Plant defence responses to volatile alert signals are population-specific

Xoaquín Moreira, William K. Petry, Johnattan Hernández-Cumplido, Stéphanie Morelon and Betty Benrey

X. Moreira (xmoreira1@gmail.com), Misión Biológica de Galicia (MBG-CSIC), Apdo. 28, ES-36080 Pontevedra, Galicia, Spain. – W. K. Petry, Dept of Ecology and Evolutionary Biology, Univ. of California, Irvine, CA 92697-2525, USA. – J. Hernández-Cumplido, S. Morelon and B. Benrey, Inst. of Biology, Laboratory of Evolutionary Entomology, Univ. of Neuchâtel, Rue Emile-Argand 11, CH-2000 Neuchâtel, Switzerland.

Herbivore-induced volatiles are widespread in plants. They can serve as alert signals that enable neighbouring leaves and plants to pre-emptively increase defences and avoid herbivory damage. However, our understanding of the factors mediating volatile organic compound (VOC) signal interpretation by receiver plants and the degree to which multiple herbivores affect VOC signals is still limited. Here we investigated whether plant responses to damage-induced VOC signals were population specific. As a secondary goal, we tested for interference in signal production or reception when plants were subjected to multiple types of herbivore damage. We factorially crossed the population sources of paired *Phaseolus lunatus* plants (same versus different population sources) with a mechanical damage treatment to one member of the pair (i.e. the VOC emitter, damaged versus control), and we measured herbivore damage to the other plant (the VOC receiver) in the field. Prior to the experiment, both emitter and receiver plants were naturally colonized by aphids, enabling us to test the hypothesis that damage from sap-feeding herbivores interferes with VOC communication by including emitter and receiver aphid abundances as covariates in our analyses. One week after mechanical leaf damage, we removed all the emitter plants from the field and conducted fortnightly surveys of leaf herbivory. We found evidence that receiver plants responded using population-specific 'dialects' where only receivers from the same source population as the damaged emitters suffered less leaf damage upon exposure to the volatile signals. We also found that the abundance of aphids on both emitter and receiver plants did not alter this volatile signalling during both production and reception despite well-documented defence crosstalk within individual plants that are simultaneously attacked by multiple herbivores. Overall, these results show that plant communication is highly sensitive to genetic relatedness between emitter and receiver plants and that communication is resilient to herbivore co-infestation.

Many organisms exchange signals with conspecifics that serve a variety of functions from warning of danger to facilitating resource acquisition (Schultz 2002). The utility of signals – critical to understanding how such signals and responses could evolve – depends on the receiver's ability to interpret the information content of the signal and on the capacity and reliability of the medium carrying the signal (Shannon 1948). Although in biology the study of communication has long been restricted to animals (Grafen 1990, Mateo 2003), a compelling body of evidence demonstrates that plants emit and respond to signals in the form of volatile organic compounds (VOCs; >30 species in 15 families; reviewed by Heil and Karban 2010, Heil 2014, Karban et al. 2014a). The utility of herbivore-induced VOCs to receiver plants is clear – many plants can activate defensive pathways in anticipation of herbivore attack and reduce tissue damage (Karban and Maron 2002, Karban 2008, Karban et al. 2012, 2013, 2014a, b, Pearse et al. 2013, Zakir et al. 2013, Heil 2014, Erb et al. 2015). Regardless of whether VOC emissions are 'sent'

to other plants, intra-plant signals, or merely by-products of damage that receivers eavesdrop upon (Peñuelas and Llusiá 2004), casting these interactions in a basic communication theory framework (Shannon 1948) reveals critical gaps in our understanding of volatile chemical ecology. For example, we currently lack an understanding of the factors mediating VOC signal interpretation by receivers and the degree to which damage or feeding by multiple herbivore species affect VOC signals (Rodriguez-Saona et al. 2003, Desurmont et al. 2014).

Defence can be costly (Stamp 2003), thus optimal defence theory predicts that receiver plants should only respond to VOCs that indicate a threat of damage. Work by Karban and colleagues supports this hypothesis by demonstrating that plant identity can mediate responses to VOCs. Specifically, emission of VOCs by sagebrush *Artemisia tridentata* plants induced herbivore resistance on neighbouring sagebrush and tobacco *Nicotiana attenuata* plants but not on three other neighbouring herb species (Karban et al. 2000,

2004). Likewise, aggregations of genetically similar neighbours may attract additional herbivores through resource concentration (Root 1973) and selectively favour genetically closely related groups that use VOC signalling (Heil 2014). Genetic relatedness effect on VOC signalling has been demonstrated in sagebrush where VOCs emitted by clones and close relatives of the receiver induced a stronger protective effect than VOCs emitted by other genotypes (Karban and Shiojiri 2009, Karban et al. 2013, 2014b, Pearse et al. 2013), however it remains unclear whether plant intraspecific recognition is a general phenomenon.

Most plants are sequentially or simultaneously attacked by a wide range of herbivores from different feeding guilds which vary in traits such as diet breadth, feeding mode, and tissue specialization (Stam et al. 2014). We might predict that co-infestation induces VOC blends containing information about each herbivore, and that receivers in turn respond additively to each component of the signal. However, these predictions depend critically on assumptions about the capacity of volatile communication channels and the physiology of both volatile production and defensive response. The channel must have sufficient capacity to carry herbivore-specific signals without interference and emitters must be able to produce and receivers must be able to parse diverse volatile blends. Furthermore, channel capacity alone is not sufficient, as plant responses to one herbivore may impact both its volatile and defence response to another herbivore. For example, phloem-sucking/piercing herbivores (e.g. aphids) commonly induce the salicylic acid (SA) defence pathway whereas leaf chewing herbivores (e.g. caterpillars and beetles) typically induce jasmonic acid (JA) and/or ethylene pathways (Thaler et al. 2012). Cross-talk between these defence signalling pathways is common within individual plants (Pieterse et al. 2006), thus the ability of plants to produce or respond to VOCs from multiple herbivores may be compromised. However, if co-infestation by leaf-chewing and sap-feeding herbivores inhibits plant production of VOC signals or if co-infestation on receivers supresses their response to volatile alert signals have not been studied yet.

Our main goal was to test for population-specificity of plant–plant communication. Additionally, we also tested for interference in signal production or reception when plants were subjected to multiple types of damage. We carried out a field experiment using wild-growing accessions of the annual legume *Phaseolus lunatus* (Fabaceae), a species known to communicate herbivore damage via VOC cues (Kost and Heil 2006, Heil and Silva Bueno 2007, Heil and Adame-Álvarez 2010) and to experience cross-talk between JA and SA defence pathways (Zhang et al. 2009). We assigned pairs of plants (i.e. emitters and receivers) that had varying abundances of aphids to treatments that factorially crossed the population sources of the plants (same versus different population) and damage group of the emitter plants (control versus mechanical damage). One week after applying the mechanical damage treatment, we removed all the emitter plants from the field and conducted fortnightly leaf damage and aphid surveys on the receiver plants. We specifically addressed the following questions: 1) do experimentally damaged plants increase herbivore resistance in genetically-related neighbouring plants more strongly than in non-genetically-related individuals? And 2) based on

an expected cross-talk between salicylic and jasmonic acid defensive pathways, does aphid abundance on the emitter and receiver plants affect the strength of VOC-induced defences against chewing herbivores? By addressing these questions, our work advances a more complete understanding of the nature, magnitude, and specificity of plant communication and how co-infestation by multiple herbivores can influence these patterns.

Material and methods

Natural history

We studied plant communication in lima bean *Phaseolus lunatus*, an annual legume found in natural populations along the Pacific coast from Mexico to South America (Freytag and Debouck 2002, Heil 2004, Delgado-Salinas et al. 2006). At our field site 15 km northwest of Puerto Escondido, Oaxaca, Mexico (Fig. 1), *P. lunatus* germinates between June and July and flowers at the beginning of October. Seeds are produced during November and December then disperse in January and February (Freytag and Debouck 2002).

Phaseolus lunatus plants are simultaneously attacked by a diverse set of insect herbivores at our field site, including the leaf chewers *Spodoptera eridania* (Lepidoptera: Noctuidae), *Diabrotica balteata* (Coleoptera: Chrysomelidae) and *Cerotoma ruficornis* (Coleoptera: Chrysomelidae) and one sap-feeder (an unidentified aphid species) (Moreira et al. 2015a). The unidentified aphid species (Hemiptera: Aphididae) is usually tended by the ant *Solenopsis geminata* (Hymenoptera: Formicidae), which feed upon the aphid's sugary waste (so called 'honeydew') and may provide protection to aphids from natural enemies and competitors (Styrsky and Eubanks 2007). This ant species readily attacks non-aphid herbivores on *P. lunatus* and also visits extrafloral nectaries of *P. lunatus* to collect their sugary secretions (Hernández-Cumplido et al. 2015).

Experimental set-up

In early October 2014, we collected seeds from 5–10 wild *P. lunatus* plants growing in four populations along the Pacific coast of Oaxaca, Mexico (Fig. 1). We sowed seeds individually in 5-l pots with a mixture composed of native soil and peat moss. After emergence, we placed all plants in nylon mesh field cages (6′ × 6′ × 6′, 20 × 20 mesh) to prevent leaf herbivory for four weeks. During this time aphids and their mutualistic ants colonized 70% of the plants. Shortly after aphid infestation, we counted the number of leaves (52.5 ± 4.1), aphids (111.6 ± 24.3) and ants (18.4 ± 2.3) on each plant, and transferred 40 pairs of plants to the field site (Univ. del Mar, Puerto Escondido Campus, Oaxaca, Mexico, 15°55′26.4″N, 97°09′02.0″W; Fig. 1).

We placed pairs of aphid-infested plants – half where both were from the same population and half from different populations – in an array with 3 m between neighbouring pairs of plants on 26 December 2014. We randomly selected one plant from each pair to be the volatile emitter and the other to be the receiver. Emitters and receivers were placed 10 cm apart in alternating orientations, and their
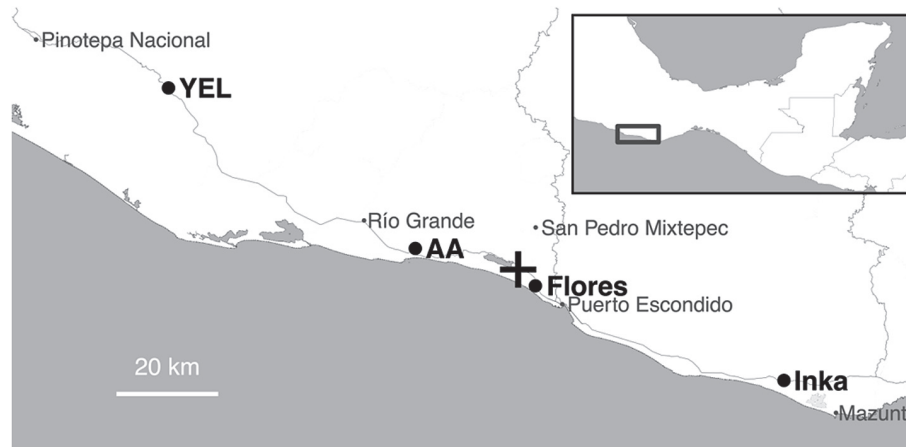
Figure 1. Spatial layout of the experimental site (+ symbol) and *Phaseolus lunatus* source populations used for the experiment (large circles with names).

canopies were individually trained on a wooden pole to avoid physical contact. Previous work in this system and others suggest that plant VOCs dissipate at distances beyond 60 cm (Karban et al. 2006, Heil and Adame-Álvarez 2010), and wild *P. lunatus* plants frequently grow at similar densities (Moreira unpubl.). This separation between plants reduces the likelihood of aphid movement from emitters to receivers.

On the following day, we factorially crossed the population source treatment with mechanical damage to the emitter (control versus damaged). We damaged half of the emitter plants by pressing a wire brush through 25% of the plant's leaves, resulting in an average damage of approximately 15 holes per cm^{-2} (Heil and Silva Bueno 2007). Three days later, we repeated this procedure on the same leaves. The other half of the emitter plants remained undamaged and served as a control. Determining the specificity of plant–plant communication among populations requires a standardized amount and type of damage. Although not identical to natural herbivore damage where plants receive a wide range of herbivore cues other than damage that affect plant responses, mechanical damage 1) is a useful mimic of natural chewing herbivory (reviewed by Karban et al 2014a), 2) induces volatile emissions in beans (Heil and Silva Bueno 2007), and 3) can be applied similarly to all damaged plants. Isolating the effect of tissue damage in this way is advantageous because it avoids the complications of selecting a single herbivore species to impose damage. The most important herbivore species, the additional elicitors they produce, and plant responses to these different species may differ among source populations, but the uniting factor is that all herbivore species damage plant tissue. One week after the second round of damage (5 January 2015), we removed all the emitter plants from the field, leaving the receivers in place. We conducted fortnightly surveys of leaf herbivory and aphid and ant abundance (16 January, 26 January and 9 February), counting the number of total leaves, the number of herbivore-damaged leaves and the number of aphids and ants on receivers.

Statistical analyses

We used the number of damaged leaves on the receiver – a bioassay of receiver resistance in response to emitter vola-tiles – as the dependent variable, with observations at each time point (2, 4 and 6 weeks after field experiment establishment) treated as a random effect nested within plant identity. The effects of population sources (same versus different populations), emitter treatment (damaged versus control), the interaction of population sources and emitter damage treatment on the number of damaged leaves were considered as fixed factors. Repeated measure fixed effects included the number of aphids and ants on the emitters and receivers and the total number of leaves on the receivers (to control for differences in total leaf output) as covariates. We controlled for ant effect as these herbivore mutualists might increase damage, for example by enabling herbivore persistence despite lower plant quality (Mooney et al. 2012).

Prior to calculating inferential statistics for each fixed effect, we used a likelihood ratio test to evaluate the correlation structure between random effect slopes and intercepts (Scheipl et al. 2008, Bates 2010). Parsimony favours a reduced model that assumes no correlation of the random effects, but our methods allowed the possibility of their association because leaves damaged and counted early in the experiment could be retained on plants and counted again during subsequent surveys. Including a parameter to estimate the correlation between slopes and intercepts marginally improved the fit of the model (D = 3.82, DF = 1, p = 0.051). We chose the reduced model for all subsequent analyses, but the results given by the two models were qualitatively identical (Supplementary material Appendix 1 Table A1).

Because 12 receiver plants died during our experiment we also tested whether plant VOCs affected plant survival, a key fitness component in annual plants like *P. lunatus*. Our post-treatment surveys of survival did not have the temporal resolution for a full survival analysis, so we examined survival at the end of the experiment (10 weeks after treatment application). Using a logistic regression we fit survival to the individual and interactive effects of population source and emitter damage treatment with initial receiver plant size (= number of leaves) as a covariate.

To test our hypotheses about the factors mediating plant–plant communication, we used generalized linear models and repeated measures linear mixed models (LMM;

package 'LME4' Bates et al. 2014) with maximum likelihood. For all LMMs, we calculated p-values from 10 000 parametric bootstrap simulations (p_{PB}) of the deviance statistic (D) for each fixed effect term in the model (package 'AFEX'; Singmann et al. 2015). We report each fixed effect after controlling for all other fixed effects in the model (package 'EFFECTS' ver. 3.0-3; Fox 2003). All statistical analyses were conducted in R ver. 3.1.2 (<www.r.project.org>). In all cases, we provide least square means $\pm$ SE as descriptive statistics.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.d6rb0> (Moreira et al. 2015b).

Results

Naturally occurring leaf herbivores fed on all receiver plants, damaging 3.9–46.3% of leaves across all surveys. Aphids colonized all receiver plants during the experiment, each hosting a mean $\pm$ SE abundance of 148.5 $\pm$ 24.4 aphids per survey (median = 17, range: 0–2261). Ants were also found on all receivers at a mean $\pm$ SE abundance per survey of 12 $\pm$ 1.4 (median = 4, range: 0–128). Twelve receiver plants died during the 44-day post-treatment period. Data from these plants were included in our analyses for time points prior to death, thus only 18 observations of leaf herbivory and aphid abundance were lost (out of 120 possible observations, i.e. 40 receiver plants $\times$ 3 surveys).

Receivers neighbouring experimentally damaged emitters suffered 37% less leaf damage than those near undamaged

emitters, but only when the emitter was from the same source population (Fig. 2; emitter damage treatment $\times$ population source treatment, D = 4.385, DF = 1, p_{PB} = 0.049). Large plants (those with more leaves) experienced significantly more leaf damage than small plants, but had a smaller proportion of their leaves damaged (slope = 0.112; D = 56.324, DF = 1, p_{PB} < 0.001). Time was also a significant covariate – damage declined over the post-treatment period (D = 15.078, DF = 1, p_{PB} = 0.002) – likely an artefact of plant growth combined with including total leaf number as a covariate.

Aphids and ants had no significant effects on leaf damage. Aphids did not modulate the transmission or reception of volatiles produced by leaf damage (emitter damage treatment $\times$ initial emitter aphids, D = 1.984, DF = 1, p_{PB} = 0.181 in Fig. 3A and emitter damage treatment $\times$ initial receiver aphids, D = 1.612, DF = 1, p_{PB} = 0.224 in Fig. 3B, respectively). Likewise, neither aphids nor ants present during the damage surveys affected leaf damage (receiver aphids D = 0.913, DF = 1, p = 0.365 and receiver ants D = 0.988, DF = 1, p = 0.345, respectively). The Supplementary material Appendix 1 Table A1 provides a full summary of the LMM.

Thirty percent of the receiver plants (12 of 40) died by the end of the experiment. Survival was not significantly affected

Figure 3. Relationship between the number of herbivore-damaged leaves on receiver plants and the number of aphids on (A) receiver plants and (B) emitter plants during induction treatment (note log scale on x-axis). Black dots indicate undamaged emitter plants and white dots represent mechanically-damaged emitter plants (n = 20 plants in each treatment). Each dot corresponds to the mean number of leaves damaged for a single plant across all observation points.
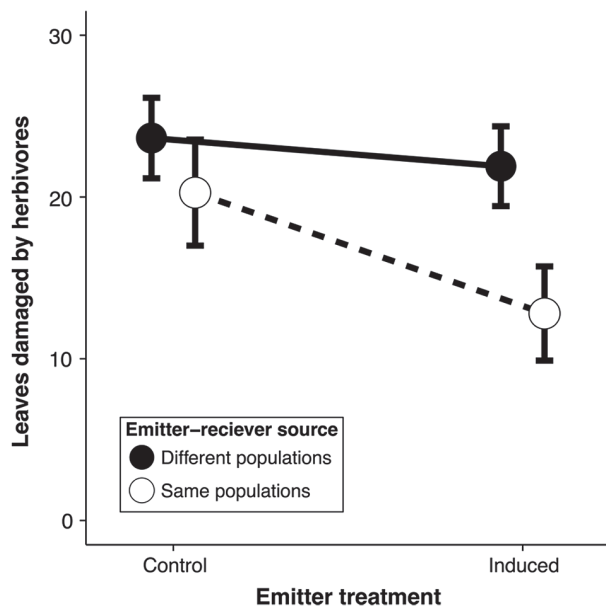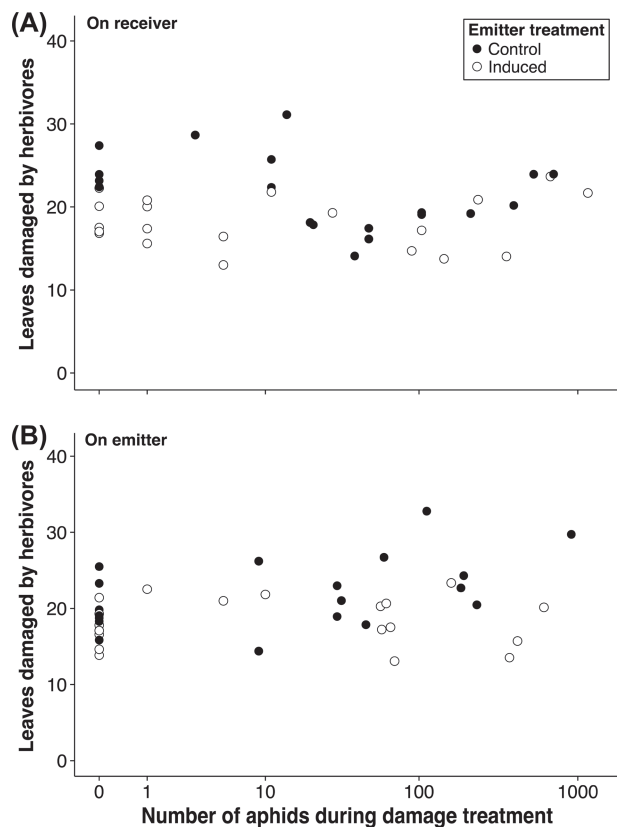
Figure 2. Effect of herbivory induction treatment to the emitter plant (control versus mechanically damaged) on the number of herbivore-damaged leaves on receiver plants. Black dots represent emitter–receiver plants from different populations and white dots represent emitter–receiver plants from the same populations (n = 10 plants).

953

by population source treatment (F = 0.901, DF = 1,35, p = 0349), damage-induced VOCs from emitters (F = 0.058, DF = 1,35, p = 0.811), nor their interaction (F = 0.015, DF = 1,35, p = 0.903). Plants that were larger at the beginning of the experiment were marginally more prone to death, but this effect was not significant (F = 2.349, DF = 1,35, p = 0.134).

Discussion

Our study demonstrates that plant–plant communication is population-specific and resilient even when other damaging herbivores are feeding on the plant. Specifically, we found that *Phaseolus lunatus* plants exposed to the VOCs of experimentally-damaged neighbours suffered less leaf damage than those exposed to undamaged plants, but only when neighbouring plants were from the same population. Additionally, we also found no evidence that contrasting types of damage (a mimic of chewing herbivory and sap feeding by aphids) altered plant communication. Overall, these results suggest that plants communicate with population-specific dialects and demonstrate that communication is resilient to herbivore co-infestation.

One of the apparent paradoxes of plant communication is how plants that emit or receive public VOC signals avoid exploitation by competitors (Heil 2014). One potential solution to this problem is volatile specificity where emitters can privatize their signals and receivers avoid costly defence induction when signals are less likely to indicate a true threat of damage. Additionally, it is also possible that receivers prime their defences rather than producing induced defences upon receipt of information on the risk of attack (Sugimoto and Arimura 2013). In particular, plant communication between individuals might have originally evolved as an adaptive by-product process for which plants can co-ordinate their own systemic responses (Karban et al. 2014a). In other words, selection might favour plants that perceive and adaptively respond better to cues from their own tissues and this bias might in turn provoke that these plants will be also very responsive to cues from related neighbours. Recent studies have reported that volatiles emitted by some herbivore-damaged parts of a plant induce resistance at undamaged sites of the same plant (i.e. within-plant signaling) and may do so more effectively than signals carried by the vascular system (Frost et al. 2007, Heil and Silva Bueno 2007, Heil 2014). This might be especially important in many plant species (including *P. lunatus*) with limited vascular connections among modules (e.g. branches and roots) where signaling of an herbivore attack by VOCs would be faster to overcome long distances than vascular signaling.

Here we show the first evidence to our knowledge of population-level specificity of volatile communication, consistent with the existence of VOC 'dialects'. Our results complement a compelling demonstration of chemotype variation and its consequences for plant communication within a population of sagebrush (Karban et al. 2014b). These authors observed that volatiles emitted by damaged sagebrush plants were characterized into two heritable chemotypes (dominated by either thujone or camphor) and that following leaf damage individuals of the same chemotype communicated more

effectively than individuals of differing chemotypes (Karban et al. 2014b). The ecological consequences of chemotypes are not clearly understood for any plant species, but some previous studies have reported that the chemotypes of *Thymus vulgaris* (Lamiaceae) have strong effects on the preferences and performances of herbivores (Linhart and Thompson 1995, Linhart et al. 2005). Although the volatiles emitted by *P. lunatus* are known to exhibit geographic variation (Ballhorn et al. 2008), further studies should address if there are contrasting chemotypes in our *P. lunatus* populations and if responding to individuals of similar chemotypes might provide a mechanism to differentiate 'local' conspecifics from 'foreign' individuals.

The existence of population-specific VOC dialects raises questions about the drivers and functional implications of this variation. First, the variation may be plastic or genetic. Our study was conducted in a common environment and thus we argue that the driver of dialect variation is likely to have a heritable component. The patterns of inter-population dialects that we observed are not sufficient to implicate adaptation or rule out drift. Replicating our common garden approach at the seed source populations could be used to search for adaptive advantages of different dialects. Plant-produced volatiles are not only used by other plants, but also attract herbivores (Carroll et al. 2006) and predators (Turlings et al. 1990, 1995) that may directly or indirectly select upon volatile blends. Constraints on VOC signals may also include abiotic factors such as temperature, which may influence which compounds are able to volatilize and serve as a signal (Peñuelas and Llusia 2003). Moreover, pairing inter-population studies like ours with studies of intra-population chemotype variation (Karban et al. 2014b) holds great promise to understand the scales at which kin-, population- and species-specific signals evolve and persist.

Although it is well-known that herbivores can induce cross-talk between salicylic and jasmonic acid pathways (Pieterse et al. 2006, Thaler et al. 2012), we found that co-infestation (by aphids and a mimic of leaf chewers) on emitters did not inhibit their ability to produce signals nor did co-infestation supress responses to volatile cues. These results strongly suggest that when *P. lunatus* emitter plants are co-infested by multiple herbivores can induce highly specific VOC blends that receivers (even co-infested) can identify, discriminate and use to respond accordingly. One plausible explanation for this pattern is that the cross-talk between both defence signalling pathways might depend on herbivore density (Zhang et al. 2009, Kroes et al. 2015). For example, a recent study by Kroes et al. (2015) observed that the growth rate of a caterpillar that fed on the leaves of *Arabidopsis thaliana* plants increased at a low aphid density, but it decreased on plants colonized by aphids at a high density. Alternatively, it is also possible that aphids did not change the VOC blend, that aphids changed the VOC blend but plants were unable to respond to aphid-specific volatile signals, or that aphids altered the VOC blend but the composition of the caterpillar signature still stood out and was perceived by the neighbour.

Our data suggest that communication in plants can evolve specificity akin to language dialects, similar to those observed in the songs of birds and other animals (Podos and Warren 2007). Recent studies have begun to explain the

mechanisms by which plant communication evolves and persists, thus confronting a multi-decade controversy with data. Moving forward, we argue that the study of biological communication will be well served by drawing inspiration from communication theory and leveraging the unique attributes of plants to achieve general synthesis across taxa.

References

Ballhorn, D. J. et al. 2008. Tradeoffs between direct and indirect defences of lima bean (*Phaseolus lunatus*). – J. Ecol. 96: 971–980.

Bates, D. 2010. lme4: mixed-effects modeling with R. – <http://lme4.r-forge.r-project.org/book/>.

Bates, D. et al. 2014. lme4: linear mixed-effects models using Eigen and S4. R package ver. 1.1-7.<http://cran.r-project.org/package=lme4>.

Carroll, M. J. et al. 2006. Attraction of *Spodoptera frugiperda* larvae to volatile from herbivore-damaged maize seedlings. – J. Chem. Ecol. 32: 1911–1924.

Delgado-Salinas, A. et al. 2006. Phylogeny of the genus *Phaseolus* (Leguminosae): a recent diversification in an ancient landscape. – Syst. Bot. 31: 779–791.

Desurmont, G. A. et al. 2014. Alien interference: disruption of infochemical networks by invasive insect herbivores. – Plant Cell Environ. 37: 1854–1865.

Erb, M. et al. 2015. Indole is an essential herbivore-induced volatile priming signal in maize. – Nat. Commun. 6: 6273.

Fox, J. 2003. Effect displays in R for generalised linear models. – J. Stat. Softw. 8: 1–27.

Freytag, G. F. and Debouck, D. G. 2002. Taxonomy, distribution and ecology of the genus *Phaseolus* (Leguminosae-Papilionoideae) in North America, Mexico and Central America. – BRIT Press, Fort Worth, TX.

Frost, C. J. et al. 2007. Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. – Ecol. Lett. 10: 490–498.

Grafen, A. 1990. Do animals really recognize kin? – Anim. Behav. 39: 42–54.

Heil, M. 2004. Induction of two indirect defences benefits Lima bean (*Phaseolus lunatus*, Fabaceae) in nature. – J. Ecol. 92: 527–536.

Heil, M. 2014. Herbivore-induced plant volatiles: targets, perception and unanswered questions. – New Phytol. 204: 297–306.

Heil, M. and Adame-Álvarez, R. M. 2010. Short signalling distances make plant communication a soliloquy. – Biol. Lett. 23: 843–845.

Heil, M. and Karban, R. 2010. Explaining the evolution of plant communication by airborne signals. – Trends Ecol. Evol. 25: 137–144.

Heil, M. and Silva Bueno, J. C. 2007. Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. – Proc. Natl Acad. Sci. USA 104: 5467–5472.

Hernández-Cumplido, J. et al. 2015. Induced floral and extrafloral nectar production affect ant–pollinator interactions and plant fitness. – Biotropica doi: 10.1111/btp.12283.

Karban, R. 2008. Plant behaviour and communication. – Ecol. Lett. 11: 727–739.

Karban, R. and Maron, J. 2002. The fitness consequences of interspecific eavesdropping between plants. – Ecology 83: 1209–1213.

Karban, R. and Shiojiri, K. 2009. Self recognition affects plant communication and defense. – Ecol. Lett. 12: 502–506.

Karban, R. et al. 2000. Communication between plants: induced resistance in wild tobacco plants following clipping of neighboring sagebrush. – Oecologia 125: 66–71.

Karban, R. et al. 2004. The specificity of eavesdropping on sagebrush by other plants. – Ecology 85: 1846–1852.

Karban, R. et al. 2006. Damage-induced resistance in sagebrush: volatiles are key to intra- and interplant communication. – Ecology 87: 922–930.

Karban, R. et al. 2012. Long-term demographic consequences of eavesdropping for sagebrush. – J. Ecol. 100: 932–938.

Karban, R. et al. 2013. Kin recognition affects plant communication and defence. – Proc. R. Soc. B 280 20123062.

Karban, R. et al. 2014a. Volatile communication between plants that affects herbivory: a meta-analysis. – Ecol. Lett. 17: 44–52.

Karban, R. et al. 2014b. Deciphering the language of plant communication: volatile chemotypes of sagebrush. – New Phytol. 204: 380–385.

Kost, C. and Heil, M. 2006. Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. – J. Ecol. 94: 619–628.

Kroes, A. et al. 2015. Density-dependent interference of aphids with caterpillar-induced defenses in *Arabidopsis*: involvement of phytohormones and transcription factors. – Plant Cell Physiol. 56: 98–106.

Linhart, Y. B. and Thompson, J. D. 1995. Terpene-based selective herbivory by *Helix aspersa* (Mollusca) on *Thymus vulgaris* (Labiatae). – Oecologia 102: 126–132.

Linhart, Y. B. et al. 2005. A chemical polymorphism in a multitrophic setting: thyme monoterpene composition and food web structure. – Am. Nat. 166: 517–529.

Mateo, J. M. 2003. Kin recognition in ground squirrels and other rodents. – J. Mammal. 84: 1163–1181.

Mooney, K. A. et al. 2012. The tri-trophic interactions hypothesis: Interactive effects of host plant quality, diet breadth and natural enemies on herbivores. – PLoS ONE 7: e34403.

Moreira, X. et al. 2015a. Specificity of induced defenses, growth, and reproduction in lima bean (*Phaseolus lunatus*, Fabaceae) in response to multispecies herbivory. – Am. J. Bot. 102: 1300–1308.

Moreira, X. et al. 2015b. Data from: Plant defence responses to volatile alert signals are population-specific . – Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.d6rb0>.

Pearse, I. S. et al. 2013. Interplant volatile signaling in willows: revisiting the original talking trees. – Oecologia 172: 869–875.

Peñuelas, J. and Llusia, J. 2003. BVOCs: plant defense against climate warming? – Trends Plant Sci. 8: 105–109.

Peñuelas, J. and Llusiá, J. 2004. Plant VOC emissions: making use of the unavoidable. – Trends Ecol. Evol. 19: 402–404.

Pieterse, C. M. et al. 2006. Signaling in plant resistance responses: divergence and cross-talk of defense pathways. – In: Tuzun, S.

and Bent, E. (eds), Multigenic and induced systemic resistance in plants. Springer, pp. 166–196.

Podos, J. and Warren, P. S. 2007. The evolution of geographic variation in birdsong. – Adv. Stud. Behav. 37: 403–458.

Rodriguez-Saona, C. et al. 2003. Volatile emissions triggered by multiple herbivore damage: beet armyworm and whitefly feeding on cotton plants. – J. Chem. Ecol. 29: 2539–2550.

Root, R. B. 1973. Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). – Ecol. Monogr. 43: 95–124.

Scheipl, F. et al. 2008. Size and power of tests for a zero random effect variance or polynomial regression in additive and linear mixed models. – Comput. Stat. Data Anal. 52: 3283–3299.

Schultz, J. C. 2002. Shared signals and the potential for phylogenetic espionage between plants and animals. – Integr. Comp. Biol. 42: 454–462.

Shannon, C. E. 1948. A mathematical theory of communication. – Bell Syst. Tech. J. 27: 379–423.

Singmann, H. et al. 2015. afex: analysis of factorial experiments. – R package ver. 0.13-145.

Stam, J. M. et al. 2014. Plant interactions with multiple insect herbivores: from community to genes. – Annu. Rev. Plant Biol. 65: 689–713.

Stamp, N. 2003. Out of the quagmire of plant defense hypotheses. – Q. Rev. Biol. 78: 23–55.

Styrsky, J. D. and Eubanks, M. D. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. – Proc. R. Soc. B 274: 151–164.

Sugimoto, K. and Arimura, G. 2013. Maize plants prime anti-herbivore responses by the memorizing and recalling of airborne information in their genome. – Plant Signal. Behav. 8: e25796.

Thaler, J. S. et al. 2012. Evolution of jasmonate and salicylate signal crosstalk. – Trends Plant Sci. 17: 260–270.

Turlings, T. C. J. et al. 1990. Explotation of herbivore-induced plant odors by host-seeking parasitic wasps. – Science 250: 1251–1253.

Turlings, T. C. J. et al. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. – Proc. Natl Acad. Sci. USA 92: 4169–4174.

Zakir, A. et al. 2013. Herbivore-induced plant volatiles provide associational resistance against an ovipositing herbivore. – J. Ecol. 101: 410–417.

Zhang, P.-J. et al. 2009. Whiteflies interfere with indirect plant defense against spider mites in Lima bean. – Proc. Natl Acad. Sci. USA 106: 21202–21207.

Appendix 1

Table A1. Summary tables for leaf damage linear mixed models with (a) correlated and (b) uncorrelated random effects. p-values were calculated from 10 000 parametric bootstraps (p_{PB}) of the deviance statistic (D). Outcomes of hypothesis tests were congruent with one exception: the main effect of 'emitter treatment' was significant in this model, though the interpretation is unaffected because this term is included in a significant interaction effect.

Effect	DF	Correlated		Uncorrelated		Congruence
		D	p_{PB}	D	p_{PB}	
Emitter treatment	1	5.08	**0.039**	3.46	0.074	no
Population source treatment	1	20.86	**<0.001**	19.94	**<0.001**	yes
Initial receiver aphids	1	1.03	0.356	1.05	0.332	yes
Initial emitter aphids	1	1.71	0.227	1.92	0.180	yes
Receiver aphids	1	1.83	0.224	0.91	0.365	yes
Receiver ants	1	0.40	0.564	0.99	0.345	yes
Receiver leaves	1	57.23	**<0.001**	56.32	**<0.001**	yes
Time	1	10.10	**0.002**	15.08	**<0.001**	yes
Emitter treatment × Population source treatment	1	5.76	**0.024**	4.38	**0.049**	yes
Emitter treatment × Initial receiver aphids	1	1.73	0.227	1.61	0.224	yes
Emitter treatment × Initial emitter aphids	1	1.34	0.289	1.98	0.181	yes

Table A2. Summary tables for linear mixed models of aphid recruitment to receivers with (a) correlated and (b) uncorrelated random effects. p-values were calculated from 10 000 parametric bootstraps (p_{PB}) of the deviance statistic (D). Outcomes of all hypothesis tests were congruent between models.

Effect	DF	Correlated		Uncorrelated		Congruence
		D	p_{PB}	D	p_{PB}	
Emitter treatment	1	2.31	0.170	2.31	0.141	yes
Population source treatment	1	0.02	0.927	0.02	0.904	yes
Initial receiver aphids	1	0.22	0.695	0.22	0.640	yes
Source population of receiver	3	12.57	**0.014**	12.57	**0.009**	yes
Receiver size (no. leaves)	1	1.88	0.231	1.88	0.194	yes
Time	1	1.37	0.325	1.37	0.273	yes
Emitter treatment × Population source treatment	1	3.19	0.126	3.19	0.082	yes

Table A3. Summary tables for linear mixed models of ant recruitment to receivers with (a) correlated and (b) uncorrelated random effects. p-values were calculated from 10 000 parametric bootstraps (p_{PB}) of the deviance statistic (D). Outcomes of all hypothesis tests were congruent between models.

Effect	DF	Correlated		Uncorrelated		Congruence
		D	p_{PB}	D	p_{PB}	
Emitter treatment	1	0.42	0.562	0.26	0.651	yes
Population source treatment	1	1.34	0.308	1.19	0.328	yes
Receiver aphids	1	7.49	**0.010**	7.99	**0.007**	yes
Source population of receiver	3	4.88	0.284	5.06	0.257	yes
Receiver size (no. leaves)	1	8.98	**0.005**	8.58	**0.008**	
Time	1	1.64	0.196	1.68	0.211	yes
Emitter treatment × Population source treatment	1	0.79	0.441	0.69	0.453	yes