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The Role of VOCs in Within-Plant Signaling and the Role of VOCs and Plant-Based Resources in Tritrophic Interactions in *Datura wrightii*

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ABSTRACT OF THE DISSERTATION

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by

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When damaged by herbivores, plants often produce and emit a suite of volatile organic compounds (VOCs). Research has shown a variety of functions for these VOCs in defense against herbivores across many plant species. In Chapter One, I outline the characteristics and the induction of VOCs. I then discuss the two main functions of VOCs, its role in within-plant signaling and its concurrent effect with plant-based resources on the predation and the colonization of an omnivorous predator.

In Chapter Two, I examined the role of VOCs as within-plant signals in *Datura wrightii* (Solanaceae) following damage by the herbivorous beetle, *Lema daturaphila* (Coleoptera: Chrysomelidae). Experimentally, I expose (receiver) leaves to VOCs emitted by a damaged (emitter) leaf on the same plant. I then applied herbivore damage to receivers and measured the subsequent VOC production from each leaf. I analyzed the VOCs produced by receivers based on exposure to VOCs from the emitter, and the amount of vascular connectivity that each receiver had with the emitter. I found that VOC exposure increased subsequent emissions in receivers in general; and particularly in receivers having no direct vascular connection to the emitter. These findings indicate the importance of VOCs as an airborne damage signal in *D. wrightii* in response to herbivory.

In Chapters Three and Four, I present my research on the concurrent effect of VOCs and plant-based resources on the predation of *L. daturaphila* and the colonization of *D. wrightii* by the omnivorous predator, *Geocoris pallens* (Hemiptera: Geocoridae). Through field and greenhouse experiments, I manipulated the presence of VOCs and plant-based resources at the host plant, *D. wrightii*. I then measured the effects of these manipulations on the rate of predation and the density of *G. pallens*. Both attractants positively increased the density of *G. pallens*, but plant-based resources strongly decreased predation of *L. daturaphila* by *G. pallens*. These findings indicate that different types of plant attractants may produce independent effects on predation vs. colonization in omnivores. Together, the research presented in this dissertation illustrate that VOCs emitted by *D. wrightii* play an integral part of the overall response to herbivory.

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CHAPTER ONE: A GENERAL INTRODUCTION ON THE ROLE OF VOLATILE ORGANIC COMPOUNDS IN WITHIN-PLANT COMMUNICATION AND THE ROLE OF VOLATILE ORGANIC COMPOUNDS AND PLANT-BASED RESOURCES IN TRITROPHIC INTERACTIONS

ABSTRACT

I review the induction, production, and emission characteristics of volatile organic compounds (VOCs) produced by plants following damage by herbivores and how these compounds may vary based on different types of inducers and ecological factors. I then introduce and discuss the two important functions of VOCs in the overall plant response to herbivory that I investigated for my dissertation. First, I review the role of VOCs in within-plant communication, the topic of chapter two. Secondly, I discuss the role of VOCs as a type of resource cue for omnivore predators in tritrophic interactions, and how their effects on predators may be influenced by the presence of plant-based resources, the topic of chapters three and four. Finally I present and discuss my dissertation objectives and how they address outstanding issues in the research field.

BACKGROUND

Volatile organic compounds: When under attack from herbivores, plants employee many tactics in order to defend against or resist further damage by insects. Physiologically, trichomes, bark, resin, as well as toxic secondary compounds serve to directly deter herbivory. More interestingly, aside from these direct lines of defense, many plant species are also able to produce and emit a suite of volatile organic compounds (VOCs) following insect damage. It has been suggested that this type induced responsive may be a cost-saving method of producing and deploying plant defenses in comparison to the constitutive production of defenses (Baldwin, 1998; Heil & Baldwin, 2002; Karban & Maron, 2002). The discovery of these VOCs has led to a tremendous amount of research regarding their induction, production, emission characteristics, and more importantly, their function in overall plant defense against herbivory.

In addition to herbivores, that induce the plant to produce and emit VOCs by damaging and removing plant tissue (Hare, 2007; Girling & Hassall, 2008), many species of plants are also inducible through abiotic mechanical wounding (Reymond *et al.*, 2000; Banchio *et al.*, 2005; Mithofer *et al.*, 2005; Bricchi *et al.*, 2010; Ueda & Matsuda, 2011). For example, leaves that were either damaged via cutting with scissors or perforated with small holes (5 cm) all emitted significantly more VOCs compared to the control groups (Banchio *et al.*, 2005; Ueda & Matsuda, 2011). Moreover, abiotic factors such as light intensity, extreme temperatures, high or low air humidity, and soil humidity have also been experimentally demonstrated to affect VOC emissions in plants (Gouinguene & Turlings, 2002; Pio *et al.*, 2005). Finally, many researchers have also used plant hormones to artificially induce plants into producing and emitting VOCs. The jasmonate plant hormone family has been widely used with success as artificial inducers, both as jasmonic acid (JA) and its ester, methyl jasmonate (MeJA) (Dicke *et al.*, 1999; Hare, 2007; Moraes *et al.*, 2009). The usage of these hormones has been crucial in many studies on VOCs due to the ability to precisely control the level of induction through measured applications of the hormonal inducers, an otherwise challenging task when administering damage using insect herbivores. In addition, the method of applying plant hormones to induce plants also eliminates potential differences in the amount of remaining leaf tissue following herbivory by insects, which may then confound the variation in VOC emission.

Following induction, the production and emission of VOCs in plants varies greatly with respect to several factors. One source of variation in VOC blends produced by conspecific plants appears to be due to the genetic variation among them. When comparing the VOC blends among six maternal families of Horsenettle (Solanaceae) that were all selfed and outcrossed, significant effects were detected both for inbreeding as well as for family lines. These results showed that inbreeding led to an overall decrease in the quantity of VOC produced and also qualitatively altered the VOC blend produced. Based on these findings, the researchers concluded that genetic variation for VOC production exist in plants and inbreeding depressing might serve to enhance these variation (Delphia *et al.*, 2009). Research on *Datura wrightii* (Solanaceae) also revealed quantitative variation (3.6- to 32- fold total VOCs) in the VOCs emitted among eight genetic lines following induction via herbivory or MeJA (Hare, 2007). Lastly, researchers found that among six varieties of JA-treated rice crop, the total VOC production differed between 2.84 to 3.53 times, and the VOC blends of these six varieties could roughly be divided up into three groups qualitatively (Lou *et al.*, 2006).

Another factor that may affect VOC production and emission is plant ontogeny. Work on *D. wrightii* showed that the VOC blends decreased quantitatively over the growth season of the plant such that VOC emissions were high when the plants were young and growing vegetatively but declined as the plants matured and begin to produce flowers and fruits. Moreover, this effect of ontogeny was separated from a seasonal effect through the partial restoration of VOC emission when older plants were cut back later in the growth season to encourage vegetative growth (Hare, 2010). Lastly, there is also limited evidence to suggest that differences in insect feeding guilds (Rodriguez-Saona *et al.*, 2003), and to a lesser extent, insect species (Agrawal, 2000) may at times lead to differences in the VOC blends emitted by some plant species following herbivory. However, conflicting experimental results also suggest that VOC blends in other plant species to be unaffected by variation in herbivore species and feeding methods (Steidle & van Loon, 2003; Hare & Sun, 2011b).

The role of VOCs as a damage cue in plants: VOCs emitted by herbivore damaged plants have been experimentally shown to convey damage information to other conspecific plants. This type of information transfer was initially discovered by two research groups and has since been shown across a variety of plant species (Baldwin & Schultz, 1983; Rhoades David, 1983; Baldwin *et al.*, 2006; Campos *et al.*, 2008; Arimura *et al.*, 2010; Das *et al.*, 2013). Typically, the exposure to VOCs emitted by damaged plants prepares the exposed plants against herbivory through 1) the upregulation of their own defensive measures preemptively and/or 2) the increased or heightened expression of their own defensive measures following herbivore damage. Finally, these information transfers has also been experimentally observed between a few pairs of plant species grown in close proximity as well (Karban, 2001; Glinwood *et al.*, 2004; Karban *et al.*, 2004; Karban *et al.*, 2014).

These cases of information transfer between plants initially puzzled researchers as the emitter plant in each case may be conferring both a competitive advantage, as well as a fitness advantage, to the eavesdropping receiver plants through the emission of VOCs following herbivory by revealing the presence of herbivores to competitors. In this context, it appears that the emission of VOCs by the herbivore damaged plant may be maladaptive and should therefore be selected against in evolutionary time. Instead, many recent reviewers now suggest that the emission of VOCs by damaged plants may be a form of within-plant communication and that the intended receivers of the damage information conveyed through VOCs are the undamaged parts of the same plant. As support for this theory, reviewers have pointed to a ubiquitous characteristic in most cases of information transfer between and within-plants: the effective range of VOCs to induce receiver plants is often very short (between 10 – 60 cm), and most plant tissue present in this range tend to belong to the emitter plant (Karban *et al.*, 2003; Karban *et al.*, 2006; Heil & Adame-Alvarez, 2010). In addition, the orthostichy patterns in many plant species indicate that certain leaves in those plants may be vascularly disconnected from other leaves on the same plant. One important implication as a result of these vascular limitations is that a vascularly transmitting damage signal may then be unable to reaching parts of the undamaged plant due to these restrictions (Orians, 2005; Heil, 2010). In these cases, an airborne damage signal may be more beneficial as it is able to convey the damage information to undamaged parts of the plant (Heil, 2010).

In summary, VOCs emitted following herbivory may in part function as an airborne damage signal to ready undamaged parts on the same plant of future herbivory. This airborne damage signal may be an important substitute in plants that are vascularly restricted such that the translocating damage signal may not be able to reach all parts of the plant. This role as an airborne damage signal in within-plant communications may in part reinforce the production and emission of VOCs across plants, and the benefit of this signal function may in part offset the cost of releasing herbivore presence information to nearby competitors.

The role of VOCs in tritrophic interactions: Many instances of antagonistic interactions between plants and herbivore insects in nature may also involve interactions with insects from the third trophic tier, such as omnivore predators and parasitoid insects. Typically, these tritrophic interactions between the plant, its herbivore, and the natural enemy of the herbivore results in an overall negative impact on the herbivore, as they are consumed or parasitized by the natural enemy. Conversely, these interactions directly benefit the predator or parasitoid, and may at times also indirectly benefit the plant. VOCs emitted by damaged plants have been shown experimentally to attract some insect predators. These predators may in turn attack suitable herbivores on those plants (Dicke & Baldwin, 2010). Initially, the function of VOCs to attract predators was theorized by some researchers to potentially increase the fitness of plants that emitted VOCs following herbivory (Dicke & Sabelis, 1988; Dicke, 1999). However, few empirical studies have detected the necessary increase in plant fitness to justify the adaptive value of these compounds as a form of indirect defense against herbivory (van der Meijden & Klinkhamer, 2000; Baldwin *et al.*, 2006; Dicke & Baldwin, 2010; Hare, 2011; Karban, 2011; Kessler & Heil, 2011, but see Kessler & Baldwin, 2004). Moreover, recent attempts to corroborate the effect of plant volatile cues on predator behaviors between the laboratory and field environments using identical (or similar) species have at times either failed to attract the same predators in the field as in the laboratory, or other times failed to attract more predators compared to undamaged controls in the field (Bernasconi Ockroy *et al.*, 2001; Hunter, 2002; Hare & Sun, 2011a). In addressing the disparity between theoretical predictions and empirical results, some authors have suggested that the simplification of ecological context in moving from a field environment into the laboratory may alter the way that VOCs are perceived by insect predators, which may in turn elicit artificial predation responses (van der Meijden & Klinkhamer, 2000; Hunter, 2002; Kigathi *et al.*, 2009).

Additional ecological cues – plant-based resources: When Price *et al*. (1980) first formalized the field of tritrophic interactions among plants and arthropods, the authors suggested a diverse suite of ecological factors that could potentially influence the behavior of predatory and parasitic insects. Two categories of plant products were explicitly referred to as natural enemy attractants: volatile chemical cues and nutritional exudates produced by plants. Indeed, omnivory has been shown to be widespread in insects (Agrawal, 2003; Eubanks, 2005; Hunter, 2009), and are well-represented among natural enemies (Coll & Guershon, 2002). So although insects are certainly an important component of the omnivore diet and should therefore be expected to affect the evolution of foraging behaviors in predators, behaviors that enhance the acquisition of plant-based resources are just as likely to be under selection in omnivore (Singer & Bernays, 2003).

Much research work to date has been conducted on the effects of plant-based resources on omnivores. Increases in fecundity and/or survival (Delima & Leigh, 1984; Limburg & Rosenheim, 2001; Begum *et al.*, 2006; Robinson *et al.*, 2008; Lundgren & Seagraves, 2011) as well as in density (McMurtry & Scriven, 1966a; McMurtry & Scriven, 1966b; Cottrell & Yeargan, 1998b; Eubanks & Denno, 2000; Rebek *et al.*, 2005) of predators are often observed when comparing experimental treatment groups with available plant-based resources vs. those without. Not only has the presence of these resources on host plants affected predator survival and density, but similar effects on omnivores have also been detected when the resources were instead on nearby non-host plant species (Naranjo & Stimac, 1985; Cottrell & Yeargan, 1998b; Pumarino *et al.*, 2012). However, experiments examining the effect plant-based resources on predation in omnivore predators suggest that the initial attraction of natural enemies to plant resources as detected through density increase may not be indicative of the ultimate predation or prey suppression strength of that predator (McMurtry & Scriven, 1966a; McMurtry & Scriven, 1966b; Cottrell & Yeargan, 1998a; Lundgren, 2009; Seagraves *et al.*, 2011; Calixto *et al.*, 2013; Urbaneja-Bernat *et al.*, 2013). Therefore, it appears that the presence of plant-based resources may produce conflicting effects on density and predation in omnivore predators.

In summary, although there are ample data on the effects between VOCs and predators in the laboratory context, the strength of these associations is still unclear in a more complex field environment. This point is particularly important when the presence of plant-based resources in the field is considered, and especially how those resources may interact with VOCs to affect the overall behavior and decision-making process in omnivore predators. One potential outcome as a result of the combination of volatile cues and plant-based resources is that combination may improve the overall quality of the host plant as perceived by the predatory insect, thereby increasing predator attraction and herbivore removal. Alternatively, predator attraction to volatile cues instead may be offset by the utilization of the available plant-based resources, leading to the reduction in overall herbivore removal on the host plant independently of the insect predator's attraction to plant volatiles and resources.

DISSERTATION OBJECTIVES

In chapter two, I achieved three central research objectives. First I performed a set of experiments to determine whether the exposure to VOCs produced by damaged emitter leaves would induce undamaged receiver leaves without explicit local herbivory using the native, undomesticated perennial, *Datura wrightii*, and its main local herbivore, the leaf beetle, *Lema daturaphila* (Coleoptera: Chrysomelidae). Previous work on maize and lima beans showed that undamaged plant tissues may become induced and emit VOCs and/or produce other form of defenses measures following only exposure to VOCs from damage plant tissue, without any damage (Engelberth *et al.*, 2004; Heil & Silva Bueno, 2007). Secondly, in the same set of experiments, I also determined if the response following exposure to VOCs from damaged emitter leaves of *D. wrightii* and *L. daturaphila* herbivory locally would increase the strength of, or “prime”, the subsequent VOC emission of the receiver leaves. When primed through previous VOC exposure, plants that are subsequently damaged emit or produce heightened levels of defensive responses. For example, following exposure to VOCs from damaged conspecifics, maize seedlings that were then attacked produced higher quantities of two defensive compounds (Ali *et al.*, 2013).

Finally, I conducted the final set of experiments in order to isolate the effect of the vascular signal from the effect of VOCs on the subsequent production and emission of VOCs in *D. wrightii*. As previously discussed above, the current theory in the persistence of VOC eavesdropping between-plants may be that VOCs act as airborne signals for within-plant communication due to the restrictions found in the vasculature of many plant species. Therefore, with these experiments, I tested the theory that VOCs may substitute for the vascular signal in communicating potential herbivory to yet-undamaged parts on the same plant that are vascularly disconnected from the source of the damage.

In chapters three and four, I simultaneously examined the effects of VOCs emitted by *D. wrightii* and plant-based resources, specifically pods on *Lotus strigosus* (Fabaceae), as originally proposed by Price *et al.* (1980) on the colonization and predation of the omnivorous predator, *Geocoris pallens* (Hemiptera: Geocoridae). With this question, I proposed that because of the nutritional value of *L. strigosus* pods, *G. pallens* (particularly during colonization) should be influenced by both VOCs as well as the presence of pods. Furthermore, I concluded that although that both emission of VOCs and the presence of plant-based resources appear to increase the density of *G. pallens*, their effects on predation in the omnivore were conflicted. Experimental evidence suggests that although VOCs improve predation against herbivores in omnivores (Hare & Sun, 2011a), the presence of plant-based resources may reduce per capita predation in omnivores (Eubanks & Denno, 2000).

In chapter three, I addressed the above questions using a multiple year, field experiments in order to measure the effects of VOCs emitted by *D. wrightii* and pods produced by *L. strigosus* on *G. pallens* under a natural ecological context as called for by recent reviewers discussed above. In particular, I had two central research objectives; 1) I wanted to quantify the effect of both VOCs and pods on the density and 2) predation of *G. pallens*. From the results of these experiments, I addressed overall how these two factors impacted the tritrophic interaction among *D. wrightii*, *L. daturaphila*, and *G. pallens*.

Because my experimental findings in the field were unable to show clearly how the two cue types may have interacted to impact the predation of *G. pallens* in concert due to the rapid changes in the VOC emission rates, I conducted another set of experiments in the greenhouse in order measure the same two responses in a more controlled environment. In conducting these experiments described in chapter four, I again examined the specific impact of both the plant-based resource produced by *L.*

strigosus and VOC cues emitted by *D. wrightii* in concert on the recruitment of and more importantly, the predation of *L. daturaphila* by, *G. pallens*.

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CHAPTER TWO: THE ROLE OF VOCS IN WITHIN-PLANT SIGNALING AND SUBSEQUENT VOC EMISSION FOLLOWING HERBIVORY IN *DATURA WRIGHTII*

ABSTRACT

The production and emission of volatile organic compounds (VOCs) is common across plants and has been implicated in a wide variety of defensive functions. One function is as an airborne signal to indicate damage within-plant. Following the perception of VOCs emitted by tissue damaged by herbivores, intact parts of the plant may then produce defensive responses in preparation for additional damage by herbivores. One benefit of an airborne signal over a vascular signal may be the airborne signal's ability to reach parts of the plant that are not vascularly connected to the damaged tissue due to the pattern of leaf growth (orthostichy).

In a set of manipulative experiments, I differentially exposed undamaged (receiver) leaves to VOCs emitted following localized herbivory at another leaf (emitter) on the same plant. I then used herbivores to damage receivers and measured the subsequent local VOC production at each leaf. Finally, I elucidated the potential vascular connections between emitter and receiver leaves. I analyzed the VOCs produced by receivers based on 1) exposure to airborne VOCs from the emitter and 2) vascular connectivity to the emitter in order to discern the relative contribution of each signal pathway in the formation of the overall systemic defensive response. I found that airborne exposure primed subsequent VOC production and specifically primed the responses of

receiver leaves having no direct vascular connection to the emitter. These findings illustrate the importance of VOCs as airborne signals to reach intact parts of the plant without direct vascular connection to damaged tissue.

INTRODUCTION

When damaged by herbivores, many species of plants produce and emit a suite of volatile organic compounds (VOCs) (Karban & Baldwin, 1997; Kessler & Heil, 2011). Research into the function of these compounds suggest that the production and emission of VOCs may be associated with the attraction of natural enemy insects (predators and parasitoids) that may in turn suppress populations of herbivores on the threatened plant (Dicke & Sabelis, 1988; Dicke & Baldwin, 2010). Additionally, these compounds have been shown to directly repel female herbivores as measured through flight and oviposition bioassays (De Moraes *et al.*, 2001), thus suppressing oviposition and further damage from herbivores. Finally, VOCs have also been implicated in other plant processes such as the formation of root microbial community and allelopathy (reviewed in: Metlen *et al.*, 2009). Due to the prevalence of the indirect interactions between many species of natural enemies and plants, VOCs are often considered to be form of indirect resistance deployed by plants against herbivory. Although the effectiveness of herbivore removal by natural enemies has been empirically measured and experimentally demonstrated many times, the resultant impact on plant fitness remains largely unclear, most likely because of the difficulty in measuring plant fitness (Dicke & van Loon, 2000; Meijden & Klinkhamer, 2000; Heil, 2008; Hare, 2011; Karban, 2011). Therefore, the evolutionary maintenance of induced VOCs by plant species may be based more than just on their ability to attract natural enemies alone.

Aside from the above putative functions, components of the VOC blend produced by many plant species under attack from herbivores have also been shown experimentally to function as important signals that may contain information regarding damage. The initial discovery of such transfer of information was made simultaneously by two research groups. Rhoades (1983) observed a reduction in the performance of the tent caterpillar, *Malacosoma californicum*, experimentally placed on undamaged willow trees (*Salix sitchensis*) that were near damaged trees. Rhoades suggested that the undamaged trees were able to induce their own resistance against herbivores through the perception of nearby herbivory via “airborne pheromonal substances”. In other words, undamaged trees were able to prepare their resistance and defensive efforts to confront potential future damage from herbivores. Likewise, Baldwin and Schultz (1983) also found that undamaged poplar (*Populus x euramericana*) and maple (*Acer saccharum*) placed near damaged conspecifics produced following exposure elevated levels of phenolics, a compound group with putative herbivore resistance properties. Although such between-plant information transfer were initially met with skepticism, research in the subsequent 30 years has shown that this phenomenon to be prevalent across many plant taxa (Baldwin *et al.*, 2006; Campos *et al.*, 2008; Arimura *et al.*, 2010; Das *et al.*, 2013, summarized in Table 2.1). The induction and activation of defensive and resistance responses following the perception of nearby herbivory may be a cost-saving method for plants to prepare for potential future herbivory without wasting resources in maintaining defensive and resistance responses in the absence of actual herbivory (Baldwin, 1998; Heil & Baldwin, 2002; Karban & Maron, 2002).

In particular, tomato plants (*Solanum lycopersicum*) sprayed with the methyl ester of jasmonic acid (MeJA) or exposed to mechanically damaged branches of sagebrush (*Artemisia tridentate*), which is known to produce MeJA, in turn began to synthesize proteinase inhibitors, a type of defensive compound (Farmer & Ryan, 1990). The herbivorous mite, *Tetranychus urticae* (Trombidiformes: Tetranychidae), also avoided oviposition on cotton seedlings (*Gossypium hirsutum*) that were previously exposed to VOCs from mite damaged conspecifics (Bruin *et al.*, 1992). Molecular work on lima beans (*Phaseolus lunatus*) showed the activation of five separate defense genes when undamaged plants were exposed to the VOCs of conspecific plants that were damaged through herbivory (Arimura *et al.*, 2000). Maize seedlings (*Zea mays*) exposed to green leaf volatiles (GLVs) or VOCs induced by caterpillars (*Spodoptera exigua*, Lepidoptera: Noctuidae) produced by damaged conspecifics in turn emitted higher levels of two VOC products, jasmonic acid (JA) and sesquiterpenes, when compared to previously unexposed controls (Engelberth *et al.*, 2004). Furthermore, the emission of JA and sesquiterpenes appears to last for up to five days following exposure (Ali *et al.*, 2013). In sagebrush, VOCs from mechanically damaged conspecifics reduced the level of herbivory in the exposed plants compared to plants that were blocked from all airborne cues by being bagged (Karban *et al.*, 2006). Moreover, the long-term effects of such exposure suggested that seedling survival was improved by proximity to damaged neighbors (Karban *et al.*, 2012).

In addition to conspecific between-plant information transfer, researchers have also found several instances of information transfer between different plant species. For example, barley plants (*Hordeum vulgare*) exposed to either thistle (*Cirsium* spp.), nightshade (*Solanum nigrum*), or *Chenopodium album*, were all less likely to be selected for oviposition by the aphid, *Rhopalosiphum padi* (Hemiptera: Aphididae) (Glinwood *et al.*, 2004; Ahman & Ninkovic, 2010). In the case of sagebrush, VOCs emitted by damaged plants are capable of inducing a suite of defensive responses including defensive enzymes and polyphenol oxidase in wild tobacco (*Nicotiana attenuata*) plants. Over time, the effects of such VOC exposure and induction translated to increased flowers and seed-capsules in years with average levels of herbivory for the wild tobacco plants (Karban, 2001; Karban & Maron, 2002; Karban *et al.*, 2004). Lastly, the direct effect of VOCs emitted by clipped sagebrush was ruled out as blends did not repel wild tobacco herbivores (Karban & Baxter, 2001). Interestingly, the interspecific information transfer between sagebrush and wild tobacco appears to be unique as several other species of naturally co-occurring plants were unaffected by damaged sagebrush VOCs in terms of resistance against subsequent herbivory following exposure (Karban *et al.*, 2004).

By and large, the response of plants to VOCs can be organized into two non-mutually exclusive categories: 1) immediate synthesis of defenses following exposure, and/or 2) synthesis of defenses only following localized damage. Following exposure to VOCs emitted by damaged plant tissue, some plant species immediately begin to produce defensive and resistance responses against further herbivory. For example, changes in both the expression levels of defensive genes as well as extrafloral nectar emissions were detected locally in lima beans following exposure to VOCs (Arimura *et al.*, 2000; Heil & Silva Bueno, 2007; Heil & Adame-Alvarez, 2010). Similarly, intact maize seedlings produced more VOCs and JA systemically than controls after just being exposed to GLVs, *S. exigua* caterpillar induced VOCs, or several synthetic components from the VOC blends individually [(*Z*)-3-hexenal, (*Z*)-3-hexen-1-ol, and (*Z*)-3-hexenyl acetate] (Engelberth *et al.*, 2004). Instead of immediate synthesis, other plants may require localized damage in addition to the previous exposure in order to initiate the synthesis of defenses.

In either case, once induced, the subsequent response of previously VOC exposed plants often yields consistently heightened levels of, or “primed”, defensive and resistance responses compared to previously unexposed plants. For example, in maize seedlings, the relative levels of both trypsin and cysteine protease inhibitors, two groups of proteins with putative functions in herbivore resistance, were significantly higher in the treatment that was previously exposed to VOCs *and* subsequently damaged when compared to unexposed but similarly damaged seedlings (Ali *et al.*, 2013). These results indicate that VOC exposure prepared the defensive system in maize to initiate a stronger response when damaged in comparison to plants that were only damaged but blocked from exposure to VOCs.

Some researchers initially suggested that these cases of information transfers between plants were ways for plants to actively signal or talk to each other in order for the damaged emitter to alert the undamaged receiver of potential herbivory in the near future. However, when considered in an evolutionary context, it was counterintuitive why damaged plants would signal nearby undamaged competitors of potential oncoming herbivory (Baldwin *et al.*, 2006; Karban *et al.*, 2014). Rather, such transfers would, in essence, only benefit the undamaged receiver and place a potentially substantial fitness cost on the damaged emitter. By contrast, recent reviews now suggest that receiver plants are, in fact, eavesdropping on the VOC cues produced by the emitter plants (Heil & Karban, 2010; Karban *et al.*, 2014). Yet, the issue remains: if receiver plants are exploiting VOC cues from emitter plants in order to gain a competitive advantage over emitter plants, then why do plants still emit VOCs?

One potential explanation for the seemingly maladaptive emission of VOCs is that the emission and perception of VOC cues may be a form of within-plant communication. For example, leaves of poplar hybrid plants (*Populus deltoides* x *nigra*) that were exposed to VOCs emitted by nearby damaged leaves on the same plant produced higher levels of defensive response following herbivore damage (Frost *et al.*, 2007). Select lima bean shoots that were exposed to VOCs produced by damaged shoots on the same plants increased in the production of extrafloral nectar, a defensive response that attracts natural enemies (Heil & Silva Bueno, 2007).

In addition, the typical distance over which plant cues are effective provides insight in regards to the intended receiver of the information. Heil and Adame-Alvarez (2010) found that the effect of VOC cues on inducing extrafloral nectar production and anti-pathogenic responses in lima beans were only detected in receiver shoots that were within 50 cm of the emitter, and that this distance also represented the cutoff where there was more (>50%) emitter plant tissue vs. tissue belonging to a conspecific plant. The authors suggest the short effective distance in airborne cues in lima beans indicates that plants are largely trying to signal themselves. Likewise, *A. tridentata* VOCs were only effective at inducing conspecific plants at a distance of 60 cm apart or less (Karban *et al.*, 2006). The interspecific induction of *N. attenuata* by *A. tridentata* requires that the plants to be even closer (10 cm or less) (Karban *et al.*, 2003). These findings again illustrate the short distance over which VOCs from damaged plants are active, and suggest that the production of these VOCs may therefore only be intended as a within-plant signal, leaving any incidence of between-plant information transfer as eavesdropping by fortuitous receivers.

Finally, in several related studies, researchers were able to detect a significant reduction in the level of herbivory suffered by sagebrush plants that were exposed to VOCs from damaged (genetically identical) clones vs. conspecifics (Karban & Shiojiri, 2009). VOCs blend composition were more similar among sagebrush clones in comparison to conspecifics (Ishizaki *et al.*, 2012). These findings suggest that between-plant information transfers may be an extension of within-plant signaling and may be enhanced by self-recognition.

Some plants have been shown to have vascularly transmitted damage signals in addition to VOCs. For example, work on tomatoes (Farmer & Ryan, 1990; Ryan, 2000) and lima beans (Arimura *et al.*, 2000) have identified JA, abscisic acid (ABA), ethylene, and systemin as putative compounds that can already be utilized by plants to induce defensive responses following damage (summarized in Table 2.1). However, the presence of the airborne signal may be advantageous for plants when considering the resistance response beyond the local site of actual herbivory, or the systemically-acquired resistance (SAR). First, most plants exhibit some form of vascular restrictions due to orthostichy or the pattern of leaf growth; potential vascular signals may not reach all parts of the plant, or at least not at the same rate. Orians (2005) suggest that due to the species specific vascular restrictions, proximately located leaves may in fact be in opposite orthostichies and therefore vascularly disconnected. Given such, the emission of and the ability to perceive signals that are unconstrained by vasculature may offset limitations in plant species with restrictive vascular connectivity (Orians, 2005; Heil, 2010). Therefore, a potential airborne damage signal may in turn overcome such vascular restrictions to induce resistance and defensive responses. Additionally, airborne signals may be a much faster method of information transfer in comparison to vascular signals, and could therefore reach and prepare undamaged parts of the plant before the herbivores move from the original damage site (Heil & Ton, 2010). Given these advantageous aspects of airborne within-plant signaling, it is likely that the evolutionary persistence of VOCs may in part be due to their function as a signal of damage to other parts of the same plant.

Locally in Riverside, the Jimsonweed, *Datura wrightii* (Solanaceae), is mainly attacked and colonized by the specialist herbivore, *Lema daturaphila* (Coleoptera: Chrysomelidae) (Hare, 2007). *D. wrightii* emits a blend of VOCs following herbivory by *L. daturaphila* but the production of volatiles is highly variable with respect to ontogeny and decreases to cessation over the course of the field season (Hare, 2010). Interestingly, this reduction in VOC production does not appear to affect predation against *L.*

daturaphila by its main local natural enemy, *Geocoris pallens* (Hemiptera: Geocoridae); predation remains high over the field season (Hare & Sun, 2011a). Additionally, preliminary work on *D. wrightii* has revealed that leaves grow in a spiral phyllotaxis which leads to opposite orthostichies for leaves 1, 4, and 6 above the source leaf. Such orthostichy leads to poor vascular connectivity of these proximate leaves to the damaged leaf as demonstrated by dye uptake assays (Fig. 2.1). Given the ontogenetically driven changes in VOC emission and the vascular restrictions found in *D. wrightii*, it is highly likely that VOCs partially may be selected as a potential airborne within-plant signal in young and vulnerable *D. wrightii* plants during the period of initial herbivore colonization.

In the following experiments, I 1) quantified subsequent VOC production as a resistance response to within-plant signaling in the form of VOCs previously emitted by a locally damaged emitter leaf; 2) determined whether this subsequent VOC production at the receiver site was immediately synthesized or required localized damage; 3) and finally, I isolated and measured the potential contribution of airborne within-plant VOC signaling vs. vascular transduction signaling to subsequent VOC production and emission in *D. wrightii*.

METHODS

Two sets of experiments were conducted for this study. I conducted the first set of experiments (hereafter: within-plant signaling experiments) in order to detect the potential of within-plant signaling in *D. wrightii* and then to determine whether the response to such signaling required localized damage (objectives one and two in the introduction). The follow up experiments (hereafter: airborne vs. vascular signal experiments) were conducted to quantify the contribution of the airborne VOCs vs. the vascular signal in response following the perception of damage elsewhere within-plant (objective three).

Plants and herbivores: All *D. wrightii* plants utilized for this study were germinated from seeds of the fifth-generation backcross progeny of the MVV6 and MVV8 lines. Both lines were created initially using seeds collected from wild plants found locally (Moreno Valley, CA), and therefore expected to be maintained solely under natural selection. Previous experiments also show these lines to be highly inducible following herbivory by *L. daturaphila* and using MeJA (Hare, 2007). Seedlings were maintained in greenhouses equipped with high-pressure sodium and metal halide lamps to provide supplemental lighting (14L:10D light cycle). Light intensities averaged 1250 ± 39 μmol m^{-2} s^{-1} for the photosynthetically active wavelengths at plant height at midday. Greenhouse temperatures ranged between 15° and 35°C. Plants were kept in the greenhouses until they had developed eight to twelve true leaves (approx. four to five mo.). For plants used in the airborne vs. vascular signal experiments, after the first four true leaves were developed, the apical meristems were removed to remove apical dominance and to impose a specific pattern of subsequent leaf development (see "Vascular Connectivity in *D. wrightii*" below).

Adult *L. daturaphila* beetles were obtained from a colony maintained year-round on *D. wrightii*. The beetle colony was periodically supplemented with field collections from the nearby UCR Agricultural Operations field site in order to alleviate any potential inbreeding. An individual *L. daturaphila* adult was used only once during an experiment.

VOC collection procedures: All experiments were conducted in a growth room with 14L:10D light cycle and 26°C day and 20°C night temperatures. Four plants (per block) were all placed in large individual cages (44 x 44 x 74 cm) constructed from PVC pipes, covered with polyethylene plastic sheets, and sealed to prevent accidental between-plant signaling. Each cage was constructed with ports to allow for the insertion of all VOC collection tubing.

The general induction and VOC collection procedures were similar in both experiments and also matched closely with our previous laboratory collections (Hare & Sun, 2011b). To induce each leaf prior to volatile collection, a pair of adult *L. daturaphila* beetles was confined to the leaf using a small mesh cloth cage (15.5 x 11 cm) for 48 hrs. Following the planned period of herbivory, the adults were then removed along with any frass or eggs. The usage of the mesh cloth cage permitted all emitted VOC compounds to disperse and reach receiver leaves within a cage but also prevented VOC collection at the emitter leaf. Therefore, to estimate the VOC production at the emitter leaf, the fourth plant in each block was designated as the emitter control plant was in each experimental block. VOCs were collected from an herbivore-damaged leaf on each emitter control plant that matched closely both the age and size of the emitter leaves on the experimental plants.

To collect VOCs from individual leaves, aeration chambers were made from polyester cooking bags (Terinex, Bedford, UK) as described in Hare (2007); however, each full size cooking bag was cut smaller (20 x 25 cm) and then heat-sealed to accommodate the smaller size of individual leaves in this study. Briefly, an aeration chamber was placed over a leaf and secured at the base of the leaf with a twist-tie. Filtered air through activated charcoal was pushed into the chambers through PTFE tubing, and then pulled through a VOC trap via vacuum suction through PVC tubing. Flow rates were independently regulated with flow meters at 0.5 L min^{-1} (Aalborg, Orangeburg, NY, USA). VOC traps were constructed from borosilicate glass tubing and filled with 25 mg of Super-Q (Alltech, State College, PA, USA). Following 24 hrs of active VOC collection, the trap was removed, wrapped in aluminum foil, and stored at -20 ºC until elution. Simultaneously, a new trap was put in place if further collection was planned. At the end of each experimental block, the remaining portion of each experimental leaf was removed from the plant. Each leaf was digitized using a scanner in order to estimate the amount of herbivory that took place using ImageJ (Rasband, 1997-2014). The leaves were then placed in a drying oven at 80 ºC for 48 hrs in order to obtain dry weights.

Within-plant signaling: To address the first two study questions, I used the following experimental design (schematic in Fig. 2.2). Following 24hrs of volatile collection prior to damage, a single leaf on each of the four plants was designated as the emitter leaf and damaged by a pair of adult *L. daturaphila* as described above. At the same time, four leaves on each of the three treatment plants (the last plant being the emitter control) were selected as receiver leaves. Out of these four leaves, two were placed in individual aeration chambers to prevent any VOC exposure from the emitter leaf; VOC collection was also started on these blocked receivers. The other two leaves were left unbagged and presumably exposed to potential VOCs from the emitter. Unlike the blocked receivers, it was not possible to collect VOCs from these exposed receivers while they were unbagged. A total of five blocks were completed.

The *L. daturaphila* adults were permitted to damage the emitter leaf for 48 hrs and were then removed, but the VOC exposure was allowed to continue for an additional 24 hrs following the secession of damage. Following the final day of exposure, the two exposed receivers were then also placed in individual aeration chambers to start VOC collection. Two adult *L. daturaphila* each were then added to one of the blocked receivers and one of the exposed receiver leaves. No beetles were added to the other two receivers, the beetles were permitted to feed on the receivers for 48 hrs before being removed and VOC collection was conducted across all four leaves for a total of six days following the initiation of herbivory at the receivers. Through the use of these four treatment levels (airborne exposure crossed with herbivory), I was able to independently detect 1) potential airborne within-plant signaling and 2) whether any response to such signaling required localized damage.

Vascular Connectivity in D. wrightii: I performed a series of dye uptake experiments using a solution of 0.25% w/v Rhodamine blue in water to partially map the orthostichy in *D. wrightii* and identify leaves that may be vascularly connected vs. disconnected from the emitter in order to address the third question proposed in the study.

I was able to impose a particular growth pattern on *D. wrightii* plants by removing the apical meristem early on in development to form a plant with two pairs of opposing leaves at the same height with a fifth older leaf beneath. Using plants grown in this configuration, I then removed the lower leaf on the plant with a clean razorblade underwater and introduced the solution of Rhodamine blue in water at the cut site (n=4). I assessed the pattern of dye uptake after 16 hrs as representing the orthostichy in *D. wrightii* grown in this particular configuration. The results of these experiments are shown in Fig. 2.3. I found that the leaf pairs above the emitter and in the same vertical plane as the emitter were connected to the emitter while the opposing leaf pairs were not.

Airborne vs. vascular signal: Although the overall experimental design was similar to the within-plant signaling experiments, I adjusted the treatment groups in order to ask the third question. In these experiments, all (four) receiver leaves on all (three) treatment plants within each block were damaged during the receiver damage bout as I was no longer interested in whether the subsequent VOC emission occurred immediately following exposure or required localized damage. However, the VOC exposure pattern over time was similar to those from the within-plant signaling experiments. Lastly, I also considered leaf connectivity when segregating receiver leaf treatments. Therefore, I again had four treatment levels for receiver leaves, comprised of two volatile exposure treatments (exposed vs. blocked) crossed with two vascular connection treatments (connected vs. disconnected). Finally, I reduced 1) the total emitter-to-receiver exposure time to 48 hrs (from 72 hrs, herbivory duration remained at 48 hrs), and 2) the receiver VOC collection period to five days following the initiation of herbivory as the quantity of VOCs collected on the sixth day was minimal (see Fig. 2.4, herbivory duration remained at 48 hrs). No other changes were made to any other herbivory or VOC collection procedures. Following the completion of each experimental block, the vascular connection of each receiver leaf to the emitter leaf on each experimental plant was confirmed via the dye uptake procedure detailed in the previous section prior to leaf removal for imagining and drying.

VOC analysis: VOCs were eluted from traps into 250 µl glass sleeves in autosampler vials using 150 µl of CH_2Cl_2 containing 4ng/µl of the internal standard, 1-bromoheptane, and then sealed with a PTFE-lined rubber septa cap as per Hare (2007). One µl of each sample was injected into and then analyzed with a Hewlett-Packard 5890 gas chromatograph equipped with a DB-5 column (30 m, 0.25 mm ID, 0.25 µm film thickness, J & W Scientific, Folsom, CA, USA) and an autosampler following parameters described previously (Hare, 2010). Most compound identifications were made using authentic standards from Sigma-Aldrich and other suppliers. The remaining compounds were identified with standards following methods in Hare (2007). Peak quantification was made using Agilent ChemStation® software based on comparison between peak heights and the internal standard in units of $ng{\bullet}g^{-1}$ leaf (dry weight)$\bullet h^{-1}$.

Statistical analysis: I conducted repeated measures ANCOVAs on $\log_{10}(X+1)$ transformed total VOC values using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc. 2011) for both experiments because VOCs were repeatedly collected from each leaf each day. Unique leaf numbers were used as the repeated measures subject and the experimental day was repeated. Percent herbivory was the covariate.

In both experiments, the experimental day factor describes the time course of the experiment with day = 0 being the point in time when adult *Lema* were introduced onto the receiver leaves (removed on day = 2, or 48 hrs later). Similarly, either day = -3 or day = -2 (within-plant signal and airborne vs. vascular signal experiments, respectively) was when adult *L. daturaphila* were introduced onto the emitter leaves (removed 48 hrs later in both experiments, day = -1 for within-plant signaling experiments and day = 0 in airborne vs. vascular signal experiments).

For the within-plant signaling experiment, I included 1) experimental day, receiver leaf treatment, and airborne exposure treatment as fixed effects, and 2) all two- and three-way interactions between the fixed effects and covariate were also included. 3) Lastly, I also included block as a random factor. To decompose the interactions, I performed a follow up analysis using the BY function in PROC MIXED.

For the airborne vs. vascular experiments, daily VOC data from a total of eight experimental blocks were analyzed together and included 1) the experimental day and receiver leaf treatment (four combinations of the airborne exposure + vascular connectivity treatment to receiver leaf groups, and the emitter control) as the fixed factors, 2) the interaction between the fixed factors, 3) block was again included as a random factor. Finally, I also requested a set of *a priori* contrasts in the statistical analysis to compare the VOC production between treatment groups of interest.

RESULTS

Within-plant signaling experiments: I detected significant variation in between receiver leaf treatments that were damaged by herbivory vs. those that were undamaged ($F_{1,341} = 30.76$, $P < 0.001$, Fig. 2.4). These findings first and foremost suggest that there is an absence of any immediate VOC emission response to potential within-plant signaling as undamaged receivers remained uninduced regardless of the type of exposure treatment administered.

I also detected a significant effect of the interaction between airborne exposure and receiver damage ($F_{1,466} = 5.28$, $P < 0.022$). Receiver leaves that were exposed to VOCs and then subsequently damaged themselves produced a stronger VOC response, or were primed, when compared to damaged receivers that were blocked from prior airborne exposure to VOCs. Finally, I also found that the amount of VOCs produced by damaged receiver leaves was significantly higher compared to the amount of VOCs emitted by damaged emitters as well ($F_{1,171} = 4.75$, $P = 0.031$, Fig. 2.4).

Due to the overwhelming effect of local damage on receiver leaves (Fig. 2.4), I performed a follow up analysis by restricting the dataset to include only damaged receiver leaves and found that there was significant effect of experimental day ($F_{9,382} = 3.04$, $P = 0.002$, Fig. 2.4). These findings are expected as VOC production increased then dropped over time in damaged receiver leaves but not undamaged receiver leaves over the course of the experiment, a induced emission pattern seen in previous experiments (Hare & Sun, 2011b).

Airborne vs. vascular signal: Overall VOC production varied significantly over time ($F_{4,207} = 15.29$, $P < 0.001$) and with treatment ($F_{4,207} = 11.38$, $P < 0.001$). The significance of the day factor indicated that VOC emission from all leaves changed over time as expected following local herbivory, with day = 2 and day = 0 being the peak emission days (receivers and emitter control leaves, respectively, Fig. 2.5), see also Hare and Sun (2011b). The significance of the treatment factor indicated that there were differences in the amount of VOCs emitted following damage among different treatment groups of receiver leaves and/or emitter control leaves; these differences were further explored with planned contrasts (Table 2.2 and described in detail below). Lastly, I did not detect a significant interaction between day and treatment ($F_{4,207} = 0.64$, $P = 0.853$), this indicated that all treatment groups followed a similar pattern of VOC emission over the time course (following emitter control treatment timescale transformation, Fig. 2.5 – timescale untransformed in figure).

Receiver leaves that were both vascularly connected to the damaged emitter leaf of the same plant as well as exposed to the airborne VOCs produced by that damaged emitter leaf produced the highest level of VOCs following localized damage over the course of VOC emission days when compared to other receiver treatments and emitter controls ($P < 0.001$ & $P = 0.004$ – contrasts A & C, respective, Table 2.2 & Fig. 2.6).

Receiver leaves that were either vascularly connected to the damaged emitter leaf or exposed to VOCs emitted by the damaged emitter leaf on the same plant, but not both, emitted a comparatively intermediate level of VOCs following damage ($P < 0.001$ & $P = 0.004$ – contrasts A & C, respectively, Table 2.2 & Fig. 2.6). Furthermore, the VOC emissions by these two receiver treatment groups did not differ statistically ($P = 0.943$, contrast D, Table 2.2 & Fig. 2.6).

Finally, receiver leaves that were both vascularly disconnected from the emitter leaf as well as blocked from VOCs emitted by the damaged emitter leaf on the same plant produced the lowest level of VOCs following damage when compared with the other three receiver treatment ($P < 0.001$, contrast A, Table 2.2 & Fig. 2.6). More importantly, the quantity of VOCs produced by leaves in this receiver treatment group did not differ significantly from that of the emitter control ($P = 0.462$, contrast B, Table 2.2 & Fig. 2.6).

DISCUSSION

VOC compounds emitted by damaged *D. wrightii* leaves function as an airborne within-plant signals to induce subsequent VOC emissions across other parts of the plant following localized damage. These VOC signals increased the strength of, or primed, the subsequent VOC emission in damaged leaf tissue following exposure. Furthermore, the relative contribution of the airborne VOC signal was comparatively similar to the vascular signal in inducing subsequently damaged leaves following exposure to either signal, thereby suggesting that it is highly likely that VOCs serve as a damage signal to prepare undamaged parts of *D. wrightii* that are otherwise vascularly poorly connected to the damage source.

VOCs function as an airborne damage signal in D. wrightii: Locally, the pattern of infestation by *L. daturaphila* on *D. wrightii* plants typically progresses from small, isolated damage left by wandering adults, followed by oviposition after females have located the appropriate plant, to finally the emergence of larvae. Compared to adults, young larvae hatch simultaneously and feed in groups to produce much stronger and more importantly, sustained damage (pers. obs.). Given the life history of the main herbivore on *D. wrightii*, the appearance of minor damage probably serves a reliable indicator of more sustained and widespread damage in the near future. The presence of a primed VOC response in *D. wrightii* therefore seems appropriate when considered in the context of the life history of its specialist herbivore.

It has been shown that exposure to MeJA increase the accumulation of proteinase inhibitors in both tomatoes and wild tobacco when plants are in airborne contact with this compound (Farmer & Ryan, 1990). In *D. wrightii*, it has also been experimentally demonstrated that plants are highly inducible via external MeJA application, and exposure led to full subsequent VOC emission without the necessity for actual herbivory (Hare, 2007). Therefore, it appears that *D. wrightii*, like other Solanaceae, may have retained both the ability to perceive airborne MeJA as well as the ability to produce and emit VOCs immediately following exposure to it. Interestingly, *D. wrightii* is also capable of a more subtle VOC emission pattern in that following exposure to the full

VOC blend from damaged nearby leaf tissue, the exposed leaves do not immediately emit VOCs, but rather primes the subsequent VOC emission should they also receive localized damage.

Aside from herbivory and airborne VOCs, plants have also been shown to be inducible by many abiotic factors. Specifically, mechanical damage has been shown to elicit volatile production in several plant species (Reymond *et al.*, 2000; Banchio *et al.*, 2005; Mithofer *et al.*, 2005; Bricchi *et al.*, 2010; Ueda & Matsuda, 2011), along with other environmental parameters such as light intensity, temperature, air and soil humidity (Gouinguene & Turlings, 2002; Pio *et al.*, 2005). Taken together, these study results strongly suggest that many plants in nature are perpetually induced.

In a recent review by Heil and Karban (2010), the authors stated specifically that the problem of autoinduction or the runaway process of reciprocal induction as a future question in moving our understanding of airborne signaling forward. One potential solution may be for plants to stop producing the signal that elicits the full VOC emission response altogether and switch to a mechanism that only readies rather than activates additional VOC emissions in anticipation of an herbivory attack in the near future as a cost-saving mechanism (Karban & Maron, 2002; Karban *et al.*, 2012). Here, I demonstrate the blend of VOCs emitted by damaged *D. wrightii* leaf tissue induces a potentially cost-saving response to herbivory.

VOCs and vascular signal contribute similarly to subsequent VOC emissions: Considering the pattern of response in Fig. 2.6, several key properties emerge for both the airborne VOC damage signal and the putative vascular signal and their ability to induce and prime subsequent VOC emissions in *D. wrightii*:

1) Damaged receiver leaves that were exposed to both signals in turn express the highest emission of VOCs following local damage. In a natural setting, I expect that the rates of atmospheric diffusion for each chemical component in a VOC blend to be dependent on the distance from the emitter leaf such that the receiver leaves close to the emitter leaf should all receive airborne VOC signals. At the same time, most of the receiver leaves near the emitter should also be vascularly connected to the emitter (with the exception of the leaf occupying the "1" position in Fig 2.1). Therefore, all leaves close to the emitter should be exposed to VOCs and the majority also to the vascular signal as well. Leaves that are closest to the emitter also have the highest probability of being damaged in the near future as *L. daturaphila* larvae tend to defoliate *D. wrightii* plants based on proximity to the leaf on which eggs were first oviposited. Together, these findings suggest that in *D. wrightii*, leaves that receive the most complete suite of damage signals and in turn produce the highest VOC response following local herbivory were also those that were highly threatened in terms of proximity to previously damaged leaf tissue.
2) Damaged receivers that were exposed to one form of the damage signal produce an intermediate level of VOC response following local herbivory. In *D. wrightii*, preliminary dye uptake assays found that vascular connectivity between leaves varies based on their pattern of growth such that some proximate leaves may

actually be disconnected from a nearby source (such as leaf “1” in Fig. 2.1). Here, I further elucidated the vascular connectivity pattern among a set of receiver leaves and identified particular leaves that were vascularly disconnected from the emitter source (Fig. 2.2). Therefore, these findings strongly suggest that for the receiver leaves that are close to the emitter but are vascularly disconnected from the emitter, airborne VOCs function as a substitution damage signal as previously suggested by Heil (2010) to overcome the orthostichy limitations in plants. Through the perception of these airborne VOCs, these otherwise uninformed leaves can in turn prime their response in preparation of potential herbivory in the future. For the plant as a whole, this substitution damage signal allows for more leaves exposed to potential herbivory to be primed for herbivory than would otherwise be possible through vascular connections alone.

3) Finally, leaves that were excluded from both vascular signals and airborne VOCs in turn produced a VOC response that was similar to that of leaves that were damaged on plants that did not suffer any prior herbivory. This final, low tier of response in these uninformed leaves reiterate by comparison the priming effect that both the airborne and/or vascular damage signal has on the subsequent VOC response that I detected earlier in the within-plant signaling experiments.

CONCLUSION

I demonstrate that VOCs produced by damaged *D. wrightii* leaf tissue can function as an airborne substitution damage signal to reach leaves that are vascularly disconnected from the damage source. When considered in the context of the overall induced resistance for plants, the benefit of such an airborne signaling mechanism becomes two-fold. First, much like other induced resistance mechanisms, it would lower the cost of maintaining the entire plant in a perpetual state of readiness against herbivory through the constant production of constitutive defense and resistance measures. Secondly and more importantly, this airborne mechanism may allow for more of the plant to become resistant to herbivory through the transmission of the damage signal to parts of the plant that may be vascularly disconnected from the damage source.

However, because of the inherent problem that signalers are unable to control which nearby plants receives the signal, this airborne signaling mechanism is therefore open to exploitation by eavesdropping neighbors (Otte, 1974). In fact, it is highly likely that the cases of conspecific and interspecific between-plant information transfer documented most likely evolved first as a form of within-plant communication (Karban & Maron, 2002; Karban *et al.*, 2004; Heil, 2009). Although potentially maladaptive should *D. wrightii* emit VOCs in the presence of nearby plants that are capable of eavesdropping, I demonstrate in this study that the persistence VOCs in this plant species may be due in part to its function as an airborne damage signal in preparing the entire plants against repeated bouts of damage that are characteristic of the local specialist herbivore.

Table 2.1 Plants that exhibit potential form(s) of airborne information transfer. The experimental induction or elicitation methods, resistance and defensive responses, and any putative local and/or systemic signals are also reported.

	Elicitation Method(s)	**Experimentally Measured Resistance/Defense Response(s)**	**Putative Local Vascular Damage Signal(s)**	**Putative Systemic Damage Signal(s)**	**Forms of Airborne Communication**	**References**
Maize	Herbivory (BAW), synthetic compounds, wounding+BAW regurgitant	VOCs, GLVs		GLVs, (*Z*)-3-hexenal, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate	Intraspecifically between-plant	Engelberth et al., 2004
Poplar	Herbivory (gypsy moth), mechanical wounding	VOCs, Wound-induced mRNAs		VOCs	Within-plant	Davis et al. 2007
Tomatoes	Airborne MeJA, Topical ABA and MeJA application, Mechanical wounding + systemin, *Artemisia tridenta* leaves	PINs, PPO	Prosystemin-Systemin, ABA, JA, Ethylene	ABA, MeJA	Intraspecifically between-plant	Stratmann 2003, Ryan 2000, Sun et al. 2011, Farmer & Ryan 1990, Odonnell et al. 1996, Penacortes et al. 1995
Barley	VOC exposure	Herbivore - aphid (*Rhopalosiphum padi*) colonization preference			Intraspecifically between-plants (different cultivars), interspecifically between-plants (induced by nightshade and the common weed)	Ahman & Ninkovic 2010, Glinwood et al. 2004
Lima Beans	VOC exposure	defense gene expression, VOCs, EFNs	JA	VOCs, (*E*)-b-ocimene	Intraspecifically between-plants, within-plants, Interspecifically between-tobacco-lima bean-lima bean	Arimura et al. 2000, Arimura et al. 2012, Heil & Silva Bueno 2007
Cotton	VOC exposure	Herbivore oviposition rate, predator attraction		VOCs	Intraspecifically between plants	Bruin et al. 1992
Tobacco	TMV, Mechanical wounding, Sagebrush VOCs	Secondary viral infection (lesions), Root nicotine, Herbivory, Polyphenol oxidase, 5 year study: seed-capsules, flowers	MeJA	MeSA, MeJA	Interspecifically between plants (receiver of sagebrush VOCs)	Park et al. 2007, Karban 2001, Karban & Maron 2002
Sagebrush	VOC exposure	Herbivory by generalist and mammals, herbivory by specialist chrysomelid (unaffected by induction), Long term: survival (no effect), inflorence, seedling survival		VOCs	Intraspecifically between plants, Within plants, Interspecifically between plants (emitter to wild tobacco, but could not induce: *Lomatium dissectum*, *Lupinus polyphyllus*, *Valeriana californica*)	Karban 2001, Karban et al. 2004, Karban et al. 2012

*BAW – beet armyworm, ABA – abscisic acid, MeJA – methyl jasmonate, JA – jasmonic acid, MeSA – methyl salicylate, VOC – volatile organic compound, TMV – tobacco mosaic virus, GLV – green leaf volatiles, PIN – proteinase inhibitors , PPO – polyphenol oxidase, EFN – extrafloral nectary

Table 2.2 Planned contrasts among receiver leaf treatment groups and emitter control leaves.

Contrast	**Num DF**	**Den DF**	**F Value**	**Pr > F**
A. Airborne & Vascular + Airborne or Vascular Signal Treatments vs. No Signal Treatment + Emitter Control	1	207	33.39	**<.001**
B. No Signal Treatment vs. Emitter Control	1	207	0.54	0.462
C. Airborne & Vascular Signal Treatment vs. (both) Airborne or Vascular Signal Treatments	1	207	8.32	**0.004**
D. Airborne vs. Vascular Signal Treatment	1	207	0.01	0.943

Figure 2.1 Schematic of vascular connections in *D. wrightii* has a spiral phyllotaxis with leaves growing at a constant divergence angle of ~137.5 º. Vascular connections were traced using a 0.25% w/v solution of Rhodamine Blue dye in water fed via an exposed petiole for 16 hours. Leaves that are growing vertically or longitudinally from the treated leaf were directly connected (leaves 2, 3, and 5, here shown as dark veins). Leaves 1, 4, and 6 were vascularly disconnected from the source leaf (shown with white veins). Schematic optimized to minimize overlap between leaves.

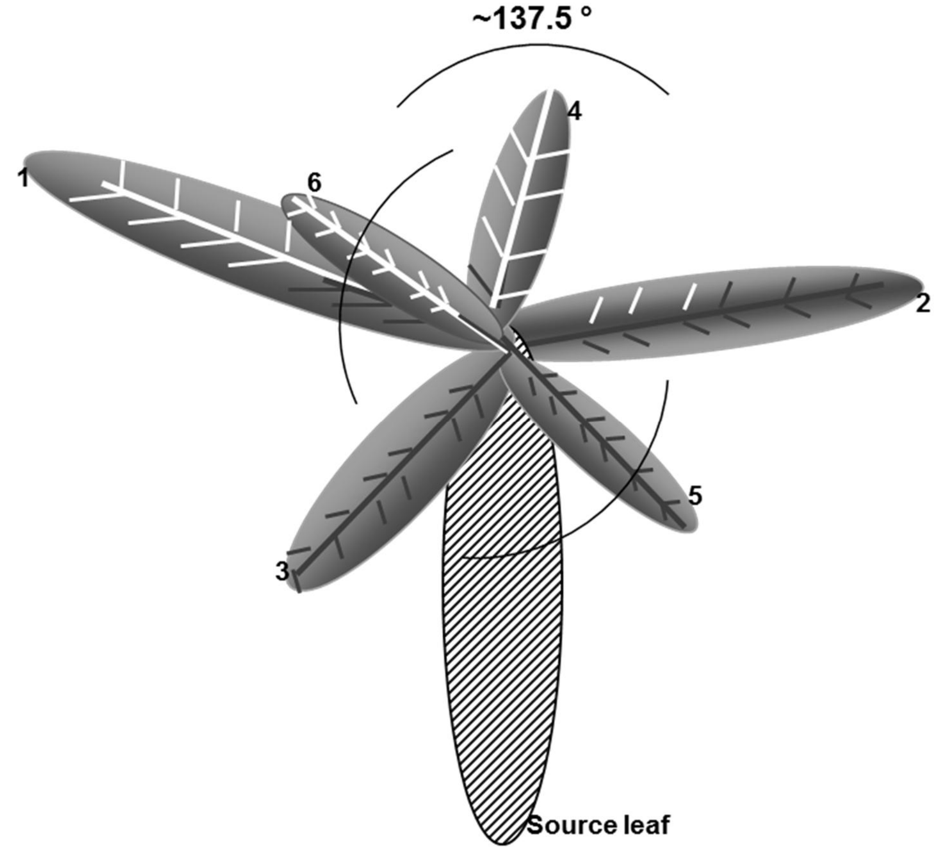


Figure 2.2 Partial schematic of the design for the within-plant signaling experiments. Day -3 represents the start of damage for the emitter leaf, followed by a 72 hours exposure period between the emitter and select receivers. Day 0 represents the start of the select receiver damage, which lasts for 48 hours while VOC collection continues for six days beyond day 0.

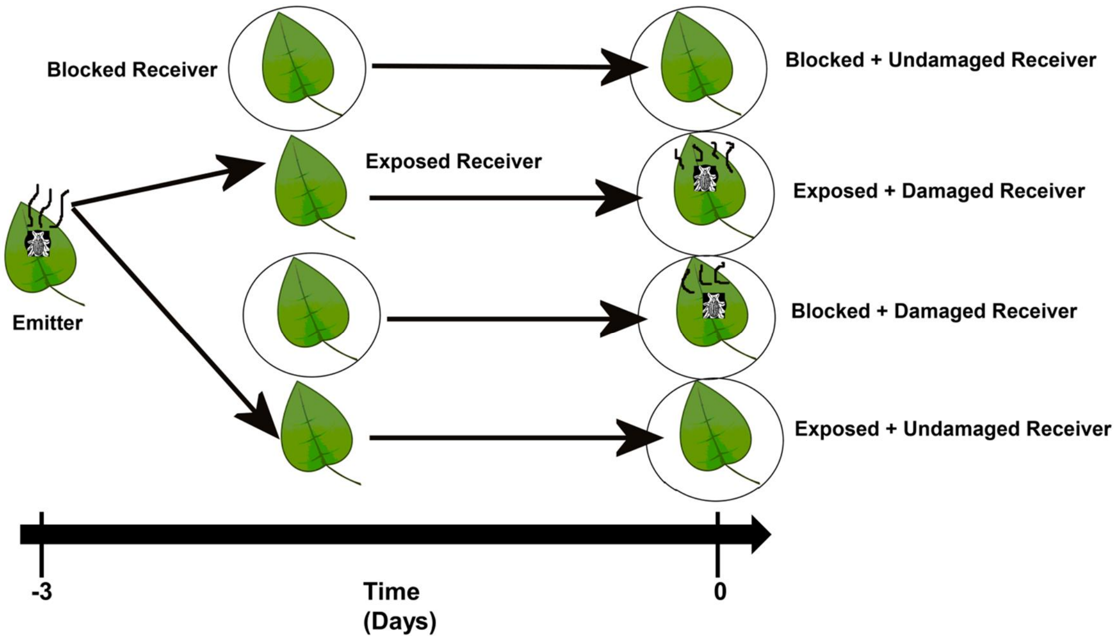


Figure 2.3A Schematic of the orthostichy pattern from the Rhodamine Blue dye trace uptake experiment. The arrow represents the site of dye introduction. Dark veins (C1 & C2) show uptake of the dye from the source and open veins (D1 & D2) show the lack of dye uptake. **2.3B** Actual photo of one experimental replicate. The arrow again indicates the site of the dye introduction. Pink dye can be seen in the bottom leaf pair (C1 & C2) indicating dye uptake and vascular connectivity, whereas the top leaf pair (D1 & D2) is free of dye and therefore vascularly disconnected.

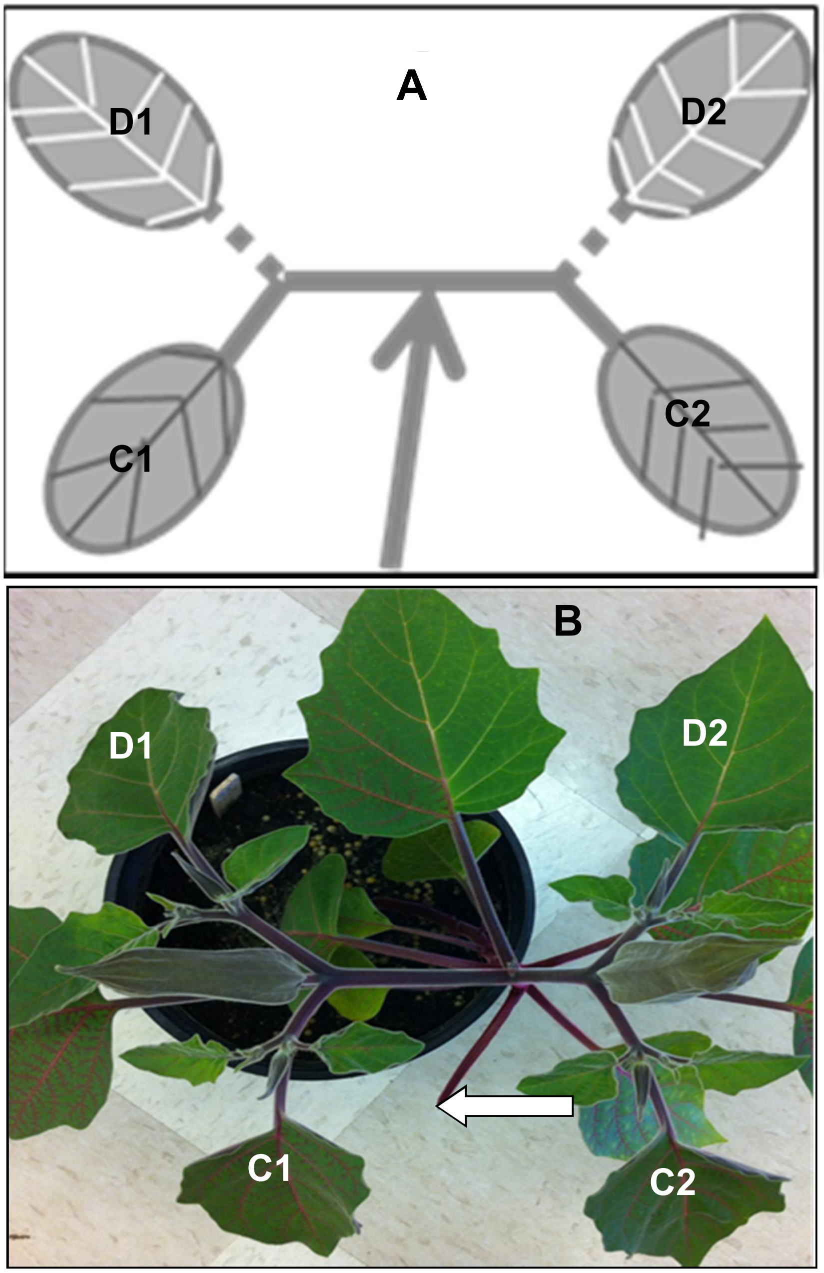


Figure 2.4 Within-plant signaling experiment: total VOC production ± SE for (damaged and undamaged) VOC exposed receiver leaves and emitter control leaves.

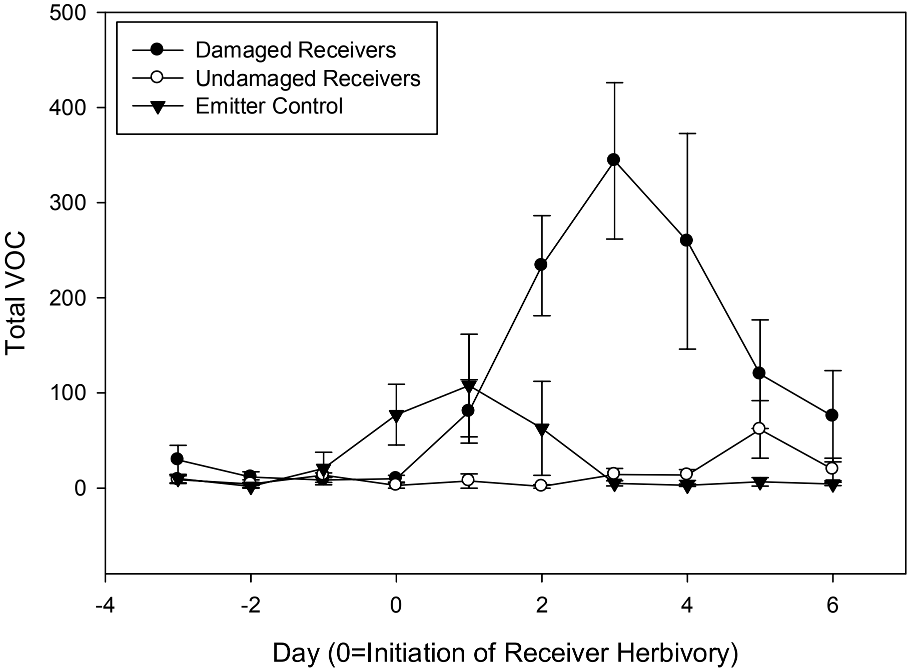


Figure 2.5 Airborne vs. vascular signal experiment: $\log_{10}$ transformed total VOC production ± SE based on receiver leaf treatment. Emitter control was also included.

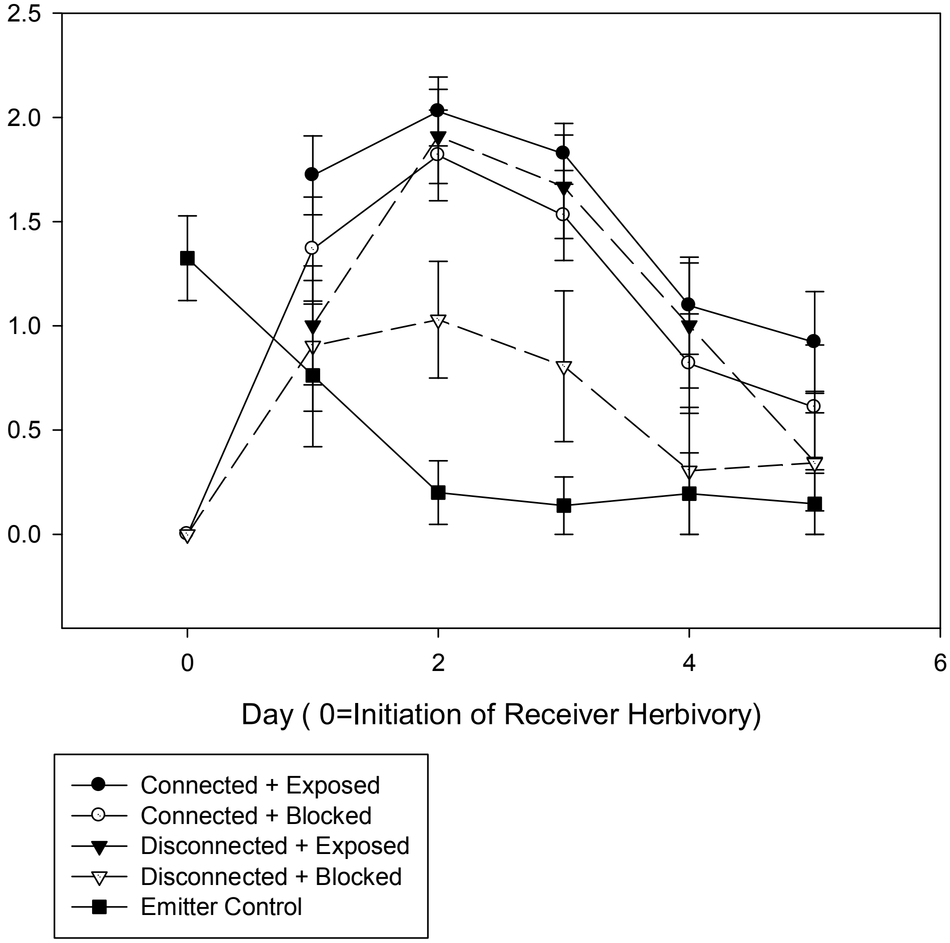
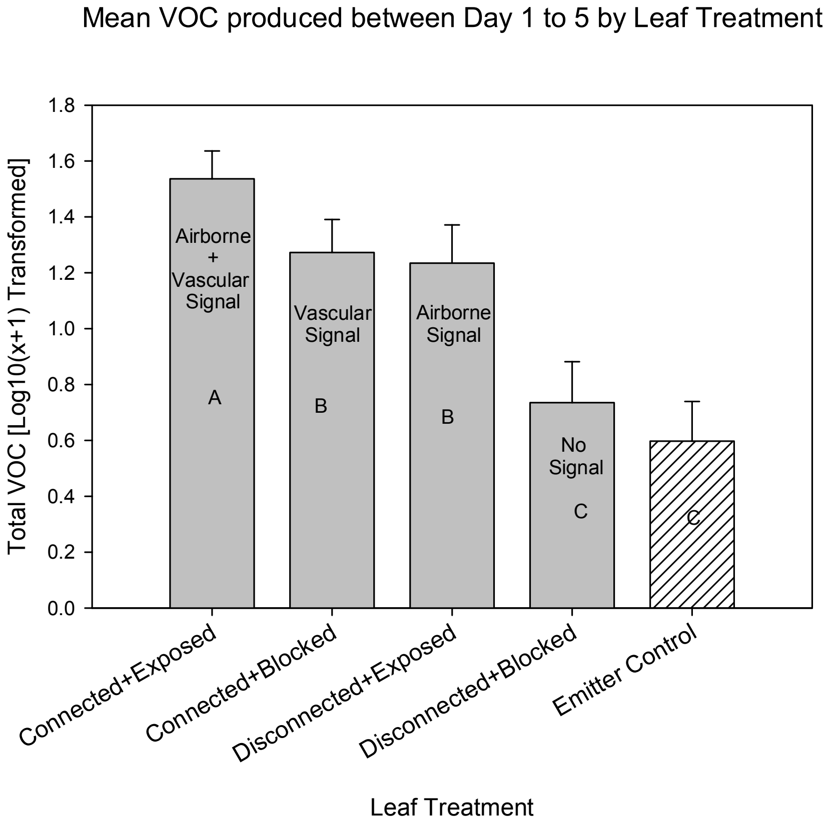


Figure 2.6 Airborne vs. vascular signal experiment: average $\log_{10}$ transformed total VOC production $\pm$ SE based on receiver leaf treatment. Emitter control was also included. Different letters indicate significant differences in planned contrast with Tukey's HSD adjustment.



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CHAPTER THREE: THE CONCURRENT EFFECTS OF A PLANT-BASED RESOURCE AND HERBIVORY-INDUCED PLANT VOLATILES ON THE COLONIZATION AND PREDATION OF AN OMNIVOROUS INSECT PREDATOR IN THE FIELD

ABSTRACT

Many plants emit a blend of volatile organic compounds following herbivory. These blends sometimes attract omnivorous insect predators in search of herbivorous insect prey. Many types of plant tissues are consumed by and may also attract omnivorous insects. Therefore, herbivory-induced plant volatiles and plant-based resources are both capable of serving as cues to facilitate antagonistic interactions between omnivorous and herbivorous insects through the attraction of the former. However, because volatiles represent information while plant-based resources are actual food items, each may be perceived and utilized differently by omnivores. I hypothesized that 1) plant-based resources may interact with herbivory-induced volatiles to increase the attraction of omnivores to damaged plants but 2) also reduce subsequent predation against herbivores by the same omnivores due to their consumption of plant-based resources. Here, I performed a series of field experiments over two consecutive years coupling an omnivore–herbivore-host plant natural tritrophic system with a locally co-occurring plant that provided additional resources to the omnivore. I simultaneously manipulated the presence of 1) herbivory-induced volatiles and 2) plant-based resources, and subsequently measured the effects of these manipulations on 1) the rate of predation against the herbivore and 2) the local density of the omnivorous predator. Data were analyzed using ANOVA and ANCOVA models.

Plant-based resources had a sustained negative effect on predation by the omnivore, while volatiles had a transient positive effect, resulting in an overall conflicting effect omnivore. Local density of the omnivorous insect on the other hand was enhanced both by volatiles and plant-based resources. These findings indicate that plant-based resources can positively augment or interfere with the potential effects of co-occurring induced volatiles on individual aspects of omnivorous insect behavior. Research into how different plant cues are perceived and utilized by omnivorous insects, especially when encountered in unison, can provide valuable information regarding the indirect interactions between plants and predatory insects as well as the manipulation and implementation of these insects as biocontrol agents in agricultural systems.

INTRODUCTION

Plants may utilize a suite of structural and chemical defenses to directly deter herbivores. Moreover, research on plants and predatory insects has also revealed many indirect interactions between the first and third trophic tiers that may facilitate arthropod predators to attack and remove herbivorous insects from plants (Bergman & Tingey, 1979; Price *et al.*, 1980; Vet & Dicke, 1992). Price *et al.* (1980) first suggested that both plant-based resources and volatile organic compounds (VOCs) may “operate directly on natural enemies” to facilitate the attraction of predatory insects to damaged plants. More recently, Kessler and Heil (2011) additionally stated that, although VOCs represent an information-based plant indirect resistance trait, plant-based resources, on the other hand represent a resource-based indirect defense trait. Accordingly, information-based traits and plant-based resources may be perceived and utilized differently by insect predators depending on the dietary breadth of the predator and ecological context.

Many species of predatory insects are omnivorous and feed from more than one trophic tier (Agrawal, 2003; Singer & Bernays, 2003; Lundgren & Harwood, 2012). In turn, many plants produce resources that attract omnivorous arthropod predators and are used as food: leaves, seeds, floral and extrafloral nectar, pods, and pollen (McMurtry & Scriven, 1966a; Naranjo & Stimac, 1985; Wei & Walde, 1997; Cottrell & Yeargan, 1998a; Eubanks & Denno, 1999; Bronstein *et al.*, 2006; Frank *et al.*, 2011; Lundgren & Harwood, 2012; Pumarino & Alomar, 2012; Amaral *et al.*, 2013). Increases in fecundity and/or survival (Delima & Leigh, 1984; Limburg & Rosenheim, 2001; Begum *et al.*, 2006; Robinson *et al.*, 2008; Lundgren & Seagraves, 2011) as well as in density (McMurtry & Scriven, 1966a; McMurtry & Scriven, 1966b; Cottrell & Yeargan, 1998b; Eubanks & Denno, 2000; Rebek *et al.*, 2005) of predators are often observed when comparing experimental treatment groups with available plant-based resources vs. those without. Not only has the presence of these resources on host plants affected predator survival and density, but similar effects on omnivores have also been detected when the resources were instead on nearby non-host plant species (Naranjo & Stimac, 1985; Cottrell & Yeargan, 1998b; Pumarino *et al.*, 2012). The increases in survivorship suggest that omnivory and resource switching may permit greater survival when prey is scarce

(Naranjo & Stimac, 1985; Lundgren & Seagraves, 2011; Pumarino & Alomar, 2012), and the density increases indicate potential attraction (Pumarino *et al.*, 2012) and population growth (Cottrell & Yeargan, 1998a) of predatory arthropods as a result of having access to these resources. Together, these findings suggest that behaviors that enhance the acquisition of plant-based resources may be common among omnivorous predators (Singer & Bernays, 2003).

In addition to resources, most plants also produce and emit a suite of VOCs when damaged by herbivores (Karban & Baldwin, 1997; Agrawal & Karban, 1999; Hare, 2011; Kessler & Heil, 2011). When exposed to these volatiles, some insect predators are attracted to the emitting plants, and may then attack suitable herbivores on those plants (Dicke & Baldwin, 2010). Initially, the emission of these compounds was considered by some to be an adaptive response by plants to actively recruit insect predators (Dicke & Sabelis, 1988; Dicke, 1999). However, few empirical studies have detected the necessary increase in plant fitness to justify the adaptive value of these compounds as a form of indirect defense against herbivory (van der Meijden & Klinkhamer, 2000; Baldwin *et al.*, 2006; Dicke & Baldwin, 2010; Hare, 2011; Karban, 2011; Kessler & Heil, 2011, but see Kessler & Baldwin, 2004). Recent attempts to corroborate the effect of plant volatile cues on predator behaviors between the laboratory and field environments using identical (or similar) species have at times either failed to attract the same predators in the field as in the laboratory, or other times failed to attract more predators compared to undamaged controls in the field (Bernasconi Ockroy *et al.*, 2001; Hunter, 2002; Hare & Sun, 2011a). In addressing the disparity between theoretical predictions and empirical results, some reviewers have suggested that the simplification of ecological context in moving from a field environment into the laboratory may alter the way that VOCs are perceived by insect predators, which may in turn elicit artificial responses by predators (van der Meijden & Klinkhamer, 2000; Hunter, 2002; Kigathi *et al.*, 2009).

Given these inconsistencies, the effects of volatile cues on the behaviors of insect predators may depend strongly on the predator species and the ecological context. Kessler and Heil (2011) suggest that information-based plant indirect resistance traits and resource-based indirect defense trait in plants should experience different selection pressures based on differences in physiological and ecological costs. More importantly, traits in each category also may be perceived and utilized differently by insect predators. Therefore, the presence of additional plant cues, such as nearby plant-based resources, may interact with volatile cues to affect the overall behavior and decision-making process of insect predators. In fact, when the natural environment is considered, one potential outcome is that combination of volatile cues and plant-based resources is an improvement in the overall quality of the host plant as perceived by the predatory insect, thereby increasing predator attraction and herbivore removal. Alternatively, attraction of predators to volatile cues instead may be offset by the utilization of the available plant-based resources, leading to the reduction in overall removal of herbivores on the host plant independently of the attraction of predators to plant volatiles and resources. Previous results suggest that the initial attraction of natural enemies to plant resources as detected through increases in density may not be indicative of the ultimate magnitude of predation by the predator (McMurtry & Scriven, 1966a; McMurtry & Scriven, 1966b;

Cottrell & Yeargan, 1998a; Lundgren, 2009; Seagraves *et al.*, 2011; Calixto *et al.*, 2013; Urbaneja-Bernat *et al.*, 2013).

Based on the predictions above, I designed and carried out a set of related studies in both natural field and controlled greenhouse environments in order to examine specifically the impact of both an informational and a resource-based cue on the recruitment and predation in an omnivorous predator. By studying how these plant-based cues may indirectly affect the direct antagonistic interactions between predator and prey insects, I aim to clarify and bring to the foreground how traditionally background factors that are a part of the ecological context can exactly impact predator-prey dynamics in tritrophic systems.

Locally, *Geocoris pallens* (Hemiptera: Geocoridae) is the main predator of the specialist herbivore, *Lema daturaphila* (Coleoptera: Chrysomelidae), and both species colonize Jimsonweed, *Datura wrightii* (Solanaceae) (Hare, 2007). *G. pallens*, along with its congeners, represent a family of well-known omnivore that consumes many types of plant resources as well as insect species (Dunbar & Bacon, 1972; Tamaki & Weeks, 1972; Crocker & Whitcomb, 1980). When lima bean pods were offered to a conger species, *G. punctipes*, per-capita predation against herbivores decreased while density levels increased in comparison to pod-free treatments (Eubanks & Denno, 2000). Such results indicate a potential independence between the effects of plant resources on predation and density in this species.

Moreover, the dynamics of VOC emission of *D. wrightii* and its indirect influence on the predation by *G. pallens* have been well characterized in the tritrophic interaction among *G. pallens*, its herbivore, and the host plant. VOC production varies with ontogeny in *D. wrightii* and decreases to cessation over the course of the field season (Hare, 2010). Interestingly, whereas predation by *G. pallens* appears to be positively influenced by herbivore-induced VOCs when they are emitted by young *D. wrightii*, the subsequent reduction in VOC emissions as *D. wrightii* age do not negatively impact *G. pallens* predation, which remains high over the rest of the field season (Hare & Sun, 2011a). Presumably, VOCs emitted by herbivore-damaged *D. wrightii* serves as an initial colonization cue for *G. pallens*, but is neither present nor required once the predators have colonized the host plant. Given both the variation in *G. pallens* predation with respect to VOCs as well as the variation in predation of *G. punctipes* in the presence of plant-based resources, the co-occurring influence of both plant resources and volatile cues may produce unpredictable effects on the colonization of plants by *G. pallens* and subsequent predation.

For the field study described in this chapter, I conducted the following experiments to address the question of how both volatile cues and plant-based resources may interact to affect the behavior of an omnivorous insect predator using a natural model system. By quantitatively and qualitatively manipulating the availability of both VOCs on the local host, *Datura wrightii*, and plant-based resources provided by *Lotus strigosus* (Fabaceae) in concert, I measured the variation in 1) predation and 2) density of the omnivorous insect predator, *Geocoris pallens*, in the field in order to gain insight into how these variations translate into differences in the colonization and predation of *G. pallens*.

METHODS

Study system: The native, undomesticated perennial, *Datura wrightii*, was utilized as the host plant. Locally, *D. wrightii* is primarily attacked by the herbivore, *Lema daturaphila*, a specialist leaf beetle. Following herbivory by *L. daturaphila*, damaged *D. wrightii* emits a suite of VOCs (Hare, 2007; Hare & Sun, 2011b). In turn, the insect predator, *Geocoris pallens*, is attracted to the VOCs produced by *D. wrightii* and may colonize the plant. Subsequently, *G. pallens* will attack and consume eggs as well as early larval stages of *L. daturaphila* on colonized *D. wrightii* plants (Hare & Sun, 2011a). In addition to *L. daturaphila*, other *Geocoris* spp. have been known to feed on a wide range of insects (Crocker & Whitcomb, 1980; Hagler & Cohen, 1991), as well as many types of plant-based resource such as pollen, extrafloral nectar, seeds, bean pods, and leaves (Dunbar & Bacon, 1972; Tamaki & Weeks, 1972; Cohen & Debolt, 1983; Naranjo & Stimac, 1985; Eubanks & Denno, 1999).

The native *Lotus strigosus* provides a suitable plant-based resource for *G. pallens*. In southern California, *L. strigosus* co-occurs with *D. wrightii*, and the small pods produced by the prostrate herb were readily fed upon by *G. pallens*. Furthermore, *G. pallens* fed *L. strigosus* pods survived longer than those under the starvation treatment in laboratory bioassays (9.65 ± 1.34 days vs. 5.85 ± 1.71 days, respectively).

Experimental design and plot establishment: Natural experiments were conducted using field plots in Agricultural Operations near the University of California, Riverside campus over two consecutive field seasons (2012 and 2013) in order to test for consistency in the predation response over time. A single (ca. 20 x 46m) plot was established in 2012 for the first set of predation experiments, and two similarly sized plots were established the following year for both the second set of predation experiments as well as the density experiments. All field plots were adjacent to a four-year old population of *D. wrightii* plant that hosted overwintering populations of both *L. daturaphila* and *G. pallens*; this area was maintained under natural (i.e. irrigation-, insecticide-, and herbicide-free) conditions since establishment.

D. wrightii seeds were planted in December of the previous year; seedlings were maintained in greenhouses before being transplanted into field plots in April at the four-to-six leaf stage. *L. strigosus* seeds also were germinated in December (methods in Sachs *et al.*, 2010) then maintained in greenhouses over winter before being co-transplanted with *D. wrightii*. During transplantation, experimental patches were constructed either of a singly transplanted *D. wrightii* plant or of a *D. wrightii* plant and a *L. strigosus* plant co-transplanted in close proximity (3-5cm). *D. wrightii*-only patches were supplemented with green colored twist-ties in order to mimic the structural complexity of *L. strigosus* but lacked pods produced by mature *L. strigosus.* There was no significant effect of the number of pods present at each *D. wrightii* + *L. strigosus* replicate on *G. pallens* predation in 2012 ($F_{1,62} = 2.974$, $P = .090$, range: 104-378 pods), so I instead used the presence or absence of *L. strigosus* pods (produced starting in May of both years) in experimental patches represented the discrete treatment levels of the plant-based resources.

Twelve alternating experimental patches were established within each row in a given field plot; six patches were constructed on the north end of the row and the other six at the south end. Within each row patches were spaced 3m apart, and the mid-row distance (large gap between north and south patch groups) was approx. 10m. Experimental rows were established in pairs and every pair of experimental rows was separated by an empty row. Each experimental row pair contained 24 patches. Once transplanted, all plant patches were protected against natural insect colonization and herbivory with whole plant cages (1m x 1m mesh sleeve cages with 4mm^2 mesh openings) that were secured around circular plastic fence frames buried around each experimental patch. Furrow irrigation was administered twice per week for three weeks in order to establish plants; no further watering took place over the summer to ensure natural field conditions.

VOC induction, collection, and analysis: To generate variation in VOC induction and emissions from plants in each experimental block, cages were removed from all patches within the block and half of the *D. wrightii* plants (12/24 plants) were experimentally damaged with *L. daturaphila* adults confined to a group of terminal leaves (range: 6-10 leaves) on each plant using a small cloth cage. Similar-aged leaves on the rest of the *D. wrightii* plants remained undamaged but were also caged, both treatments lasted for 48hrs (similar to methods in Hare & Sun, 2011a). These manipulations constituted the volatile induction treatment. To avoid accidental plant induction (through incidental herbivore infestation and abiotic factors), induced VOCs were collected from focal leaves on all *D. wrightii* plants in the experimental block following the herbivory (or control) treatment in order to quantify volatile production. Because I physically excluded *G. pallens* from plants until the start of herbivory treatment, pre-damage (baseline) VOC production on experimental plants could not influence subsequent *G. pallens* attraction and predation prior to herbivory treatment and was therefore not measured.

Detailed methodology of field VOC collection was previously described in Hare and Sun (2011a). Succinctly, a polyester cooking bag (Terinex, Bedford, UK) with a borosilicate glass trap filled with 25mg of Super-Q (Alltech, State College, PA, USA) was placed over the treatment leaves of each experimental *D. wrightii* plant and sealed around the leaf branch using a twist tie. Filtered air was then continuously passed through the bag and trap using a push-pull system driven by two 12V portable air pumps (Model # MOA- P125-JH, Gast Manufacturing, Benton Harbor, MI, USA) powered by a 12V deep cycle marine battery. Airflow through each bag was independently regulated with a pair of flow regulators (Aalborg Instruments, Orangeburg, NY, USA) at 1.0 l/min. Two aerations were conducted on each experimental block over the course of a single day; all 12 *D. wrightii* plants on either the north or south end of a block were aerated for 90min in each aeration. Across all experiment dates, the first aeration was conducted between ~10.00h-11.30h and the second between ~13.00h-14.30h; the starting order between north and south patch groups was alternated between dates.

Following each aeration, VOC traps were collected, wrapped in aluminum foil, and stored at -20ºC until elution. After the removal of the aeration bag from treatment leaves on each plant, the twist tie was re-wrapped around the branch to identify the treatment leaves for subsequent assay. Following either the predation or density experiments (described above), all treatment leaves were harvested and dried in the lab (at 80 ºC for 48hrs) to obtain leaf dry weight.

VOCs were eluted from traps into 250 µl glass sleeves in autosampler vials using 150 µl of CH_2Cl_2 containing 4ng/µl of the internal standard, 1-bromoheptane, and then sealed with a PTFE-lined rubber septa cap. One µl of each sample was analyzed with a Hewlett-Packard 5890 gas chromatograph equipped with a DB-5 column (30 m, 0.25 mm ID, 0.25 µm film thickness, J & W Scientific, Folsom, CA, USA) following methods described in Hare (2010). Compound identifications were made with authentic standards purchased from Sigma-Aldrich and associated suppliers, or obtained from Dr. J. G. Millar (methods: Hare, 2007). Peak quantification, in units of $ng \cdot g^{-1}$ leaf (dry weight)$\cdot h^{-1}$, was made using Agilent ChemStation® software based on comparison between peak heights and the internal standard. In 2012, VOCs were collected from five experimental blocks, while eight blocks were used in 2013. In total, VOCs were collected from 305 plants after accounting for accidental sample losses.

Predation experiments: To measure the effect of the experimental manipulations on predation in *G. pallens* under natural setting, I collected and used natural egg masses attached to the ovipositional leaves from *D. wrightii* plants outside of experimental plots. I then used a cork borer to punch out leaf disc (1 cm dia.) containing the egg mass, and stored them under refrigeration for no more than two days until the bioassay. I only selected and used egg masses that had between 10-25 eggs, but did not attempt to further standardize egg number per mass to avoid unnecessary manipulation of the egg masses that may then impact their resistance to wind and high temperatures in the field. Following VOC collection, two leaf discs with egg masses were individually attached to the underside of two treatment leaves on each *D. wrightii* plant within the experimental block using a synthetic resin glue (UHU Adhesives, Saunders Mfg. Co., Readfield, ME, USA; preliminary experiment showed no evidence of *G. pallens* being attracted to or repelled by the glue). leaf disc on each plant was then enclosed in a 'closed' clip cage constructed from a spring hair clip and a 2.5 cm dia plastic vial glued to a rubber washer (1.8 cm I.D., 2.5 cm O.D.); the cap on each vial was cut out and covered with brass screen to provide ventilation. The other egg mass on each plant was enclosed in an 'open' cage constructed from the hair clip and rubber washer only. After 48hrs in the field, I removed the egg masses and leaves to measure damage under the microscope in the lab. Because egg mortality in 'closed' cages varied significantly in terms of sample date ($F_{9,226} = 2.70$, $P = 0.005$), I used mean 'closed' cage egg mortality values for each sample date to correct for baseline egg mortality at each open cage during the same sample date (Hare & Sun, 2011a).

Field density experiments: To measure the effect of the experimental manipulations on *G. pallens* density in the field, the number of *G. pallens* in each patch was counted at 24hrs and 48hrs following aeration. For each experimental patch, a circular ring (30cm dia.) was constructed from a thin strip of paper and placed at the base of the patch and all visible *G. pallens* in the focal area (on plants or soil) within the ring were counted; no attempt was made to search for hidden *G. pallens* within plant foliage to avoid disturbance to visible specimens. Each patch was monitored for 90s and any *G. pallens* that moved out of the focal area were subtracted from the patch count total.

Statistical analysis: Total VOC concentration was analyzed by year using a two-factor restricted maximum likelihood (REML) ANOVA using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc. 2011), the model included the experimental date and induction (damaged or undamaged) as fixed effects, as well as the two-way interaction between the fixed effects.

I performed a three-factor REML ANCOVA on the proportional egg mortality data from the predation experiments using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc. 2011). I used 1) *L. strigosus* pod presence and experimental date as the fixed effects, 2) total VOCs as a covariate, 3) the experimental year as a random factor, and finally, 4) I also included all two- and three-way interactions among the fixed effects and the covariate. Significant ($P < 0.05$) two-way interactions were decomposed using the SLICE option in the PROC MIXED procedure. Nonsignificant interactions were deleted from the final model.

I conducted a repeated measures three-factor restricted maximum pseudo-likelihood (Residual PL) ANCOVA on the *G. pallens* count data from the density experiments. Because the data sets consisted of count values, I used the PROC GLIMMIX procedure in SAS 9.3 (SAS Institute Inc. 2011) with a log-link function and a Poisson distribution to better fit the data set. I again used 1) *L. strigosus* pod presence and experimental date as the fixed effects, 2) total VOCs as a covariate, and 3) all two- and three-way interactions between the fixed effects and covariate were also included. Lastly, 4) because each patch was repeatedly counted at two time points, unique patch identification numbers were used as the repeated measures subject. Nonsignificant interactions were deleted from the final model.

RESULTS

VOC production: Overall volatile production varied both with respect to experimental date and herbivory treatment across both years (all $P \leq 0.001$, Fig. 3.1). In each year, VOC emissions were higher during the month of June and decreased significantly by late June to July (both $P < .0001$, Fig. 3.1), and herbivore damaged plants produced more VOCs than undamaged control plants (both $P \leq 0.004$, Fig. 3.1). More importantly, the interaction between experimental date and herbivory treatment was also significant in each sample year (both $P \leq 0.002$, Fig. 3.1), showing that pattern of VOC induction among plants damaged by herbivores vs. undamaged plants changed with respect to date. In 2012, herbivore damaged *D. wrightii* plants emitted more than four times the VOCs compared to undamaged plants on 4 June, and VOC emissions by damaged plants were more than eleven fold higher than control plants on 13 June ($F_{1,127} = 12.36$, $P < 0.001$ and $F_{1,127} = 24.63$, $P < 0.001$, respectively, Fig. 3.1A). Subsequent sampling did not detect significant differences in VOC production between the damaged and control plants (all $P \geq 0.092$, Fig. 3.1A). In 2013, VOC production patterns varied similarly. During the first two sample dates, 6 June and 19 June, VOC production between damaged and undamaged plants differed by more than eight and three fold ($F_{1,144} = 29.56$, $P < 0.001$

and $F_{1,144} = 12.03$, $P <$s 0.001, respectively, Fig. 3.1B). VOC production no longer differed during the subsequent six dates (all $P \geq 0.617$, Fig. 3.1B).

Predation by G. pallens: The presence of *L. strigosus* pods consistently reduced predation against *L. daturaphila* eggs across all sample dates in both sample years (e.g. 0.78 ± 0.03 vs. 0.51 ± 0.02, $F_{1,193} = 12.52$, $P < 0.001$, Fig. 3.2). Predation was affected differently by the available VOC over time as detected through the significant interaction between the total VOC covariate and experimental date ($F_{9,193} = 3.62$, $P < 0.001$, Fig. 3.3). In both years, the total VOC concentration increased *L. daturaphila* egg mortality in June, but by July, VOC emissions were nearly absent comparatively and no longer influence *G. pallens* predation, which independently remained at high levels (not shown). These findings were similar to results previously described in Hare and Sun (2011a). The pattern of reduction in predation strength due to *L. strigosus* pods did not interact with the presence and subsequent decline of VOC cues.

Mean proportional *L. daturaphila* egg mortality due to predation ranged from 0.58 ± 0.04 to 0.73 ± 0.02 in 2012 and 0.51 ± 0.03 to 0.77 ± 0.01 in 2013; in general, predation rates increased over the season in both years ($F_{9,193} = 13.02$, $P < 0.001$ Figs. 3.2 and 3.3), and the range of mortality was greater in June relative to July for both years. Finally, the mean proportional natural *L. daturaphila* egg mortality in ‘closed’ cages ranged from 0.03 ± 0.01 to 0.06 ± 0.02 in 2012 and 0.05 ± 0.01 to 0.10 ± 0.02 in 2013, these mortality values mostly increasing over the sampling seasons as well (not shown).

Density of G. pallens: The presence of *L. strigosus* pods positively influenced *G. pallens* colonization; this effect was once again significant across all sample dates ($F_{1,47} = 25.71$, $P < 0.001$, Fig. 3.4). Total VOCs once again interacted significantly with sample date to affect *G. pallens* density ($F_{2,47} = 3.48$, $P = 0.039$), indicating differences in the colonization of *G. pallens* due to VOC emission across the three sampling date. On 26 June, density correlated positively with VOC emission (Fig. 3.5), although on the latter two sample dates, 10 July and 24 July, reduced levels of VOC emission (three fold and nine fold reduction compared to 26 June, respectively) no longer influenced *G. pallens* density (not shown). Finally, although mean *G. pallens* density in the field decreased from 1.70 ± 0.22 to 0.98 ± 0.13 over the season, the variation was not significant ($F_{2,47} = 2.60$, $P = 0.085$).

DISCUSSION

The effect of plant-based resource on the interaction between VOCs and omnivorous natural enemies: Although VOCs (or plant damage) and *L. strigosus* pods both enhanced the density of *G. pallens*, the two factors had conflicting effects on predation. Furthermore, although the plant-based resource influenced *G. pallens* over the entirety of the field experiment, the effect of VOCs on *G. pallens* was transient and restricted to earlier sample dates, similar to previous results (Hare & Sun, 2011a).

The presence of *L. strigosus* pods reduced predation against *L. daturaphila* eggs in both field seasons. In contrast, VOC cues only increased predation in June. Because volatiles have been shown to also play a significant role in influencing predation patterns in *G. pallens* during the earlier portion of the *D. wrightii* field season (Hare & Sun, 2011a), it might have been possible that predation was influenced by both VOC cues and plant-based resources at that point in the field season. But as the production of VOCs rapidly decreases over the field season, this interactive effect was not detected in this study. The variation in predation over the remaining sample dates across both years can be attributed to the presence or absence of *L. strigosus* pods.

Plant-based resources may be a necessary dietary component for some species of omnivores (Lundgren & Seagraves, 2011) and may be consumed by other omnivores as nutritionally inferior food resource (Lundgren & Harwood, 2012). The presence of *L. strigosus* pods in this study may have reduced the necessity for *G. pallens* to consume *L. daturaphila* eggs. Moreover, the potential preference for herbivorous prey may even be reduced following the consumption of plant-based resources, leading to a shift in preference against predation (Weber & Lundgren, 2011).

The influence of diet breadth on natural enemy in other systems: Plant-based resources were capable of producing positive effects on both predation and density in natural enemies (Amaral *et al.*, 2013). Interestingly, several studies have also found opposing effects of plant-based resources on generalist predation and density. For example, Seagraves *et al.* (2011) found that although the addition of sucrose enhanced local coccinellid density across all sites, these density increases did not necessarily translate to higher control against aphids. Similarly, predation by *Nesidiocoris tenuis* (Hemiptera: Miridae) against eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae) was reduced with the addition of sucrose solution in mixed diet bioassays (Urbaneja-Bernat *et al.*, 2013). And when mixed diets consisting of both pollen and several herbivore prey species were offered to the generalist predator, *Orius insidiosus* (Anthocoridae), longevity, ovipositional period length, fecundity, and survival increased, but predation was unaffected (Calixto *et al.*, 2013). Finally, Wei and Walde (1997) saw that the presence of pollen from *Typha latifolia* (Typhaceae) significantly decreased the predation rate of *Typhlodromus pyri* (Acari: Phytoseiidae), a predatory mite, against its prey, *Panonychus ulmi* (Tetranychidae), but only at high (prey) densities. These findings again suggest the need for herbivore prey may be relaxed or reduced following the consumption of plant-based resources (McMurtry & Scriven, 1966a).

Together, these differences between the two factors present an interesting dichotomy (Kessler & Heil, 2011); although information-based plant indirect resistance traits such as VOCs enhanced both the predation and colonization by *G. pallens*, a resource-based indirect defense trait (in this case, *L. strigosus* pods) instead reduced predation while also increasing colonization. These findings indicate that indirect resistance and defense traits may produce independent conflicting variation on the predation and colonization of an omnivorous natural enemy.

Basis of omnivory: It is clear from this study that the omnivorous nature of *G. pallens* influenced both its colonization and foraging. Moreover, these findings suggest that omnivory, defined as feeding from more than one trophic level (e.g. Agrawal, 2003), has important implications for various behavioral characteristics of omnivores. Although the evolution and maintenance of omnivory in arthropods itself remains largely understudied and poorly understood, recent research has produced many testable hypotheses regarding the causes and consequences of omnivory in arthropods. These hypotheses on omnivory can be divided into three major categories: those that are based upon resources, physiology or behavior.

Resource requirements may drive and maintain omnivorous feeding. Although extensively studied across many insect taxa (Mead *et al.*, 1994; MacRae & Croft, 1997; Bommarco, 1999; Stamp, 2001), the problem of prey limitation underlies the problem of restrictions in the amount of fundamental resources available to insect predators such as amino acids, proteins, and carbohydrates that may in turn affect performance. Therefore, predators may turn to omnivory (i.e. feeding on plants) in order to supplement the limiting resource. In terms of nitrogen, predatory insects typically need a higher nitrogen intake compared to herbivores (Fagan *et al.*, 2002); the additional nitrogen requirements may be met through ingesting nitrogen-rich components of plants in addition to insect prey (Denno & Fagan, 2003). In terms of carbohydrate limitations, Denno and Fagan (2003) argue that as prey items become more scarce in relation to the searching energy cost, predators may instead become limited in terms of energy, which may also be alleviated by ingesting carbohydrates (i.e. sugars) from plants.

The nutritional requirements of *Geocoris* spp. are somewhat debatable; Cohen (1985) found that an artificial diet high in nitrogen (18.7% protein : 9.9% fats : 3.1% carbohydrate) produced the best results in the mass rearing of *G. punctipes*. Yet, results of another study found a diet consisting of *Oecophylla smaragdina*, (Hymenoptera:

Formicidae) pupae and fresh twigs that was high in carbohydrates with intermediate levels of nitrogen (17.8% protein : 8.2% lipid : 29.1% carbohydrate) was capable of rearing *G. ochropterus* (Mukhopadhyay & Sannigrahi, 1993). Nevertheless, both diet studies suggest that *Geocoris* spp. may have nitrogen and/or carbohydrate requirements that could be met with supplemental plant feeding.

Physiologically, omnivorous arthropods differ both from herbivores as well as from carnivores, with specialized morphological and enzymatic adaptations to allow for the consumption of both plant and animal resources. Certain morphological characters such as stylets and digestive tracts are unique and often feature aspects found both in predators and herbivores (Goodchild, 1966; Cohen, 1996). It has been theorized that the ability of certain herbivores to consume and digest plant tissues rich in nitrogen (seeds and pollen) may have been an evolutionary precursor to predation (Cobben, 1979; Sweet, 1979). Indeed, when tested in a meta-analysis across several family-level groups with both herbivore and omnivorous insects, Eubanks *et al.* (2003) demonstrated a statistically significant correlation between the consumption of specialized plant parts and omnivory, indicating the potential of preadaptation in herbivores that consumed nitrogen rich plant tissues to become omnivorous. When considered in light of these results, the fact that granivory is well represented in both the superfamily *Lygaeoidea* as well as to a lesser degree in its subordinate family, *Geocoridae*, could certainly be inferred as evidence for the evolutionary onset of omnivory across most *Geocoris* species.

Aside from resource limitation and physiological adaptations, several hypotheses have also been raised regarding various behavioral adaptations that may select for the evolution of and/or maintenance of omnivory. Of particular interest to *Geocoris* is the theory that omnivory may be advantageous in terms of avoiding predators during foraging. Work on omnivorous ant species suggests predator avoidance may lead to switching food consumption and omnivory (Nonacs & Dill, 1990; Folgarait & Gilbert, 1999). There has been some evidence that all life stages of *Geocoris* spp. are attacked and consumed by lynx spiders in the laboratory (Guillebeau & All, 1989). Locally, jumping spiders, *Habronattus pyrrithrix*, (Araneae: Salticidae) are present in the field (Gassmann & Hare, 2005) so predator avoidance may factor into the foraging behavior of these insects. When considered in the context of the experimental system used for this study, *L. daturaphila* eggs are typically oviposited on younger leaves and unopened flowers and therefore away from the soil surface; whereas the pods of *L. strigosus* were fairly prostrate to the soil surface due to the phenology of the plant. Therefore, predation risk may have indeed been a factor in the foraging choices made by *G. pallens*.

CONCLUSION

I demonstrated that, whereas plant-based resources and VOCs emitted by the damaged host plant may increase the density of an omnivorous natural enemy, the presence of the same plant-based resource may also negate the expected density-dependent increase in predation against the herbivore prey. Because volatile cues in *D. wrightii* are largely transient over the field season such that their production is limited to the earlier portion of plant development, these compounds may function as cues for utilization by *G. pallens* during initial host plant colonization and prey location (Hare & Sun, 2011a).

The presence of a suitable plant-based resource in addition to the presence of potential insect prey (detected through the presence of VOCs) may increase the quality or value of a specific plant patch, thereby increase the density both through colonization as well as subsequent reproduction (McMurtry & Scriven, 1966a; McMurtry & Scriven, 1966b; Eubanks & Denno, 2000).

In contrast, the presence of *L. strigosus* plant-based resources appeared to have continuously reduced predation of herbivore eggs but simultaneously increased patch colonization rates and density throughout the experimental period and may therefore function as a more persistent cue against emigration. More importantly, the presence of the plant-based resource cue produced variable effects on the predation of *G. pallens* and altered the antagonistic predator-prey dynamic between *G. pallens* and *L. strigosus*.

Broadly, the findings from this study may have important implications for the application of omnivores as biocontrol agents in agriculture. The usage of resource lures and/or lure plants in order to bolster the density of desirable natural enemies is a common practice in many agriculture operations. However, as shown here, the presence of informational cues may attract omnivore predators and thereby increase the subsequent predation on the herbivore pests, leading to the desirable biocontrol outcome. Other types of resource cues may similarly attract omnivores but instead offset the increase in predator density with a total reduction in the predation strength, causing a potential net decrease in the overall control of pests. Therefore, such interactions must always be carefully researched on a case by case basis in order to lead to the successful control of pest insects.

Figure 3.1 field *D. wrightii* VOC collection – mean (± S.E.) total VOC (ng·g^{-1}leaf (dry wt.) ·h^{-1}) produced by previously damaged (48hrs) or undamaged field plants. X-axis constructed using common Julian date scale, exact calendar date for each sample listed above total VOC concentrations.

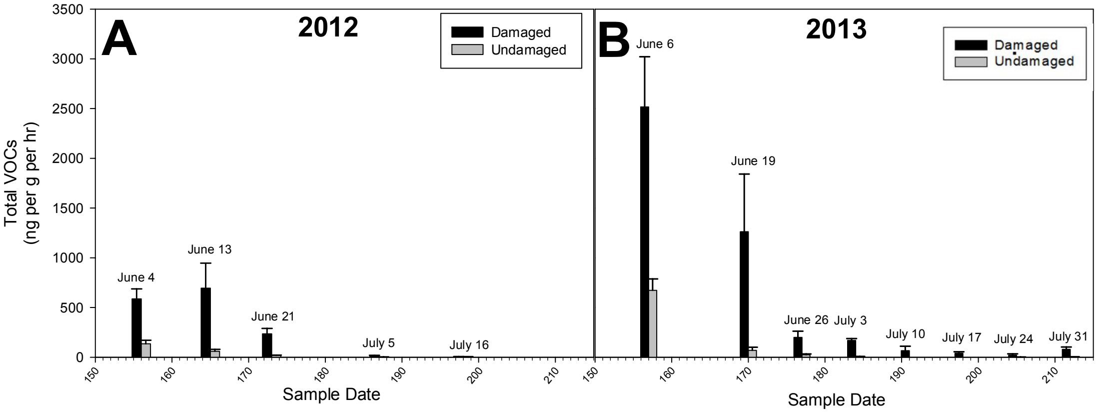


Figure 3.2 *G. pallens* predation in the field measured through the proportion of corrected mortality (± S.E.) based on the presence of nearby *L. strigosus* pods. Filled circles: predation on plants with nearby pods, open circles: predation on plants without nearby pods.

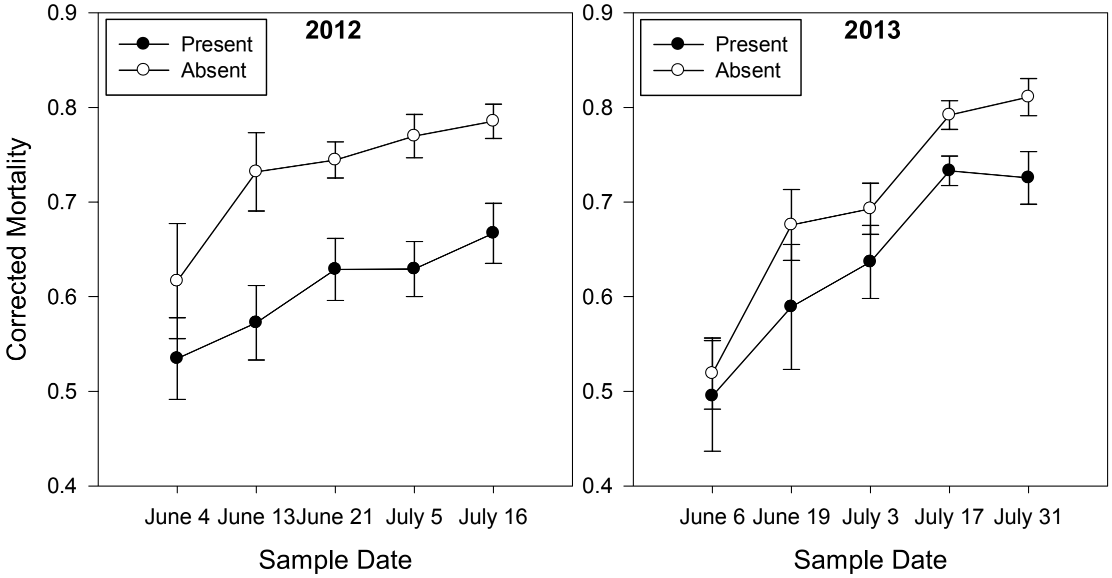


Figure 3.3A *G. pallens* predation in the field measured through the proportion of corrected mortality over field produced VOCs (ng$\cdot$g^{-1}leaf (dry wt.) $\cdot$h^{-1}) collected on each sample date in June of 2012, **3.3B** and 2013. Note difference in x-axis scale between years.

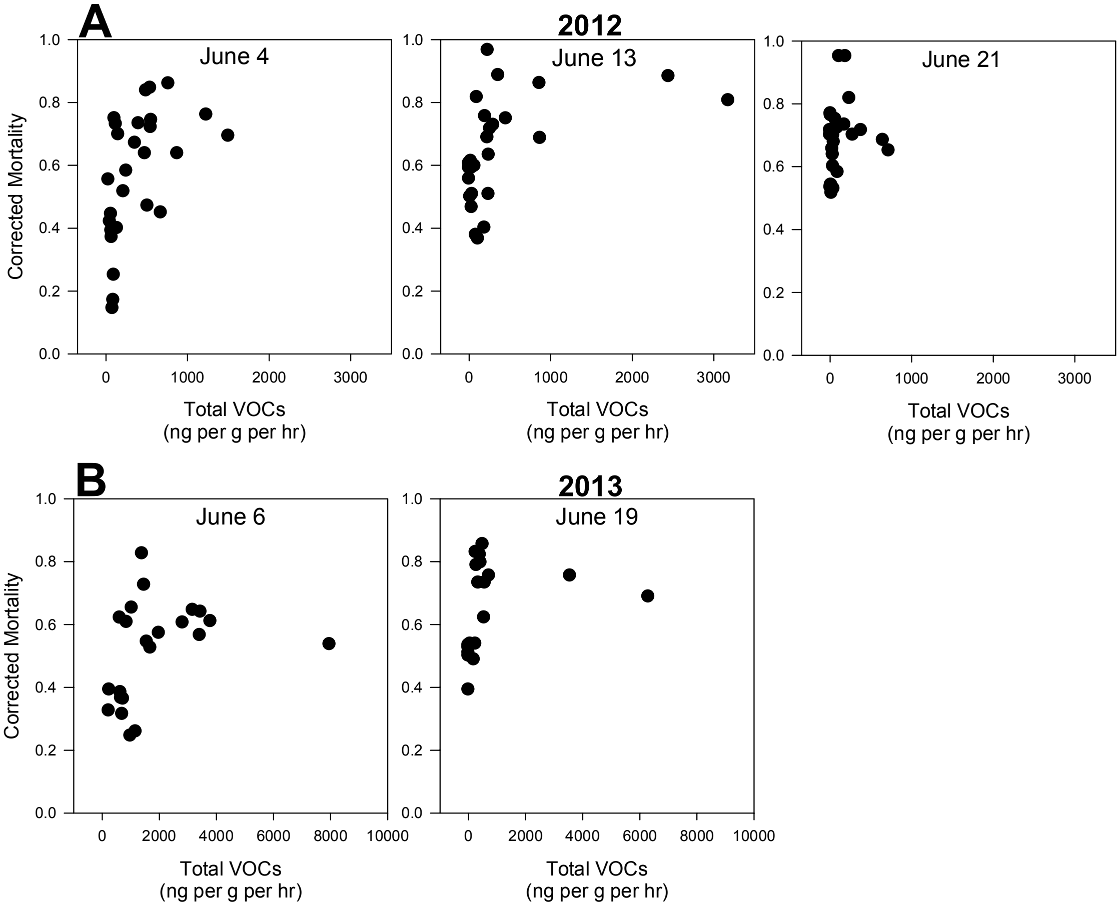


Figure 3.4 *G. pallens* density (± S.E.) based on the presence of nearby pods. Filled circles: *G. pallens* count in patches with pods, open circles: *G. pallens* count in patches without pods.

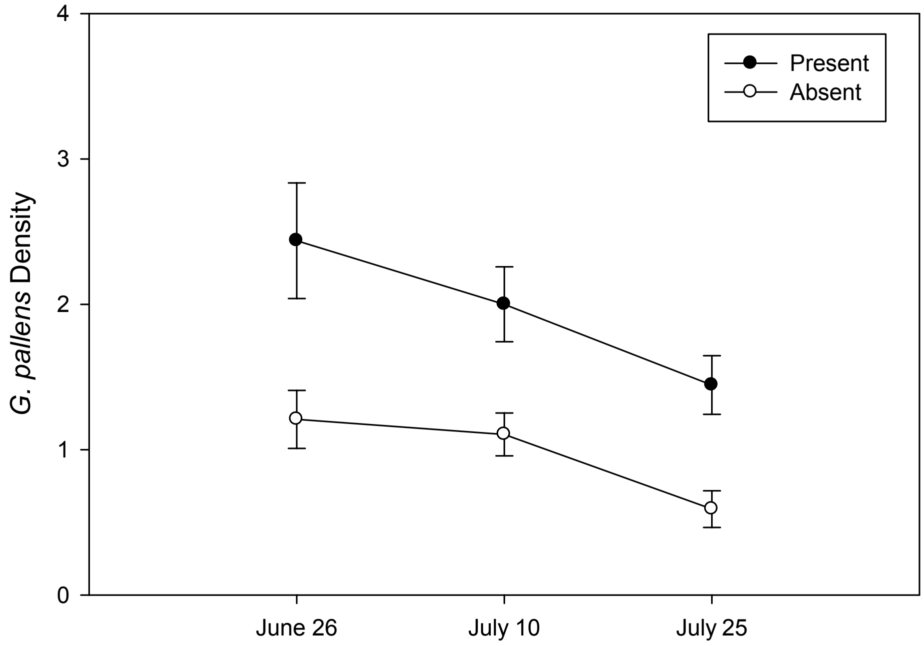
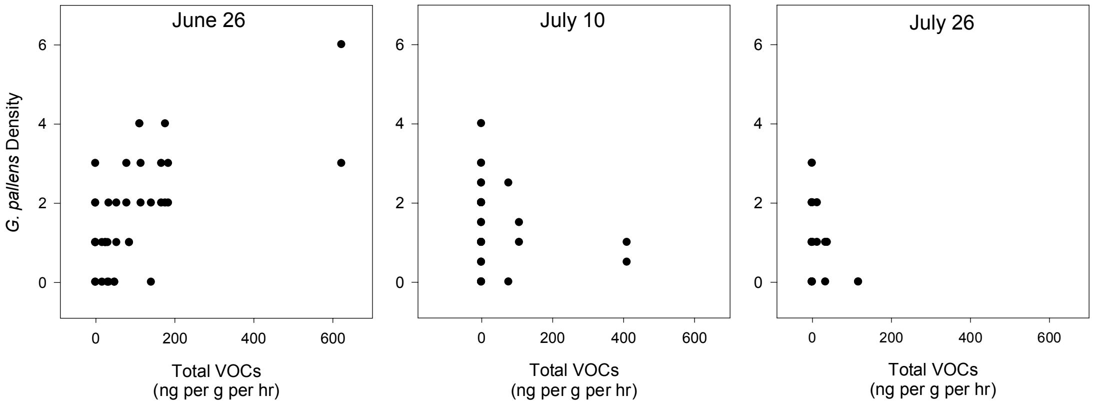


Figure 3.5 *G. pallens* density over field produced VOCs (ng·g^{-1}leaf (dry wt.) ·h^{-1}).



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CHAPTER FOUR: THE CONCURRENT EFFECTS OF A PLANT-BASED RESOURCE AND HERBIVORY-INDUCED PLANT VOLATILES ON THE COLONIZATION AND PREDATION OF AN OMNIVOROUS PREDATOR INSECT IN THE GREENHOUSE

ABSTRACT

Based on the results of the experiments described in Chapter Three, I conducted a set of follow-up experiments in a controlled greenhouse environment in order to evaluate the combined effects of both volatile organic compounds and plant-based resources on the recruitment and predation of an omnivorous predatory insect. Using methodology similar to that previous utilized in the field, I differentially damaged *Datura wrightii* (Solanaceae) that were individually planted in pots with or without *Lotus strigosus* (Fabaceae) in order to simultaneous generate variation in both plant damage (proxy for volatile emission) as well as in the availability of plant-based resources. I then released the omnivorous predator, *Geocoris pallens* (Hemiptera: Geocoridae), into the experimental cage and measured the resultant damage to herbivore eggs as well as the final distribution pattern of *G. pallens* following 48hrs of exposure.

G. pallens predation against herbivore eggs decreased strongly in the presence *L. strigosus* pods again, however, results from this study indicate that this reduction to predation ignores the typically positive effect that volatiles had on both predation and density. The effect of volatiles on predation in the absence of *L. strigosus* pods was

positive, and lastly, the presence of both factors also had positive effects on recruitment (density), these results were similar to those found in the field. These results further reinforce the independent effect that plant attractants may have on colonization vs. predation of an omnivorous insect. Finally, these studies together illustrate how just two types of background cues that make up part of the ecological context in nature can have a strong indirect effect on the predator-prey dynamics within a tritrophic system.

INTRODUCTION

Omnivorous insects that consume both insect prey and plant-based resources often respond to different types of plant cues while foraging. For example, volatile organic compounds (VOCs) produced by many plant species following herbivore damage have been experimentally shown to attract predatory insects (Vet & Dicke, 1992; Hare, 2011). The recruited predators may then attack and consume the herbivores on the emitting plants. Vet and Dicke (1992) suggested that the ability to detect and use these plant VOCs as informational cues evolved to supplement the pheromonal cues emitted by prey insects. They reasoned that VOCs should be present in the natural environment at a higher concentration and therefore would be more likely to be detected by predators at a greater distance away from the source when compared with insect pheromones due to the size difference between plants and insects. However, insect-produced pheromones should, on the other hand, be more reliable indicators of the presence of suitable prey than VOCs produced and emitted by damaged plants (Vet & Dicke, 1992). Ensuing research has indeed found that the production and emission of VOCs may function as an indirect resistance against herbivory in some plant species (Dicke & van Loon, 2000). The emission of VOCs after damage by herbivores may even increase the overall fitness of some emitting plants (Kessler & Baldwin, 2004; Schuman *et al.*, 2012); although, most studies to date either did not directly measure or failed to detect significant changes in plant fitness (Dicke & Baldwin, 2010). Due to the apparent lack of adaptive value associated with the attraction of predators by plants, the detection and utilization of these VOC cues by predatory insects have now been largely recognized as exploitative in nature, suggesting that behaviors associated with informational cues should be highly plastic, context dependent, and only exhibited when they benefit the insects (Allison & Hare, 2009; Dicke & Baldwin, 2010; Kessler & Heil, 2011). Lastly, additional research has also demonstrated that although the production and emission of VOCs following herbivory appear to be commonplace across different plant taxa, it is also a highly context-dependent process that varies both in the emission rate and the quality of VOCs produced with respect to plant ontogeny (Hare, 2010) as well as abiotic factors such as UV, soil composition, and climate (Gouinguene & Turlings, 2002; Kigathi *et al.*, 2009; Dinh *et al.*, 2013).

Alternatively, certain plant products such as pollen, nectar, and seeds may also attract omnivorous insects due to their nutritional value in the omnivorous diet (Naranjo & Stimac, 1985; Bronstein *et al.*, 2006; Lundgren & Harwood, 2012). Studies on the recruitment of omnivorous predators via these plant-based resources indicate that the presence of these resource can increase the overall density of predators on or near the source plant (Eubanks & Denno, 1999; Frank *et al.*, 2011). However, recent studies have also indicated that the increase in omnivore density might not necessarily result in a reduction of the number of herbivores that are typically consumed by these predators (Lundgren, 2009; Seagraves *et al.*, 2011; Urbaneja-Bernat *et al.*, 2013). This absence of increase in herbivore predation in association with the increase in predator density may be due to the fact that the plant-produced resources may function as nutritional substitutes for insect prey (Nonacs & Dill, 1990).

Individually, there has been extensive research on the effect of information cues or plant-based resources on natural enemy behavior. Yet, there has been no study on combined effects of both types of attractants on insect predators. In nature, it is likely that both plant-based resources and VOC cues will co-occur given the heterogeneity of plant species found in most naturally assembled plant patches. It is therefore probable that both attractants will be perceived and utilized simultaneously by omnivorous insects. I propose these experiments to examine the interactive effect of both types of attractants on the colonization and predation of an omnivore predator in a controlled environment.

I hypothesize that both the plant-based resources and VOC cues may increase the recruitment to, and the colonization of, the host plant. I predict that although the presence of VOC cues will increase the predation of the omnivorous predator against the herbivore prey, the co-occurrence of a plant-based resource will reduce the overall predation against the herbivore prey. Although the presence of these two attractants may lead to an overall increase in omnivore predator density, the two attractants may instead produce a conflicting result on the predation against the herbivore prey by the omnivore.

In the field, my experimental findings suggested that there was indeed an overall positive influence of both plant-based resource and VOC cues on the colonization of the omnivorous predator to the host plant patch. Similarly, my results indicated that VOC cues increased predation by the omnivore early when plants were young and VOC emission rates were high following damage. Plant-based resources reduced omnivore predation against herbivores consistently across time, resulting in a reduced rate of predation across the sample dates. Although both set of results matched my initial predictions, the VOC emissions by damaged field diminished rapidly and restricted the simultaneous presence of both attractants in the experiments to the earlier sample dates of both years, therefore also limiting the exposure of the omnivore to both attractants.

In these experiments, I instead used a temperature-controlled greenhouse in order to examine the specific impact of both plant-based resource and VOC cues in concert on the recruitment and more importantly, the predation, of the omnivore predators. I chose to utilize a much more controlled experimental environment and a shorter experimental duration in order to ensure that both types of attractants would be present and detected in combination by the omnivore, and that I would be able to understand based on the exposure to both attractants in concert.

METHODS

Study system: I used *Datura wrightii* as the host plant species for these experiments. In Riverside, the specialist leaf beetle, *Lema daturaphila* (Coleoptera: Chrysomelidae), is the main herbivore of *D. wrightii*. Young *D. wrightii* plants emit a suite of VOCs following herbivory by *L. daturaphila* (Hare, 2007; Hare & Sun, 2011b). *Geocoris pallens*, a local omnivorous predator, has been shown experimentally to use these VOCs in order to locate and attack the eggs as well as young larvae of *L. daturaphila* on *D. wrightii* (Hare & Sun, 2011a). Many *Geocoris* spp. also have been shown experimentally to be generalist omnivores, consuming many species of insects (Crocker & Whitcomb, 1980; Hagler & Cohen, 1991), as well as many types of plant-based resources such as pollen, extrafloral nectar, seeds, bean pods, and leaves (Dunbar & Bacon, 1972; Tamaki & Weeks, 1972; Cohen & Debolt, 1983; Naranjo & Stimac, 1985; Eubanks & Denno, 1999).

I selected the native *Lotus strigosus* as a source of the plant-based resource. *L. strigosus* has an overlapping natural distribution with *D. wrightii* (Sachs *et al.*, 2009), and produces small pods that are fed upon by *G. pallens* in preliminary laboratory assays. Laboratory bioassays showed that the consumption of these pods increased survival in *G. pallens* when compared with the starvation treatment (9.65 ± 1.34 days vs. 5.85 ± 1.71 days, respectively), suggesting that pods are a suitable alternate resource.

Experiment procedures: I conducted experiments in a temperature controlled greenhouse using large insect cages (72cm x 72cm x 72cm) constructed from mesh and PVC. I placed four individually potted *D. wrightii* plants in each cage and then placed a set of fitted particleboards over the lip the pots to create a floor approximately flush with the base of the plants. The boards were then interlocked to prevent *G. pallens* from going beneath the floor of the cage. Of the four potted *D. wrightii* plants, two pots contained a single *L. strigosus* plant that was co-transplanted with the *D. wrightii*, while the other two pots only contained a single *D. wrightii* plant each. During an experiment, I added loosely bundled green plastic twist-ties to the base of the single *D. wrightii* plant pots in order to simulate the spatial complexity of *L. strigosus* without the presence of the pods. Each cage represented one experimental replicate. The study was conducted over eight experimental blocks; each block consisted of four to five cage replicates.

Prior to starting an experiment, I used *L. daturaphila* adults to differentially damage two out of four *D. wrightii* plants in each replicate cage (each plant received two adults in small mesh cages for 48 hrs). The damage treatment was always administered to a single *D. wrightii* plant and one *D. wrightii* plant in a co-transplant pot. Leaves of the other two *D. wrightii* plants were enclosed in empty mesh cages as controls for the cage effect. The position of the potted plants, as well as the damage and control treatments was randomized in each cage replicate. I generated four treatment combinations through these manipulations, presence of *L. strigosus* pods crossed with herbivore damage to *D. wrightii* to measure the effect of the plant-based resource and VOCs emitted by damaged plants on 1) the predation against *L. daturaphila* by and 2) the distribution of *G. pallens* across plants. Here I assumed that herbivore damage served as a proxy for VOC production (Hare, 2007; Hare & Sun, 2011a).

To measure the predation strength of *G. pallens*, I collected *L. daturaphila* egg masses still attached to the ovipositional leaves from natural *D. wrightii* plants outside of our experimental field plots. I then removed extra eggs from these collected egg masses until all were standardized to ten eggs per mass. Unlike the conditions described for the field experiments, these egg masses would not be exposed to the harsh field environment in the greenhouse, it was possible to standardize the total number of eggs per mass with careful handling without compromising the integrity of the remaining eggs or the adhesiveness of the egg mass. Similar to Hare and Sun (2011a), using a cork borer, a leaf disc (1 cm dia.) containing the egg mass was then punched out from each leaf containing a mass. Egg masses were stored under refrigeration for no more than two days until the bioassay. At the start of each bioassay, a leaf disc containing an egg mass was attached to the underside of the treatment (or control) leaf of each *D. wrightii* plant within the cage using synthetic resin glue (UHU Adhesives, Saunders Mfg. Co., Readfield, ME, USA; preliminary experiment showed no evidence of *G. pallens* being attracted to or repelled by the glue). I then released 20 field-caught *G. pallens* (starved for 24 hrs) into each of the cages and allow the experiment to proceed for 48 hrs. In each experimental block, I also setup one cage without releasing *G. pallens* in order to measure the baseline mortality levels of *L. daturaphila* eggs in cages in the greenhouse.

At the end of the experimental period, I first recorded the quadrant (based on the nearest plant) of all *G. pallens* individuals that were seen from outside of the cage with minimal disturbance. I then opened the cage and removed the leaves with *L. daturaphila* eggs in order to count the number of eggs destroyed by *G. pallens*. All experimental egg masses were brought back into the lab and examined under a microscope. Eggs that were fed upon by *G. pallens* were easily identified by their shriveled appearance. I recorded the proportion of eggs that were destroyed by *G. pallens* predation in each egg mass.

Statistical analysis: I conducted two separate analyses for the data collected. For the effect of the experimental manipulations on *G. pallens* predation, I performed an ANOVA on the proportion of *L. daturaphila* eggs that were destroyed using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc. 2011) using *L. strigosus* pod presence and *D. wrightii* damage as fixed effects. The two-way interaction between the fixed effects and the experimental replicate nested within experimental block as the random effect were included in the original model. Lastly, I requested multiple comparisons of the significant two-way interaction term (see results) with Tukey's HSD adjustment.

Because the density data from the experiments consisted of count data, I used the PROC GLIMMIX procedure in SAS 9.3 (SAS Institute Inc. 2011) with a log-link function and a Poisson distribution to improve the fit of the data set. I included *L. strigosus* pod presence and *D. wrightii* damage as the fixed effects. I also included the two-way interactions between the fixed effects, and the experimental replicate nested within experimental block was included as the random effect.

RESULTS

Predation by G. pallens: the presence of *L. strigosus* pods significantly reduced the mortality rates of *L. daturaphila* egg ($F_{1,99}$ = 100.78, P <0.001, Fig. 4.1A). More importantly, there was a significant interaction between the presence of *L. strigosus* pods and *D. wrightii* damage ($F_{1,99}$ = 12.39, P <0.001). Multiple comparisons showed that *L. daturaphila* eggs on damaged, single *D. wrightii* plants suffered the highest proportional mortality (0.60 ± 0.03), followed by eggs on undamaged, single *D. wrightii* plants (0.50 ±

0.03). By contrast, all eggs on *D. wrightii* plants co-transplanted with *L. strigosus* suffered significantly less mortality regardless of the damage of the *D. wrightii* plants (0.30 ± 0.02 on damaged plants, 0.35 ± 0.02 on undamaged plants, Fig. 4.1A). Over the course of these experiments, proportional *L. daturaphila* egg mortality in experimental cages ranged between 0.30 ± 0.02 to 0.60 ± 0.03, but *L. daturaphila* eggs in control cages without *G. pallens* did not suffer any mortality.

Density of G. pallens: both the presence of *L. strigosus* ($F_{1,99} = 42.99$, $P < 0.001$, Fig. 4.1B), and the herbivore damage to *D. wrightii* ($F_{1,99} = 7.01$, $P = 0.009$, Fig. 4.1B) significantly affected *G. pallens* density. Treatments with damaged *D. wrightii* and the presence of *L. strigosus* pods had the highest recruitment of *G. pallens* (5.1 ± 0.3), followed by treatments with undamaged *D. wrightii* that were also co-transplanted with *L. strigosus* (4.5 ± 0.3). Both single *D. wrightii* plant treatments had fewer *G. pallens* comparatively to previous treatment groups. Damaged, single *D. wrightii* plant treatments recruited more *G. pallens* in comparison to undamaged, single *D. wrightii* plant treatments (3.1 ± 0.3 vs. 2.1 ± 0.2, Fig. 4.1B). Overall *G. pallens* density ranged from 2.1 ± 0.3 to 5.2 ± 0.3.

DISCUSSION

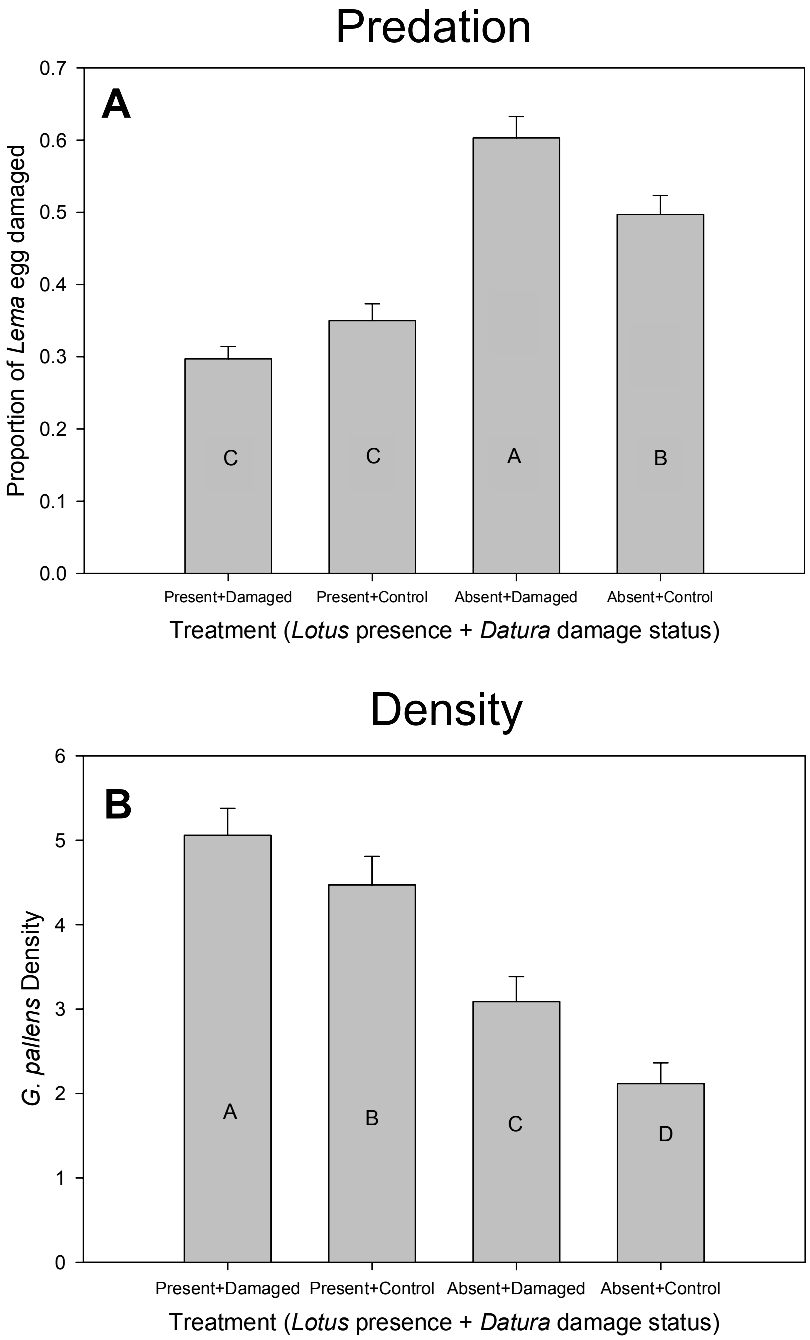
In these experiments, plant-based resources and damage as a proxy of VOCs had conflicting effects on *G. pallens* predation against *L. daturaphila.* In the greenhouse environment, the interaction between plant damage and predation was completely disrupted by the presence of a plant-based resource, indicating that the presence and consumption of *L. strigosus* pods reduced the predation and consumption of *L. daturaphila* by *G. pallens* (Fig. 4.1A). More importantly, the significant interaction between the two effects in the model suggested that the pattern of response was not additive across treatments. It was clear that *L. daturaphila* egg mortality was similar between damaged and undamaged *D. wrightii* plants in the presence of *L. strigosus* pods (Fig. 4.1A), suggesting that the presence of a plant-based resource here reduced predation, and also negated the positive effect of any VOCs emitted by the damaged host plants on omnivore predation behavior.

Considering the effects of the two factors on both predation and density simultaneously, these findings demonstrate that the presence of the plant-based resource is capable of decoupling the normal relationship between *G. pallens* predation of *L. daturaphila* eggs and *G. pallens* recruitment through the emission of VOCs produced by damaged *D. wrightii* plants. In cages without *L. strigosus*, VOC emission resulted in an increase in *G. pallens* density on *D. wrightii*, which presumably then increased predation against *L. daturaphila* eggs (Fig. 4.1). However, although *G. pallens* densities were also positively affected by both the presence of *L. strigosus* pods alone or in combination with VOCs (Fig. 4.1B), predation in each of those treatments was instead reduced, regardless of the *G. pallens* density. This unexpected pattern indicates the independence between density and predation in *G. pallens*, and suggests a potential preference switch to the plant-based resource.

As discussed in the Chapter Three, a possible mechanism for the usage of an inferior resource may be predator avoidance. In nature, *Geocoris* spp. are typically very cryptic even while hunting and consuming herbivore prey (Hagler & Naranjo, 1994), most likely due to the threat of being attacked themselves by other predators such as spiders (although this was not directly observed in the field). Therefore, the availability of an alternate resource may reduce the rate of consumption of a more high risk resource that might potentially expose these omnivores to the potential predators. Due to the prostrate nature of the *L. strigosus* plants and its pods, *G. pallens* may have preferentially stayed under the cover provided by the plants and fed on the pods as much as possible to minimize the need to search for *L. daturaphila* egg masses in the taller *D. wrightii* plants. Studies using other *Geocoris* spp. have shown that they may use a combination of inferior plant foods/insect prey in order to obtain the nutritional requirements necessary to complete development in the absence of an nutritionally superior resource (Eubanks & Denno, 1999).

Together with the results of the previous chapter, these experiments show that the predator-prey dynamics between an omnivorous predator such as *G. pallens* and its herbivore prey can be drastically changed indirectly based on the presence of suitable alternate resource (Holt & Lawton, 1994). In particular, although *G. pallens* density increased through the presence of an alternative resource, the pods actually alleviated the predation pressure against the main herbivore on the host plant. These findings illustrate the importance of identifying the potential of alternate resources and the resource preferences of a generalist omnivorous insect, especially when it is commonly used as a natural enemy. For example, the practice of mix cropping often uses non-crop plants in order to attract beneficial natural enemies into the crop plant to reduce the density of an agricultural pest. This study and that of Chapter Three suggest pest management planners may need to account for the preference or the consumption rates of both the targeted herbivore as well as the plant-based resource to ensure that the herbivore pest will remain the preferential resource regardless of the alternative resource present. These precautions ensure that any increases in natural enemy density as a result of the alternative resource presence are not accompanied by the reduction in predation against the targeted herbivore.

Figure 4.1A *G. pallens* predation in the greenhouse measured through the proportion of *L. daturaphila* egg damaged (± S.E.) 48 hrs following introduction. Different letters represent significant differences between treatment groups. **4.1B** *G. pallens* density (± S.E.) 48 hrs following release into bioassay cage with treatments that varied based on herbivory and nearby plant resource.



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CHAPTER FIVE: CONCLUDING REMARKS ON THE ROLE OF VOLATILE ORGANIC COMPOUNDS IN WITHIN-PLANT COMMUNICATION AND THE ROLE OF VOLATILE ORGANIC COMPOUNDS AND PLANT-BASED RESOURCES IN TRITROPHIC INTERACTIONS

SUMMARY

Within-plant communication: in Chapter Two I first demonstrated that the volatile organic compounds (VOCs) emitted by the perennial, *Datura wrightii* (Solanaceae), following damage by its local herbivore, *Lema daturaphila* (Coleoptera: Chrysomelidae), are capable of functioning as an airborne signal to communicate damage to intact parts of the plant. This experimental demonstration of within-plant communication using this undomesticated and native plant species adds to the current research field and suggests that this role of VOCs as airborne damage signals has been maintained under natural selection and represents an important component of its overall function in the response to herbivory in *D. wrightii*.

Secondly, I demonstrate that previous exposure to VOCs “primes”, or increases the total output of, subsequent VOC emission in *D. wrightii* following damage by *L. daturaphila.* These results illustrate two important points regarding the response to herbivory in *D. wrightii*. One, these findings indicate that following the perception of nearby herbivory, *D. wrightii* plants dedicate additional resources over and above the amount utilized for the initial VOC emission response into the preparation of additional

VOC emissions against further herbivory. More importantly, these results also demonstrate that in *D. wrightii*, the response to repeated herbivory is prepared but not immediately deployed following the perception of damage. Following the current theory on induced vs. constitutive defense and resistance in plants, this suggest that the pattern VOC emissions in this species may be one that has been selected to balance the cost and benefit of dedicating additional plant resources toward responding to repeated bouts of herbivory (Baldwin, 1998; Heil & Baldwin, 2002; Karban & Maron, 2002).

Finally and most importantly, I demonstrated that VOCs are capable of functioning as a substitution for the vascular damage signal in *D. wrightii* to prime the subsequent secondary VOC emission following herbivory by *L. daturaphila*. These results validate the theory put forth by many others that the intended receivers of the airborne damage signal may be parts of the plant that are vascularly disconnected from the source of the initial damage (Karban *et al.*, 2003; Karban *et al.*, 2006; Heil & Adame-Alvarez, 2010). These findings therefore also lend support to the idea that the role of VOCs as airborne damage signals in within-plant communications may in part reinforce their production and emission across plants, and that the benefit of this substitutive signal function may outweigh the consequences of inadvertently notifying nearby plants to the presence of herbivores.

Concurrent effects of VOCs and plant-based resources on Geocoris pallens: In Chapters Three and Four, I demonstrated that both VOCs emitted by *D. wrightii* and pods produced by *Lotus strigosus* (Fabaceae) were capable of increasing the density of an omnivorous predator, *Geocoris pallens* (Hemiptera: Geocoridae), on *D. wrightii*. These findings indicate that the information cue and the plant-based resources both were capable of influencing the colonization of *G. pallens*. In addition, these results also indicated that plant-based resources found in the environment near *D. wrightii* (produced by co-occurring plants in this case) positively increased the density of *G. pallens* on the *D wrightii* itself, beyond the period when VOCs were actively emitted by this host plant.

More interestingly the two factors had conflicting influences on the predation strength of *G. pallens* against *L. daturaphila*; these results were both shown individually in chapter three and concurrently in chapter four. Natural enemy attractants in plants that differ based on whether they provide information or resources *independently* augmented the colonization vs. the predation in *G. pallens*. In particular, while the presence of either attractant enhanced *G. pallens* colonization, the presence of pods shifted predation pressure away from *L. daturaphila*. To contrast the results on colonization, these findings indicate that the consumption of the plant-based resource decreased the predation of *L. daturaphila* by *G. pallens*. When considered in light of omnivory, these results suggest that the presence of a suitable plant-based resource simultaneously increased the quality of a host plant but also reduced necessity of the omnivore to seek out and consume prey, potentially due to the greater risk associated with finding prey (Nonacs & Dill, 1990; Folgarait & Gilbert, 1999).

Moreover, the results in chapter four further indicate that the presence of the plant-based resource was not only capable of reducing predation by *G. pallens* against *L. daturaphila* in the field, but that when presented concurrently with VOCs, it was capable of disrupting the positive effects of VOCs on predation seen previously in chapter three and earlier studies. These findings highlighted the value of the plant-based resource and the VOC cues for *G. pallens*. Although both attractants may attract and recruit omnivores, the resource is capable of altering the typical role that VOCs have in tritrophic interactions between *D. wrightii*, *L. daturaphila*, and *G. pallens*.

Overall role of VOCs in the response to herbivory in D. wrightii: Although not limited to these functions alone, I established that VOCs can function as an airborne damage signal to prime the plant against additional herbivory as well as to inform vascularly disconnected parts of the plant of potential herbivory. I also show that in its role as an information signal, it is highly context dependent because the final impact on natural enemy colonization and more importantly, predation, depends largely on the surrounding ecological cues found in the natural environment. In conclusion, with this body of work, I demonstrated that VOCs produced and emitted by *D. wrightii* play an integral part of the plant's overall response to herbivory by *L. daturaphila*.

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