlying sex specificity of plant communication, but also the molecular mechanisms responsible for this specificity. For example, specific changes in the expression of different resistance genes on receiver male and female plants may play a pivotal role in sex specificity of plant–plant communication. Finally, we also encourage further studies in natural conditions to investigate how herbivore enemies (e.g., vertebrate and invertebrate predators, parasitoids) can identify specific herbivore-induced VOCs emitted by male and female plants and interpret them as cues of herbivore presence. Assessing how communication influences the extended community and the different pathways through which such effects take place will yield insight into the ecological relevance of this phenomenon.

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