VOLATILES, PLANT-PLANT INTERACTIONS AND INSECT HERBIVORY: LESSONS FROM OLD-FIELD COMMUNITIES THAT INFORM COMMUNITY ECOLOGY

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VOLATILES, PLANT-PLANT INTERACTIONS AND INSECT HERBIVORY: LESSONS FROM OLD-FIELD COMMUNITIES THAT INFORM COMMUNITY ECOLOGY

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Plants interact with numerous organisms in their communities, including neighboring plants. In large part, these interactions are mediated by blends of volatile organic compounds (VOCs) that plants emit into their headspaces, which may inform other organisms by reflecting plants' physiologies and the biotic and abiotic stresses they are experiencing. However, while the ubiquity of VOC-mediated plant-plant interactions is well established, their mechanisms, ecological outcomes and evolutionary trajectories remain largely uncharacterized. Collectively, these experiments seek to close some of these knowledge gaps, using tall goldenrod (*Solidago altissima*) and its diverse herbivore community as a model system.

Chapter 1 reviews the literature on plants' responses to herbivores and VOCs from neighboring plants – highlighting numerous parallels between the molecular mechanisms and ecological outcomes underlying induced responses to herbivores and VOC-mediated plant-plant interactions. We emphasize the need to conduct mechanistic and ecological studies that directly compare plants' responses to VOCs vis-à-vis herbivory.

Chapter 2 examines the effects of VOC-mediated plant-plant interactions on

herbivore performance and behavior. It reveals that these interactions may result in VOC-exposed and herbivore-damaged plants becoming of equally bad food quality for subsequent herbivores – reducing feeding and increasing between-host movement – in spite of the fact that induced leaf chemistry of damaged and exposed plants is not identical.

Chapter 3 explores whether VOC-mediated plant-plant interactions have specific effects on different herbivores. We demonstrate that herbivore-induced VOCs elicit specific chemical changes in exposed plants, and that these chemical changes have specific effects – making VOC-exposed plants more or less resistant to subsequent herbivores.

Chapter 4 explores whether VOC emission and perception vary across plant genotypes. We find that genotypes vary in their abilities to emit and perceive VOCs, and that receivers may induce resistance or susceptibility to subsequent herbivores, or not respond, depending on the genotypes of VOC-emitting plants.

Collectively, these experiments reveal that the identity of a plant's neighbors affects available information and its resistance state in its community. The observed specificity and genotypic variation in VOC-mediated plant-plant interactions suggests that it is worth exploring the costs and benefits of these interactions in the context of the genetic structure of plant populations, and the diversity/composition of the herbivore community.

BIOGRAPHICAL SKETCH

Kimberly grew up in Saint Louis, Missouri. From the start, she was inspired by the outdoors – her parents frequently took her on nature walks, and encouraged her to collect everything from rocks and shells, to flowers and insects. These early collections quickly kindled her fascination with the natural world – exploring the diverse, beautiful and bizarre organisms in it. They also inspired Kimberly to always be a learner – never accept that you know everything about the natural world, and always keep open eyes and an open mind.

Before long, it wasn't easy to keep "Huckleberry Kim" inside – she spent her free time exploring the woods around her house with her friend Kimberly Hause, and especially loved helping out on the family farm with her maternal grandparents, Ralph and Mary Gerber. Her aunt and uncle, Jan and John Kinar, also kindled her early interest in plant and insect ecology – taking her to the zoo, butterfly house, and botanical gardens; marveling in the diversity of plants and insects around the world; and inviting her to join them on an inspiring trip to the rainforests of Costa Rica.

In seventh grade, Kimberly was selected to participate in a school field trip to the Grand Teton National Park – the first major step in her path towards becoming a community ecologist. In the Tetons, she quickly became inspired by both the geological and ecological history of the region, and was especially touched by the tension she observed between the development of Jackson Hole as a tourist destination, and its position right in the middle of elk migratory routes. After this experience, she became interested in learning not only about science, but also about society and culture – seeking to understand situations in which development and

preservation of the natural world are at odds with one another, and striving to work at the interface – finding ways to enable both interests to coexist more peacefully.

During her time as an undergraduate at Carleton College, she was able to delve whole-heartedly into understanding this tension. Her coursework, research projects and summer internships enabled her to study anthropology, sociology, biology and chemistry – learning the nuts and bolts of scientific research, while showing her that she enjoys thinking and working at the interface of multiple disciplines.

As a participant in the Semester in Environmental Science Program at the Marine Biological Laboratory in Woods Hole, MA, she learned to measure numerous aspects of ecosystem health and function. In particular, her independent research project at the culmination of the semester (focusing on the effects of cranberry farming on stream ecosystem health and function), not only revealed the deleterious impacts of agriculture on stream ecosystems, but also challenged her to find ways to mitigate them by working with restoration groups. It also placed her in the middle of a social debate over whether to preserve cranberry bogs for their historical significance or restore them back to flowing, productive stream ecosystems to support the active fishing community. Kimberly found that she thrived on grappling with these kinds of challenges, conveying scientific data to both scientists and non-scientists, and providing data that helped to inform community decisions. This inspired her to pursue another off-campus study opportunity in Australia during her senior year at Carleton in which she worked with local water quality associations to provide science/policy recommendations on issues of coral bleaching and eutrophication.

Kimberly's Environment and Technology Studies professor at Carleton,

Tsegaye Nega, also inspired her interest in issues at the interface of conservation and development. Specifically, his "Biodiversity, Conservation, and Development" class challenged her to think critically about the issues she explored in her travels, and simultaneously inspired her to work towards finding solutions. It also made her interested in and critical of the United States' system of agriculture, and kindled her interest in studying natural plant defenses against insects to identify and promote more sustainable agricultural practices.

Kimberly's time as a graduate student with Dr. Andre Kessler at Cornell University solidified her interest in studying the chemical ecology of plant-insect and plant-plant interactions to identify promising strategies that can be applied in agricultural systems. She particularly enjoyed opportunities to study rainforest ecology in Costa Rica, agricultural policy in Washington DC, and to do applied agricultural research on *Sorghum bicolor* in collaboration with the International Center of Insect Physiology and Ecology in Mbita Point, Kenya. Kimberly is excited to continue the work she started at Cornell as a Research Entomologist with Monsanto Company in Union City, Tennessee where she will study the evolution of resistance to transgenes, identify promising strategies to mitigate resistance evolution, and disseminate this information to farmers and policy-makers.

"Under-commit and over-achieve" – Monica Geber

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Dedicated to Jim and Susan Morrell, for kindling my curiosity and love of the outdoors, for encouraging me to travel the world to pursue my interests, for supporting me in countless ways throughout this journey, and for encouraging me to both be myself and find myself. And to Mark Narby – none of this would have been possible without your constant love and encouragement.

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I would also like to thank my major committee members for helping me in fundamental ways throughout my tenure as a graduate student. Specifically, I would like to acknowledge Dr. Robert Raguso for being both a teacher and a mentor throughout my time at Cornell. I appreciate his willingness to teach me how to run and analyze volatile organic compounds, and for providing intellectual and emotional support whenever I needed it. It's rare to find someone as supportive and with as open a door as Rob, and I feel so lucky to have worked with him these past six years. I also want to thank Dr. Anurag Agrawal for continually challenging me to place my work in a larger ecological/evolutionary context, for always being willing to ask me the tough questions, and for teaching me so much about professional development and grant-writing. I appreciate the fact that he provided me with pointed feedback during all of the most important moments in my thesis, and I know I have grown a great deal as a

person and as a scientist from working with him. Finally, working with Dr. Jennifer Thaler has truly been a pleasure. I most appreciate her positive outlook and enthusiasm, for teaching me so much about work-life balance, for her openness and support, and for helping me to continue to think across disciplines and place my work in a larger context. Collectively, I could not have asked for a better committee – they provided just the right balance of support and critique, and helped me grow as a person and as a scientist in countless ways.

In addition to my committee, I am especially grateful to Dr. Monica Geber for serving as an important mentor throughout my time at Cornell. From my first day as a graduate student, she helped me identify and articulate research topics that I am passionate about, and taught me so much about grant proposal writing and effective science communication. I appreciate that I could always talk to her, about any topic personal or professional. And I continue to appreciate her message that success in graduate school (and life) means taking the time to look back and realize how far you've come. I owe so much of the confidence that I gained during my time in graduate school to my interactions with Monica.

Additionally, this dissertation would not have been possible without the help of numerous field and laboratory assistants, including Marta del Campo, Justin Proctor, Ellen Fagan, Leslie Decker, Amy Ericksen, Kayleigh Chalkowski, Ja Young Kim, Jose Luis Caldo Primo, and David Tian. I could not have asked for more enthusiastic, passionate, engaged, and hard-working students, and it was truly a pleasure to work with all of them. I also appreciate the countless times my family and friends stepped in to help with various aspects of my fieldwork – my family members Tom, Ginny and

Susan Morrell, and my fiancée Mark Narby all willingly helped me propagate thousands of rhizomes and conduct field experiments whenever I needed a hand. Their ongoing support, both logistically and emotionally, means a great deal to me.

I could not have asked for a more supportive lab community during my time at Cornell University. Thank you especially to Robert Bode, Stuart Campbell, Maya Lim, Akane Uesugi, Rayko Halitschke, Mark Sarvary, Marta del Campo, and Kaori Shiojiri for countless memories and experiences, and for teaching me so much about science. Working with all of you has been a pleasure! In addition to my lab community, I am also grateful to the Plant Interactions Group (PIG) – a weekly discussion forum about all topics plant, insect, or chemical ecology that taught me a great deal over the years, and also provided constructive feedback at all phases of the work associated with my Ph. D. dissertation.

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Biology-An Ecological Approach course, Cornell's Environmental Policy Processes course in Washington DC, and the Semester in Environmental Science Program at the Marine Biological Laboratory. All of these courses were incredibly transformative experiences for me, both as a person and as a scientist, and I am so grateful to all of the scientists who continue to make these courses available to future graduate and undergraduate students.

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I feel blessed to have incredibly loving and supportive parents, Jim and Susan Morrell. Thank you for raising me to be an inquisitive and curious person, for encouraging me to travel the world and experience other ecosystems and cultures, for making me self-reliant, hard-working and empathetic, and for encouraging me to pursue a wide range of interests – from art to music to science – from an early age. I appreciate your guidance, and also your willingness to give me independence, and your unending love and support as I figured out who I want to be.

And last, but certainly not least, I want to thank my fiancée, Mark Narby, for being the most supportive, loving and encouraging partner I could have ever dreamed of finding. None of this work would have been possible without him, and I am so grateful to have him by my side as we start a new chapter together in Tennessee.

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LIST OF ABBREVIATIONS

ABBREVIATION:	MEANING:
VOC	Volatile organic compound
MAPK	Mitogen-activated protein kinase
GLV	Green leafy volatile
MT	Monoterpene, 10-carbon volatile
SQT	Sesquiterpene, 15-carbon volatile
RGR	Herbivore relative growth rate
RUE	Herbivore resource use efficiency
SPI	Serine protease inhibitor
CPI	Cysteine protease inhibitor
TPI	Trypsin proteinase inhibitor
IR	Induced resistance
IS	Induced susceptibility
NMDS	Non-metric multidimensional scaling
ANOVA	Analysis of variation
PERMANOVA	Permutational analysis of variation
PE	Prediction Error, in Random Forest analysis
BE	Bootstrap Error, in Random Forest analysis
RE	Resubstitution Error, in Random Forest analysis
ER	Error Rate at Random, in Random Forest analysis
HPLC	High-performance liquid chromatography
CA	Caffeic acid derivative
CO	Coumaric acid derivative
FL	Flavonoid derivative
DTA	Diterpene acid
GC-MS	Gas chromatography-mass spectrometry

CHAPTER 1

THE SCENT OF DANGER: VOLATILE-MEDIATED INFORMATION TRANSFER AND DEFENSE PRIMING IN PLANTS

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ABSTRACT

Volatile organic compounds (VOCs) are constantly emitted by plants, and play a major role in information transfer between plants and other organisms. One of the consequences of VOC-mediated information transfer is that plants 'warn' neighboring plants when they are attacked – enabling these neighbors to prime defense responses in anticipation of future attack. Priming refers to a memory effect in which previous exposure to a stimulus (i.e. VOCs) enables plants to respond faster and more strongly when presented with a future stimulus (i.e. herbivory, pathogen infection). In recent years, our knowledge of how VOCs are perceived by plants, and the broad-scale phenotypic changes they induce, has grown dramatically. Multiple plant species seem to prime defense responses following exposure to herbivore-induced VOCs. However, the mechanisms underlying priming remain speculative. Here we highlight recent advancements in our understanding of stress perception by plants and discuss hypotheses of what might be happening on a molecular level in primed plants. Furthermore, we discuss ecological consequences of priming responses such as consequences for plant competitive interactions, and interactions with mutualistic and antagonistic animals.

INFORMATION IN THE PLANT HEADSPACE

Plants constantly emit blends of volatile organic compounds (VOCs) into their aerial environments; however, these emissions are characterized by remarkable context-dependency. VOC blends vary qualitatively and quantitatively with the stressor (or combination of stressors) that a plant is experiencing, such as drought (Tariq et al. 2013), herbivory (Tariq et al. 2013), and infection (Piesik et al. 2011), as well as with the genotype of the damaged plant (Degen et al. 2004), the herbivore species doing the damage (Turlings et al. 1998), and the intensity and

feeding duration of herbivory (Copolovici et al. 2011). In this way, VOC blends reflect a plant's current physiological state in the context of its biotic and abiotic environment, and thereby provide information to any organism that can perceive and process it (Dicke and van Loon 2000).

Many organisms use the information contained in VOC blends. Predators and parasitoids of herbivores may be attracted to the VOCs emitted by plants that their prey/host is feeding on, facilitating "information-mediated indirect resistance" (Kessler and Heil 2011). Similarly, hyper-parasitoids (fourth trophic level) use herbivore-induced plant VOCs to locate their hosts (Poelman et al. 2012), further revealing that VOC-mediated information transfer can mediate complex ecological interactions and potentially compromise effects of interactions at lower trophic levels. Pollinators are attracted to floral VOC blends, but may be repelled by floral and vegetative VOC blends from herbivore-damaged plants (Kessler and Halitschke 2009). Herbivores also respond directly to VOCs – sometimes avoiding and other times aggregating on herbivore-damaged plants (Zakir et al. 2013). Most important to this article, plants respond to VOCs from their damaged neighbors (*plant communication*) by inducing the expression of resistance mediating traits presumably in anticipation of future herbivory (Heil and Karban 2010). While it is now well-established that VOCs can transmit information about a plant's current state of stress, the specific mechanisms of information transfer as well as the costs and benefits to plants of informing other organisms (especially neighboring plants) remain speculative.

PLANTS USING VOC-MEDIATED INFORMATION

To understand the costs and benefits of plant communication, researchers have focused

on understanding its chemical mechanisms and ecological consequences. Interestingly, there are striking similarities in the mechanisms by which plants respond to herbivory and VOCs from neighboring plants (e.g. both involve "damaged-self recognition", Heil 2009). However, while herbivory is more often shown to **directly induce** plant responses, exposure to VOCs from damaged neighbors seems more likely to **prime** plants for stronger or faster responses when an herbivore actually attacks (Engelberth et al. 2004, Kessler et al. 2006). Moreover, direct induction and priming by VOCs both involve a transcriptional and/or metabolic response, but the phenotypic change is only considered direct induction if it results in affected plants increasing production of defense metabolites and becoming chemically-similar to herbivore-damaged plants (Baldwin and Schultz 1983, Farmer and Ryan 1990). On the other hand, priming is evident if exposure to VOCs followed by subsequent attack results in a faster or stronger chemical phenotypic change in comparison to a plant that was damaged by a herbivore but was not previously exposed to a VOC cue (Engelberth et al. 2004, Kessler et al. 2006).

Some have speculated that because plants primed by exposure to VOCs from damaged neighbors seem to arrest their responses until they are actually damaged, priming is a cost-saving mechanism by which plants can use information from neighboring plants but save on the cost of producing expensive defense compounds until they need them for defense (Kessler et al. 2006). This general observation, as well as studies on plant pathogen resistance, led to the hypothesis that plant responses to external stressors are regulated through thresholds above which plants change into a different metabolic state (Conrath 2011). Thus, the transcriptional and/or metabolic changes associated with VOC-mediated priming prepare plants for enhanced responses, which in turn result in yet another metabolic reconfiguration of the plant when it is presented with an additional stimulus (e.g. herbivory).

To date, studies documenting either priming or direct induction following exposure to VOCs from neighboring plants remain rare. VOC-mediated priming has been shown in corn, and tobacco plants next to damaged sagebrush (Engelberth et al. 2004, Kessler et al. 2006; Figure 1.1), whereas VOC-mediated direct induction has been suggested in poplar, sugar maple, and tomato (Baldwin and Schultz 1983, Farmer and Ryan 1990). Although priming and direct induction are typically discussed as alternative plant response strategies, important questions remain about their comparative mechanisms and resultant ecological consequences.

Figure 1.1 Wild tobacco *Nicotiana attenuata* (in front, left) and sagebrush, *Artemisia tridendata* (in back, right), are model plants in the study of volatile organic compound (VOC)-mediated plant-plant interactions. *Nicotiana attenuata* has been shown to prime plant responses after exposure to damaged sagebrush VOCs exemplifying interspecific plant-plant interaction (Karban 2007, Kessler et al. 2006). *Artemisia tridentata* has been show to induce the strongest resistance responses after being exposed to VOCs from damaged plants of the same genotype suggesting that VOC-mediated information transfer is primarily a within-plant signal transduction mechanism (Karban and Shiojiri 2009). (Photo: André Kessler)

Since these studies measured relatively few chemical response variables over a limited time frame, it remains unclear whether priming and direct induction represent two distinct response mechanisms or different states along a response continuum. Likewise, it remains unclear whether plant species will either consistently prime or consistently directly induce its defenses in response to a given stress, or whether priming exhibits stressor- and context-dependent plasticity. Answering these questions has strong implications for the functional interpretation of VOC-mediated plant responses because the physiological and/or ecological context in which the interactions are played out shapes the relative costs and benefits of traits associated with priming versus direct induction. Thus, in the following paragraphs, we explore the current state of our knowledge about priming and direct induction in VOC-meditated plant-plant interactions at three levels: stress perception; downstream responses to stress; and the ecological consequences of these whole-plant responses.

MECHANISMS OF VOC PERCEPTION

In the past decade, our understanding of the molecular mechanisms of stress detection by plants has improved dramatically. Generally, stress perception takes place in the apoplast, where perception of a stressor triggers responses that relay this information to the cell's interior. A central component of plants' perception of biotic stress involves the detection of foreign (non-self) molecules in the apoplast such as pathogen-associated molecular patterns, chemical elicitors in herbivores' salivary excretions, or changes in VOC emission (Heil 2009).

Interestingly, when responding to the VOCs from a neighboring plant or salivary elicitors from a feeding herbivore, plants use a similar mechanism to relay the presence of a stressor to the cell's interior. The process starts with altered calcium ion fluxes and/or potassium-channel

mediated plasma membrane depolarizations, which act in a neuron-like fashion to trigger mitogen-activated protein kinase (MAPK)-dependent phosphorylation cascades, changes in phytohormone production, the production of reactive oxygen species and/or nitrous oxides that connect diverse signaling pathways, the repression and/or activation of transcription factors, and ultimately changes in the expression of defense-related genes (Wu and Baldwin 2010; Figure 1.2).

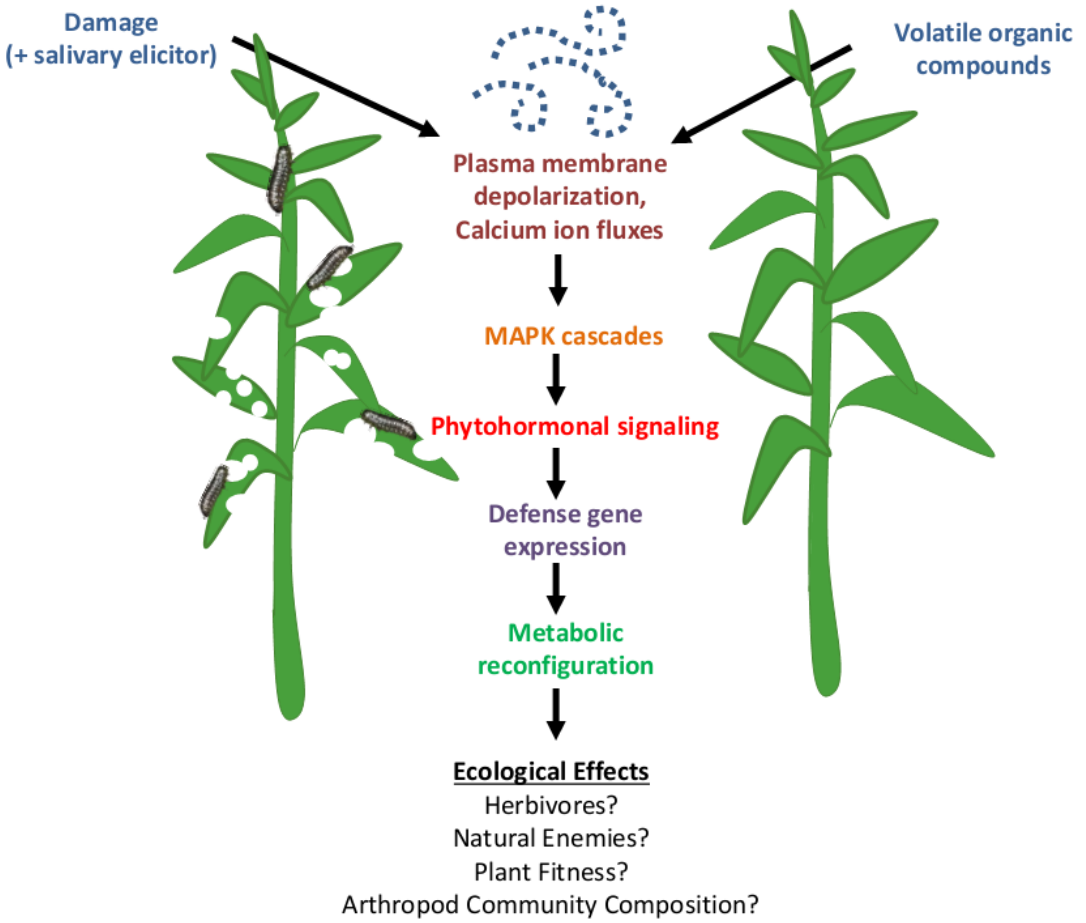

Figure 1.2. Information transfer and plant responses after plant exposure to herbivory (mechanical damage and herbivore-derived chemical elicitors) and volatile organic compounds (VOCs) from damaged neighboring plants. Current knowledge seems to suggest that similar mechanisms of signal perception, signal transduction and resulting metabolic changes apply when plant responses are triggered by exposure to herbivory or by herbivore-induced VOCs. Thus, in response to both stimuli, plants are expected to either directly induce resistance or prime the plant for stronger and/or faster responses when an additional stimulus is applied. Plants' response strength (e.g. priming vs. direct induction of resistance) is a function of intensity and persistence of the stimulus (e.g. herbivore or VOC).

So how, then, do plants capture information about the identity of a stressor and initiate appropriate responses to combat it? Plants seemingly begin tailoring their responses to a specific stressor as early as with the interaction with the cell membrane and associated ion channels, and it seems likely that the magnitude of the perceived stress is captured in these early events – plants elicit stronger initial responses to damage plus salivary secretions than to damage alone (Bricchi et al. 2010). Similarly, only herbivory (not mechanical damage) results in plasma membrane depolarization and calcium fluxes, and herbivory produces reactive oxygen species and nitrous oxides faster and in higher amounts (Bricchi et al. 2010). Moreover, the time of onset of plasma membrane depolarization varies with the identity of the stressor – in *Arabidopsis thaliana*, it is much more rapidly induced by insect herbivores (30min-6 hours) than by pathogens (16 hours), and is highly correlated with the regulation of vastly different suites of genes (Bricchi et al. 2012).

But how do these mechanisms compare when a plant is responding to an actual herbivore versus VOCs from a damaged neighbor? Although studies have not directly compared properties of the early responses elicited by VOCs, mechanical damage and herbivory, it is clear that the composition of the VOC blend affects the time of onset, magnitude and duration of membrane depolarization and calcium fluxes in much the same way that herbivore salivary secretions do. Some VOCs, such as green leafy volatiles (GLVs), cause stronger membrane depolarization than terpenoids (Zebelo et al. 2012). And while GLVs induce calcium ion fluxes, terpenoids seem to have no effect (Zebelo et al. 2012). As with herbivory, exposure to the VOCs from a damaged plant elicits specific changes in gene expression in exposed plants (Godard et al. 2008, Kessler et al. 2006), suggesting that the mechanism by which exposed plants capture and synthesize the information in VOC blends is remarkably similar to the mechanism used to detect stressors like

herbivores or pathogens and involves a similar degree of specificity.

Interestingly, there seem to be dramatic differences in how fast membrane depolarization and calcium fluxes occur in response to different stressors. Although studies documenting the timing of these responses were in two different plant species (Bricchi et al. 2012, Zebelo et al. 2012), it is surprising that responses to herbivores and pathogens were observed on the order of minutes-to-hours, whereas responses to herbivore-induced VOC blends were on the order of seconds. Future studies should elucidate whether this observation holds true within a species and, if it does, what the ecological implications of such a rapid response to VOCs might be.

In tandem, these studies suggest that the time of onset, magnitude and duration of these early events enable plants to capture and transmit specific information about a stressor to the interior of the cell for further signal transduction and induction of metabolic changes. Amazingly, even in the earliest stages of stress perception and response, plants tailor their responses to particular stressors – possibly facilitating specific downstream regulation of directly induced or primed transcriptional and metabolic responses.

HOW DO PLANTS PRIME RESISTANCE?

While the studies above present mechanisms for how plants respond to VOCs and biotic stressors, it remains unclear how the information initially perceived through membrane depolarization and altered calcium fluxes gets propagated throughout the cell and the plant and, more specifically, how the molecular mechanisms underlying priming and direct induction compare.

Although early plant responses to VOCs and herbivores seem comparable, the broad-scale phenotypic changes, which may include changes in the expression of known defense

metabolites and changes in VOC emission (Wu and Baldwin 2010), that come next seem to depend on the plant species. Downstream, some plant species seem to exhibit comparable phenotypic responses to herbivores and herbivore-induced VOCs from neighboring plants. These plants respond by inducing the production of metabolites (i.e. phenolics, proteinase inhibitors) that directly increase their resistance to the stressor (Baldwin and Schultz 1983, Farmer and Ryan 1990).

On the other hand, other plant species exhibit different broad-scale phenotypic responses depending on whether they experience herbivory versus exposure to VOCs. For instance, while they induce the production of defense metabolites directly following herbivory, they seem to arrest the defense elicitation process at some point prior to the production of defense compounds when exposed to VOCs, and only finish eliciting defenses when a subsequent herbivore starts feeding. For example, Kessler et al. (2006) found that while tobacco plants exposed to damaged sagebrush had measurable changes in *trypsin proteinase inhibitor* (*TPI) synthase* activity but no observable induction of TPIs in response to exposure; tobacco plants exposed to damaged sagebrush *and then subsequently damaged* by herbivores produced TPIs significantly faster than tobacco plants that had previously received damage but not VOC exposure.

Although current paradigm presents VOC priming and direct induction of resistance traits as alternative response strategies, all mechanistic information available to date suggest that priming and induction are two related outcomes - they result from similar signaling mechanisms but are simply triggered by different stimulus intensities and persistence – in one case, we get a plant response to VOCs that proceeds fully to completion (i.e. production of putative defense compounds), and in the other case, we get a plant response that seems to be arrested at some point prior to the production of defense compounds. Although the detailed mechanisms still

remain to be elucidated, they likely fall in two distinct but functionally non-mutually exclusive categories:

(A) Downstream responses *change proportionately* with the strength of the incoming information (e.g. concentration of VOCs, extent of damage). For example, the more VOC compounds that interact with the plasma membrane, the greater and longer the depolarization and the stronger the response.

(B) Defense elicitation is more of an on/off response related to *certain thresholds* of incoming information. For instance, the amount of incoming information (e.g. VOCs, damage) only triggers a downstream response when a certain threshold level is reached. In this case, we would expect priming to result from a receptor-type interaction with the VOC followed by subsequent amplification of the signal.

Regardless of the mechanism, we expect a plant's response strength and whether or not a specific resistance trait is directly induced or primed, to depend on the intensity and persistence of the signal. Specifically, the intensity should be a function of the amount of VOCs interacting with the cell membrane, their structural properties and the interactions between different compounds (e.g. synergistic, antagonistic effects). The persistence of the signal is a function of the time a plant is exposed to a compound or compound mixture and with what rate these compounds degrade over time.

Interestingly, published work seems to suggest that either mechanism is plausible. For instance, in *Arabidopsis*, priming is associated with an accumulation of mRNA and inactive proteins of MAPKs (Beckers et al. 2009). Upon challenge, these enzymes are activated and linked to enhanced defensive responses against the stressor (Beckers et al. 2009). These findings seem most in line with mechanism A because, presumably, the more inactive MAPK proteins

that are recruited following VOC exposure, the stronger the defense response the plant can elicit following herbivory. Similarly, we might expect plants to accumulate enzymes involved in particular defense metabolite pathways in response to a mild stimulus such as VOC emission, which would then result in faster and stronger defense elicitation once the substrate of these enzymes is freed upon damage.

In contrast, other studies seem consistent with the idea of a threshold-based defense elicitation mechanism. They suggest that priming is associated with epigenetic modifications that, in effect, put plant responses on hair-trigger alert (Kim and Felton 2013). These modifications effectively lower the threshold required to induce a transcriptional response to a stressor, resulting in faster transcription of genes and therefore a faster and stronger defense response to a subsequent stressor.

WHY MIGHT PLANTS PRIME RESISTANCE?

The regulatory parallels between plants' responses to herbivory and VOCs as we know them so far are striking. Although it is tempting to conclude that common regulatory mechanisms indicate that the resulting plant responses have similar ecological functions, we still need to close significant gaps in our understanding of how plants perceive and process VOCs to answer this question.

Some important avenues to pursue in the future include: what structural features of VOCs explain the magnitude of early perception and subsequent defense gene expression? How is the perception of information linked to the expression and strength of plant defense responses (e.g. gradual vs. threshold-mediated induction of defense responses)? Can we identify any specific receptors for VOC compounds? And how do the signaling cascades leading to priming and direct

induction of resistance traits differ? This latter question has to be answered individually for the expression of different defense metabolites in order to understand specific and systematic differences between responses to direct damage and the exposure to equivalent damage-induced VOC emission.

Furthermore, it is critical to answer these questions in a comparative framework (e.g. comparing responses in different genotypes and species) to clarify the hypotheses that need to be addressed and develop the tools (e.g. breeding populations, genetically transformed plants, etc.) to address these hypotheses. Developing an understanding of phenotypic variation in plant responses to VOCs in general and priming responses in particular, will also allow us to directly address questions about the evolutionary origin of these responses and their ecological functions (Heil and Karban 2010). Currently most of the hypotheses regarding the functions of VOC-mediated plant-plant interactions are based on experiments that show a mechanism, namely that plants exposed to a damaged neighbor's VOC emission and damaged plants become equally resistant and more resistant than plants that were not exposed to VOCs from a damaged neighbor. However, an ecological function can only be indicated if natural variation of and natural selection on the trait, VOC-mediated resistance induction, can be demonstrated. Karban and Shiojiri (2009) conducted a first approach towards a functional analysis of plant-plant interactions, and found that sagebrush genotypes (*Artemisia tridentata*) respond most strongly to the VOCs from damaged plants that are of their same genotype. This finding led to the hypothesis that VOC-mediated information transfer may have evolved as a within-plant signal to overcome vascular limitations of signal transduction in branched plants. Consequently, neighboring plants perceiving and utilizing this information are likely "eavesdropping" and may gain a competitive advantage over their damaged neighbors by readying their defenses and

potentially increasing their investment in increased competitive ability. Conversely, plants exposed to damaged neighbors may suffer reduced performance if subsequent herbivory is of little probability (e.g. functional allelopathy, Karban 2007). In such cases priming may benefit plants by enabling them to respond to the information in VOC blends without the cost of producing costly defense metabolites (Kessler et al. 2006). As an alternative to the within-plant-signaling hypothesis, plants that grow in dense populations may gain benefits from exchanging information about herbivory and inducing resistance in their neighbors by so generating patches of resistant plants that reduce the probability of additional herbivory and/or the return of herbivores to this patch. In this scenario both the senders and receivers will benefit from the information exchange. No such case has been reported thus far and re-emphasizes the need for a comparative approach to the study of plant communication.

Similarly, a comparative approach could provide insights into the relative importance and ecological function of direct vs. primed induction of plant response to plant VOCs. While both priming and direct induction might elicit responses that differ mechanistically, it is possible that the responses may nonetheless be equally effective at combating the stressor. Researchers have already begun to think about applications of priming responses through the exposure of crop plants to resistance-inducing or priming VOCs. As our understanding of the mechanisms and functions of plant-plant information transfer grows, scientists may soon learn to control information transfer between plants in order to improve pest control technologies in agricultural systems.

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CHAPTER 2

PLANT COMMUNICATION IN *SOLIDAGO ALTISSIMA*: KEEPING HERBIVORES ON THE MOVE

In review for publication in *Functional Ecology* as: Kimberly A. Morrell and André Kessler. Plant communication in *Solidago altissima*: Keeping herbivores on the move.

ABSTRACT

Plant communication has been documented in over 35 plant species spanning 16 families to date; however, the underlying mechanisms through which it shapes plants' ecological interactions remain less clear. Using a combination of field/laboratory bioassays and leaf chemical analyses in the tall goldenrod (*Solidago altissima*)-leaf beetle (*Trirhabda virgata*) system, we tested the hypothesis that volatile organic compound (VOC)-mediated plant communication affects the performance, feeding and movement behavior of herbivores by changing plants' chemical phenotypes. We found that plant communication accelerates herbivore movement between host plants while simultaneously reducing herbivory. This suggests that a major ecological function of plant communication can be to limit herbivore loads by keeping herbivores on the move between host plants. Moreover, despite qualitative and phenological differences in plants' chemical responses to herbivory-induced VOCs and feeding herbivores, it is interesting that herbivores nonetheless perceive herbivore-damaged plants and plants exposed to VOCs from damaged neighbors as being of equivalently poor quality. In a larger context, this study suggests that by enlarging the spatial scale at which induced resistance affects the distribution of plant chemical phenotypes in plant populations, plant communication alters the movement behavior and performance of herbivores.

KEYWORDS: volatile organic compounds, plant-to-plant signaling, herbivore movement behavior, herbivore performance, plant defense chemistry

INTRODUCTION

Plants constantly emit blends of volatile organic compounds (VOCs) into their

headspaces, and the chemical composition of VOC blends conveys information about a plant's species/genotype and the biotic and abiotic stresses it is presently experiencing (reviewed in Dudareva et al. 2006; Dicke and Baldwin 2010). Plant VOC emissions vary qualitatively and quantitatively with drought (Funk et al. 2005), light levels (Kegge et al. 2013), temperature (Duhl et al. 2008), ozone stress (Pellegrini et al. 2012), pathogen infection (Oluwafemi et al. 2012) and herbivory (Dicke and Baldwin 2010). Additionally, numerous organisms perceive and respond to the information contained in VOC blends, including pollinators, herbivores, predators, parasitoids, hyper-parasitoids, and neighboring plants (Dicke and Baldwin 2010; Heil and Karban 2010; Poelman et al. 2012).

Understanding the mechanisms underlying these ecological interactions remains difficult because organisms may respond *directly* to the VOCs, and/or *indirectly* to the changes that those VOCs induce in the community (i.e. induced changes in plant chemistry, or in the composition of the arthropod community). For instance, herbivore-induced VOCs may directly attract or repel subsequent herbivores (Dicke and Baldwin 2010), while VOC-mediated information transfer between plants (hereafter plant communication) may indirectly affect plants' subsequent levels of herbivory (Heil and Karban 2010).

Although plant communication was once highly controversial (Fowler and Lawton 1985), it is now considered a widespread and ecologically relevant phenomenon (Karban et al. 2014). In response to VOC cues from damaged neighbors, "exposed" plants can change their chemotypes and thereby reduce the feeding and performance of subsequent herbivores (Heil and Karban 2010; Karban et al. 2014); however, the mechanisms of resistance remain less clear. Studies have documented reduced herbivore survival/performance (Kessler et al. 2006; Yoneya et al. 2014) and reduced herbivory (Heil and Karban 2010) on exposed plants, but very few have investigated

whether and how plant communication affects herbivore behavior and dispersal. VOC-mediated plant communication has been shown to affect herbivores in four major ways. Specifically, exposed plants may:

A. Prime or directly induce changes in leaf chemistry that directly affect their quality for subsequent herbivores (*active*; e.g. Baldwin and Schultz 1983; Farmer and Ryan 1990; Kessler et al. 2006)

B. Take up and convert VOCs into toxins that may be used against subsequent herbivores (*active*; Sugimoto et al. 2014)

C. Prime or directly induce changes in VOC emission that affect their perceived quality to subsequent herbivores (*passive*; Engelberth et al. 2004)

D. Adsorb/re-release VOCs from neighboring plants onto their leaf cuticular surfaces, affecting their perceived quality (*passive*; Choh et al. 2004; Himanen et al. 2010)

Within the framework of hypothesis A, these experiments test if VOC-mediated plant communication in tall goldenrod, *Solidago altissima*, induces chemical changes in exposed plants and affects herbivore performance and behavior.

Herbivore-induced plant responses have been well-documented at the level of defense-related gene expression (e.g. Baldwin et al. 2001), defense compound production (e.g. Bode et al. 2013), and VOC emission (e.g. Dicke and Baldwin 2010); as well as in bioassays measuring herbivore survival, performance, and locomotion/feeding behavior (reviewed in Karban 2011). Although less well understood, plants' responses to herbivore-induced plant VOCs, seem very similar mechanistically (Morrell and Kessler 2014); however, the degree to which the ecological effects of plant responses induced directly by herbivory are comparable to those induced by VOCs from neighboring plants remain unclear, and are therefore a major focus of this study.

The effect of induced resistance on herbivore locomotion depends on the spatial extent of the response – localized induced resistance increases within-plant locomotion, whereas systemic induced resistance increases between-plant and between-patch movement (Hoy et al. 1998; Tiffin et al. 2006; Underwood et al. 2011; Hamback et al. 2014). Herbivores are also sensitive to *heterogeneity* in plant defenses (including herbivore-induced variability) and move more within heterogeneous plants and plant patches (Karban 2011).

Moreover, a particular induced response can affect herbivore species in different ways, depending on their mobility and selectivity (Karban 2011). Herbivore mobility influences the time scale over which plant defenses affect herbivore behavior – mobile herbivores increase their feeding and locomotion on poor-quality hosts (e.g. Hoy et al. 1998; Fernandes et al. 2011). Herbivore selectivity governs how herbivores respond to variation in tissue quality, the criteria they use to select hosts, how intensely they feed, their frequency of movement within and between host plants, and the distribution of their damage in the population (Karban 2011). While herbivores clearly respond to herbivore-induced variation in plant traits, it is not yet clear whether plant communication-induced variation in plant traits has similar effects on herbivores' performance, feeding and movement behavior.

To better understand the importance of plant communication in shaping plant-herbivore interactions, we addressed four questions: (1) does plant communication affect herbivores' movement behavior and performance? (2) Do VOCs mediate plant communication? (3) How do the defense phenotypes of damaged and exposed plants compare? And (4) what is the spatial extent of plant communication?

We addressed these questions using tall goldenrod (*Solidago altissima*) – a dominant perennial species in the old-field Asteraceae forb community native to northeastern North

America. Since *S. altissima* is clonal, neighboring plants in the population are often the same species and genotype. Additionally, insect herbivory is an important selective force affecting *S. altissima*'s competitive dominance – at high herbivore loads, seed set and stem growth are severely inhibited (Root 1996; Carson and Root 2000). Thus, any factor (behavioral or performance-related) that reduces or maintains low herbivore pressure should positively affect plant fitness.

Moreover, *S. altissima* is associated with a diverse arthropod community (Maddox and Root 1987, 1990; Root and Cappuccino 1992). One of these herbivores, the leaf beetle larva *Trirhabda virgata* (Coleoptera:Chrysomelidae) is a mobile, selective, specialist whose feeding is significantly negatively correlated with *S. altissima*'s fitness (Meyer 1993; Meyer and Root 1993; Meyer 1998). *Trirhabda* is sensitive to induced resistance (Hufbauer and Root 2002), exhibits frequent locomotion between host plants (Goodwin and Fahrig 2002), and induces changes in VOC emission and leaf chemistry (Kessler and Morrell 2010; Bode et al. 2013; Uesugi et al. 2013). Although it is unclear whether *S. altissima* plants communicate, the high density of the plant community, the clonal structure of *S. altissima*, and the high intensity of herbivory shared among several plant species (Carson and Root 1999, 2000) suggest a potential value of plant communication in maintaining the low herbivore pressure observed >75% of the time (Root 1996). Additionally, *Trirhabda*'s high mobility, selectivity, and impact on *S. altissima*'s fitness, suggest that any plant trait that increases *Trirhabda*'s between-plant locomotion may benefit *S. altissima*.

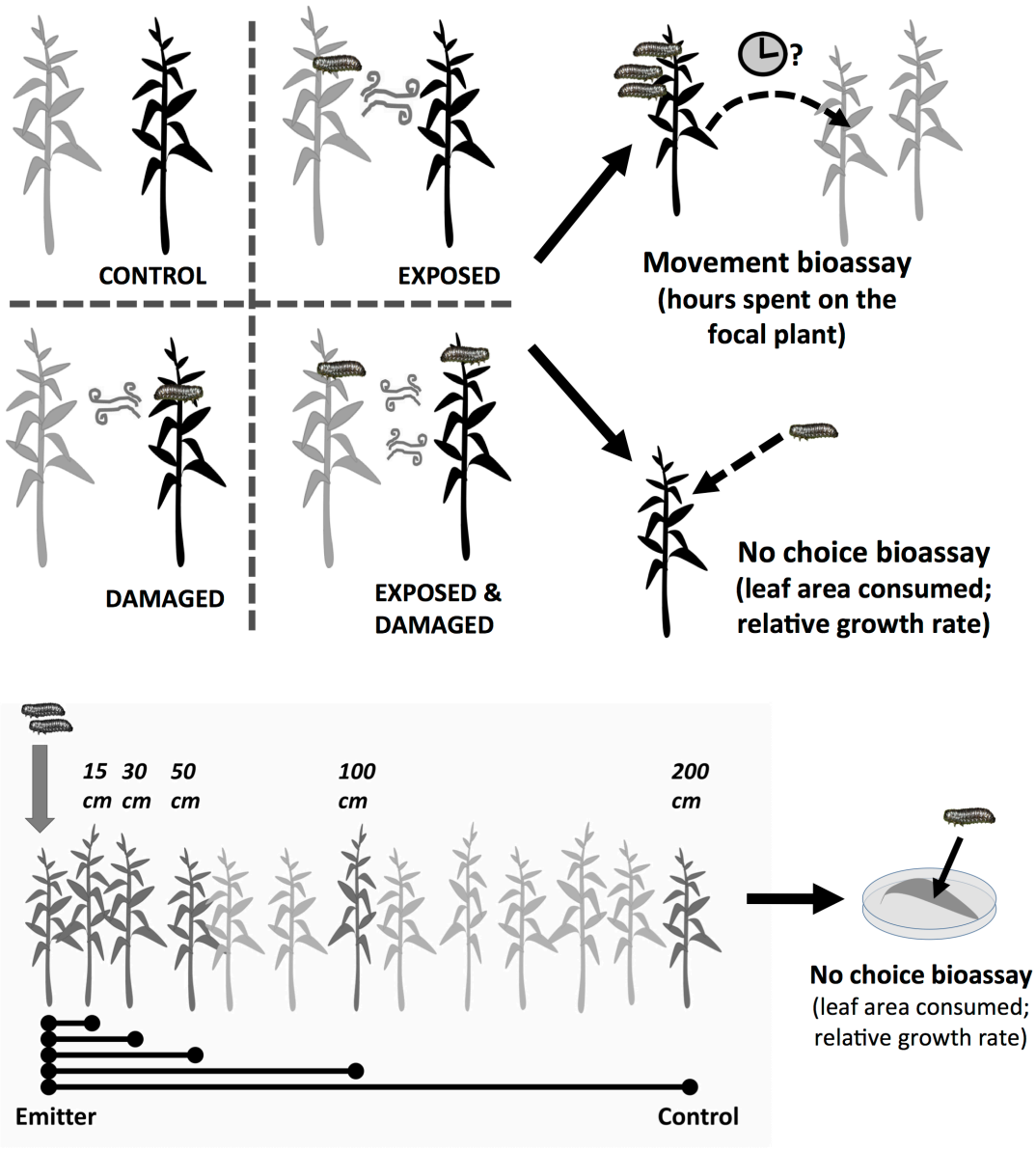

Figure 2.1 We used a 2x2-factorial experimental design with damage by *Trirhabda* ( ) and exposure to VOCs from damaged plants as factors (upper). Black plants indicate "focal" plants measured in bioassays; gray plants ("neighbors") facilitated treatment application. In *Experiment 1*, the time the *Trirhabda* spent on the focal plant indicates the effect of host quality on herbivore behavior; in *Experiment 2*, we measured herbivore performance. We conducted *Experiment 4* (lower) in a natural old-field community where we damaged random focals (0cm), and performed no-choice bioassays on focals and neighbors 15cm, 30cm, 50cm, 100cm and 200cm away.

MATERIALS AND METHODS

Treatments

To test whether *Solidago altissima* plants communicate and whether this affects plant chemistry and herbivores, we employed four treatments (control, exposure, damage, exposure + damage) in a 2x2-factorial design with exposure and damage as independent factors (Figure 2.1). We applied these treatments to pairs of plants, either naturally standing next to each other in the field (*Experiment 1*), or in pots placed next to each other (*Experiment 2-3*). One plant in the pair was designated the "focal" on which core measurements were performed, and the other its "neighbor" that received treatments to which focals were exposed. Control plants (focals and neighbors) experienced no herbivory over the course of the treatment period; focal plants in the damaged treatment were damaged with 2-3 *Trirhabda* larvae for a continuous period of 4-7 days; and exposed focals were next to neighboring plant that was continuously damaged by 2-3 *Trirhabda* larvae. In the exposed + damaged treatment, both the focal and neighbor were damaged by 2-3 *Trirhabda*. In order to test for a priming effect, we began the exposure treatment 24 hours before starting the damage treatment.

To specifically test whether chemical phenotypic changes in the focal plant affect the performance and behavior of our bioassay *Trirhabda*, we removed the neighbor plant prior to starting the bioassays. This eliminated the possibility that our bioassay insects responded to the VOC blends from neighbors (instead of the chemistry of focals), which is known to occur (Bernasconi et al. 1998; De Moraes et al. 2001).

Experiment 1: Does plant communication influence herbivore movement behavior in the field?

We set up the four treatments (control, exposure, damage, exposure + damage) described above in an old-field plant community in the Finger Lakes region of New York State (Whipple

Farm, 42.487895, -76.429448). We selected 40 pairs of *S. altissima* plants along a linear transect through the old-field (n=10 focal-neighbor pairs per treatment). Every 10 meters, we selected four pairs of plants (1 experimental block) in the NE, SE, NW and SW directions so that each plant pair was at least 1m apart. Each plant pair in a block was randomly assigned one of the four treatments (Figure 2.1). We enclosed the plant pairs in breathable mesh sleeve bags (Breather^{TM}, Palm Tree Packaging), and designated one plant the focal and the other its neighbor.

To create the treatments, we applied three 2nd-instar *Trirhabda* larvae to the neighbors (for exposed or exposed + damaged pairs) on May 14, 2010, and to the focals (for damaged or exposed + damaged pairs) on May 15, 2010 (Figure 2.1). We allowed *Trirhabda* to feed freely for 7 days, which is typical for 2nd-instar larvae (André Kessler, *personal observation*). After the treatment period, we removed the *Trirhabda* and neighbors to cease exposure, removed the mesh bags, and added three 2nd-instar bioassay *Trirhabda* per focal. We counted the number of *Trirhabda* remaining on the focals approximately every 4 hours during the day for the next 48 hours.

We repeated the experiment in 2011, as described above, and applied the treatments on June 7-8, 2011 using three 3rd-instar *Trirhabda* larvae. We allowed the treatment larvae to feed for 4 days, as is typical for this later instar (André Kessler, *personal observation*). After the period of initial damage and/or exposure, we removed the treatment larvae, neighboring plants, and mesh bags, and applied three 3rd-instar bioassay larvae. Approximately every 6 hours during the day for the next 72 hours, we counted the number of *Trirhabda* remaining on each focal.

We analyzed 2010 and 2011 larval retention data separately in R (version 2.15.1, package "lme4") using repeated measures ANOVAs (*glmer*) with binary responses indicating the presence (yes/no) of individual bioassay larva on each focal. We included our treatments

(Exposure*Damage) and time as factors in the model, and larval identity and plant genotype as random factors.

Experiment 2: Do VOCs mediate plant communication in S. altissima*?*

Because the rhizome connectivity of plants used in *Experiment 1* was unknown, our field behavioral bioassays could not differentiate whether undamaged neighboring plants responded to information from vascular or VOC cues. To test whether VOCs mediate plant communication, we performed an additional experiment with potted *S. altissima*, eliminating vascular connectivity.

We propagated three *S. altissima* genetic clones, one from Whipple Farm (2010) and two from Durland Preserve (42.437931, -76.398380; 2011), by clipping 3cm pieces of rhizome containing an apical meristem and a root, and placing them vertically (root-down) in flats of Metro-Mix 360® soil. We submerged the flats in water, and used humidity domes to keep the cuttings moist. Once they developed new roots and young leaves, after approximately six days, we uncovered the flats, removed the submerging trays and began watering twice daily and fertilizing once weekly. We transplanted 5cm propagules into 25cm-diameter pots and grew them to 15cm over the course of 8 weeks (greenhouse: 23^{o}C day/19^{o}C night; 16/8-hour light regime). The *Trirhabda* larvae used in these experiments were collected from natural field populations in Ithaca, NY.

During June-July 2010, we placed one emitter and four receivers (potted; of the same genetic clone) into each of eight 35cmx70cmx35cm plexiglass chambers. Computer fans circulated the air in the chambers and incoming air was filtered (using ORBO^{TM} 32 small activated charcoal traps, Supelco®), as in Kessler et al. (2006). We began our exposure treatments (using two 3rd-instar *Trirhabda*) 24 hours prior to starting our damage treatments

(Figure 2.1). After four days of damage, we removed plants and treatment larvae and enclosed the plants in mesh bags. We excised and froze leaves of similar developmental stages (first fully developed leaves, at positions five and six below the apical meristem) in liquid Nitrogen, and stored them at -80^{o}C for chemical analysis (see *Experiment 3*). One 3rd-instar bioassay *Trirhabda* larva was applied to the fourth position leaf and allowed to feed freely on the entire plant. After three days, we measured leaf area consumed (cm^2 using ImageJ® 4.13), larval relative growth rate (RGR; mass gain/initial mass), and larval resource use efficiency (RUE; relative growth rate/leaf area consumed).

We repeated this experiment twice more in June 2011, under field conditions in a common garden (East Ithaca, 42.440795, -76.470926). Prior to the experiment, the field site was plowed so that no other plants were within five meters of the treated plants. Potted focals and neighbors (genotype constant within an experiment) were placed 15cm apart, with at least two meters between plots. We applied two 3rd-instar larvae to appropriate focals and neighbors to apply our treatments (as in Figure 2.1). After four days of treatment application, we excised the fourth position leaf, hydrated the petiole with moist flower foam, and placed each leaf in a petri dish with a 3rd-instar bioassay larva. We determined leaf area consumed, larval RGR and RUE 72 hours later.

In a meta-analysis of these experiments, we compared leaf area eaten, RGR, and RUE across our treatments using ANOVAs in R. We used a log-transformation to normalize all performance data, and included damage (yes/no), exposure (yes/no), and environment (field/greenhouse) as interactive factors, and experiment number (A/B/C) and block as random factors.

Experiment 3: How do the induction phenotypes of exposed and damaged plants compare?

To compare the induction phenotypes of exposed, damaged, and exposed + damaged plants, we analyzed the phenolic content and serine (SPI) and cysteine (CPI) protease inhibitor activity of leaves harvested from *Experiment 2*. We measured phenolic chemistry to characterize the general induction phenotype of *S. altissima* because it reflects a large range of compounds is thereby a proxy for plant metabolic response without *a priori* assumptions of resistance-mediating functions. We also measured CPI and SPI activity because of their well-studied resistance effects on Coleopteran herbivores, including *Trirhabda* (Bode et al. 2013).

We measured induced changes in phenolic secondary metabolites using the protocol described by Keinanen and colleagues (2001). Frozen leaf samples (~100-200mg leaf tissue) were extracted in 1mL 90% MeOH using a FastPrep® tissue homogenizer, and the supernatant was analyzed for phenolic secondary metabolites by high-performance liquid chromatography (HPLC) on a Hewlett Packard 1100 series HPLC with a Gemini C18 reverse-phase column (3µm, 150mm x 4.6mm; Phenomenex, Torrance, CA, USA), using an acidic water/acetonitrile elution system as follows: 0-6min, acetonitrile increased from 0-12%; 6-10min, from 12-18%; and 10-30min, from 18-58%, with a flow rate of 1mL/min and an injection volume of 15uL. We quantified phenolic compounds at 320nm and calculated the concentration of individual phenolics by dividing peak area by the mass of leaf tissue in the sample.

SPI and CPI activity mg^{-1} protein were measured using the micro plate assays developed by Bode et al. (2013). Briefly, we extracted our frozen leaf tissue samples (~100-200mg leaf tissue) in 1mL 25mM sodium phosphate buffer (pH 7.2) (containing sodium chloride, EDTA, phenylthiourea, diethyldithiocarbamate, and polyvinylpolypyrrolidone) using a FastPrep® tissue homogenizer.

We quantified the protein in the supernatant with a Bradford assay (Bode et al. 2013) by comparing the total absorbance of our samples at 595nm to the total absorbance of a standard immunoglobulin dilution series on a Synergy HT multi-detection micro plate reader (Bio-Tek).

SPI activity was determined by conducting an enzyme-substrate reaction with our leaf samples and 0.25mg/mL trypsin in a 0.1 M TRIS (pH 7.6) reaction buffer. We added N-benzoyl-DL-arginine-b-naphtylamide in dimethyl sulfoxide as a substrate, and allowed the reaction to proceed for 20 minutes. We subsequently analyzed total sample absorbance at 540nm before and after a dye reaction with p-dimethylamino-cinnamaldehyde in ethanol on the plate reader. We determined SPI activity (mg SPI activity mg^{-1} protein) by comparing sample activity to a standard curve from soybean trypsin protease inhibitor standards.

We used a similar enzyme-substrate reaction to measure CPI activity, except we conducted the reaction with 0.25mg/mL papain (not trypsin) in a 0.25 M sodium phosphate reaction buffer (pH 6.0) with EDTA (Bode et al. 2013). CPI activity was calculated as percent of inhibited papain activity in the sample relative to a positive control (containing only papain and buffer) per milligram of protein.

The concentration of individual phenolic compounds, mg SPI activity mg^{-1} protein, and percent CPI activity mg^{-1} protein were analyzed across treatments in R using ANOVAs with exposure, damage, and hours post treatment as interactive factors. We normalized phenolic, SPI and CPI data using a log-transformation (+0.005).

Experiment 4: What is the spatial extent of plant communication?

To test the spatial extent of plant communication in *S. altissima*, we compared herbivore performance on a damaged plant and its neighbors at varying distances in the field.

On June 28, 2011, in a forest edge old-field community (Beebe Lake; 42.450658, -

76.473616), we selected 32 random focal plants, at least five meters apart, enclosed the top third of the plant in a mesh sleeve bag, and added two 3rd-instar *Trirhabda*. After four days of continuous feeding, we harvested fully expanded leaves (at position seven below the apical meristem) from focals, and neighbors 15cm, 30cm, 50cm, 100cm, and 200cm from the focal (Figure 2.1), each in a randomly chosen direction to account for variable wind direction.

These distances were selected because *S. altissima* plants stand an average of 16.2cm from their neighbors, so 15cm represents an immediate neighbor, and 50cm represents a neighbor three plants away. In naturally-occurring old-field populations, *S. altissima*'s mean rhizome length is 14cm, but exhibits high variability (≥6-54cm), suggesting that *S. altissima* is not necessarily related to its immediate neighbors (Cain 1990).

During collection and the bioassays, we hydrated the harvested leaf petiole in moist flower foam. In the lab, we scanned the leaves to determine initial damage, and put each leaf in a Petri dish together with one 3rd-instar *Trirhabda* larva. We allowed the larvae to feed for three days, after which we re-scanned the leaves for tissue consumption measurements and determined the final mass of the larvae. As in *Experiments 1-2*, we calculated leaf area consumed, larval RGR and RUE. In R, we log-transformed all response variables to normalize, performed generalized linear models (*glm*) across all distances, and compared focals and neighbors at each distance using one-tailed paired *t*-tests.

RESULTS

Does plant communication influence herbivore movement behavior?

Across the movement period, the number of *Trirhabda* larvae remaining on the focal plants decreased significantly with time for all treatments in both years (**2010**: z=-8.14,

P<0.0001; **2011**: z=-3.43, P<0.0001; Figure 2.2).

Additionally, focals that had previously received damage, were exposed, or had been exposed + damaged all experienced significantly sharper declines in the number of *Trirhabda* remaining than controls. Across the movement interval, we observed significant effects of damage (**2010**: z=-2.99, P=0.003; **2011**: z=-5.84, P<0.0001), exposure (**2010**: z=-2.59, P=0.01; **2011**: z=-5.84, P<0.0001), and an exposure*damage effect (**2010**: z=3.02, P=0.003; **2011**: z=5.71, P<0.0001) compared with controls.

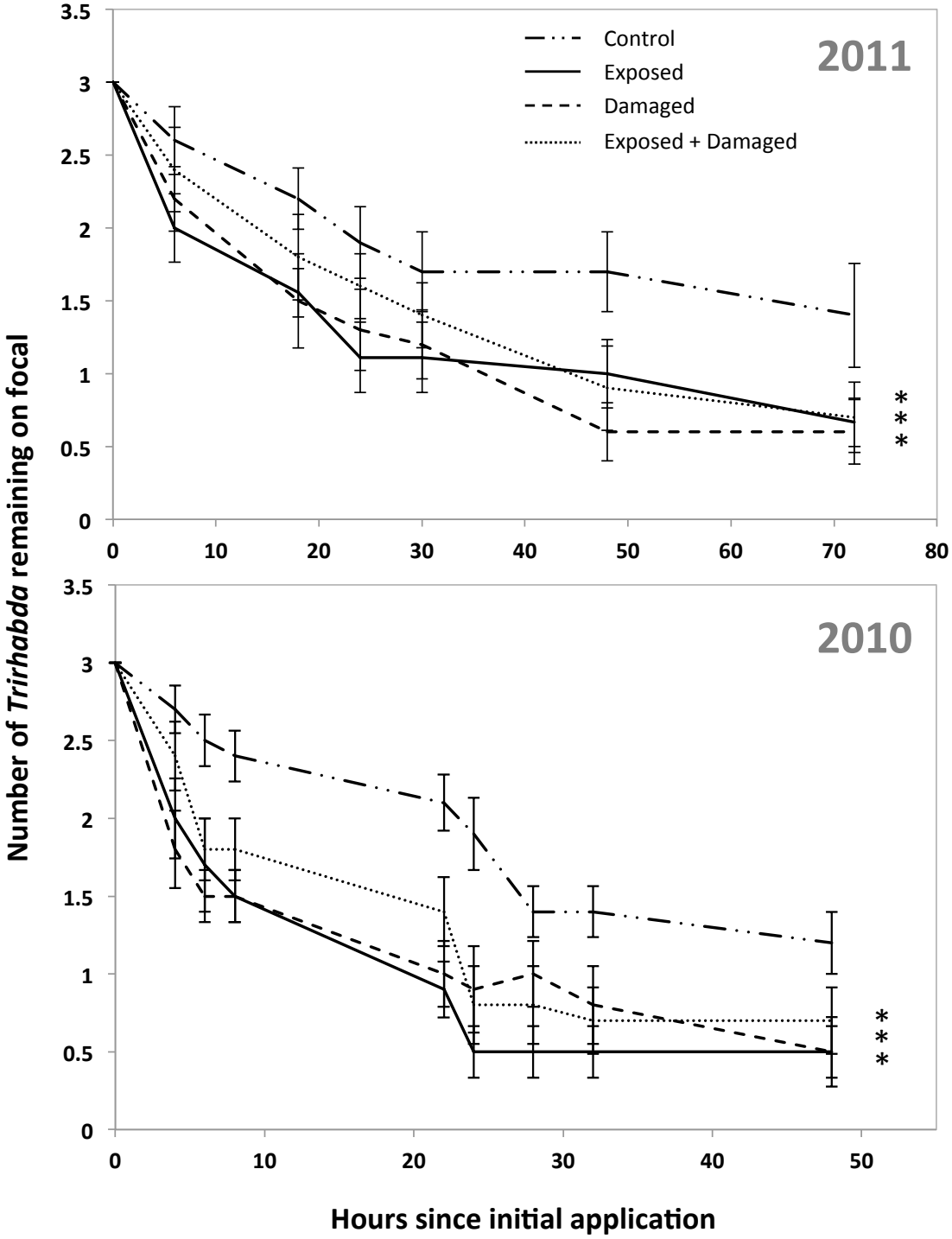

Figure 2.2 The number of *Trirhabda* remaining on control, exposed, damaged, and exposed + damaged plants as a function of time since the initial application of 3 Bioassay *Trirhabda*. This experiment was repeated in June 2010 and July 2011. Bars shown represent standard error, and stars indicate that the linear regression for a treatment across time points was significantly different ($P < 0.001$) from the control regression.

Do volatile organic compounds mediate plant communication?

Consistent across one greenhouse and two field experiments, the leaf area consumed by *Trirhabda* larvae was significantly negatively affected by exposure ($F_{1,100}$=6.39, P=0.01) and damage ($F_{1,100}$=9.47, P=0.003) (Figure 2.3). We also observed a significant exposure*damage effect on leaf area consumed ($F_{1,100}$=5.34, P=0.02).

Trirhabda RGR was significantly negatively affected by damage ($F_{1,100}$=4.28, P=0.04), but not exposure ($F_{1,100}$=0.0072, P=0.93), and we did not observe an exposure*damage effect on RGR ($F_{1,100}$=0.95, P=0.33). Additionally, exposure ($F_{1,98}$=4.41, P=0.04) significantly affected larval RUE, but damage ($F_{1,98}$=0.11, P=0.75) and exposure*damage ($F_{1,98}$=0.46, P=0.50) did not.

Environment (greenhouse, field) was a significant predictor of RGR ($F_{1,100}$=8.19, P=0.005), and leaf tissue consumption ($F_{1,100}$=162.33, P<0.0001), but not RUE ($F_{1,98}$=0.24, P>0.05). RGR were significantly higher in the greenhouse (1.12±0.13mg, N=37) than in the field (0.29±0.07mg, N=70). Similarly, larval leaf tissue consumption was significantly higher in the greenhouse (11.76±1.29cm^2, N=37) than in the field (1.32±0.18cm^2, N=70). In contrast, larval RUE was significantly higher in the field (0.51±0.49mg/cm^2, N=70) than in the greenhouse (0.16±0.03mg/cm^2, N=37).

Additionally, there was a significant damage*environment effect on RUE ($F_{1,98}$=10.13, P=0.002), and marginally significant damage*environment effects on leaf tissue consumption ($F_{1,100}$=3.37, P=0.07) and RGR ($F_{1,100}$=3.84, P=0.053), suggesting that plants responded differently to damage in the greenhouse than in the field.

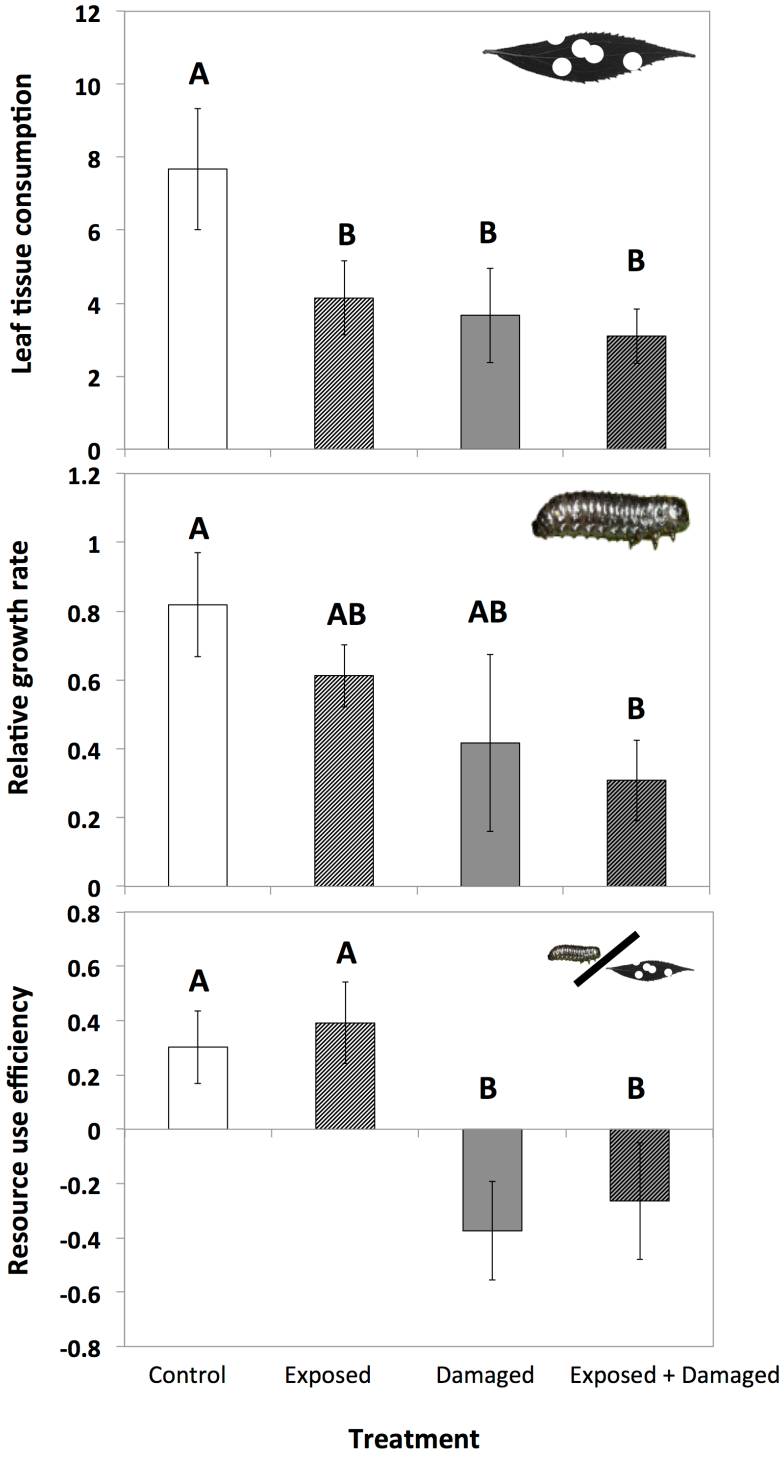

Figure 2.3 The effect of prior damage and/or exposure on the average (±SEM) leaf area consumed (cm^2), relative growth rates (RGR), and the resource use efficiency (RUE; relative growth rate/leaf area eaten) of *Trirhabda* across one greenhouse and two field experiments using potted plants. Damage, exposure, and exposure*damage significantly affected leaf area consumed. Damage significantly affected RGR, and exposure significantly affected RUE. The environment in which the study was conducted (field/greenhouse) significantly affected all performance measures. Letters reflect results of Tukey's Honestly Significant post-hoc tests.

Do exposed plants and damaged plants have similar induction phenotypes?

Both damage and exposure elicited significant changes in plant chemistry; however, the chemotypes induced by damage and exposure differed by treatment and over time. The treatment effects were subtle – none of our treatments elicited an increase in total phenolics (ANOVA: $P>0.05$), and damage elicited a significant, albeit slight, increase in the number of phenolic compounds produced (ANOVA: $F_{1,75}=7.14$, $P=0.009$).

Although total phenolic production and compound diversity did not differ considerably across treatments, exposure and damage significantly affected the overall chemical phenotype of *S. altissima*. A MANOVA incorporating CPIs, SPIs, and 29 phenolic compounds from three compound classes (10 caffeic acid (CA) derivatives, eight coumaric acid (CO) derivatives, six flavonoids (FL), and five unknown compounds) showed significant effects of exposure ($F_{1,29}=6.32$, $P<0.0001$), damage ($F_{1,29}=2.11$, $P=0.02$), and time post treatment application ($F_{4,128}=1.72$, $P=0.001$).

MANOVAs also revealed significant effects of damage, exposure and time point on the induction of classes of phenolic compounds – CA derivatives showed significant induction with exposure ($F_{1,50}=4.55$, $P=0.0001$), time point ($F_{4,212}=1.77$, $P=0.005$), and exposure*time point ($F_{4,212}=1.64$, $P=0.01$). Similarly, CO derivatives showed significant induction with exposure ($F_{1,52}=3.70$, $P=0.002$), damage ($F_{1,52}=2.18$, $P=0.04$), and time point ($F_{4,220}=1.74$, $P=0.01$). FLs, collectively, were not induced, and unknown compounds showed significant effects of exposure ($F_{1,55}=6.19$, $P=0.0001$), time point ($F_{4,232}=1.84$, $P=0.02$), exposure*time point ($F_{4,232}=1.89$, $P=0.01$), and damage*time point ($F_{4,232}=1.67$, $P=0.04$).

Subsequent ANOVAs on individual phenolic compounds showed that 19 were (marginally) significantly affected by damage and/or exposure for at least one time point. They

included CA, CO, and FL derivatives, and some unknown phenolic compounds (Figure 2.4). Moreover, the number of compounds that exhibited statistically significant responses increased from one compound at 24 hours, to 19 compounds at 96 and 120 hours.

We observed a wide range of effect types, so we classified phenolics into five classes based on their responses to exposure/damage, including: (**1**) induction or suppression by exposure (exposure effect); (**2**) induction or suppression by damage (damage effect); (**3**) an interactive effect of damage and exposure; (**4**) induction or suppression by exposure as well as by damage (both effects, not necessarily the same directions); and (**5**) non-responding (Figure 2.4). Specifically, 14 compounds exhibited **pattern 2**: induction by damage, 12 compounds exhibited **pattern 1**, five had interactive effects of damage and exposure (**pattern 3**), and three had separate damage and exposure effects (**pattern 4**). Interestingly, all three compounds that were affected by damage and exposure (CA at 17.34min, FL at 18.42min, unknown at 12.75min) were induced by exposure, but suppressed by damage. Exposure seemed to elicit an earlier response than damage – the number of compounds exhibiting exposure effects (**pattern 1**) peaked at 72-96 hours, whereas the number of compounds exhibiting damage effects (**pattern 2**) peaked at 120 hours.

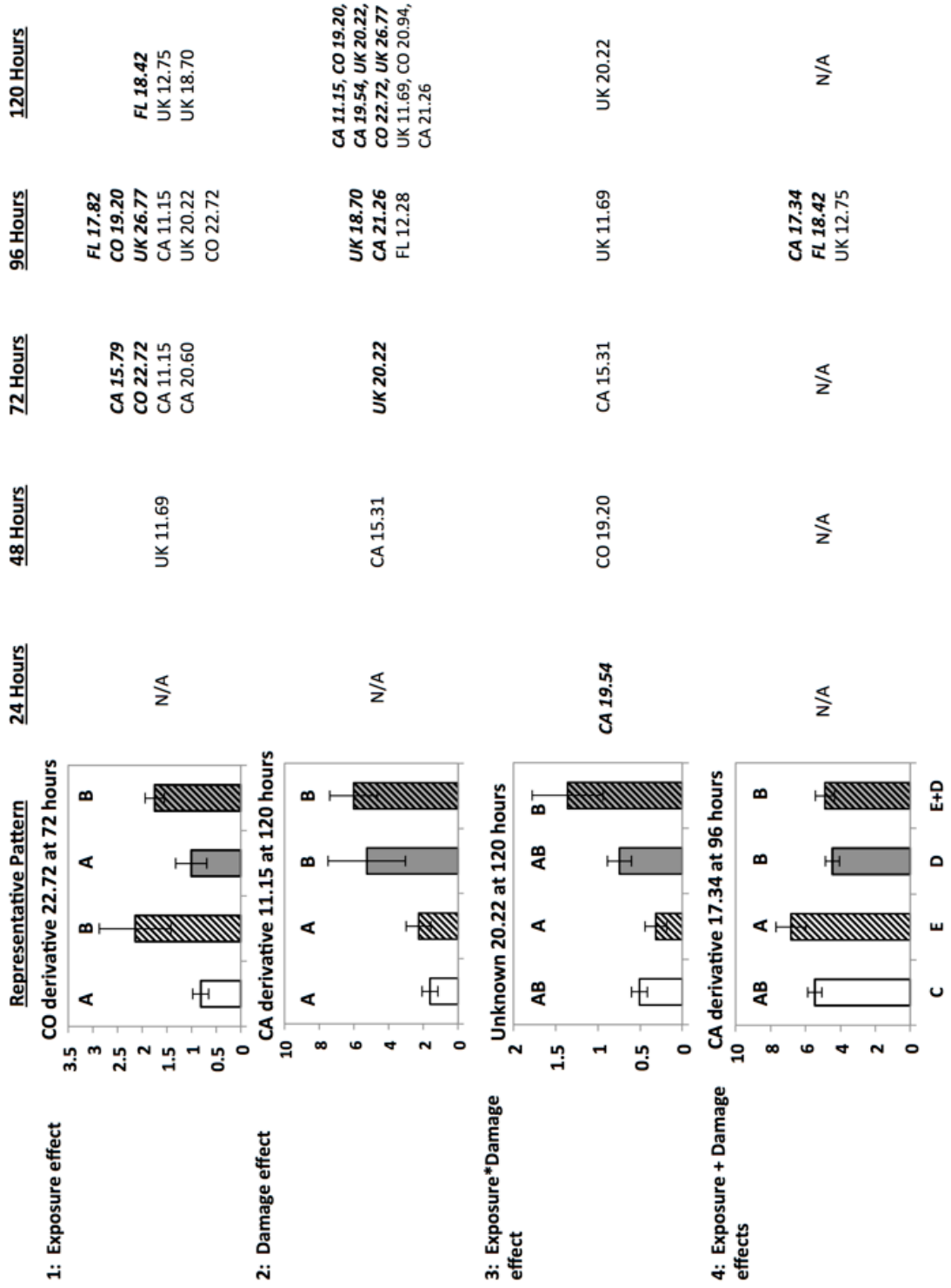

Figure 2.4 Phenolic compounds are differentially induced by damage and/or exposure, and their production varies with *S. altissima*'s induction phenology. We classified induction patterns into 4 classes depending the significance of ANOVAs of exposure and/or damage at particular time points. Compounds significantly affected by exposure and/or damage ($P<0.05$) are in ***bold***; marginally-significantly induced compounds ($P<0.1$) are normally-printed. Compounds within a class do not show consistent effect types, and plants respond to exposure faster than damage.

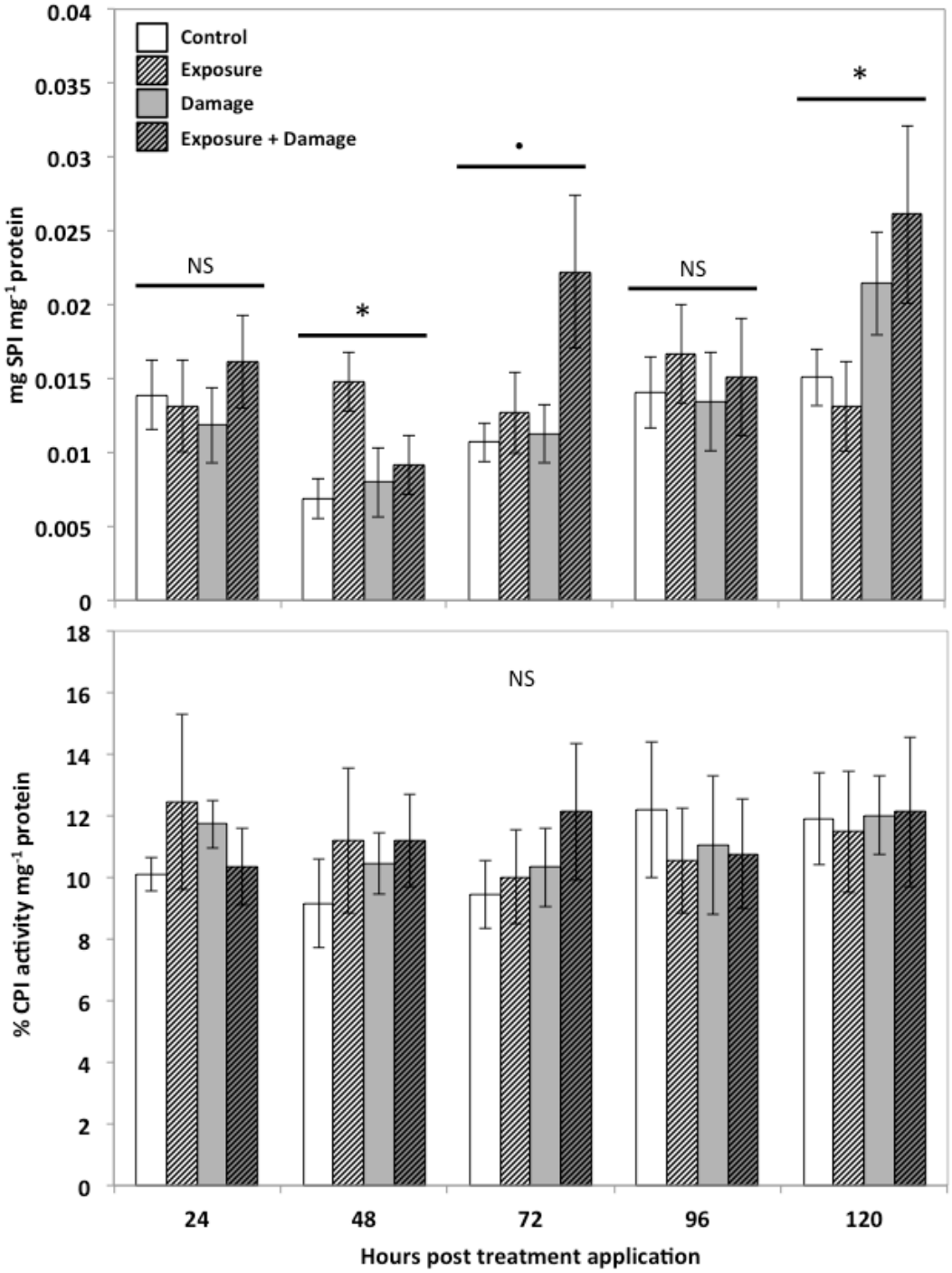

Figure 2.5 Over the treatment interval, serine proteinase inhibitors (SPIs) were significantly induced by exposure ($F_{1,60}$=4.62, P=0.04) and time ($F_{4,60}$=5.11, P=0.001). Specifically, SPIs were induced by exposure 48 hours ($F_{1,12}$=6.51, P=0.03) and 72 hours ($F_{1,12}$=3.79, P=0.08), and induced by damage 120 hours ($F_{1,12}$=7.32, P=0.02) post treatment application. Cysteine proteinase inhibitors (CPIs) were not induced (P>>0.05 for all effects). Asteriks indicate significance of ANOVAs at particular time points (• P<0.1; * P<0.05).

SPIs were significantly induced by damage (**pattern 2**; ANOVA: $F_{1,60}$=4.62, P=0.04) and time point ($F_{4,60}$=5.11, P=0.001); however, there was no significant effect of exposure, damage, and/or time on CPI activity (**pattern 5**; ANOVA all effects: P>0.05; Figure 2.5).

What is the spatial extent of plant communication in S. altissima*?*

The response strength of *S. altissima* to information from neighboring plants varied with distance and was spatially limited. Larval RUE was significantly positively affected by distance from the focal plant (GLM: t=2.35, P=0.02), and larval RGR showed a similar, marginally significant trend (GLM: t=1.77, P=0.08).

Paired t-tests suggested that RGRs of *Trirhabda* larvae on damaged focals were not statistically different from RGRs on exposed plants 15cm (df=24, t=-0.074, P=0.47) away, but were significantly higher 30cm (df=20, t=-2.13, P=0.02), 50cm (df=22, t=-1.93, P=0.03), 100cm from damaged focals (df=18, t=-3.42, P=0.002) and 200cm (df=22, t=-2.39, P=0.01) (Figure 2.6).

We did not observe significant differences in the leaf area consumed by *Trirhabda* on damaged and exposed plants 15cm (df=24, t=0.87, P=0.80), 30cm (df=20, t=-1.29, P=0.11), 50cm (df=22, t=-0.64, P=0.26), 100cm (df=19, t=-0.29, P=0.39) away; however, leaf consumption was marginally-significantly higher on exposed plants 200cm (df=22, t=-2.01, P=0.06) from the focal (Figure 2.6).

Finally, the RUE of *Trirhabda* larvae on damaged focal plants was not significantly different from larval RUE on exposed plants 15cm away (df=24, t=-0.95, P=0.18). However, it was marginally significantly higher 30cm (df=20, t=-1.56, P=0.07), and significantly higher 50cm (df=22, t=-2.38, P=0.01), 100cm (df=19, t=-2.46, P=0.01), and 200cm (df=22, t=-2.01, P=0.03), from the damaged focal (Figure 2.6).

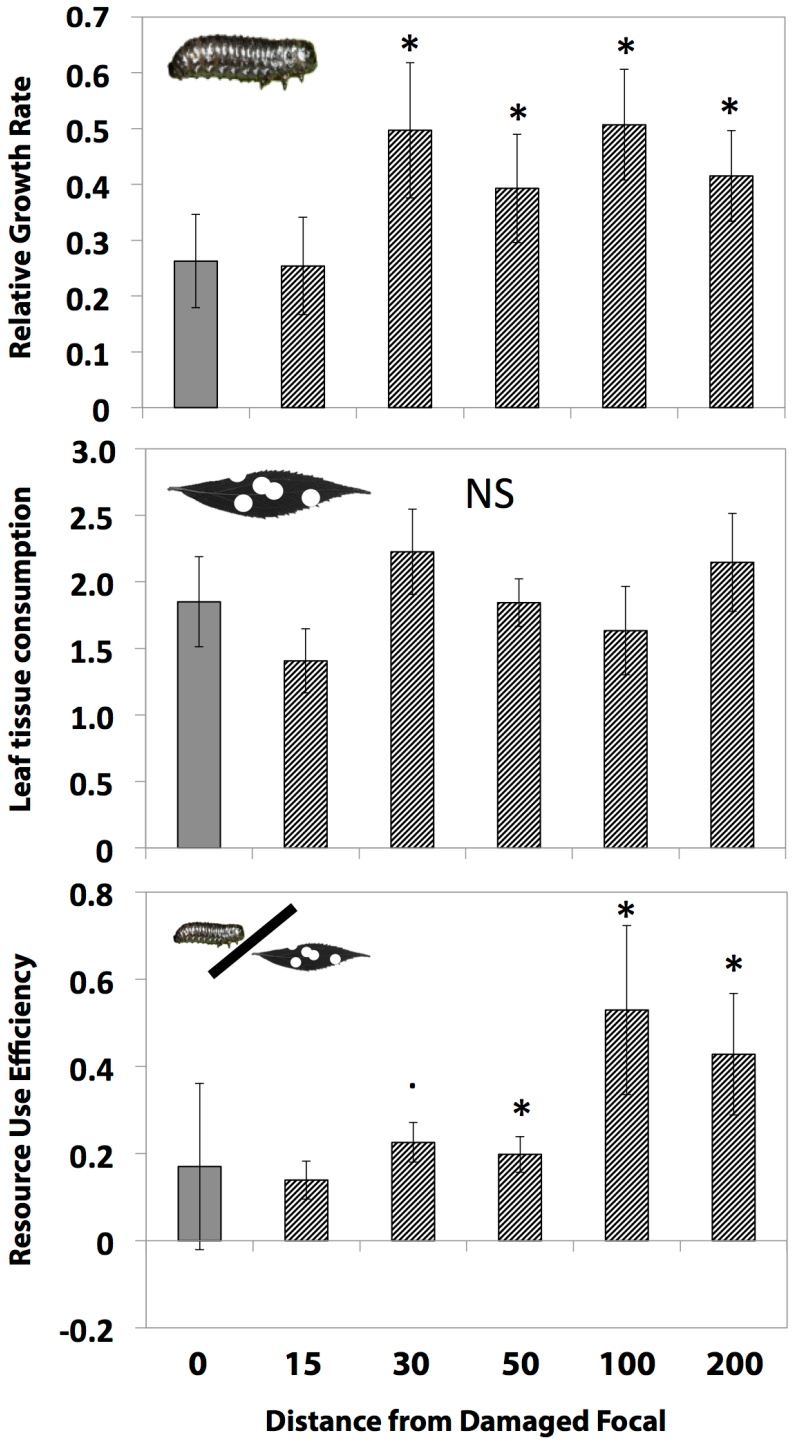

Figure 2.6 Relative growth rates (RGR), leaf area consumed (cm^2), and resource use efficiency (RUE) of *Trirhabda* on undamaged plants (± S.E.M.) at varying distances (cm) from a damaged focal (0cm). Across the distance interval, we observed a marginally significant effect of distance on larval RGR, and a significant effect of distance on larval RUE. There was no significant effect of distance on leaf tissue consumption. Asterisks indicate significance of one-tailed paired *t*-tests (• $P<0.1$; * $P<0.05$).

DISCUSSION

VOC-mediated plant communication has been demonstrated in over 35 plant species spanning 16 families (reviewed in Heil and Karban 2010; Karban et al. 2014), and this study adds *Solidago altissima*, an herbaceous perennial forb in the Asteraceae, to this list. The results of this study support past findings that VOC-mediated plant communication is a widespread, yet spatially restricted, phenomenon that affects herbivores by inducing chemotypic changes in exposed plants. Specifically, consistent with prior studies, our results indicate that localized plant communication (Barbosa et al. 2009) affects herbivore performance (Yoneya et al. 2014) and feeding (Heil and Karban 2010); and that VOCs are sufficient to elicit "enhanced defense phenotypes" in neighboring plants that limit subsequent herbivory (Karban et al. 2014).

In addition, our experiments shed light on three additional functional aspects of plant communication: that plant communication affects herbivore dispersal behavior, differential secondary metabolite production in exposed and damaged plants, and that herbivore performance and behavior reflect differences in plant chemotypes. Additionally, we show that exposure followed by damage (priming) synergistically and antagonistically affects individual compound production, but not herbivore performance or behavior.

Plant communication increases herbivore locomotion

We observed accelerated herbivore locomotion from herbivore-damaged plants. This was predicted for an herbivore like *Trirhabda* because mobile, selective herbivores move more frequently within and between herbivore-induced plants (e.g. Fernandes et al. 2011), and benefit by maximizing their time spent feeding on un-induced tissues (Paschold et al. 2007; Karban 2011). Interestingly, in *S. altissima*, plant communication reduces herbivore residence times on exposed and herbivore-damaged plants *to a similar degree.* In this way, exposure and subsequent

damage does not synergistically affect *Trirhabda* locomotion, suggesting that plant chemotypic responses are either directly induced or very rapidly primed after herbivory (Kessler et al. 2006). This indicates that herbivore damage and exposure to VOCs have similar ecological effects on herbivore behavior in the *S. altissima* system.

Plant communication negatively affects herbivore performance

In addition to leaving exposed and/or damaged plants more quickly, no-choice bioassays indicate that *Trirhabda* experiences reduced performance on exposed and damaged chemotypes. However, although *Trirhabda* consumes significantly less leaf tissue in exposed, damaged, and exposed + damaged plants, its RGR and RUE were only significantly reduced by damage, suggesting that exposed and damaged plants are not of equivalent quality to herbivores.

There are two major factors that may explain the discrepancy in our performance and behavior measures. First, adsorption/re-release of VOCs from the leaf cuticular surface of exposed plants (initially shown in Choh et al. 2004) may repel *Trirhabda*, but testing this explicitly was beyond the scope of this study. Alternatively, though not mutually-exclusively, exposure and damage may induce distinct chemotypes that differentially affect *Trirhabda*'s performance and behavior (Bode et al. 2013; Uesugi et al. 2013). Our herbivore movement and performance data generally seem to support this hypothesis, and are consistent with a study showing that "sub-lethal" plant defenses differentially affect *Trirhabda*'s feeding and growth rate (Wise et al. 2006).

Exposure and Damage Induce Different Chemotypes

Measuring CPIs, SPIs and phenolic secondary metabolites revealed that *S. altissima* chemotypes change in response to exposure and damage; however, the damaged and exposed chemotypes nonetheless differ qualitatively, and over *S. altissima*'s induction phenology.

Moreover, the chemotypic differences reflect changes in multiple individual compounds, from many compound classes, suggesting that the damaged and exposed chemotypes result from a broad metabolic reconfiguration that includes direct induction, suppression, and priming of individual compounds.

By extension, our results indicate that, rather than categorizing plant responses to VOCs or herbivory as induced or primed, it is more appropriate to view priming as an intermediary stage on a continuum from no response to an immediate response to a perceived stressor. Since different compounds may be differentially regulated along this continuum, it is valuable to characterize plant responses using multiple compounds/compound classes over a range of time points after treatment.

In spite of these differences in chemistry, it is remarkable that *Trirhabda* responds to exposed and damaged plants so similarly (both in locomotion and leaf tissue consumption). This suggests that differences in chemistry may not necessarily translate into measurable differences in ecological effects on herbivores and that it is unlikely that one single compound fully explains resistance to *Trirhabda*. Although the approach used in this study did not allow us to correlate herbivore behavior with the induction of individual compounds directly, the fact that we see comparable effects on herbivore behavior despite differences in the exposed and damaged chemotypes suggests that it may be worthwhile to investigate herbivore-induced changes in suites of compounds, rather than individual compounds, as candidates for resistance. It also supports the hypothesis that variation in plant traits, in and of itself, contributes to resistance by making the plant a "moving target" for herbivores (Adler and Karban 1994) – encouraging between-host locomotion of herbivores due to the physiological challenges of adapting to highly-variable plant chemistry.

Finally, the high degree of congruence between our movement and performance measures, and the clear chemotypic changes observed in spite of eliminating vascular connectivity between plants, all reveal that VOCs are sufficient to mediate plant communication in *S. altissima*. This result supports numerous other studies demonstrating VOC-mediated plant communication (Karban et al. 2014). Nevertheless, it is well worth testing whether utilizing multiple signaling mechanisms (e.g. vascular, adsorption/re-release) facilitates different and/or stronger chemotypic responses.

Plant Communication is Spatially Limited

Consistent with our findings, all studies to date suggest that responses to VOCs are spatially restricted – plants within 10-60cm of the induced plant respond (reviewed in Barbosa et al. 2009). Similarly, in old-field communities, the spatial extent of plant communication is 15-30cm, between one *S. altissima* ramet and its immediate neighbors.

There are numerous alternative (non mutually-exclusive) hypotheses for why plant communication is spatially restricted. First, there are known physical limitations on aerial VOC transport, including air currents, temperature, plant canopy density, and the presence of atmospheric constituents (e.g. O_3) that degrade certain VOCs (Kuroyanagi et al. 2012).

Alternatively, because VOCs are not 'private signals' (Gershenzon 2007), there may be selection on plants to limit VOC emission, minimizing the spread of the cue (Heil and Bueno 2007). For example, studies suggest that there may be genotypic constraints on VOC emission and perception, and that plant communication elicits the strongest responses in plants of the same genotype (Karban and Shiojiri 2009). Because *S. altissima* is clonal, it is possible that our observed decline with distance simply results from plants being less likely to be of the same genetic clone, which is known to vary across populations (Cain 1990). Moreover, it is worth

exploring the importance of other ecological functions of inducible VOC emissions (e.g. indirect resistance) in this system.

Overall, in a system with a dense plant population and a major herbivore that is selective and mobile, plant communication can have strong ecological effects that go beyond the effects of induced resistance and move herbivores between the plants in the population. Plant communication in tandem with induced resistance seems to allow *S. altissima* to exhibit a response large enough to encourage *Trirhabda* to leave its current host, but localized enough to make the move cost-effective (there are better quality hosts available ≥30cm away). Thus, when damaged and exposed plants become of comparable bad/repellent quality, *Trirhabda* quickly moves on to more palatable (control) plants, leaving limited damage behind. By constantly keeping herbivores on the move, plants can minimize the cumulative effect of herbivory on their growth and reproduction. Specifically in *S. altissima*, our results may help to explain the observation that herbivore loads on individual plants are low more than 75% of the time (except during outbreaks), and that at these low loads, herbivory has no measurable effect on *S. altissima*'s fitness (Root 1996). Thus, in dense populations where plants have many conspecific neighbors, plant communication and strong induced resistance reduce the probability that any given plant will experience high levels of damage.

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CHAPTER 3

SPECIFICITY OF PLANT-PLANT COMMUNICATION: VOLATILES INDUCE SPECIFIC PLANT CHEMICAL RESPONSES THAT AFFECT HERBIVORE HOST CHOICES

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ABSTRACT

Specificity of induced plant responses to herbivory, which include specific changes in the emission of volatile organic compounds (VOCs), has well-documented effects on plant-herbivore interactions and arthropod community composition. However, it remains unclear whether VOC-mediated exchange of information between plants (plant-plant communication) exhibits a similar degree of specificity. Using tall goldenrod (*Solidago altissima*) and two specialist leaf-chewing herbivores (*Dichomeris leuconotella* and *Trirhabda virgata*), we compared the degree of specificity in plants' responses to VOCs from herbivore-attacked neighbor plants. We found that VOC blends induced by *Dichomeris* and *Trirhabda* herbivory were compositionally different from the VOC profile of control plants as well as from each other, indicating that other organisms in the community (such as neighboring plants) could gain information about the identity of a potential future attacker. Accordingly, plants responded in a specific manner to VOCs from neighboring plants as they did to herbivore damage, as indicated by leaf defense metabolite production (diterpene acids, serine protease inhibitors). Moreover, plants' chemical responses to VOCs and herbivory were functionally equivalent and affected the host choice of subsequent herbivores in species-specific ways. Overall, our results indicate that plants elicit chemical responses in response to VOCs and feeding herbivores, and that VOC-mediated information transfer between plants induces specific chemical phenotypes that affect herbivore behavior.

KEYWORDS: Induced resistance, plant-plant communication, specificity of elicitation, specificity of effect, *Trirhabda virgata*, *Dichomeris leuconotella*, *Solidago altissima*

INTRODUCTION

Induced responses to herbivory have been documented in hundreds of plant systems, and have well-characterized, albeit diverse, effects on interactions between herbivore species (Van Zandt and Agrawal 2004b, Voelckel et al. 2004, Uesugi et al. 2013) and arthropod community composition (Thaler et al. 2001, Van Zandt and Agrawal 2004a, Viswanathan et al. 2005). Inducible responses are thought to provide plants with distinct advantages over a constitutive expression of resistance traits in that they enable plants to save on the costs of producing defense metabolites until they are actually needed for defense (e.g. Zangerl 2003), and herbivores often exhibit limited abilities to adapt their physiologies to rapid changes in their host's quality (Adler and Karban 1994, Karban 2011).

Thus, in order to manage the diverse challenges that plants face, including attraction of pollinators or natural enemies, defending against herbivores and pathogens, competing with neighboring plants, and dealing with abiotic stress, plants constantly assimilate information from their environments in order to detect and respond appropriately to environmental changes (Kessler 2015). Moreover, just as a given suite of defenses is not always sufficient to combat both generalist and specialist herbivores (Agrawal 2000, Ali and Agrawal 2012), so too, can one set of defense traits simultaneously confer resistance to some herbivores but increase susceptibility to others (Hare and Elle 2002). Thus, as a means to manage a complex array of stressors and respond appropriately, many plants employ some degree of specificity in their responses to different environmental challenges – tailoring their defenses to the most immediate threat (Simms and Rausher 1987). In this way, plants can use numerous mechanisms to identify immediate threats using chemical motifs, such as herbivore-associated salivary elicitors (Barrett and Heil 2012), to identify the feeding herbivore and elicit a targeted response.

The specificity in plants' induced responses to herbivores can be broadly categorized into two classes: (a) specificity of *elicitation* – different herbivore species elicit distinct chemical responses in their plant hosts, and (b) specificity of *effect* – these distinct chemical responses can have positive, negative or neutral effects on subsequent herbivores of different species (Stout et al. 1998). To understand the ecological costs/benefits and evolutionary trajectory of plant defenses, it is important to understand both facets of specificity, since a phenotype that is adaptive for a plant in one context may be maladaptive in the future.

The specific metabolic responses elicited by herbivore attack include specific changes in the composition of a plant's headspace (via herbivore-induced emission of volatile organic compounds, VOCs). Plant VOC emission is highly variable, qualitatively and quantitatively, reflecting both the species and genotype of the plant being attacked (e.g. Boom et al. 2004, Pearse et al. 2013, Karban et al. 2014a), as well as the species of the herbivore doing the damage (Pierre et al. 2011, Piesik et al. 2011, Bautista-Lozada and Espinosa-Garcia 2013). Because the information content of a plant's headspace changes in predictable ways with the overall metabolism when plants experience herbivory, it is possible that neighboring plants could gain specific information about which herbivore species are in the vicinity and thereby preemptively mount specific responses to combat them. Although numerous studies demonstrate that neighboring plants do respond to changes in VOC emission by "enhancing their defense phenotypes" (reviewed in Karban et al. 2014b), the degree of specificity (both elicitation and effect) of VOC-mediated plant communication has heretofore received limited attention.

This paper presents the results of experiments designed to address the following questions:

(1) To what degree are specific plant responses to herbivores reflected in the composition of plants' headspaces (i.e. through changes in VOC emission)?

(2) In response to changes in VOC emission, can neighboring plants elicit specific chemical responses (i.e. specificity of elicitation)?

(3) How do VOC-induced changes in plant chemistry affect the host choices of subsequent herbivores of different species (i.e. specificity of effect)?

(4) Does the information contained in VOC blends convey "honest" information about plants' current defensive state?

We address these questions using tall goldenrod, *Solidago altissima* L. (Asteraceae), and two of its specialist leaf-feeding herbivores – the leaf roller *Dichomeris leuconotella* H. (Lepidoptera: Gelechiidae), and the leaf beetle larva *Trirhabda virgata* (Coleoptera: Chrysomelidae). *S. altissima* induces distinct chemical responses to herbivores of different species, including changes in VOC emission (Kessler and Morrell 2010), leaf protease inhibitor content (Bode et al. 2013), and leaf diterpene acid profiles (Uesugi et al. 2013). Moreover, that these induced responses can have specific effects on subsequent herbivores of the same and different species (Uesugi et al. 2013). These prior studies reveal that *S. altissima*'s induced responses to herbivory exhibit both specificity of elicitation and effect. Moreover, herbivore-induced changes in VOC emission also induce neighboring *S. altissima* plants to respond by inducing changes in leaf defense chemistry and resistance (Morrell and Kessler *in review*). However, it remains unknown whether the VOCs emitted from a damaged plant induce metabolic changes and resistance in an unattacked neighbor that are specific to the attacking herbivore and thus comparable to an actually damaged plant. In a larger context, understanding the specificity of information encoded in herbivory-induced VOC bouquets and elucidating whether this information is transferred to

neighboring plants is crucial for gaining insight into the function and evolution of plant communication, and its role in shaping plant population structure and community composition.

To address these questions, we employ a 3x2-factorial design with herbivore treatment (control, *Dichomeris*, *Trirhabda*) and exposure regime (herbivore-exposed "emitters", VOC-exposed "receivers") as factors. Comparing various aspects of plant chemistry and herbivore host preferences in each herbivore treatment across exposure regimes enabled us to test whether specificity in elicitation and effect, which have previously been observed in *S. altissima*'s induced responses to direct herbivory, also extends to the VOC-induced responses in neighboring plants.

MATERIALS AND METHODS

Plant propagation & treatment application

S. altissima plants of a moderately inducible *S. altissima* genotype (Uesugi, personal communication) were propagated from rhizomes in late March 2013. Briefly, we clipped 3cm pieces of rhizome – each containing an apical meristem and a root, dipped the root end in Bontone® Rooting Powder (0.10% Indole-3-butyric acid), placed the cuttings root-down in 98-well flats of Metro Mix 360® soil, and submerged them in 2cm water with humidity domes to keep the cuttings moist. After 6-8 days, when the cuttings developed new leaves, we removed the humidity domes and submerging trays, and began watering twice daily and fertilizing once weekly (16/8 hour light regime; 72^{o}C day/64^{o}C night). When the cuttings were 5cm, we transplanted them into 150cm-diameter Azalea pots, and used the plants for experiments when they were 20cm tall. We chose to use potted plants in these experiments in order to eliminate the potential for signaling between plants via vascular tissues, rhizomes or root exudate cues (Heil

and Bueno 2007). The insects used for treatment application and bioassays were collected from naturally occurring field populations around Ithaca, NY.

In early May 2013, we dug 36 holes (n=12 holes/treatment) 500cm in diameter, 250cm deep, and 3.5m apart in the naturally occurring old-field community at Cornell University's Whipple Farm (42.488494, -76.429642). On May 21, we added four potted *S. altissima* plants to each hole (n=144 plants total), and bagged all plants in mesh sleeve bags (Breather®, Palm Tree Packaging) to contain treatment insects but facilitate exchange of VOC cues. Within each hole, two plants were designated "emitters" (which received one of the herbivore treatments - damage from one 2nd-instar *Dichomeris* larvae, two 2nd-instar *Trirhabda* larvae, or remained undamaged controls); and the others "receivers" – plants that were exposed to the VOC emission from the emitters, but did not experience initial herbivory. To apply the treatments, *Dichomeris* or *Trirhabda* larvae were allowed to feed freely on the designated focal plants for 1 week.

VOC collection & statistical analysis

Four days into the treatment application, on May 25, 2013, from 1030 to 1830 (8 hours), we trapped volatile organic compounds (VOCs) from all focal plants using an open-flow dynamic headspace trapping design (Kessler and Baldwin 2001). Briefly, we covered the top half of each plant (approximately 15 leaves) in 750mL polyethylene chambers, and pulled ambient air over the plants and into Orbo-32^{TM} small coconut charcoal traps (Supelco®). We eluted the traps in dichloromethane along with a tetraline internal standard, and analyzed the VOC blends via GC-MS on a Varian Saturn 2200-GC/MS/MS with a CP-8400 autosampler in splitless mode on a DB5 column (VF-5ms, 30m x 0.25mm ID, DF=0.25). Our injection temperature was 225^{o}C, and our temperature program began at 40^{o}C for 5 minutes, heated up to 180^{o}C at a rate of 10^{o}C/minute, further heated up to 220^{o}C at a rate of 40^{o}C/minute, and the final temperature was

held for 10 minutes.

We characterized peaks based on their retention times and mass spectra into the following classes of compounds: green leafy volatiles, monoterpenes, sesquiterpenes, aromatic compounds, aliphatic compounds, or unknowns. We were able to further identify a subset of compounds through comparisons of mass spectra to a Nist Library (version 2.0) and the Nist Mass Spectral Search Program (Standard Reference Data Program of The National Institute of Standards & Technology, version 2.0) as well as by comparing mass spectra and retention times with those of authentic standards. We reported the Kovats Indices of all unidentified compounds in our samples. Reported emission of individual VOC compounds represents the Ion Intensities (Mcounts) of these compounds relative to internal standards, and controlled by emission of these compounds in an average ambient control sample (empty collection chamber).

Total VOC emission was analyzed statistically in R (version 3.1.1; R Statistical Computing) using ANOVAs with herbivore treatment (Control, *Dichomeris*, *Trirhabda*) as a fixed factor. Because sample sizes were not large enough (n=4 samples per treatment, with 56 identified VOC compounds) to run MANOVAs, we used PERMANOVAs (*adonis* package in R-vegan package) to calculate an index of dissimilarity between our treatments, as a proxy for compositional changes in VOC emission. Due to low replication, and to enhance the interpretability of our results, we conducted two pairwise PERMANOVAs (control versus *Dichomeris*, control versus *Trirhabda*) to ascertain the specific compositional changes in VOC emission induced by the two herbivore species.

Additionally, we used Random Forest analyses (packages *RColorBrewer*, *randomForest*, and *varSelRF* in R) to identify a minimum set of VOC compounds that best characterizes the compositional differences between our treatments (Ranganathan and Borges 2010). Random

Forest is a particularly robust statistical method for low sample sizes and when response variables may be highly auto-correlated, which is highly likely for the VOC compounds in this analysis (Ranganathan and Borges 2010). Using Random Forest analysis, we calculated the number (out of 200 iterations) that each compound appeared in the best-fit model (see Results), as well as the mean decrease in accuracy of removing each compound from the analysis. To evaluate model strength, we also calculated the prediction, bootstrap and re-substitution errors and compared them to the error rates at random for each of the models.

Leaf chemistry analysis

To measure specificity of plant responses, we assayed two classes of defense compounds – serine protease inhibitors (SPIs) and diterpene acids (DTAs). We measured SPIs because of their well-established anti-digestive effects on many herbivores, including those attacking *S. altissima* (Bode et al. 2013). And, we measured DTAs, 20-carbon semi-volatile terpenoids, because of their well-characterized toxic and deterrent effects on the performance, behavior and growth of a wide variety of herbivore species (Langenheim 1994, Trapp and Croteau 2001, Raffa and Powell 2004), including *Trirhabda* and *Dichomeris* (Uesugi et al. 2013).

We harvested leaf samples for chemical analyses (two leaves per plant at the 5^{th} and 6^{th} positions below the apical meristem) from one emitter and one receiver in each hole immediately following the initial treatment period, on May 28, 2013. In the field, we quickly flash-froze the leaf tissue in liquid Nitrogen, and stored it at -80^{o}C until subsequent leaf chemistry analysis.

In the lab, approximately 200mg tissue from one of the leaves from each plant was ground in liquid Nitrogen and extracted in 1mL 90% methanol using a FastPrep®-24 machine (MP Biomedicals) with ~1g lysing beads (2.3mm Zirconia/Silica Beads, BioSpec Products). Subsequently, the DTA content of the supernatant was analyzed via high-performance liquid

chromatography (HPLC). Briefly, diterpene acids were identified by their absorbance spectra and retention times at 230nm, quantified using ChemStation (Agilent Technologies), and expressed as peak intensity per milligram leaf tissue in the extract (as described in Uesugi et al. 2013).

Serine protease inhibitor (SPI) activity was determined by first extracting approximately 200mg tissue from individual leafs in a 0.1M Tris-HCl buffer (pH 8.0) using a Fast Prep machine. We quantified the leaf protein content of the leaves using a Bradford Assay, and determined levels of leaf SPI activity by performing an enzyme-substrate reaction with trypsin and N-benzoyl-DL-arginine-b-naphtylamide in dimethyl sulfoxide, based on a standard soybean trypsin inhibitor standard curve (as described by Bode et al. 2013). We expressed SPI activity as milligrams of SPI activity per milligram protein.

Total DTA levels were compared across treatments and exposure regimes using ANOVAs in R. We also analyzed differences in the composition of the DTA profiles using non-metric multidimensional scaling (NMDS), PERMANOVAs (*adonis* function in R-vegan package), and MANOVAs, using herbivore treatment and exposure regime as interactive factors.

Because MANOVAs yielded significant treatment effects but not significant effects of exposure regime (see Results), we carried out subsequent ANOVAs on individual DTAs to identify specific compounds that explained the differences between herbivore treatments for each exposure regime. The ANOVAs on individual DTAs were conducted in two ways: (1) emitters and receivers were analyzed separately, using herbivore treatment as a factor; (2) emitters and receivers were analyzed together, using herbivore treatment and exposure regime as interactive factors.

We also compared SPI activity across treatments (normalized using a *log* transformation)

using an ANOVA with herbivore treatment and exposure regime as interactive factors in R.

Herbivore host choices

To ascertain the effects of past herbivory and/or plant communication on the host choices of subsequent herbivores, we conducted three-way choice assays with *Dichomeris* and *Trirhabda*. For these assays, we used excised leaves in a Petri dish arena to limit additional induction by our bioassay herbivores and thereby more accurately quantify the effects of our initial exposure and damage treatments on herbivore preferences (as in Uesugi et al. 2013).

We tested each insect species' preferences within a treatment, keeping exposure regime (herbivore-damaged emitters, VOC-exposed receivers) consistent within each replicate. To set up the choice assays, we cored 1.6cm diameter leaf disks from each of our herbivore treatment and exposure regimes and randomly assigned three disks (one from a *Dichomeris*-treated plant, one from a *Trirhabda*-treated plant, and one from a control plant of the same exposure regime) to each Petri dish (10cm diameter). The three leaf disks were placed on moist filter paper to avoid desiccation, and placed equidistant from each other and from the center of the arena. We added one 2nd instar *Trirhabda* larva to the center of each choice arena, in a direction chosen by a random number generator in Microsoft Excel. Twelve hours later, we removed the bioassay larva and scanned the leaf-disks. Leaf area eaten (%) was determined using ImageJ (version 4.3). We repeated this same procedure for 2nd-instar *Dichomeris* larvae. In all, we created 124 choice arenas = 2 bioassay herbivore species x 2 exposure regimes x 31 arenas/exposure regime.

To determine herbivore preferences we first assigned a rank to each leaf disk, in which the leaf disk that received the most damage was ranked 1, and all others were ranked 0. We then analyzed herbivore preferences using Friedman's Tests in R (function *friedman.test*). Finally, to see whether herbivore choices on herbivore-damaged emitters matched their choices on VOC-

exposed receivers, we conducted Fisher's 2x3 tests (note: for Fisher's 2x3 tests, a significant P-value indicates that the herbivores choose differently on emitters and receivers).

Honesty of VOC Blends

Due to the biosynthetic relatedness of VOC sesquiterpenes and leaf DTAs, we tested whether VOC blends convey honest information about leaf chemistry by using generalized linear models to correlate levels of VOC sesquiterpenes with leaf DTA levels. We also used generalized linear models with leaf DTA levels and average herbivore host preference (1 = disk with most damage; 0 = all other disks) to test whether leaf DTA levels inform the host preferences of *Dichomeris* and *Trirhabda*.

RESULTS

Volatile Organic Compound Emission

Dichomeris and *Trirhabda* herbivory induced changes in the blends of VOCs emitted by *S. altissima* (**Figure 3.1**). Although herbivory did not induce an overall increase in total VOC emission ($F_{2,8}$ =1.97, P=0.20), it did induce changes in the emission of some classes of VOCs – *Trirhabda* feeding elicited an overall induction of volatile sesquiterpenes ($F_{2,8}$=12.17, P=0.004; **Figure 3.2A**), and *Dichomeris* damage reduced the emission of aromatic VOCs ($F_{2,8}$=5.04, P=0.04; **Figure 3.2B**). The other classes of VOCs that we measured, including green leafy VOCs ($F_{2,8}$=0.38, P=0.70), monoterpenes ($F_{2,8}$=1.12, P=0.37) and aliphatic VOCs ($F_{2,8}$=0.90, P=0.45), were unaffected by the herbivore treatments.

Additionally, compared to control plants, *Trirhabda* herbivory significantly altered VOC blend composition (pairwise PERMANOVA: $F_{1,7}$ =6.33, R^2=0.56, P=0.03), but *Dichomeris* herbivory did not ($F_{1,8}$=0.38, R^2=0.07, P=0.91). This suggests that *Trirhabda* feeding induces a

phenotypically distinct VOC blend from control *S. altissima*, and that *Dichomeris* damage induces an intermediate VOC blend (**Figure 3.2C**).

Overall, 14 individual VOCs were sufficient to explain differences in the composition of the volatile blends of *Trirhabda*- and *Dichomeris*-damaged plants in at least 10% of Random Forest models (**Figure 3.2D**). Specifically, compared to control plants, *Trirhabda*-damaged plants induced higher levels of 9 sesquiterpenes (Model 1; **Table S3.1**); whereas, *Dichomeris*-damaged plants have elevated levels of one monoterpene and 12 sesquiterpenes (Model 2; **Table S3.1**). Additionally, compared to *Dichomeris*-damaged plants, increases in the emission of two volatile sesquiterpenes characterize the VOCs induced by *Trirhabda* damage (Model 3; **Table S3.1**). All three Random Forest models had sufficient predictive power to describe compositional differences in the VOC samples – the bootstrap (BE), prediction (PE), and classification (CE) errors associated with all models were significantly less than the error rates at random (RE) (Control vs *Trirhabda*: PE=0.082, BE=0.12, RE=0.43; Control vs *Dichomeris*: PE=0.092, BE=0.13, RE=0.43; *Dichomeris* vs *Trirhabda*: PE=0.20, BE=0.26, RE=0.50; **Table S3.1**).

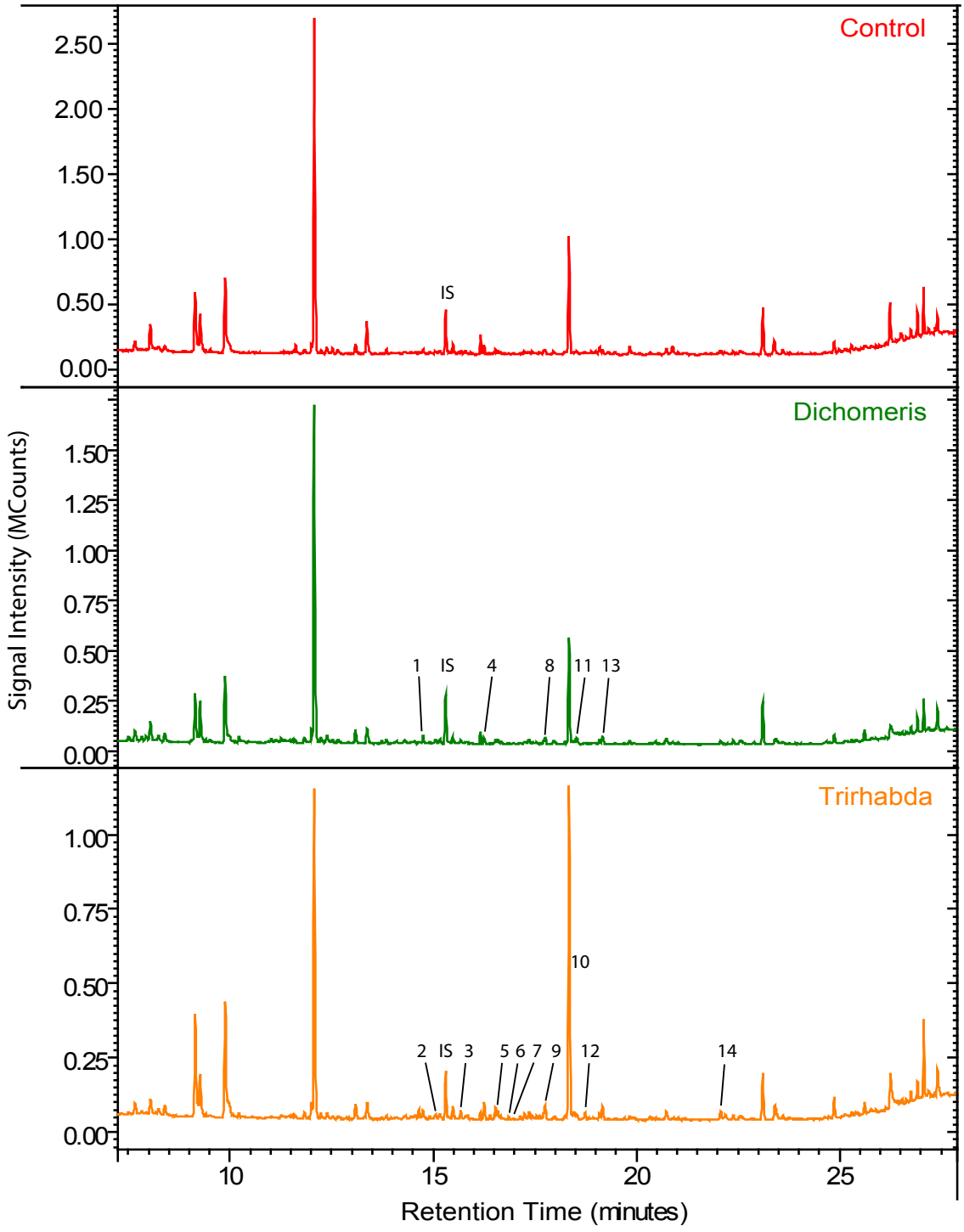

Figure 3.1 Herbivory by *Dichomeris* and *Trirhabda* induces qualitative and quantitative changes in VOC emission by *S. altissima*.

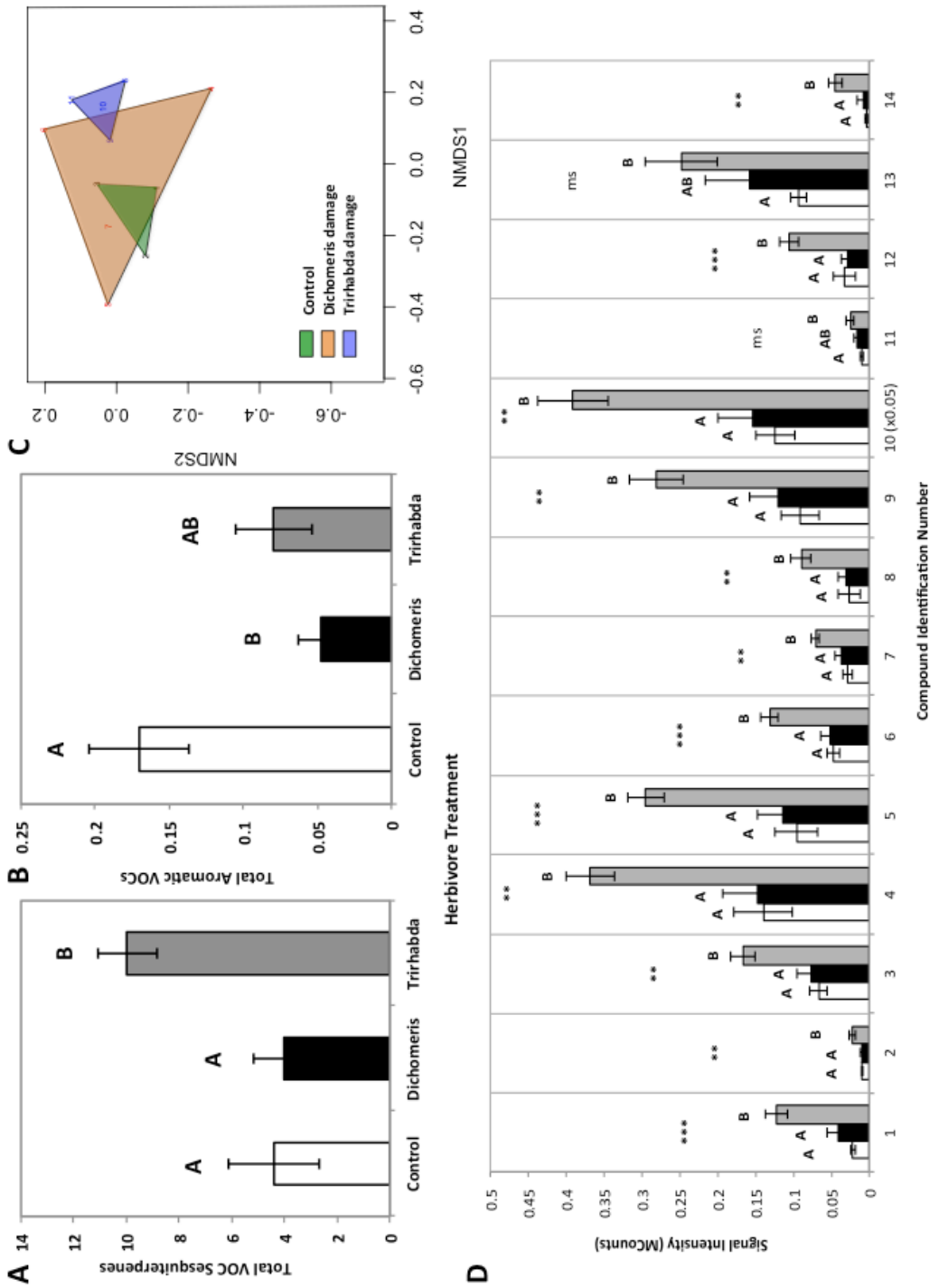

Figure 3.2A-D Herbivores induce distinct, species-specific changes in VOC emission – *Trirhabda* feeding strongly induces VOC sesquiterpenes (**A**), whereas *Dichomeris* feeding suppresses aromatic VOC emission (**B**). Additionally, *Trirhabda*-feeding induces a compositionally different VOC blend from control *S. altissima*, while *Dichomeris* damage induces a phenotypically intermediate blend (**C**). Random Forest models reveal that 14 individual VOCs, most induced by *Trirhabda* damage, characterize the compositional differences between herbivore treatments in these experiments (**D**).

ANOVAs of individual VOCs were largely consistent with the three Random Forest models – sesquiterpenes explained the bulk of the differences between treatments. In total, 22 compounds, including 17 out of 20 sesquiterpenes, one out of five aromatic compounds, two out of nine monoterpenes, and two unknown compounds were significantly affected by our treatments (**Table S3.2**). Of these, *Trirhabda* feeding significantly induced levels of all 17 sesquiterpenes, 2 monoterpenes, and both unknown compounds. Levels of aromatic compounds were generally suppressed by herbivory – more so with *Dichomeris* than with *Trirhabda* damage.

In tandem, these results reveal specificity in the VOC blends induced by *Dichomeris* and *Trirhabda* herbivory, suggesting that neighboring plants could thereby gain information from induced VOC blends and elicit specific biochemical responses.

Leaf Chemistry of Emitters & Receivers

Herbivory and exposure to VOCs significantly induced diterpene acid (DTA) production. Specifically, *Trirhabda* led to a 41% increase, and *Dichomeris* led to a 23% increase, in total DTA production in both damaged and exposed plants (ANOVA Treatment: $F_{2,59}=6.56$, $P=0.003$; **Figure 3.3A**). In addition to overall changes in DTA levels, the herbivory and VOC treatments elicited significant compositional changes in the DTA profiles of damaged and exposed plants. Both a PERMANOVA ($F_{2,59}=4.45$, $R^2=0.13$, $P=0.003$) and a MANOVA ($F_{2,59}=3.41$, $P<0.0001$) revealed significant treatment effects on leaf DTA profiles.

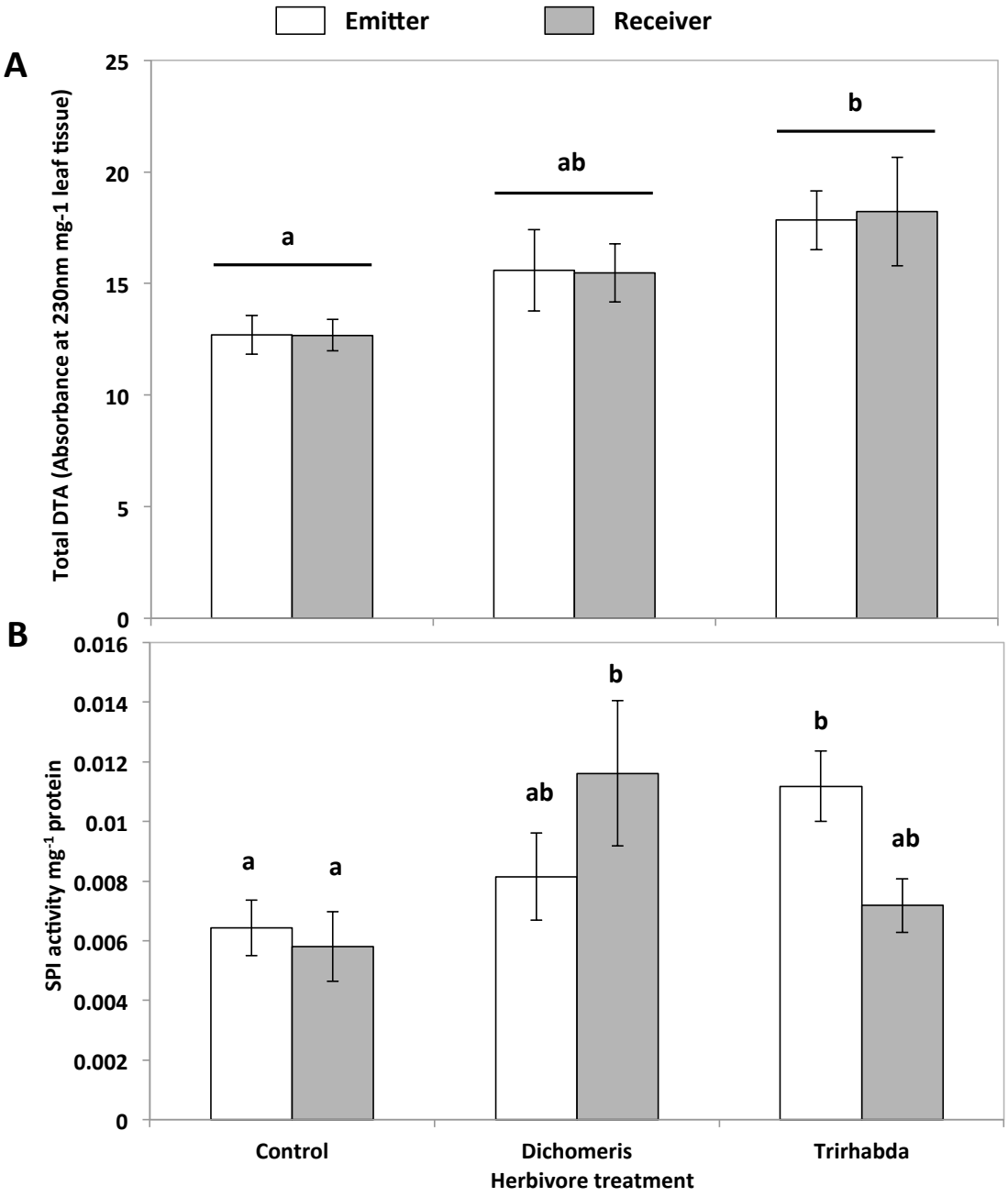

Figure 3.3A-B Total diterpene acids (DTA) in herbivore-damaged emitters and VOC-exposed receivers are significantly induced by *Trirhabda*, and moderately induced by *Dichomeris* feeding (**A**). In contrast, herbivore damage and VOCs did not induce similar production of SPIs. Receivers exposed to the VOCs emitted by *Dichomeris*-damaged plants more strongly induce SPI production than *Dichomeris*-damaged emitters, whereas *Trirhabda*-damaged emitters induced SPI production more strongly than VOC-exposed receivers (**B**).

Specifically, levels of 14 individual DTAs were affected by herbivore damage and/or exposure regime (**Figure 3.4**). Specifically, 13 DTAs were affected by herbivore damage, including those at 22.1min ($F_{2,59}$=7.09, P=0.002), 22.6min ($F_{2,59}$=8.07, P=0.0008), 23.7min ($F_{2,59}$=4.73, P=0.01), 25.1min ($F_{2,59}$=2.88, P=0.06), 26.1min ($F_{2,59}$=4.42, P=0.02), 26.6min ($F_{2,59}$=16.60, P<0.0001), 28.5min ($F_{2,59}$=25.58, P<0.0001), 29.0min ($F_{2,59}$=17.57, P<0.0001), 29.8min ($F_{2,59}$=6.38, P=0.003), 30.1min ($F_{2,59}$=4.90, P=0.01), 30.9min ($F_{2,59}$=5.37, P=0.007), 31.2min ($F_{2,59}$=3.81, P=0.03), and 33.9min ($F_{2,59}$=3.80, P=0.03). Of those DTAs affected by the herbivore treatments, *Trirhabda* damage resulted in increased production of the majority of these DTAs, and *Dichomeris* herbivory also moderately-to-significantly induced levels of many DTAs.

We did not observe an overall effect of exposure regime on the composition of leaf DTA profiles – emitters and receivers had statistically comparable DTA profiles (PERMANOVA: $F_{1,64}$=0.55, R^2=0.01, P=0.62; MANOVA: $F_{1,59}$=1.44, P=0.17). However, two individual DTAs – at 24.7min (ANOVA: $F_{1,59}$=4.73, P=0.01) and 33.9min ($F_{1,59}$=3.95, P=0.05) – were significantly induced in receivers.

Leaf serine protease inhibitor (SPI) activity was affected by treatment ($F_{2,65}$=5.32, P=0.007). Additionally, exposure regimes affected our herbivore treatments differently (Trtmt*Exposure Regime: $F_{2,65}$=3.86, P=0.03) – exposure to *Dichomeris*-induced VOCs and direct feeding by *Trirhabda* both induced significant increases; whereas *Dichomeris*-feeding and exposure to *Trirhabda*-induced VOCs only induced marginal increases in leaf SPI levels (**Figure 3.3B**).

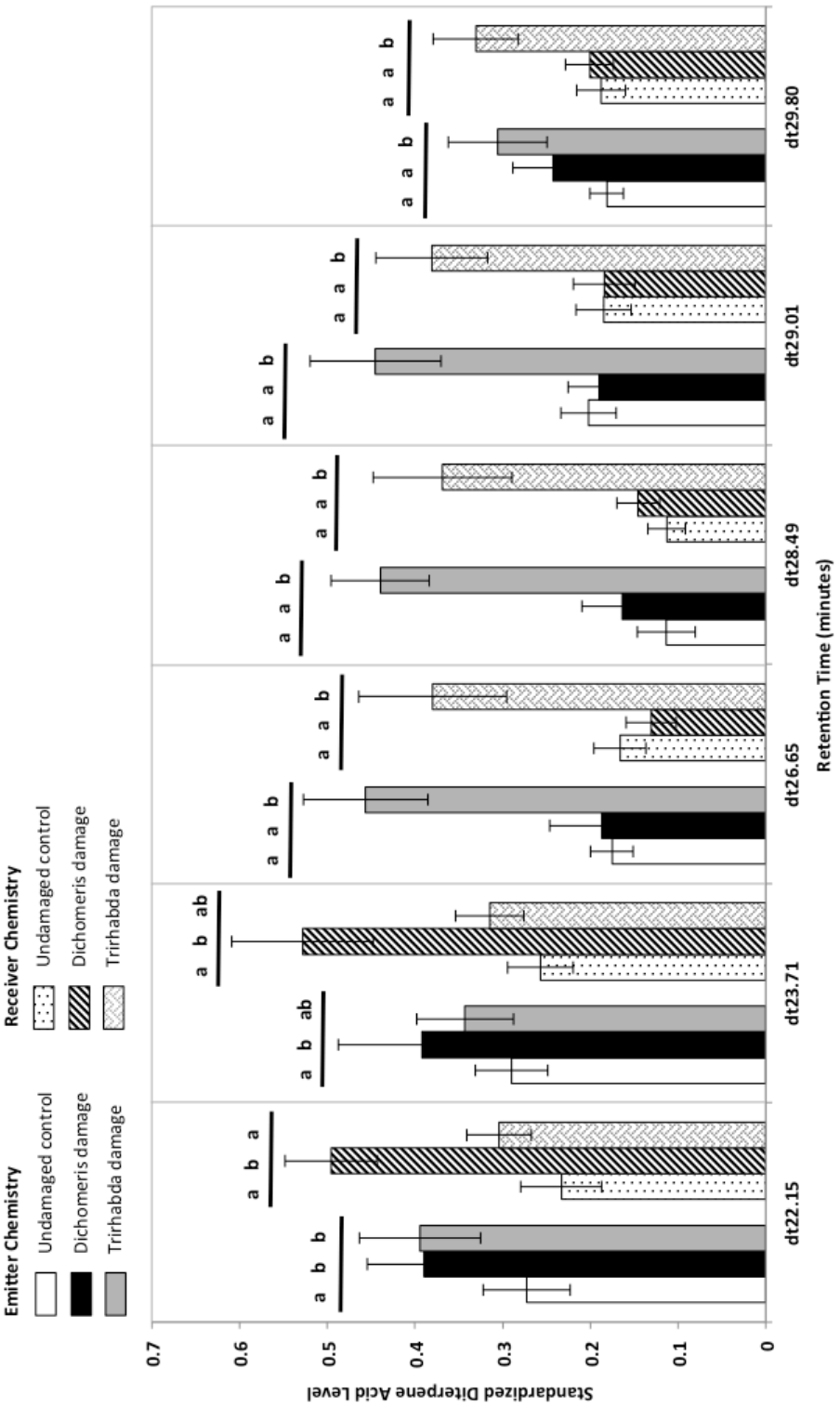

Figure 3.4 Characterizing levels of individual DTAs in emitters and receivers. Six DTAs (mean±SEM) explained the compositional differences – five were significantly induced by *Trirhabda*-feeding in both emitters and receivers, and one was significantly induced by *Dichomeris* herbivory. Letters reflect results of Tukey's Honestly Significant Difference post-hoc analyses.

Herbivore Leaf Preferences

Trirhabda larvae preferentially fed on control leaves, both in arenas with herbivore-damaged emitters (df=2, X^2=16.77, P=0.0002) and with VOC-exposed receivers (df=2, X^2=15.85, P=0.0004; **Figure 3.5, upper**).

In contrast, *Dichomeris* larvae preferentially fed on plants that have previously been damaged by other *Dichomeris* (df=2, X^2=8.58, P=0.01), or exposed to the VOCs of a *Dichomeris*-damaged plant (df=2, X^2=11.10, P=0.004; **Figure 3.5, lower**).

Honesty of VOC blends

There is a significant positive relationship between leaf DTA levels and VOC sesquiterpene emission (GLM: t=2.72, P=0.008; **Figure 3.6A**), suggesting that VOC sesquiterpenes may be honest indicators of leaf DTA levels in *S. altissima*. Furthermore, supporting *Trirhabda*'s apparent sensitivity to DTAs (Uesugi et al. 2013), *Trirhabda*'s host choices are significantly negatively affected by leaf DTA levels (GLM: t=-3.57, P=0.0009; **Figure 3.6B**) – indicating that *Trirhabda* may consider the terpenoid content of *S. altissima*'s VOC emission when choosing host plants. In contrast, leaf DTA levels do not inform host choices by *Dichomeris* (GLM: t=-0.044, P=0.97).

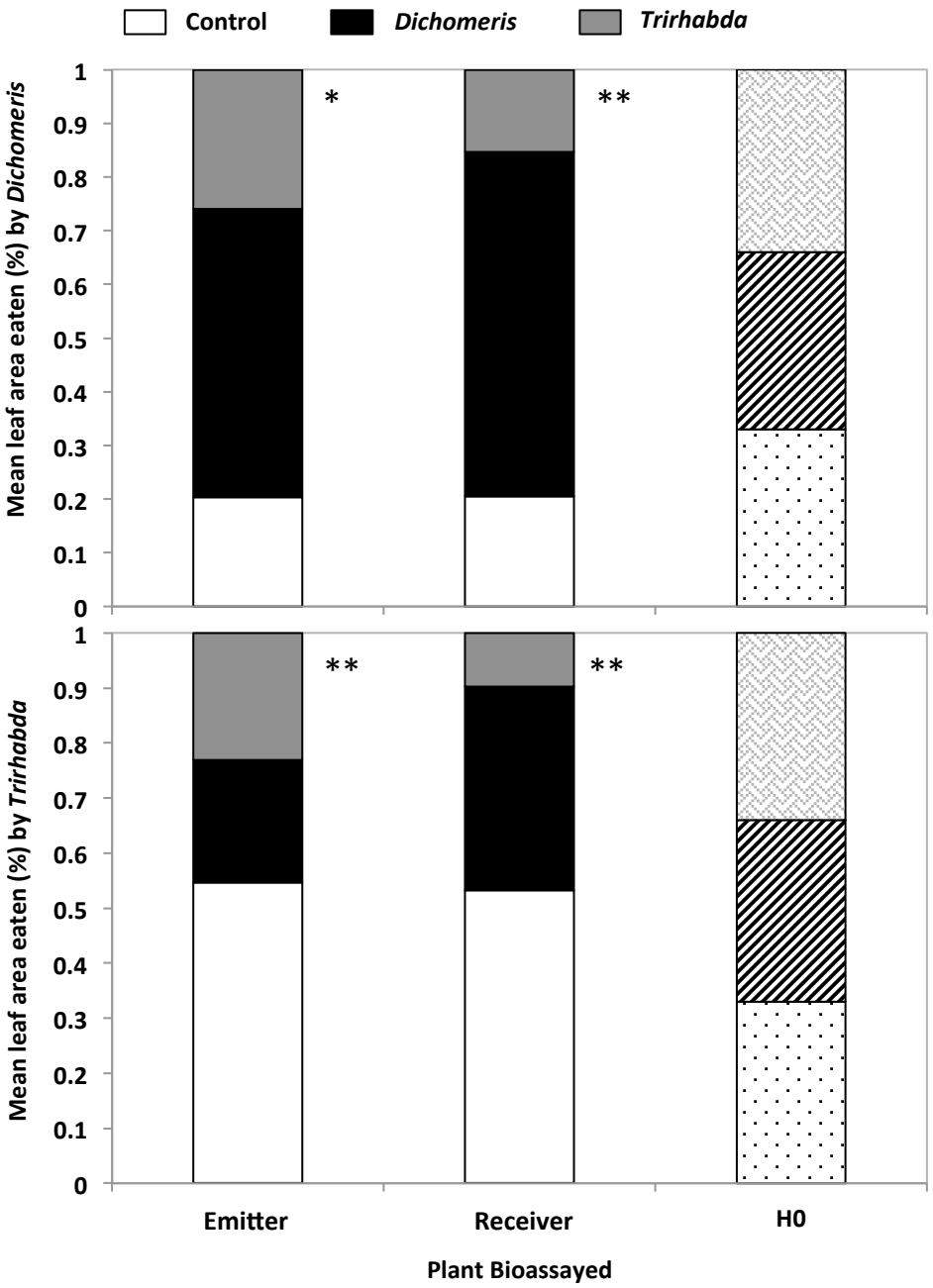

Figure 3.5 We used three-way choice assays using excised leaf disks to test the preferences of bioassay *Dichomeris* and *Trirhabda* for each of the herbivore treatments (control, *Dichomeris*, *Trirhabda*), across exposure regimes (**upper**). Both on herbivore-damaged emitters and VOC-exposed receivers, *Dichomeris* significantly preferred its own damage and/or exposure, whereas *Trirhabda* significantly preferred controls (**lower**).

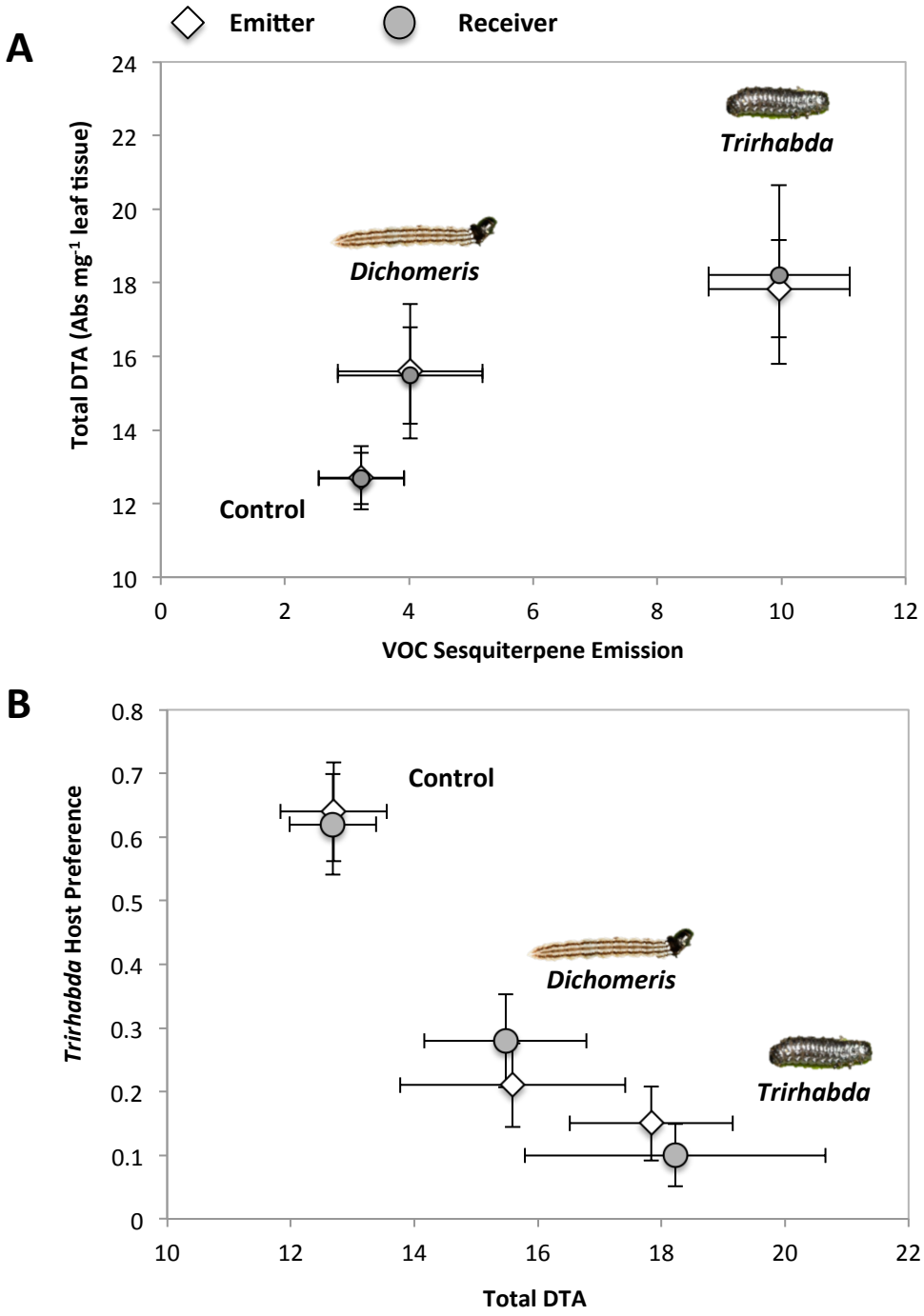

Figure 3.6A-B A significant positive relationship between VOC sesquiterpene emission and leaf DTA levels in *S. altissima* suggests that VOCs provide valid information about leaf defense levels, as posited by the aposematic fragrance hypothesis (**A**). Additionally, *Trirhabda*'s host choices are significantly negatively correlated with leaf DTA levels, suggesting that this information be useful to herbivores (like *Trirhabda*) that are sensitive to DTAs (**B**).

DISCUSSION

Overall, our results suggest that VOC blends contain specific information – both that *S. altissima* plants are being damaged, as well as by whom. We observed marked compositional changes in the VOC blends induced by herbivory in *S. altissima*, and found that these compositional changes differ depending on the species of herbivore damaging the plant. Thus, *S. altissima*'s headspace conveys information about the threat of future herbivory, and this information is available to numerous organisms – including other herbivores and neighboring plants (reviewed in Bruce and Pickett 2011, Karban et al. 2014b).

Additionally, our findings corroborate numerous studies in a variety of plant systems demonstrating that plant induced responses to herbivory are often highly specific – both in the chemical responses elicited in response to particular herbivore species (*elicitation*), and in the effects that these responses have on subsequent herbivores (*effect*; Agrawal and Heil 2012). However, going beyond the scope of these other studies, we found that in addition to mounting specific induced responses to cues associated with herbivore feeding, neighboring plants also responded specifically to the information encoded in the VOC bouquet emitted from a damaged neighbor. In *S. altissima*, DTA levels were significantly affected by herbivore treatment (control, *Dichomeris*, *Trirhabda*) but not exposure regime (emitter, receiver) – suggesting an equivalency between the chemical responses induced by herbivore-associated cues and VOCs.

Finally, our results suggest that the chemical changes induced by exposure to VOCs and to herbivore-associated cues affect the host choices of subsequent herbivores in highly similar ways. We observed species-specific variation in host choices of *Dichomeris* and *Trirhabda* – chemical changes induced by both herbivores repel *Trirhabda*, whereas *Dichomeris*-induced chemical changes are more attractive to subsequent *Dichomeris*. However, it is noteworthy that

both of these herbivore species selected host plants similarly in emitters and receivers despite marked differences in leaf chemistry. Perhaps, this is due to the fact that the emission of VOC sesquiterpenes is positively correlated with leaf DTA chemistry and negatively correlated with herbivore host choice; indicating that VOCs may be honest indicators of host plant quality to foraging *Trirhabda*.

Ecological Function of Variation in VOC Emission

Plants exhibit enormous variation in VOC emission, and this variation is widely documented, and known to result from multiple sources, such as a plant's age and identity, and the biotic (e.g. herbivores, pathogens) and abiotic (e.g. drought, nutrient availability), stressors it is experiencing (e.g. Gouinguene and Turlings 2002, Piesik et al. 2011, Pearse et al. 2013). However, while this variation is becoming increasingly well characterized, our understanding of what that variation means ecologically, and functionally, for plant-plant and plant-herbivore interactions remains largely speculative.

Speculating on the ecological functionality of VOC blend variability, an important open question is whether VOC blends contain *specific* and *honest* information about plants' current defensive state (either direct or indirect; Pearse et al. 2013), and convey this information to organisms in the vicinity. In support of this, studies have shown that the diversity (Becerra et al. 2009), composition (i.e. compound ratios; Webster et al. 2010), and overall phenotype of the VOC blend (e.g. Karban et al. 2014a) may all matter functionally to the organisms in a plant's immediate environment – including predators, parasitoids, herbivores and neighboring plants. However, the information contained in plants' VOC emission may (but need not) be honest – while one *Brassica oleracea* variety emits dose-dependent concentrations of VOCs (providing honest information to foraging parasitoids about plant herbivore loads), another variety "cries

wolf" – emitting constantly high concentrations of VOCs independent of its herbivore load (Shiojiri et al. 2010). This suggests that the genotypes and/or species in a community may vary in the degree to which their VOC emission honestly reflects their present defensive state.

Another open question involves the importance of VOC-mediated information transfer between plants and predators/parasitoids vis-à-vis information transfer between plants and neighboring plants. While numerous studies have documented VOC-mediated information transfer between plants that recruits natural enemies, there are many examples in which this does not occur (recently reviewed in Allison & Hare 2009), suggesting that it is worth considering alternative hypotheses. In light of the specificity in VOC emission by different herbivores that we observed in *S. altissima* and the fact that this VOC emission induces responses in neighboring plants (Morrell & Kessler *in review*), it is interesting to speculate about the functional ecological importance of VOC-mediated information transfer between plants, and degree to which VOC emission conveys honest information to neighboring plants. If VOC blends are specific and honest, we expect to see a strong relationship between some aspect of a plant's VOC blend and its resistance state. We also predict that specificity of resistance should be exactly transferred to the neighboring plant – inducing it to mount a specific chemical response to the anticipated attacker. However, evolutionarily, we expect honesty to be favored insofar as the sender and receiver of the information experience reciprocal benefits, but evolutionary waves of honest and dishonest signaling since cheaters only benefit if receivers still perceive the information as honest (van Baalen and Jansen 2003). Thus, regarding VOC-mediated plant-plant interactions, we would expect honest information to be favored evolutionarily if plant-plant information transfer is an important outcome of VOC emission, there are reciprocal benefits for emitters and receivers.

In light of the biosynthetic relatedness of *S. altissima*'s terpenoid profile, which includes volatile mono- and sesquiterpenes, and semi-volatile diterpene acids (DTAs), and the diversity of toxic to anti-digestive effects of terpenoids on herbivores (Langenheim 1994, Powell and Raffa 1999, Trapp and Croteau 2001, Powell and Raffa 2003), our findings that VOC terpenoids are significantly positively correlated with leaf DTA levels, and that leaf DTA levels are negatively correlated with *Trirhabda*'s host choices is noteworthy. This suggests that VOC sesquiterpenes have the potential to provide honest information to neighboring plants and herbivores about leaf DTA levels.

Specificity of Elicitation: Plants Respond Specifically to Herbivore Elicitors & VOCs

Consistent with past studies, *S. altissima* plants respond specifically to feeding by herbivores of different species (specificity of elicitation). This corroborates recent findings by Uesugi and colleagues (2013) in *S. altissima*, and also a variety of studies conducted in other plant species (reviewed by Agrawal and Heil 2012). One hypothesis states that this specificity is adaptive because it may enable plants to induce very targeted, and thus more efficient, responses to particular attackers (Agrawal and Heil 2012).

Perhaps the similar degree of specificity that we observed in the chemical responses to herbivore feeding and VOC exposure is indicative of similar molecular mechanisms by which plants perceive VOCs and herbivores. The mechanism by which plants perceive herbivore damage is becoming increasingly well characterized. It involves "damaged-self" recognition; namely, recognition of plant-derived molecules that are degraded and/or located outside of their typical location in the plant cell, or herbivore-associated elicitors (Heil 2009). When these molecules are detected, the plant triggers a signaling network involving plasma membrane

potential variations, cytosolic calcium ion fluxes, mitogen-activated protein kinase cascades and gene expression changes (reviewed in Wu and Baldwin 2009). Additionally, plants can detect specific information about the identity of the present attacker from differences in the molecular structure of elicitor classes or different herbivore feeding patterns – enabling plants to mount specific defense responses to particular attackers (reviewed in Wu and Baldwin 2009).

However, changes in VOC emission also characterize a plant's "damaged-self" phenotype, and can thereby similarly trigger signaling cascades – either in distal parts of the damaged plant, and/or in undamaged neighboring plants. Although the signaling network triggered by VOCs has not yet been as well characterized as cascades triggered by herbivore feeding, there seems to be parallels in early signaling events such as plasma membrane potential variations and cytosolic calcium fluxes (Zebelo et al. 2012). Additionally, the composition of the VOC blend mediates *specific* signaling responses: whereas GLVs elicit strong plasma membrane depolarizations and calcium ion fluxes, terpenoids elicit slightly weaker plasma membrane depolarizations and exert no measurable effect on calcium ion fluxes (Zebelo et al. 2012) – suggesting that plants have mechanisms by which to capture variation in VOC blends, relay this information to the cell's interior, and thereby mount specific chemical responses. Yet despite many similarities in plants' responses to VOCs and herbivore damage, there are nonetheless slight differences – both chemically and in terms of timing – whose mechanisms and ecological consequences are worth exploring in the future as potentially-relevant dimensions of plant response specificity (Morrell and Kessler 2014).

Specificity of Effect: Specific Plant Responses affect Herbivore Host Choices

In addition to eliciting specific chemical responses in the leaf tissue of *S. altissima*, these experiments also reveal that the chemical changes elicited by VOCs and past herbivory affect the

host choices of subsequent herbivores in species-specific ways (i.e. there is specificity of effect). While *Dichomeris* prefers hosts damaged by previous conspecifics, or host plants that were previously exposed to the VOCs of *Dichomeris*-damaged plants, *Trirhabda* prefers hosts that have not experienced previous herbivory or exposure to herbivore-induced VOCs. These findings reveal that just as the chemical changes induced by herbivory may have different effects on subsequent herbivores (Stout et al. 1998, Van Zandt and Agrawal 2004b, Uesugi et al. 2013); the same is true for the chemical changes induced by exposure to herbivore-induced VOCs in receivers.

Although we did not measure plant fitness directly, it is likely that the strong herbivore host preference behaviors that we observed in these experiments in response to induced responses and plant-plant communication may ultimately increase *S. altissima*'s fitness. Although we were unable to test this directly by comparing populations with and without plant-plant communication experimentally, a recently published model incorporating insect host choice, feeding and movement tested how the distribution of herbivory compared in these two scenarios. The model indicated that the distribution of herbivory is more evenly dispersed in populations with plant-plant communication than in populations in which plants cannot communicate (Rubin et al. *in press*). This even dispersion suggests that induced responses and plant communication act together to reduce the amount of herbivory that any individual plant in the population receives. In light of the fact that damage levels in *S. altissima* are strongly correlated with fitness, this more even distribution of herbivory should thereby increase plant fitness (Root 1996, Meyer 1998).

Conclusions

VOCs play key roles in mediating information exchange between plants and a wide

variety of organisms – including herbivores, pollinators, predators, parasitoids, hyper-parasitoids, and even other plants (e.g. Karban et al. 2014a, Andrews et al. 2007, Bruce and Pickett 2011, Poelman et al. 2012). VOCs can also influence herbivore attraction/host choice by conveying aspects of host quality and/or the location of potential mates. Yet as our collection of experiments explores, another key role that VOCs play is in conveying specific information to neighboring plants in the vicinity – information that enables neighboring plants to mount targeted responses to attackers.

In light of our findings, that herbivore-induced VOCs contain specific information that elicits specific plant responses in and informs subsequent herbivore host choices on receivers, it is interesting to speculate about the adaptive significance of specific VOC emission in plant populations, and whether specificity in VOC emission has the potential to evolve by natural selection. From an evolutionary perspective, the specificity of VOC-mediated information transfer between plants may be adaptive because VOCs from neighboring plants would likely be the best available predictors of a plant's likelihood of experiencing future herbivory. However, it also seems that different selective pressures on VOC specificity might be at work depending on the nature of the interaction between the plant emitting and the plant receiving the information. These relationships between emitters and receivers might span a spectrum ranging from allelopathy to mutualism to eavesdropping. If the interaction is *allelopathic* – namely that the emitter gains a benefit from signaling and the receiver experiences a cost – then we might expect selection to favor emitters whose VOC blends convey toxicity, and selection to favor receivers that do not respond to VOCs. In contrast, if the interaction is *parasitic* ("eavesdropping") – namely that the receiver gains a benefit from responding to information in the headspace but the emitter experiences a cost – we might expect selection on emitters to avoid emitting "honest"

information in their VOC blends (i.e. selection for information that is not correlated with their current defense state). Conversely, we would expect selection on eavesdropping receivers for highly specific responses. Finally, if the interaction between emitters and receivers is *mutualistic* – namely that both emitter and receiver gain benefits from plant communication – we would expect selection on emitters to emit VOCs that reflect their current metabolic state, and selection on receivers to respond specifically to these cues.

In *S. altissima*, it seems possible that emitters and receivers may both experience benefits from VOC-mediated plant communication. This is because plant communication and informed herbivore movement seem to act synergistically in this system – keeping herbivores on the move between host plants (Morrell and Kessler *in review*), and resulting in an over-dispersion of plant damage in the population (Rubin et al. *in press*). This over-dispersion of plant damage is noteworthy, from a plant fitness perspective, because it keeps overall levels of herbivory per plant low enough to minimize the effects of herbivore damage on plant fitness (Root 1996). However, in the future, it is well worth exploring the possibility that the specificity of defense responses that we observed in these experiments may come at a cost to receivers if *S. altissima* genotypes vary in the honesty of their VOC emission. Additionally, in light of the enormous diversity of herbivore species that feed on *S. altissima* (Maddox and Root 1990), it is worth exploring if a specific response to VOC emission comes at a cost if a different herbivore species ends up attacking the receiver next. Building on our understanding of genotypic variation in signal honesty and the potential costs of responding specifically will greatly enhance our understanding of the nature and evolutionary trajectory of the interaction between emitters and receivers in plant populations, and its effects on community composition.

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CHAPTER 4

GENOTYPIC VARIATION IN PLANT COMMUNICATION

In preparation for *Journal of Ecology* as: Kimberly A. Morrell and André Kessler. Genotypic variation in plant communication.

ABSTRACT

Evidence for volatile organic compound (VOC)-mediated plant-plant interactions and their importance in shaping plant-herbivore and plant-natural enemy interactions is rapidly growing. However, the effect of plant genotype on the strength and ecological outcome of plant-plant interactions, which is crucial to understanding the function and evolution of plant communication, remains less well characterized. Using five genotypes of tall goldenrod (*Solidago altissima*, Asteraceae), we compared plant-plant interactions across 25 combinations of emitter and receiver genotypes to test three non-mutually exclusive hypotheses regarding genotypic variation in plant-plant interactions: that VOC-mediated plant-plant interactions are constrained by A) emitter genotype, B) receiver genotype, and/or C) self-recognition by plants of the same genotype. We measured levels of herbivory by two specialist leaf-chewing herbivores (*Paria thoracica* and *Trirhabda virgata*, Coleoptera: Chrysomelidae) as proxies for response strength by neighboring (receiver) plants, and characterized the observed effects on herbivores by measuring emitter volatile chemistry and receiver leaf chemistry. Overall, emitter and receiver genotype (but not self-recognition) affect the outcome of plant-plant interactions in *S. altissima*. Additionally, receivers may induce resistance or susceptibility to subsequent herbivores, depending on herbivore identity and the source of the VOC cue. Moreover, compositional differences in emitters' volatile and receivers' leaf diterpene acid profiles, rather than the total chemotype, best characterize the genotypes of plants that induce resistance versus susceptibility. Advancing our knowledge on genotypic variation in plant-plant interactions, our finding that receiver and emitter genotype both explain observed effects on herbivores suggests that there could be reciprocal natural selection for increased or decreased plant communication within plant populations depending on the value of the information exchange and the

environment in which these interactions are played out.

KEYWORDS: volatile organic compounds, plant-plant interactions, herbivore bioassay, genotypic variation, diterpene acids, leaf chemistry, *Solidago altissima*, *Trirhabda virgata*, *Paria thoracica*

INTRODUCTION

Volatile-mediated plant-plant interactions have been shown in over 35 plant species to date (reviewed in Heil and Karban 2010, Karban et al. 2014b). Most documented examples show intra-specific information transfer (Karban et al. 2014b), although there is also evidence that VOCs can transfer information between plants of different species (e.g. Karban et al. 2000). However, in spite of increased documentation of VOC-mediated plant-plant interactions, in systems ranging from agricultural crops to grasses, shrubs, forbs and trees, the debate over whether plants *communicate* via VOC signals (implying a benefit for emitters and receivers) or simply *eavesdrop* on VOC cues (implying that receivers benefit, but emitters experience a cost) rages on.

At the heart of this debate, nicely summarized by Pearse and Karban (2013), lies the fact that limited work has sought to understand the "ecological contingencies" that may favor plant-plant interactions in ecological communities (Pearse and Karban 2013). One of these contingencies is the structure of a plant's neighborhood, both its *genetic* and *species* composition, which affect a plant's risk of experiencing herbivory as well as the availability and reliability of information from a plant's neighbors (Barbosa et al. 2009, Underwood et al. 2014, Hamback et al. 2014).

Additionally, plant-plant interactions (both intra- and inter-specific) may have diverse outcomes – ranging from induced *resistance* to induced *susceptibility* to *no response* (nicely reviewed in Karban et al. 2014b); however, it is not yet clear what ecological and evolutionary conditions lead to these different outcomes. Studies investigating associational effects in multi-species assemblages have shown that attractive species may retain herbivores and/or result in spillover effects, whereas repellent species may push herbivores to other plant patches (reviewed in Barbosa et al. 2009). Additionally, VOC-mediated plant-plant interactions may lead neighboring plants to mount defensive responses of their own – becoming more or less resistant to subsequent herbivores (Pearse et al. 2012, Kessler et al. 2006).

However, studies elucidating whether natural selection shapes plant communication, or even how genetic differences between emitters and receivers may result in these different outcomes of VOC-mediated plant-plant interactions (i.e. resistance versus susceptibility) remain absent or limited. There is some evidence that plant genotypes exhibit variation in their VOC emission profiles (e.g. Eller et al. 2012, Hare 2007) that can affect natural enemy attraction (e.g. Shiojiri et al. 2010) and plant-plant interactions (Karban and Shiojiri 2009). Specifically regarding VOC-mediated plant-plant interactions, extensive work in sagebrush (*Artemisia tridentata*) suggests that receiver plants respond most strongly to an emitter of their same genotype (Karban and Shiojiri 2009) or VOC chemotype (Karban et al. 2014a). However, this research is just beginning to graze the surface of how genotypic differences between emitters and receivers affect the likelihood of observing VOC-mediated plant-plant interactions (or associational effects), as well as the factors driving the observed range of outcomes (from no response, to resistance, to susceptibility).

Thus, in this collection of experiments, we took a neutral approach, screening 25

combinations of emitter and receiver genotypes to ask:

(1) Is information exchange between plants constrained by the genotype of the emitter, receiver, and/or by self-recognition (non-mutually exclusive)? What is the range of observed VOC-induced responses (induced resistance versus induced susceptibility versus no response)?

(2) What aspects of emitter genotypes' VOC profiles characterize the resistant versus susceptible phenotypes that they induce in receivers?

(3) What aspects of receivers' leaf chemical profiles characterize genotypes that induce resistant versus susceptible phenotypes in response to VOC cues from neighboring plants?

We addressed these questions in *Solidago altissima* L. (Asteraceae) – a clonal, perennial forb native to the Northeastern United States. *S. altissima* is attacked by a diverse and well-characterized suite of insect herbivores, including multiple species of specialist leaf-chewing Chrysomelid beetles such as *Paria thoracica* and *Trirhabda virgata* (Messina and Root 1980; **Figure 4.1**).

Additionally, *S. altissima* genotypes vary in levels of defenses to specific herbivores – genotypes tend to be susceptible to some suites of herbivores but resistant to others, independent of these herbivores' feeding guilds (Maddox and Root 1987, 1990). These differences in resistance are also correlated with plant defense chemistry, including leaf serine and cysteine protease inhibitors (Bode et al. 2013), leaf diterpene acids (Uesugi et al. 2013), and herbivore-induced VOC emission (Morrell and Kessler 2010, Morrell and Kessler *in review*). Additionally, plant genotypic diversity and arthropod species diversity are strongly positively correlated in *S. altissima* populations (Crutsinger et al. 2006), suggesting that there may be benefits to perceiving

VOC information from different genotypes as well as clones. Moreover, prior studies have established that VOC-mediated plant-plant interactions occur in *S. altissima* (Morrell and Kessler *in review*).

Figure 4.1 Two specialist leaf-chewing beetles on *Solidago altissima* include *Paria thoracica* and *Trirhabda virgata* (Chrysomelidae, Coleoptera). In insect community surveys of old-fields in Ithaca, NY, the abundances of these two beetle species are negatively correlated (Messina and Root 1980). Image rights purchased from Tom Murray.

This prior work in *S. altissima* enabled us to take a multi-pronged approach to addressing our questions – using herbivore bioassays to understand the strength of VOC-mediated plant-plant interactions across a variety of genotypes, the effects of these interactions on herbivore resistance (positive, negative, neutral), and to correlate these outcomes with the underlying leaf and VOC defense chemistry of emitters and receivers.

METHODS

Genotype Collection and Plant Propagation

In August 2009, we collected five *Solidago altissima* genotypes from three distinct, naturally occurring field populations around Ithaca NY. Genotypes 8A2 and 3D4 were collected from Whipple Farm (coordinates: 42.490358, -76.430930), 6R was collected from Dunlop Preserve (coordinates: 42.385298, -76.395879), and 9R and 14R were collected from Durland Preserve (coordinates: 42.437851, -76.398166). We maintained greenhouse populations of each genotype for four vegetative (clonal) propagation cycles before using them in these experiments.

In the greenhouse, we propagated 36 plants from each of the five genotypes (N=180 plants total) by clipping 3cm pieces of rhizomes, each containing at least one apical meristem and root. We dipped the rooting end of each rhizome in rooting promotor (Bontone® Rooting Powder, Bonide Products, Oriskany, NY) before planting it vertically in 96-well germination flats. To aid new leaf formation, the flats were submerged in 3cm water and covered with a humidity dome for 6-7 days. When young leaves began to emerge, we subsequently removed the submerging trays and humidity domes, and began to water the propagules three times daily and fertilize once weekly (greenhouse conditions: 80^{o}C daily/60^{o}C nightly; 16/8 hour light regime). When the propagules reached a height of 5cm, we transplanted them into 150cm diameter Azalea pots, and started experiments when plants reached a height of 25-30cm.

Field Preparation and Treatment Application

At Whipple Farm, in a location distinct from where genotypes 3D4 and 8A2 were collected (coordinates: 42.488487, -76.429830), we dug 30 holes – 500cm in diameter, 250cm deep, and each 4 meters from any other holes. We also mowed the vegetation surrounding the holes and plot and applied pyrethrin insecticide to limit the presence of other insects in the trial

area four weeks prior to starting field trials.

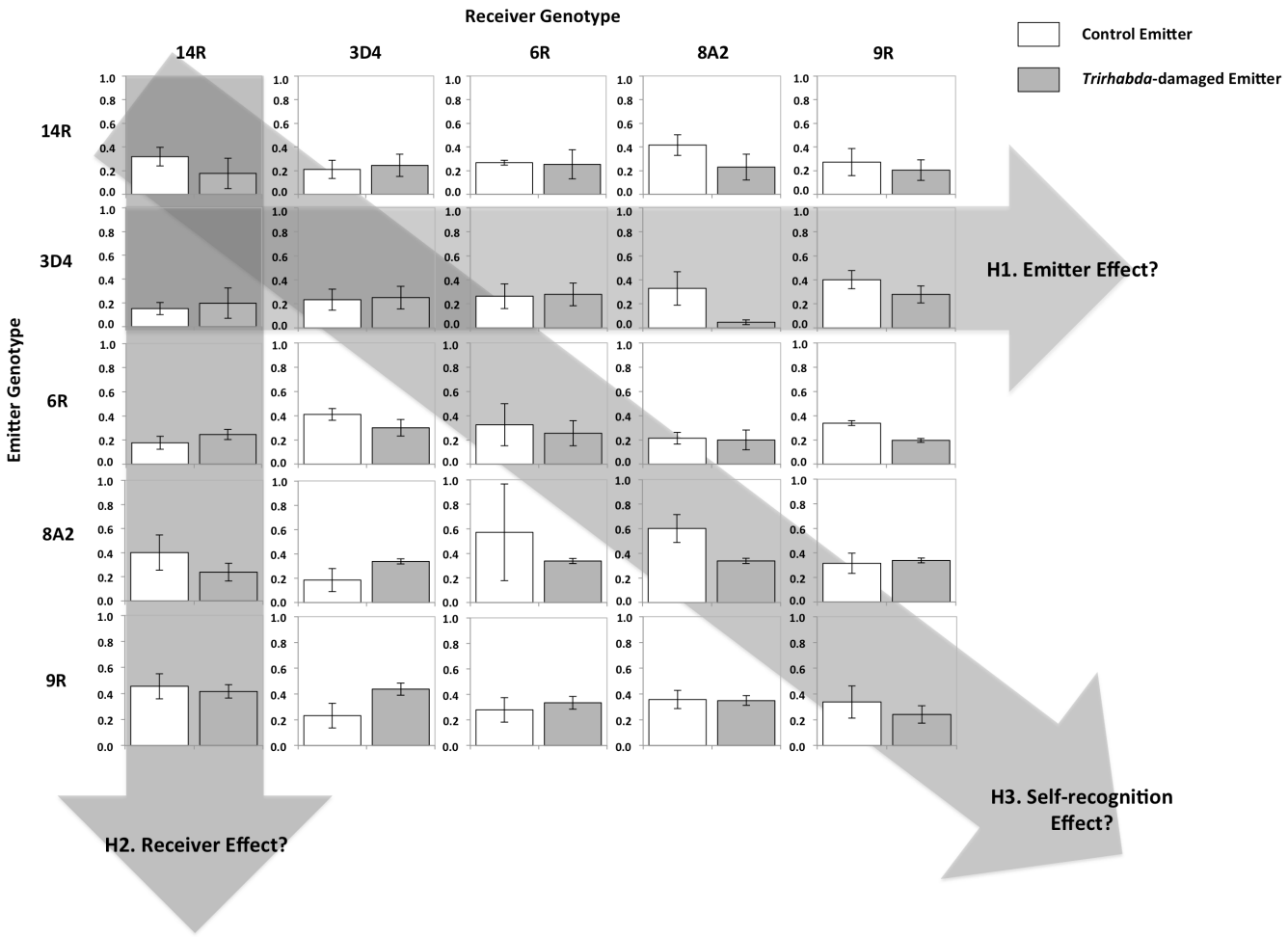

Figure 4.2 Experimental setup and hypothesis framework. To test the hypotheses that VOC-mediated plant-plant interactions are affected by emitter genotype (horizontal arrow), receiver genotype (vertical arrow), and/or self-recognition effects (diagonal arrow), we created and compared herbivore feeding levels in 25 combinations of emitter and receiver genotypes of *Solidago altissima*. Bar graphs show the average proportion of damage by *Trirhabda virgata* on receivers with control (white bars) and *T. virgata*-damaged (gray bars) emitters (± SEM) for each emitter-receiver genotype combination.

We began treatment application on June 9, 2014 by moving six potted plants – one randomly assigned "emitter" and five "receivers" (one from each of the five genotypes) – into each hole. Additionally, all plants in the experiment were covered in mesh sleeve bags (Breather^{TM}, Palm Tree Packaging, Apopka, FL) to contain the treatment insects and/or keep out other insects in the vicinity. In these experiments, emitter refers to a plant that has received a treatment (no damage (control) or *Trirhabda virgata* damage) to which receiver plants are

exposed. In this experiment, each of the five genotypes was represented as an emitter six times – three times as a control emitter, and three times as a damaged emitter (damaged by three 2nd-instar *T. virgata* larvae) – creating 25 emitter-receiver combinations (**Figure 4.2**).

Laboratory No-choice Bioassays

After nine days of treatment application, on June 18, 2014, we harvested 2 leaves, at positions 6 and 7 below the apical meristem, from each receiver plant. One leaf, at position 7, was flash-frozen in liquid Nitrogen in the field and stored at -80°C until subsequent leaf chemical analyses (described below). The petiole of the other leaf was placed in moist floral foam and brought back to the lab for no-choice bioassays.

In the lab, we used a scanner to determine the initial area of each leaf, and added it to a Petri dish along with a 2nd-instar *T. virgata* larva (initial mass 15-25mg, starved for 12 hours prior to starting no-choice bioassays). Each larva was allowed to feed freely for four days. To determine leaf tissue consumption by *T. virgata*, we re-scanned leaves and used ImageJ (version 4.3.1) to calculate the proportion of each leaf eaten.

Because the proportion of leaf tissue eaten by *T. virgata* could reflect differences in the constitutive resistance levels of each of our genotypes as well as differences in the effects of our exposure treatments, we isolated the effect of our exposure treatments by subtracting the leaf tissue consumed on a receiver exposed to a damaged emitter from average leaf tissue consumed on a receiver of the same genotype exposed to a control emitter (average control – damaged). We therefore used the resultant **Difference in the Proportion of Leaf Tissue Eaten by *T. virgata*** as our response variable in all subsequent statistical analyses.

To address the first question, whether information exchange between plants is constrained by the genotype of the emitter, receiver, or by self-recognition, we performed

generalized linear models on all receiver plants in the experiment, using emitter genotype, receiver genotype, and self (Yes/No – are emitter and receiver genotype the same) as factors (**Figure 4.2**). If we see an effect of emitter genotype on the responsiveness of receivers, we can conclude that there is some property of a particular emitter genotype that induces strong responses in receivers regardless of their genotypes. If we see an effect of receiver genotype, we can conclude that plant genotypes vary in their responsiveness to VOC signals independent of the genotype of the emitter. And if we see a self-recognition effect, we can conclude that genotypes respond strongest to VOCs from a plant of their same genotype (as in Karban and Shiojiri 2009).

Field Natural Herbivory Survey

Following the conclusion of our treatment period, on June 19, 2014, emitters were moved out of the holes for analysis of volatile organic compound (VOC) emission (see below), and the mesh bags of all receivers were removed – making the plants available to natural herbivores in the vicinity. Eight days later, on June 26, 2014, we surveyed the herbivore community and levels of natural herbivory on all receiver plants by recording the proportion of herbivore damage on each damaged leaf. Because one herbivore, *Paria thoracica* (Coleoptera: Chrysomelidae), dominated the community surveys and did the overwhelming majority of the damage to the focal receiver plants (**Figure 4.1**), we chose to measure its damage exclusively, expressed as the proportion of damaged leaves per plant. As with *T. virgata*, we also accounted for genotypic differences in constitutive resistance levels to *P. thoracica* by subtracting the average proportion of damaged leaves on receivers with a *control* emitter from the proportion of damaged leaves on receivers with a *damaged* emitter to standardize.

As before, we used generalized linear models conducted in R (version 3.1.1) with emitter

genotype, receiver genotype, and self (Yes/No) as factors to test whether the **Difference in the Proportion of *Paria thoracica*-Damaged Leaves per Plant** was affected by the genotypes of the emitters, receivers, and/or self-recognition.

Volatile Organic Compound Emission of Emitters

On June 19, 2014, from 0945 to 1645, we removed treatment herbivores and measured the volatile organic compound emission from all emitter plants using an open-flow dynamic headspace trapping design (Kessler and Baldwin 2001). Briefly, the top 20 leaves of each plant were covered with a 750mL polyethylene chamber, and air was pulled over the leaves and onto an Orbo-32 small-activated coconut charcoal (20/40) trap (100/50mg, Sigma-Aldrich LLC.) at a flow rate of 350ml/min using a 12V vacuum pump (Gast™ Manufacturing Inc., Grainger® Industrial Supply). The weather was sunny and 22-24^{o}C throughout the trapping interval.

In the lab, we added 5uL of a tetraline in hexane (90ng/uL) internal standard to each trap, and eluted them in 350uL dichloromethane. The eluate was analyzed via GC-MS on a Varian Saturn 2200 GC/MS/MS with an Agilent J&W GC Column (DB-WAX FAME, 30m x 0.25mm ID, DF=0.25) and a CP-8400 Autosampler in splitless mode. We used an injection temperature of 225^{o}C, heated the eluate from 40^{o}C-180^{o}C at a rate of 10^{o}C/minute, further heated the eluate up to 220^{o}C at a rate of 40^{o}C/minute, and held it at the final temperature for 10 minutes.

The emission of each peak was calculated as signal intensity relative to the tetraline internal standard minus the average intensity of that peak in the air controls (empty collection chamber). Peaks were identified by their retention times and mass spectra compared to a series of authentic standards as well as the Nist Mass Spectral Search Program library (version 2.0).

Because our herbivore no-choice bioassay and natural damage survey revealed significant emitter effects (see Results), we classified Emitter Genotypes by whether they induced resistance

(IR) or susceptibility (IS) in receivers. In genotypes that induce resistance, herbivores fed on these genotypes *more in the control state* than in the damaged state (difference in percent herbivory > 0). In contrast, in genotypes that induce susceptibility, herbivores fed *more in the damaged state* than in the control state (difference in percent herbivory < 0). Genotypes on which herbivores fed equally in the control and damaged state were considered non-responsive.

To characterize the VOC profiles of plants that induce resistance versus susceptibility, we compared four properties in the VOC blends of IR versus IS emitters: (a) total VOC emission in the damaged state; (b) total composition of the VOC blend in the damaged state, (c) induced dissimilarity in the VOC profile of control versus damaged emitters, and (d) individual VOCs that characterize IR versus IS emitters in the damaged state.

To address (a), we calculated Total VOC Emission in each sample by summing the emission rates of all individual compounds (standardized by the air control) in the VOC blend. We compared Total VOC Emission in emitter genotypes that induced resistance versus susceptibility using a t-test in R.

To test (b), whether the composition of VOC blend in the damaged state differs for emitters that IR versus IS, we conducted PERMANOVAs (*adonis* functions in R) with Designation (IR versus IS) as an independent factor and individual VOCs as dependent factors, and subsequently used non-metric multidimensional scaling (NMDS; R packages *lme4*, *car*, *nlme*, *MixMod*, and *vegan*) to visualize these differences.

Next, to test whether inducibility of the VOC profile affected whether emitter genotypes induced resistance or susceptibility (c), we used the *vegdist* function in R to calculate the degree of dissimilarity in multidimensional space between the average control and average damaged phenotype of each Emitter Genotype (hereafter *induced dissimilarity*). We then compared these

degrees of induced dissimilarity between emitters classified as IR versus IS, using a t-test in R.

Finally, we determined which individual VOCs characterized the blends of emitter genotypes that IR versus those that IS, (d), by conducting a Random Forest Analysis in R (packages *RColorBrewer*, *randomForest*, and *varselRF*; Ranganathan and Borges 2010). Random Forest is a bootstrapping algorithm designed to identify the smallest set of predictor variables that explain the difference between one group of samples (IR) versus the rest (IS), whose predictions are not affected by low sample size or autocorrelations between predictor variables (which is likely the case for *S. altissima*'s VOC profile). We subsequently ran t-tests on each of the predictor variables to see whether high or low emission of these compounds characterizes emitters that IR versus IS in receivers.

Leaf Chemistry of Receivers

In the lab, we extracted ~150-200mg frozen leaf samples from receivers so that we could measure leaf diterpene acids (DTAs). DTAs are a diverse class of 20-carbon semi-volatile compounds whose effects range from digestibility reducers to toxins (Langenheim 1994), are abundant in *S. altissima*, and correlated with resistance to *Trirhabda virgata* (Uesugi et al. 2013).

We measured DTAs exactly as described by Uesugi and colleagues (2013). Briefly, leaf samples were crushed in liquid Nitrogen, and extracted in 90% methanol with ~1g Zirconia/Silica lysing beads (2.3mm, BioSpec Products) on a FastPrep®-24 machine (MP Biomedicals). The supernatant was analyzed via high-performance liquid chromatography (HPLC) at 230nm (temperature program described in Uesugi et al. 2013). Subsequently, DTAs were identified by their UV absorbance spectra and retention times at the 230nm wavelength, and quantified as peak area per milligram of leaf tissue using ChemStation (Agilent Technologies).

Since our *Trirhabda virgata* no-choice bioassays revealed significant receiver effects (see Results), we subsequently asked which aspects of leaf DTA profiles characterize the resistance phenotypes of receivers that induce resistance versus susceptibility. As with the emitter effect, Receiver Genotypes were considered to induce resistance (IR) if *Trirhabda virgata* herbivory was higher on control plants than on damaged (difference in percent herbivory >0), and vice-versa. Receiver genotypes were considered non-responding if *Trirhabda virgata* herbivory was equal on receivers with control and damaged emitters.

Using the same methods described above for understanding differences in the VOC profiles of IR versus IS Emitter Genotypes, we compared IR and IS leaf DTA phenotypes of the five receiver genotypes in four distinct ways: (a) total DTA levels per milligram leaf tissue in receivers exposed to damaged emitters; (b) the composition of leaf DTA profile in receivers exposed to damaged emitters; (c) induced dissimilarity between the leaf DTA profiles of receivers exposed to control versus damaged emitters; and (d) individual DTAs that characterize the differences between receivers that respond with IR versus IS. As before, we used t-tests with classification (IR, IS) to address (a) and (c); PERMANOVAs and NMDS to test (b), and Random Forest Analyses followed by t-tests to address (d).

RESULTS

Herbivore No-choice Bioassays and Natural Herbivory Surveys

Across the 25 combinations of emitter and receiver genotypes, plant genotype affects both the strength (difference between responsiveness to a control versus a damaged emitter) and direction (resistance versus susceptibility) of receiver responses (**Figure 4.2**).

Overall, emitter genotype (GLM: t=-2.326, P=0.02) significantly affected levels of *Trirhabda virgata* herbivory (**Figure 4.3**). Emitter genotypes 14R, 3D4, 6R and 8A2 induced

resistance in receivers, whereas, 9R induced susceptibility. We also observed a significant effect of receiver genotype (t=2.965, P=0.004) on *T. virgata* herbivory. Specifically, receiver genotypes 14R, 6R, 8A2 and 9R generally induced resistance, 3D4 generally induced susceptibility to *T. virgata*.

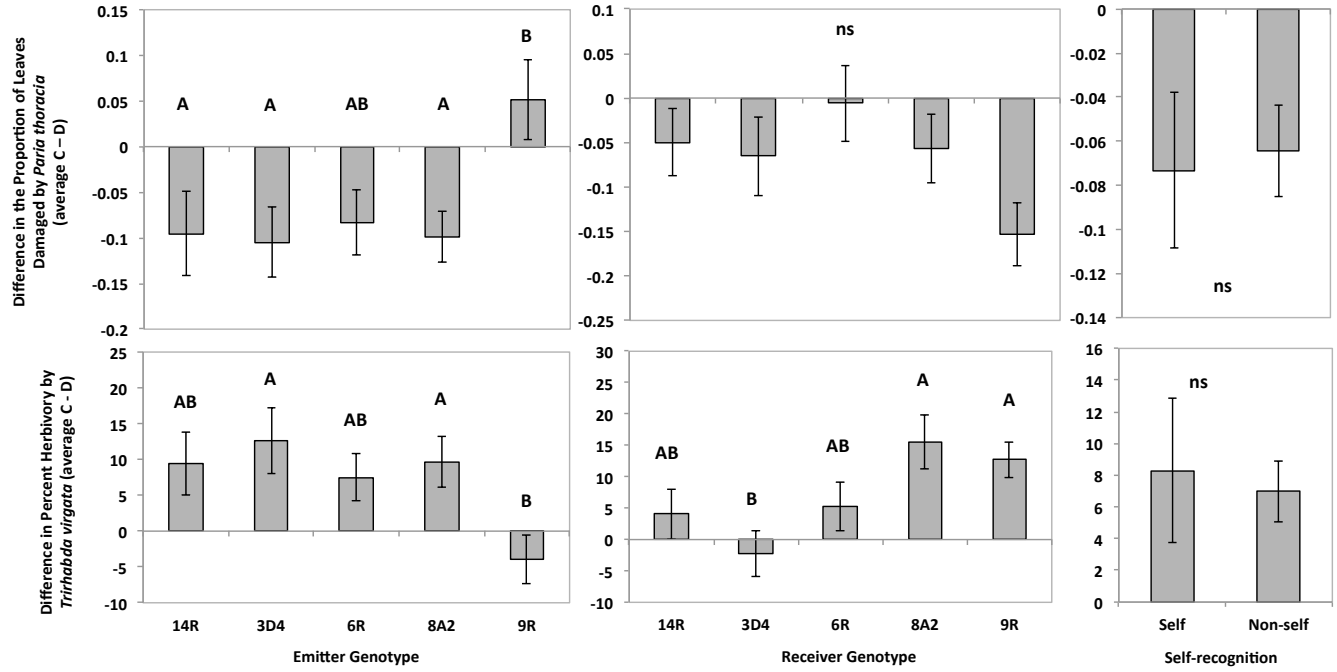

Figure 4.3 Plant genotype specific effects of plant communication on insect herbivory. Results of natural herbivory surveys with *Paria thoracica* (left three figures) and no-choice bioassays with *Trirhabda virgata* (right three figures). Each bar represents the difference (±SEM) in feeding on receiver plants with a damaged emitter minus the average feeding with a control emitter for each emitter genotype (left), receiver genotype (middle), or self/non-self combination (right). Differences greater than zero represent genotypes that induce resistance, differences less than zero represent plants that induce susceptibility, and differences overlapping zero indicate plants that do not respond to the exposure/damage treatments.

Similarly, the natural herbivory survey showed a significant effect of emitter genotype (GLM: t=2.460, P=0.02), but only a weak effect of receiver genotype (t=-1.598, P=0.11) on levels of *Paria thoracica* herbivory. Interestingly, the natural herbivory survey suggests that emitter genotypes 14R, 3D4, 6R and 8A2 all induce susceptibility to *P. thoracica*, whereas 9R induces resistance, suggesting that plants' IR and IS phenotypes are context-dependent –

depending on the identity of the feeding herbivore – in addition to the emitter genotype.

We did not observe an overall self-recognition effects on levels of herbivory by *T. virgata* (GLM: t=0.229, P=0.82) or *P. thoracica* (t=-0.236, P=0.81; **Figure 4.3**); however, some individual combinations of genotypes showed evidence of self-recognition effects (e.g. 14R and 8A2; **Figure 4.2**).

Emitter Volatile Organic Compound Emission

Although total VOC emission (*t-test*: t=-1.36, df=5.919, P=0.22; **Figure 4.4A**) and induced dissimilarity in VOC emission (t=-1.36, df=12, P=0.20; **Figure 4.4C**) did not explain the variation in emitter effects on herbivory, the composition of the VOC blend differed significantly between receivers that IR versus IS (PERMANOVA: $F_{1,15}$=2.69, R^2=0.16, P=0.03; **Figure 4.4B**). Specifically, the levels of three compounds – one monoterpene (KI-1043: t=-4.17, df=2.24, P=0.04) and two sesquiterpenes (KI-1043: t=-4.44, df=2.31, P=0.04; KI-1302: t=-11.92, df=5.93, P<0.0001) are emitted in significantly larger quantities by emitters that induce susceptibility compared to emitters that induce resistance (**Figure 4.4D**).

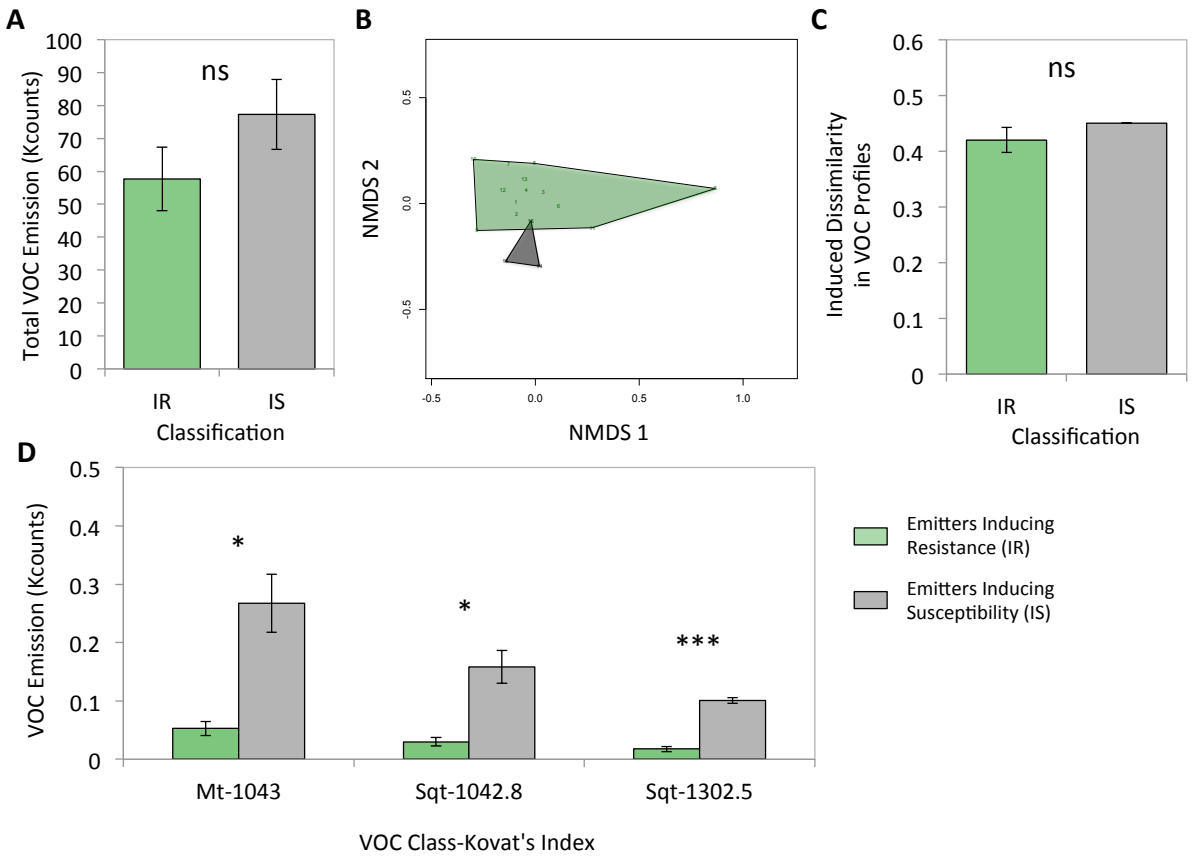

Figure 4.4 Characterizing qualitative and quantitative variation in emitter VOC chemistry. To characterize and compare the VOC chemistry of emitter genotypes that induce resistance (IR) versus susceptibility (IS), we compared Total VOC Emission (±SEM, **A**), compositional differences in the VOC blends of IR (green) versus IS (gray) emitters (**B**); induced dissimilarity (±SEM) between the control and damaged phenotypes of IR versus IS emitters (**C**); and the emission rates of individual VOCs important in characterizing IR and IS emitters in a Random Forest (**D**). Stars indicate significant results of t-tests (* P<0.05; *** P<0.0001; ns = not significant).

Receiver Leaf Diterpene Acid Profiles

Like VOC emission in the emitter genotypes, the leaf diterpene acid profiles vary for receiver genotypes that induce resistance versus susceptibility. Although total DTA levels do not differ (*t-test*: t=-0.49, df=22.03, P=0.63; **Figure 4.5A**), the composition (MANOVA: $F_{4,70}$=2.91, P<0.0001; PERMANOVA: $F_{4,70}$=2.64, P=0.06; **Figure 4.5B**) and induced dissimilarity (*t-test*: t=84.69, df=59, P<0.0001; **Figure 4.5C**) of the DTA profile of IR versus IS receivers differs considerably.

Specifically, in a Random Forest Analysis, seven individual DTAs are sufficient to

characterize the differences between IR and IS receivers (**Figure 4.5D**). Of these, three are associated with induced resistance – at 23.7 minutes (*t-test*: t=3.86, df=61.76, P=0.003), 26.5 minutes (t=4.35, df=66.94, P<0.0001), and 28.6 minutes (t=4.09, df=50.57, P=0.0002); and four are associated with susceptibility – at 25.9 minutes (t=-3.24, df=19.22, P=0.004), 30.4 minutes (t=-3.81, df=18.07, P=0.0013), 31.7 minutes (t=-6.13, df=15.94, P<0.0001) and 32.7 minutes (t=-3.22, df=18.67, P=0.005).

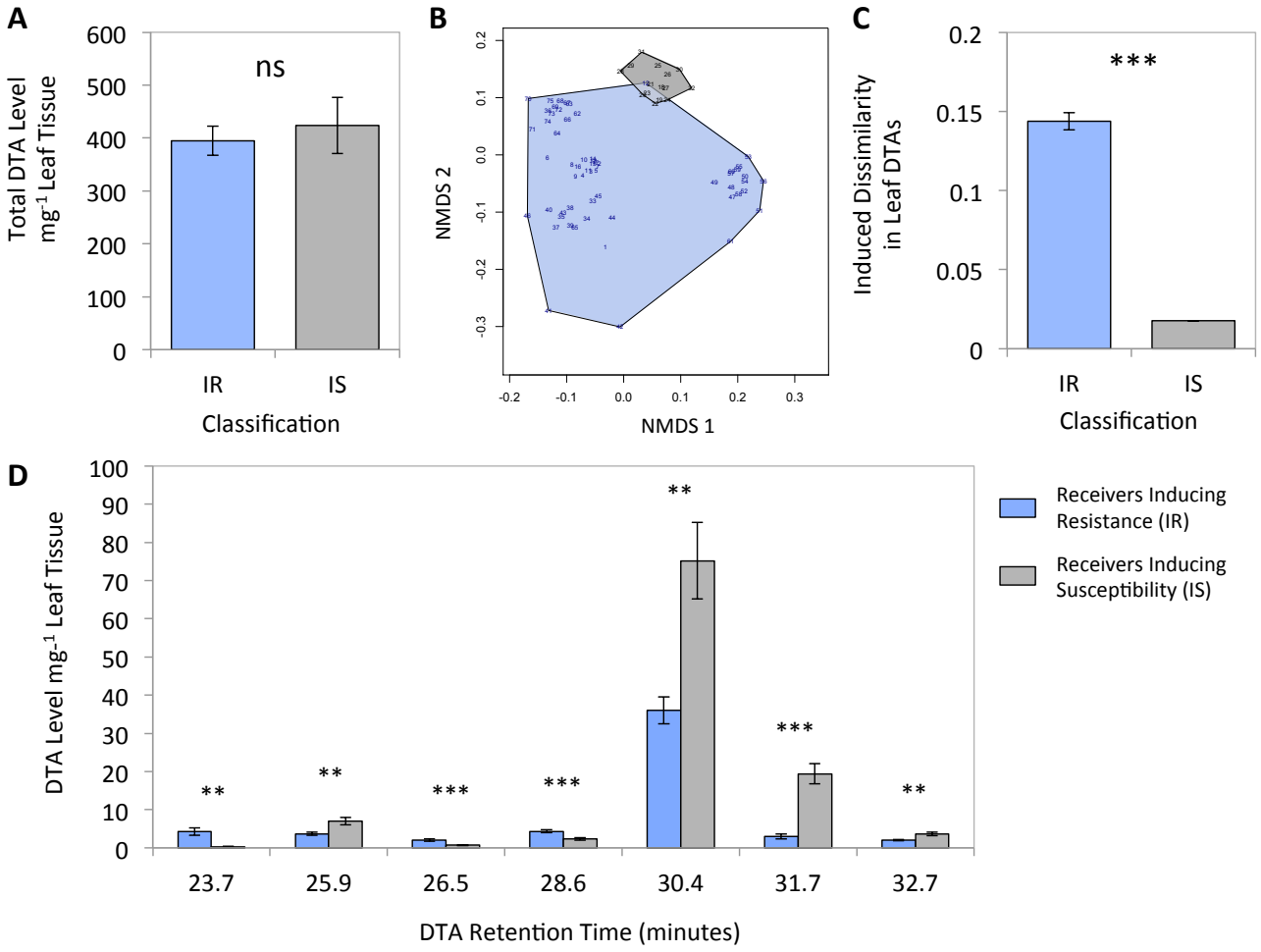

Figure 4.5 Characterizing qualitative and quantitative variation in receiver leaf chemistry. To characterize and compare the leaf chemistry of receiver genotypes that induce resistance (IR) versus susceptibility (IS), we compared Total DTA Levels (±SEM, **A**), compositional differences in the leaf DTA profile of IR (green) versus IS (gray) receivers (**B**); induced dissimilarity between the control and damaged receivers that IR versus IS (**C**); and levels of individual DTAs important in characterizing IR and IS receivers in a Random Forest (**D**). Stars indicate significant results of *t-tests* (* P<0.05; ** P<0.01; *** P<0.001; ns = not significant).

DISCUSSION

Our results indicate that plant genotypes vary in traits associated with plant-plant interactions - specifically, traits associated with both the *emission* and *perception* of VOC cues. The emitter effect suggests that receivers respond more strongly to some emitter genotypes than others, independent of their own genotype. Similarly, the receiver effect suggests that some genotypes respond more strongly to VOC cues in their environment than other genotypes, regardless of emitter genotype. And the lack of an overall self-recognition effect suggests that natural selection may act independently on traits associated with VOC emission and perception.

Across the 25 emitter-receiver combinations screened in this study, we did not find strong evidence that receivers generally responded most strongly to emitters of their same genotype. These findings are surprising since the other major set of studies demonstrating genotypic variation in plant communication, conducted in sagebrush (*Artemisia tridentata*), are most consistent with the self-recognition hypothesis (Karban and Shiojiri 2009, Karban et al. 2013, Karban et al. 2014a); however, it is important to note that these studies did not explicitly test for emitter or receiver effects.

Although we did not find overall self-recognition effects in our herbivore feeding assays, some individual genotypes (14R and 8A2) seem to respond stronger to their own VOC emission than to that of other genotypes (**Figure 4.2**). Thus, it is possible that some genotypes may have stronger self-recognition responses than others, but that generally, other traits associated with VOC emission and perception are more important in affecting plant-plant interactions in these experiments and the *S. altissima* system. In the future, it would be well-worth looking into the relatedness between the genotypes utilized in these experiments to see if there are any relatedness thresholds associated with the directions and strengths of the responses we observed

in these studies.

Another major outcome of these experiments is the finding that plant-plant interactions may induce resistance or susceptibility to subsequent herbivores, depending on the genotypes of the emitter and receiver. In general, both effects (IR, IS) have been documented across the range of species in which plant-plant interactions have been demonstrated. For instance, a recent meta-analysis of the 48 reported observations of plant communication to date (spanning ~35 plant species) found that 39 demonstrated induced resistance, eight showed induced susceptibility, and only one measured no response (Karban et al. 2014b). Similarly, of 164 reported cases of associational effects between plants of different species, the majority of cases reported associational resistance as opposed to susceptibility (Barbosa et al. 2009). Our results agree with these meta-analyses – that induced resistance and susceptibility are both possible outcomes of plant communication – but suggest that induced susceptibility and non-responses may be more common than has been previously reported. Of the 25 emitter-receiver combinations tested in this study, 9 showed induced resistance, 4 showed induced susceptibility, and 12 showed no response (as measured by *T. virgata* feeding levels; **Figure 4.2**).

It is noteworthy that *individual genotypes* can exhibit all three of these phenotypes, with the particular outcome depending on the genotype of the emitter providing VOC cues in its environment. For example, while we found that in general, receivers responded to the 8A2 emitter by inducing a phenotype that was resistant to *Trirhabda virgata*, but susceptible to *Paria thoracica* (same phenotype, different perceived quality to herbivores; Figure 4.3), the range of ecological outcomes varied with receiver genotype – receivers 8A2, 6R, and 8A2 responded to the 8A2 emitter by inducing resistance, the 3D4 emitter responded to the 8A2 emitter by inducing susceptibility, and the 9R emitter did not respond to the 8A2 emitter (Figure 4.2). This

suggests that plant-plant interactions have outcomes that are far more context-dependent than previously portrayed in the literature.

Finally, characterizing the VOC chemotypes and leaf chemical profiles of emitters and receivers that induce resistance versus susceptibility revealed considerable compositional differences that may have driven the strong herbivore bioassay results observed in these experiments. Specifically, characterizing the VOC phenotypes of the emitter genotypes in these experiments revealed that the VOC chemotypes that induce resistance in receivers are compositionally different from the chemotypes that induce susceptibility. Specifically, the enhanced emission of just three compounds, two sesquiterpenes and one monoterpene, is statistically sufficient to characterize the chemotypes of IS emitters, although the emission rates of other compounds in the VOC blend changes as well. It is also noteworthy that the VOC phenotype of emitters inducing IR was collectively much more variable than the IS phenotype, although given our sample size of one IS emitter genotype, additional experiments with multiple genotypes that IR and IS would be necessary to test whether this trend holds true across a larger number of emitter genotypes. However, our results suggest that inducing resistance versus susceptibility may be associated with particular compounds, compound classes, or specific chemical characteristics of the eliciting compounds.

Along a similar vein, the leaf DTA profiles of IR receivers are compositionally different from the profiles of IS receivers. Like the VOC phenotypes, IR receivers also exhibit leaf DTA profiles that are substantially more variable than the profiles of IS receivers (though a more extensive sampling of receiver genotypes that induce susceptibility to *T. virgata* herbivory would be necessary to conclusively test this observation). However, unlike the VOC profiles, some individual DTAs were induced and others were suppressed. In light of the fact that individual

DTAs are known to exhibit a wide range of anti-herbivore functions, ranging from deterrents and anti-digestive agents to toxins, that are not necessarily equivalent in the type and magnitude of their effects on insect herbivores (Langenheim 1994), it would be interesting to identify the modes of action of specific DTAs to more fully characterize the leaf DTA phenotypes of IR versus IS receiver genotypes. It is also possible that insect genotypes and species may exhibit variation in their abilities to resist particular classes of DTAs, suggesting that these may also be important cues driving the IR/IS leaf chemical phenotypes and resistance patterns we observed in these experiments. It is well worth testing the hypothesis that there are multiple leaf chemical phenotypes that are ecologically equivalent (either equally resistant or susceptible) to herbivores (see Uesugi et al. 2013).

Furthermore, in addition to changes in levels of individual DTAs that may have specific resistance-mediating functions and overall compositional differences between the DTA profiles of IR and IS receivers, we also observed significant induced changes in the dissimilarity of the leaf DTA profiles of IR versus IS receivers. This overall induced dissimilarity combined with the high phenotypic variability of IR receivers may also be a meaningful part of *S. altissima*'s defense strategy since it is more difficult, physiologically, for feeding insects to adapt to changing defense phenotypes than to static ones ("moving-target hypothesis"; Adler and Karban 1994, Karban 2011).

Overall, our findings support a burgeoning field of literature suggesting that plants induce responses to feeding herbivores as well as to VOC cues in their environments from neighboring damaged plants. However, as our data reveal, genotypic variation in VOC emission and perception suggests that plants can respond to the same information in *diverse* ways: they may induce resistance, susceptibility or not respond. Moreover, plants' induced responses to VOC

cues in their environments are *specific* – (1) the same induced phenotype that resists one herbivore may induce susceptibility to another (as we saw with *Trirhabda virgata* and *Paria thoracica*), and (2) the same genotype may respond differently to VOCs from *Trirhabda virgata*-damaged emitters, depending on which genotype is emitting the VOC cues.

These results shed new light on the functionality and ecological impact of inducibility in plant communities. At its most basic level, herbivore-inducible responses not only alter the frequency and distribution of defense phenotypes in plant populations, but also alter the information available in the headspace through changes in VOC emission. This information is available to all organisms in the neighborhood and, as this study reveals, result in differential effects in neighboring plants. This, in turn, can alter the movement and host-searching behavior of natural enemies (e.g. Bruin et al. 1992, Choh et al. 2004) and herbivores (Morrell and Kessler *in review*, Rubin et al. *in press*, Choh et al. 2013) – thereby affecting community composition and structure (Underwood et al. 2014). It is noteworthy that this was also an unforeseen outcome of our experiment at Whipple Farm – after the treatment application period by *T. virgata* on our emitter plants, we observed a strikingly high abundance of and damage by the leaf-chewing beetle *P. thoracica*. This was especially noteworthy given past observations that the abundances of *P. thoracica* and *T. virgata* were negatively correlated in multi-year community surveys of old-field communities (Messina and Root 1980). In tandem, these results suggest that plant-plant interactions as well as herbivore-induced VOC emission maintain variation in information and plant defense phenotypes in plant populations – driving a diverse and constantly changing suite of interactions between plants, neighboring plants, and the herbivore community (Karban 2011).

In the future, it is well worth testing the importance of plant-plant interactions – both their strength and context-dependency – in affecting the spatial structure and selective pressures

on plant neighborhoods. In this series of experiments, we controlled for plant neighborhood – every emitter plant was surrounded by an equally diverse neighborhood of five plants, one from each of five genotypes; however, in natural populations, this need not be the case. In light of the diverse receiver phenotypes that we observed, it is worth testing the hypothesis that particular assemblages of emitter and receiver genotypes may be selected for under certain conditions that depend on nutrient availability and herbivore pressure, among other conditions. This would involve mapping the results of experiments like this one, which screen resistance responses across multiple combinations of emitter and receiver genotypes, onto the spatial distribution of genotypes within plant populations to ask: (1) on average, do emitters stand next to receivers in which they induce resistance, susceptibility, or no response? (2) In emitter-receiver combinations in which receivers induce susceptibility, how is end-of-season plant fitness affected? And (3) how does the frequency of emitter and receiver phenotypes that induce resistance versus susceptibility change with ecological context in which the information is transferred? Addressing all of these questions will greatly advance our understanding of the ecological outcomes and evolutionary trajectory of plant-plant interactions, as well as our understanding of the structure and organization of ecological communities.

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APPENDIX

FIGURE S3.1 ANOVAs of individual leaf diterpene acids induced by herbivory (Trt) and/or exposure regime (Exp) yielded 14 individual DTAs that were significantly induced by our treatments. White bars represent controls, black bars represent *Dichomeris* herbivory, and gray bars indicate *Trirhabda* herbivory; additionally, solid bars represent emitters and hatched bars represent receivers. Effect type (Exp, Trt) and significance statistics (* $P<0.05$, ** $P<0.01$, *** $P<0.001$) are listed above each set of bars (mean±SEM).

FIGURE S3.1

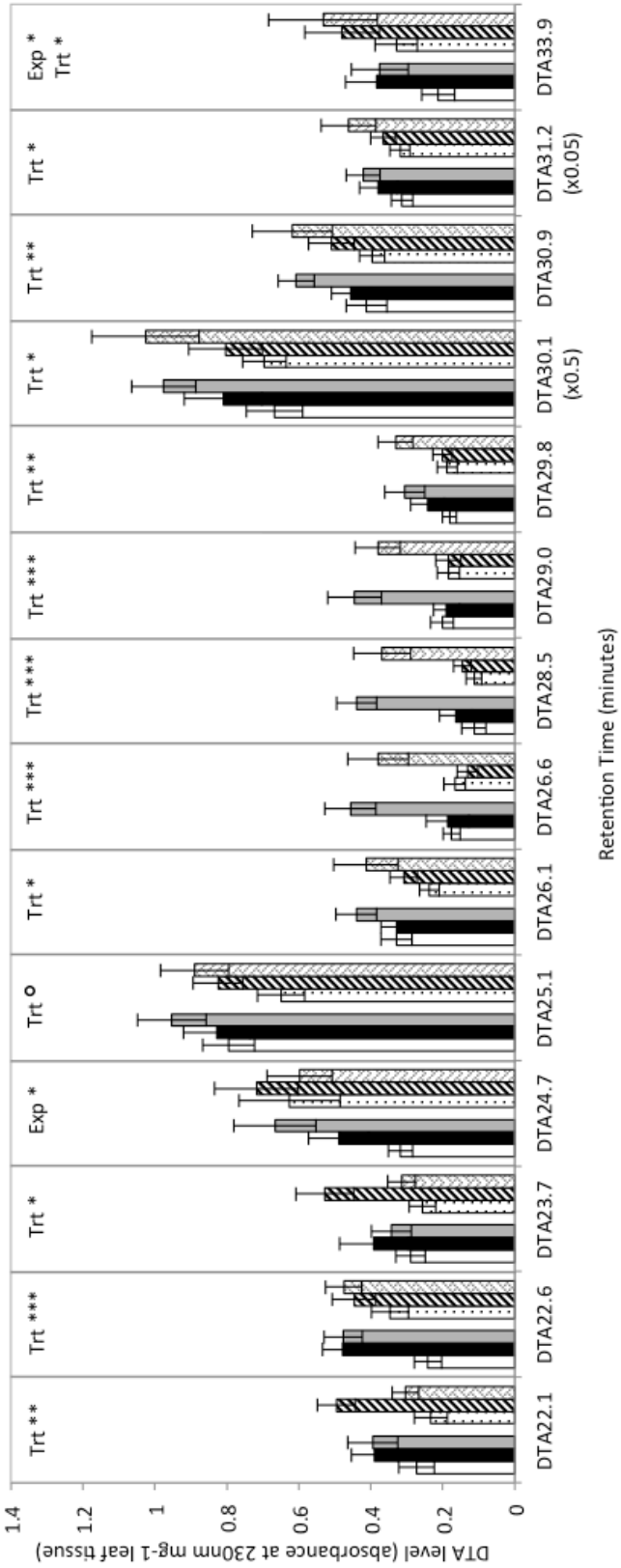
DTA level (absorbance at 230nm mg-1 leaf tissue)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Retention Time (minutes)
Trt **
Trt ***
Trt *
Exp *
Trt °
Trt *
Trt ***
Trt ***
Trt ***
Trt **
Trt *
Trt **
Trt *
Exp *
Trt *
DTA22.1
DTA22.6
DTA23.7
DTA24.7
DTA25.1
DTA26.1
DTA26.6
DTA28.5
DTA29.0
DTA29.8
DTA30.1 (x0.5)
DTA30.9
DTA31.2 (x0.05)
DTA33.9

TABLE S3.1 The results of Random Forest models designed to test which individual VOCs best characterize the differences between herbivore treatments. The table shows compound number (1-14), VOC identification (or Kovat's Index, KI), and compound class (mt=monoterpene, sqt=sesquiterpene), as well as the proportion of bootstrap iterations in which each compound was a significant predictor. Statistics indicating the overall fit of each model (which is considered good when the prediction, bootstrap and resubstitution errors are all less than the error rate at random) are also reported.

Compound #	Compound ID or KI (DB-5)	Compound Class	Proportion of Models	Error Type	Error Rate
Random Forest Model 1: Control versus *Trirhabda*-damaged *S. altissima*					
2	KI=1064	sqt	11	Prediction Error:	0.08
3	cedrene	sqt	13	Bootstrap Error:	0.11
5	KI=1212	sqt	20	Classification Error:	0.00
6	KI=1213	sqt	10		
7	KI=1223	sqt	19	Error Rate at Random:	0.43
9	KI=1250	sqt	12		
10	KI=1268	sqt	14		
12	KI=1279	sqt	14		
14	Z,E-farnesene	sqt	10		
Random Forest Model 2: Control versus *Dichomeris*-damaged *S. altissima*					
1	KI=1051	mt	24	Prediction Error:	0.09
3	cedrene	sqt	16	Bootstrap Error:	0.13
4	KI=1202	sqt	19	Classification Error:	0.00
5	KI=1212	sqt	16		
6	KI=1213	sqt	16	Error Rate at Random:	0.43
7	KI=1223	sqt	21		
8	α-gurjunene	sqt	15		
9	KI=1250	sqt	15		
10	KI=1268	sqt	15		
11	KI=1273	sqt	10		
12	KI=1279	sqt	20		
13	KI=1291	sqt	13		
14	Z,E-farnesene	sqt	16		
Random Forest Model 3: *Dichomeris*- versus *Trirhabda*-damaged *S. altissima*					
6	KI=1213	sqt	82	Prediction Error:	0.20
12	KI=1279	sqt	95	Bootstrap Error:	0.26
				Classification Error:	0.00
				Error Rate at Random:	0.50

TABLE S3.2 A summary of the 56 VOC compounds emitted by *S. altissima* (±SEM), organized by compound class, for each of the herbivore treatments employed in these experiments. Statistics on ANOVAs of the total emission of VOCs (or particular classes of VOCs) are shown for each category, and ANOVAs of individual VOC compounds, are shown with their levels of significance (* $P<0.05$, ** $P<0.01$, *** $P < 0.001$).

TABLE S3.2:

Kovats Index	Retention Time (min)	Compound Identification or m/z Fragments (% of BP)	Undamaged control VOC emission ±SEM	*Dichomeris leuconotella* VOC emission ±SEM	*Trirhabda virgata* VOC emission ±SEM	ANOVA F Statistic (P value)
Green Leafy Volatiles						
908	9.873	67(BP), 82(49)	1.42 ± 0.51	1.31 ± 0.50	1.45 ± 0.14	-
967	11.924	67(BP), 82(32)	0.018 ± 0.002	0.023 ± 0.011	0.011 ± 0.003	-
971	12.070	z3-hexenyl acetate	14.36 ± 1.50	15.73 ± 5.78	12.22 ± 1.46	-
997	13.083	z3-hexenol	0.52 ± 0.10	0.85 ± 0.39	0.55 ± 0.09	-
1040	14.328	67(BP), 82(30)	0.044 ± 0.009	0.038 ± 0.012	0.031 ± 0.005	-
1047	14.539	67(BP), 82(40)	0.049 ± 0.006	0.035 ± 0.004	0.046 ± 0.007	-
1054	14.745	z3-hexenyl isovalerate	0.12 ± 0.02	0.16 ± 0.02	0.19 ± 0.05	-
1238	17.352	83(BP), 67(73), 59(63), 82(43)	0.018 ± 0.005	0.039 ± 0.008	0.048 ± 0.018	-
1241	17.445	67(BP), 82(42)	0.071 ± 0.005	0.048 ± 0.014	0.090 ± 0.023	-
1620	23.598	67(BP), 81(41)	0.049 ± 0.008	0.038 ± 0.016	0.039 ± 0.005	-
Monoterpenes						
<900	9.136	β-myrcene	1.32 ± 0.32	1.24 ± 0.43	1.56 ± 0.20	-
908	9.874	limonene	1.24 ± 0.40	1.13 ± 0.43	1.26 ± 0.13	-
914	10.061	ocimene	0.018 ± 0.007	0.011 ± 0.004	0.014 ± 0.006	-
957	11.542	93(BP), 121(93), 91(74), 136(73)	0.071 ± 0.033	0.047 ± 0.016	0.055 ± 0.012	-
1031	14.067	59(BP), 93(71), 94(58), 175(49)	0.003 ± 0.000	0.002 ± 0.001	0.004 ± 0.001	-
1045	14.464	94(BP), 93(87)	0.002 ± 0.001	0.001 ± 0.001	0.002 ± 0.001	-
1051	14.650	121(BP), 93(76), 91(42)	0.030 ± 0.013	0.040 ± 0.016	0.12 ± 0.02	**15.44 (0.002) ****
1078	15.478	linalool	0.066 ± 0.007	0.035 ± 0.009	0.098 ± 0.021	**6.68 (0.02) ***
1100	16.163	95(BP), 93(57), 121(56), 136(48)	0.15 ± 0.06	0.096 ± 0.041	0.081 ± 0.011	-
Sesquiterpenes						
1064	15.045	105(BP), 161(99), 119(80), 85(60), 91(45)	0.012 ± 0.004	0.010 ± 0.002	0.022 ± 0.004	**4.86 (0.04) ***
1085	15.676	cedrene	0.091 ± 0.035	0.077 ± 0.020	0.17 ± 0.02	**6.86 (0.02) ***
1202	16.244	161(BP), 89(74), 105(67), 133(66), 120(66)	0.18 ± 0.06	0.15 ± 0.05	0.37 ± 0.03	**10.04 (0.007) ****
1207	16.394	133(BP), 91(84), 119(63), 105(58), 161(43)	0.008 ± 0.005	0.008 ± 0.005	0.026 ± 0.004	**6.62 (0.02) ***
1212	16.528	161(BP), 91(76), 105(46)	0.13 ± 0.05	0.12 ± 0.03	0.30 ± 0.02	**11.79 (0.004) ****
1213	16.581	133(BP), 91(66), 93(46), 105(40), 119(32)	0.062 ± 0.019	0.051 ± 0.013	0.13 ± 0.01	**14.32 (0.002) ****
1223	16.866	89(BP), 161(88), 133(86), 87(77), 97(69)	0.040 ± 0.018	0.038 ± 0.008	0.071 ± 0.006	**5.32 (0.03) ***
1232	17.147	α-humulene	0.003 ± 0.001	0.003 ± 0.000	0.006 ± 0.001	**7.31 (0.02) ***
1235	17.248	E,E-farnesene	0.033 ± 0.014	0.030 ± 0.010	0.080 ± 0.009	**9.23 (0.008) ****
1246	17.614	α-gurjunene	0.036 ± 0.019	0.031 ± 0.011	0.090 ± 0.013	**8.17 (0.01) ***
1250	17.750	161(BP), 105(34), 133(30)	0.13 ± 0.06	0.12 ± 0.04	0.28 ± 0.04	**6.95 (0.02) ***
1268	18.335	161(BP), 105(64), 91(56), 119(46), 133(34)	3.38 ± 1.33	3.09 ± 0.92	7.82 ± 0.93	**9.63 (0.007) ****
1271	18.450	161(BP), 133(49), 105(47), 89(45), 91(41)	0.036 ± 0.010	0.033 ± 0.012	0.069 ± 0.017	-
1273	18.514	133(BP), 91(82), 105(80), 85(77), 93(74)	0.013 ± 0.006	0.016 ± 0.005	0.025 ± 0.005	-
1279	18.737	91(BP), 133(96), 69(88), 161(82), 105(67)	0.042 ± 0.017	0.029 ± 0.009	0.11 ± 0.01	**16.00 (0.002) ****
1289	19.086	91(BP), 133(58)	0.038 ± 0.014	0.037 ± 0.010	0.064 ± 0.009	-
1291	19.151	161(BP), 119(66), 204(61), 105(51), 91(38)	0.13 ± 0.05	0.16 ± 0.06	0.25 ± 0.05	-
1407	19.690	161(BP), 91(36), 105(36), 59(30), 119(30)	0.026 ± 0.007	0.024 ± 0.008	0.043 ± 0.007	-
1475	22.079	Z,E-farnesene	0.005 ± 0.004	0.009 ± 0.008	0.045 ± 0.009	**10.42 (0.006) ****
1487	22.542	91(BP), 105(32), 119(20), 133(15)	0.008 ± 0.005	0.001 ± 0.001	0.008 ± 0.002	-
1620	23.598	nerolidol	0.070 ± 0.009	0.057 ± 0.024	0.058 ± 0.007	-
Aromatic Compounds						
922	10.334	105(BP)	0.008 ± 0.010	0.005 ± 0.006	0.011 ± 0.008	-
953	11.413	105(BP), 120(46)	0.003 ± 0.004	0.001 ± 0.001	0.002 ± 0.001	-
1289	19.092	methyl salicylate	0.16 ± 0.02	0.042 ± 0.010	0.067 ± 0.024	**13.21 (0.003) ****
1673	25.127	4-ethoxy-ethylbenzoate	0.038 ± 0.012	0.019 ± 0.003	0.025 ± 0.003	4.22 (0.06)
Aliphatic Compounds						
<900	7.508	71(BP), 57(84), 85(68), 70(58)	0.027 ± 0.018	0.046 ± 0.052	0.031 ± 0.012	-
918	10.210	57(BP), 71(61), 89(56)	0.013 ± 0.016	0.036 ± 0.042	0.017 ± 0.012	-
945	11.124	57(BP), 71(74), 85(49)	0.018 ± 0.009	0.021 ± 0.022	0.014 ± 0.006	-
949	11.262	57(BP), 71(97), 84(85), 85(42)	0.012 ± 0.006	0.016 ± 0.013	0.016 ± 0.003	-
1063	15.024	57(BP), 71(80), 85(50)	0.008 ± 0.007	0.003 ± 0.003	0.003 ± 0.004	-
1411	19.824	2,4-decadienal	0.24 ± 0.14	0.069 ± 0.004	0.13 ± 0.03	-
1478	22.190	57(BP), 85(44)	not detectable	0.019 ± 0.022	0.11 ± 0.09	-
Uncategorized Unknown Compounds						
949	11.264	119(BP), 91(40)	0.034 ± 0.019	0.039 ± 0.015	0.035 ± 0.013	-
969	12.004	69(BP)	0.19 ± 0.04	0.12 ± 0.02	0.22 ± 0.03	3.75 (0.07)
1032	14.114	91(BP), 119(56), 134(40)	0.008 ± 0.004	0.005 ± 0.003	0.007 ± 0.003	-
1256	17.947	95(BP), 57(73), 133(55)	0.047 ± 0.010	0.040 ± 0.003	0.069 ± 0.011	**4.78 (0.04) ***
1418	20.063	137(BP), 152(30)	0.025 ± 0.008	0.021 ± 0.008	0.017 ± 0.002	-

TABLE S4.1: Three individual volatile compounds characterize the differences between emitter genotypes that induce resistance versus susceptibility in receivers - their retention times, Kovats indices, emission rates (±SEM) in control and *Trirhabda*-damaged emitters, the results of *T-tests* between emitters that induce resistance versus susceptibility, and the error rates associated with Random Forest models.

					VOC Emission ± SEM				
					IR Emitters		IS Emitters		
Retention Time (min)	Kovats Index	% Random Forest Models	Major m/z fragments (% of BP)	Class	Control	*Trirhabda*-damaged	Control	*Trirhabda*-damaged	*T-test* Results (damaged only)
14.614	1043	53	91(BP), 161(92), 105(75), 119(33), 133(18)	sqt	0.011 ± 0.002	0.030 ± 0.008	0.034 ± 0.002	0.16 ± 0.03	df=2.25, t=-1.47, P=0.04 *
14.621	1043	47	121(BP), 93(64), 136(27)	mt	0.021 ± 0.003	0.053 ± 0.012	0.049 ± 0.007	0.27 ± 0.05	df=2.31, t=-4.44, P=0.04 *
19.757	1403	100	161(BP), 91(36), 105(36), 119(27), 133(18)	sqt	0.012 ± 0.005	0.018 ± 0.005	0.055 ± 0.029	0.101 ± 0.001	df=5.93, t=-11.92, P<0.0001 ***
Prediction Error:		0.16							
Bootstrap Error:		0.19							
Resubstitution Error:		0.00							
Error Rate at Random:		0.19							

CURRICULUM VITAE

Education

2015 **PhD Cornell University, Ecology & Evolutionary Biology**
Thesis: Volatiles, plant communication and insect herbivory: lessons from old-field communities that inform community ecology
Committee: Drs. André Kessler*, Anurag Agrawal, Robert Raguso, and Jennifer Thaler

2009 **BA Carleton College, Biology**
Graduated *magna cum laude*, with distinction on my senior thesis.
Thesis: The role of JA-SA crosstalk and coronatine in allowing *Pseudomonas syringae* to successfully infect tomato plants.

Peer-reviewed Publications

2015 Rubin, I., S. Ellner, A. Kessler and **K. Morrell**. Informed herbivore movement and plant-plant communication determine the effects of induced plant resistance in an individual-based model. *Journal of Animal Ecology*. DOI: 10.1111/1365-2656.12369.

2014 **Morrell, K.** and A. Kessler. 2014. The scent of danger: Volatile-mediated information transfer and defense priming in plants. *The Biochemist* 36(5): 26-32.

2010 Kessler, A. & **K. Morrell**. Plant Volatile Signalling: Multitrophic Interactions in the Headspace. *In* The Chemistry and Biology of Volatiles. Ed. A. Herrmann. Wiley: Chichester, 95-122.

Awards and Honors

2014 Sigma Xi "Chapter of Excellence Award" to the Cornell Chapter (2nd year in a row)
2013 Sigma Xi "Chapter of Excellence Award" to the Cornell Chapter
Winner of the 2nd Annual Grilled Cheese Competition at Cornell University
2011 First place poster; 14th Symposium in Insect-Plant Interactions (SIP 14)
2009 Received Distinction on senior thesis, Carleton College
Nominated for Phi Beta Kappa and Sigma Xi, Carleton College
2006 Best independent research project, MBL Semester in Environmental Science

Conference Poster Presentations

2014 **K. Morrell**, K. Poveda, Z. Khan, C. Midega and A. Kessler. Exploring physiological mechanisms and ecological consequences of genotypic diversity in

VOC emission: A case study using land races of *Sorghum bicolor*. *In the* Gordon Research Seminar and Conference on Plant Volatiles, Ventura Beach, California.

2013 **K. Morrell**. Plant-to-plant signaling: Can *Solidago altissima* receive specific information from its damaged neighbors? *In the* Gordon Research Conference (GRC) on Plant-Herbivore Interactions, Ventura Beach, California.

2011 **K. Morrell** and A. Kessler. 2011. Plant-plant interactions influence the spatial dynamics of an herbivore population. *In the* 14th Symposium in Insect-Plant Interactions (SIP 14), Wageningen, Netherlands. ***Received first place poster prize.***

2009 A. Kessler, **K. Morrell**, K. Poveda and R. D. Johnson III. 2009. Behavioral Ecology of Tall Goldenrod, *Solidago altissima*. *In* Cornell University-Pennsylvania State University Mini-symposium, Ithaca, NY.

Fellowship Support

2011-2014 National Science Foundation Graduate Research Fellowship ($30,000/year)
2009-2010 Presidential Life Sciences Fellowship ($30,000/year)

Research Grants

2013 NSF Doctoral Dissertation Improvement Grant ($20,000)
Cornell University Conference Travel Grant ($440)
2012 Sigma Xi Grant-in-Aid-of-Research ($400)
2011 Cornell University Research Travel Grant ($675)
Cornell University Conference Travel Grant ($675)
Biogeochemistry and Environmental Biocomplexity Small Grant ($3000)
2010 Paul Feeny Grant for Graduate Summer Research ($1000)
Orenstein Foundation Travel Grant ($750)
Organization for Tropical Studies' Ruggles Scholarship ($1000)

Research Talks

2014 "Plant-to-plant signaling: Does genotype matter?" *A seminar for the* Plant Interactions Group, a discussion-based course at Cornell University.

2013 "Plant-to-plant signaling: Emitter strength governs *Sorghum bicolor*'s responsiveness to neighboring conspecifics." *A talk for the* Sigma Xi Student Research Symposium.

"Plant-to-plant signaling: Exploring genotypic differences in volatile emission and the effects these differences have on a major herbivore of *Sorghum bicolor*." *A talk for* Cornell University's 38th Annual Ecology & Evolutionary Biology Symposium.

2012 "Specificity in plant-to-plant signaling?" *A seminar for the* Plant Interactions Group, a discussion-based course at Cornell University.

"Plant-to-plant signaling: Emitter strength governs *Sorghum bicolor*'s responsiveness to neighboring conspecifics." *A talk at the* Cary Institute for Ecosystem Ecology.

"Screening direct and indirect defenses, and plant-to-plant signaling across landraces of *Sorghum bicolor*." *A seminar for the* Plant Interactions Group at Cornell University.

2011 "Plant-to-plant signaling mediates localized associational resistance in *Solidago altissima*. *In* Cornell University's 36th Annual Ecology & Evolutionary Biology Symposium.

"Does plant-to-plant signaling influence herbivore spatial dynamics?" *A seminar for the* Plant Interactions Group, a discussion-based course at Cornell University.

"Plant induced defenses: Keeping a major herbivore on the move?" *In* Cornell University's 35th Annual Ecology and Evolutionary Biology Symposium.

2010 **"**Plant-plant communication in *Solidago altissima* and its ecological consequences." *A seminar for the* Plant Interactions Group at Cornell University.

"How *Solidago altissima* keeps a major herbivore on the run." *In the* Biogeochemistry and Environmental Biocomplexity Mini-symposium, Cornell University.

"Project Proposal: Plant-plant communication in *Solidago altissima*." *In* Cornell University's 34th Annual Ecology and Evolutionary Biology Symposium.

College Teaching Experiences

2015	BioEE1610: Ecology & the Environment, Cornell University
2014	BioEE7670: Current Topics in Evolutionary Biology, Cornell University
2010-2011	BioG1105-1106: Introductory Biology TA, Cornell University
2009	Bio 244: Genetics lab TA, Carleton College
2009	Bio 125: Genes, Evolution and Development TA, Carleton College
2008	Bio 126: Energy Flow in Biological Systems TA, Carleton College
2007	Chem 233: Introduction to Organic Chemistry lab TA, Carleton College

Research Experiences

2012 **International Center of Insect Physiology and Ecology, Kenya**
Project: Screening plant defense traits across *Sorghum bicolor* land races to broaden the push-pull system. Collaborators: Drs. Zeyaur Khan, André Kessler, Katja Poveda, Charles Midega

2008, 2009 **Summer REU with Protein Sciences Division, Monsanto Company**
Project: Designing & synthesizing genes to increase drought-tolerance of *Zea mays*. Advisors: Drs. Sonya Franklin, Jeffrey Seale, Fred Moshiri

2008, 2009 **Research assistant, Carleton College**
Project: Sequencing pigmentation genes in plateau lizards
Advisor: Dr. Matthew Rand

2007 **Research assistant, Carleton College**
Project: Monitoring expression of flowering genes in *Chamaecrista*
Advisor: Dr. Susan Singer

2006, 2007 **Summer REU with Donald Danforth Plant Science Center**
Project: Designing and expressing novel miRNA in *Arabidopsis thaliana*
Advisors: Drs. Christopher Taylor and Brad Barbazuk

2006 **Research assistant, Carleton College**
Project: Identification of SNPs in indigo snakes
Advisor: Dr. Stephan Zweifel

Workshops & Ecology Field Courses

2013 *Cornell University*: Environmental Policy Processes in Washington DC
2011 *Cornell University*: Field Ecology in Hawai'i
2010 *Organization for Tropical Studies*: Tropical Biology – An Ecological Approach
2008 *Carleton College*: Coastal Marine Ecology & Environmental Science in Australia
2006 *Marine Biological Laboratory*: Semester in Environmental Science
***** Received recognition for best independent research project

Leadership and Service

K-12 Science Outreach:

Coordinator, Expanding Your Horizons in Math & Science Conference
2012-2015 Fundraised $25,000/year for the EYH 2013, 2014, and 2015 conferences
2013 Organized and coordinated mini-conference at Lansing Correctional Facility
2012-2014 "Buddy" for young women at the conference
2011 Event Photographer

Volunteer, Interactive Science Demonstrations
2009-2013 Organizer, Presenter at a science booth at Ithaca's harvest festival (Science from the Slope)
2010-2014 Led interactive science demos at the Entomology Dept Open House (Insectapalooza)

Volunteer Teacher, GrassHOPR
2014 "Honeybee jives & flower fortresses: Inside the world of plant and animal communication;" an 8-week course for 2nd graders at South Hill Elementary School.

Cornell Graduate and Undergraduate Student Outreach:

Activities Coordinator, Sigma Xi: The Science Research Society (Cornell Chapter)

2012-2015 Organize invited lectures and mini-symposia
Coordinate science outreach for the general public
Organize and lead professional development workshops
* Recognized as a "Chapter of Excellence" in 2013

Organizer, Professional Development Workshops

2011-2013 Science Communication
2011-2013 Effective CV Writing
2011 Writing and Reviewing Small Grants
2011 How to Sell Your Science

Mentor Undergraduates

2009-2014 Mentored 6 Cornell, and 1 Swarthmore College undergraduates in research in the Kessler lab
2010-2012 Mentored 2 Cornell undergraduates in science career preparation (EnviroMentors Program)
2013 Served on a panel for an undergraduate seminar on preparing for graduate school
2012 Judged student posters at a spring research symposium (Cornell Undergraduate Research Board)

Event organizer; Graduate Student Organizations

2013 E&EB Beginning-of-year welcome picnic
2011, 2012 E&EB End-of-year gathering
2011 Biogeochemistry & Environmental Biocomplexity Fall Retreat
2010 Dinner for first-year graduate students
2009 Pizza lunch for graduate students to meet with invited seminar speakers

Professional Activities

Member: Sigma Xi (Cornell Chapter), American Association for the Advancement of Science (AAAS), Phi Beta Kappa, Mortar Board.
Reviewer: *Ecological Entomology; Proceedings of the Royal Academy of Sciences – B.*