PLANT MEDIATED INTERACTIONS BETWEEN HERBIVORES FROM DIFFERENT FEEDING GUILDS (*MYZUS PERSICAE* AND *LEPTINOTARSA DECEMLINEATA*) ON POTATO (*SOLANUM TUBEROSUM*)

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ABSTRACT

PLANT MEDIATED INTERACTIONS BETWEEN HERBIVORES FROM DIFFERENT FEEDING GUILDS (*MYZUS PERSICAE* AND *LEPTINOTARSA DECEMLINEATA*) ON POTATO (*SOLANUM TUBEROSUM*)

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Herbivory induces defense responses in plants that alter plant traits, which in turn affect herbivore fitness and behavior. Jasmonic (JA) and salicylic (SA) acid play a central role in regulating plant defenses. Induction of these pathways is closely associated with the feeding guild of the herbivore. Therefore, herbivores that share a host-plant may interact indirectly even if spatially or temporally distant. This study investigated how the co-occurrence of herbivores from different feeding guilds, *Myzus persicae* (Sulzer) and *Leptinotarsa decemlineata* (Say), could lead to differential responses in plant phytochemistry, herbivore performance and host-plant preference when feeding on potato, *Solanum tuberosum* L. In laboratory bioassays *M. persicae* performed better when feeding alone, but the presence of *M. persicae* did not impact *L. decemlineata* performance. Interestingly, when given a choice, *M. persicae* preferred host-plants that were damaged by *L. decemlineata*, while *L. decemlineata* preferred undamaged plants. A field study was conducted to evaluate the consequences of induced defenses due to multi-herbivory on tuber yield and whether laboratory performance results were consistent in an agricultural setting. Compared to laboratory bioassays, herbivore interactions were diminished in the field with no observed effect on yield. Differences in volatile emissions, glycoalkaloid, and JA/SA content are also discussed for each trial. Further studies investigating the qualitative and quantitative strength of feeding guild-plant interactions could provide a more thorough understanding of resistant traits and improve pest management products and practices.

For Alora and Abbie, of course.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1.

PLANT DEFENSES AND FEEDING GUILD INTERACTIONS IN *SOLANUM TUBEROSUM*

1.1 Plant Defense Against Herbivory

Plants exist in a dynamic world and are under constant pressures from their environment and other organisms. Phytophagous insects are a significant threat to plant survival and can attack plants using a variety of mechanisms (Howe & Jander, 2008). However, plants are not merely passive victims and have evolved resistant traits allowing them to endure and cope with such pressures. Resistant traits can include physical defenses, such as tough external cuticles and trichomes, or biochemical defenses, such as feeding deterrents, toxins, and nutrient uptake interference (Howe & Schaller, 2008). Resistance, however, is energetically costly and plants neutralize this expense by partitioning energy into either constitutive or inducible defenses (Agrawal, 2011; Karban & Meyers, 1989). Constitutive defenses are invariably expressed by plants regardless of the presence or absence of a stressor, whereas inducible defenses are activated in response to a current attack or stimulus (Karban & Meyers, 1989). To further conserve energy and to initiate an effective and appropriate response, plants can differentiate between the plethora of herbivore attacks and tailor defenses based on the identity of their attacker.

1.1.1 Phytohormone signaling Upon detection of an herbivore pest, various signal transduction pathways are activated which allow plants to tolerate, resist, or directly defend against the specific attacker. Inducible defenses can include the synthesis of toxic compounds, digestibility reducers, and other repellents or compounds that recruit natural enemies (Dicke, 1999).

Herbivore recognition and plant defense rely on herbivore derived elicitors and their induction of phytohormone signaling pathways (Walling, 2000). For example, oviposition by *Spodoptera exiqua* primes feeding-induced defenses in *Nicotiana attenuata* (Bandoly, Hilker, & Steppuhn, 2015) and secretion of salivary contents alone by *Myzus persicae* are enough to induce a localized defense response in *Arabidopsis thaliana* (De Vos & Jander, 2009).

Two major signal transduction pathways involved in plant defense are the jasmonic acid (JA) and salicylic acid (SA) pathways (Bruinsma & Dicke, 2008; Howe & Jander, 2008; Thaler, Humphreys, & Whiteman, 2012; Walling, 2000). Each pathway is responsible for a variety of plant defenses (Bruinsma & Dicke, 2008; Heidel & Baldwin, 2004). Jasmonic acid biosynthesis plays a central role in the regulation of wound responses, often corresponding to damage by chewing herbivores, while SA signaling is commonly activated in response to pathogens and phloem-feeding insects (Thompson & Goggin, 2006; Wu and Baldwin, 2009). Activation of the JA pathway is initiated when damage caused by herbivores induces the release of JA-precursors and intermediate proteins, which regulate JA biosynthesis and defense gene expression (Farmer, 2014; Turner, Ellis, & Devoto, 2002). Wound inducible (*i.e*. JA regulated) defenses can include proteinase inhibitors (Farmer, 2014; Turner et al., 2002), toxic compounds, such as glucosinolates (Farmer, 2014; Wiesner, Hanschen, Schreiner, Glatt, & Zrenner, 2013), as well as morphological defense features (Dangash, Bharillya, Jhala, & Jain, 2014; Farmer, 2014; Kobayashi, Yanaka, & Ikeda, 2010; Traw & Bergelson, 2003; Yoshida, Sano, Wada, Takabayashi, & Okada, 2009). Accumulation of pathogenesis-related proteins induces the SA pathway, which is responsible for pathogen-related defenses. This can lead to the production of antimicrobial compounds, isolation of the attack site via cell necrosis or cell wall lignification (Pieterse & Van Loon, 1999), and systemic acquired resistance (SAR) (Durrant & Dong, 2004;

Loake & Grant, 2007; Thaler et al., 2012). Contrary to the previously described hypersensitive responses utilized by plants to prevent the spread of pathogens, SAR provides long-term, or immunological, systemic defense against microbial attacks (Beckers & Spoel, 2008). Negative reciprocal crosstalk between the JA and SA pathways has been observed in many systems (Thaler et al., 2012). Induction of one pathway simultaneously inhibits the other, indicating that, depending on the nature of induction, antagonistic interactions between these pathways can influence plant defensive outcomes and, subsequently, herbivore communities (Turner et al., 2002). Crosstalk between these pathways allows plants to fine tune their response to herbivore attacks, but can also be manipulated by pests in order to compromise plant defenses and enhance pest fitness (Chung et al., 2013; Cui et al., 2005; Diezel, Von Dahl, Gaquerel, & Baldwin, 2009; Weech, et al., 2008; Zarate, Kempema, & Walling, 2007). For example, feeding by silverleaf whiteflies (*Bemisia tabaci*) on Arabidopsis plants activates the SA signaling pathway, which suppresses JA defenses through crosstalk, and maximizes whitefly fitness (Zarate et al., 2007). Plants are often attacked by a variety of herbivores, and the degree and timing of induction can also influence JA-SA interference (Thaler, Fidantsef, & Bostock, 2002; Thompson & Goggin, 2006). Identifying how signaling pathways interact under pressure by multiple herbivores and the consequences on both plant and herbivore fitness will provide insight into plant-insect coevolution and can be used for developing novel pest management strategies.

1.1.2 Volatile organic compounds Plants also respond to herbivory through the induction or suppression of volatile organic compounds (VOCs). Many VOCs are constitutively emitted by plants and are primarily comprised of terpenoids, green leaf volatiles, and fatty acid derivatives (Bruce & Pickett, 2011; Dicke, 2009; Dudareva, Pichersky, & Gershenzon, 2004). Volatile plant

emissions are not single compounds, but consist of complex blends of many compounds, that are altered qualitatively and/or quantitatively in response to herbivore damage (Dicke 1999). The role of VOCs in plant-insect interactions is multifunctional. For one, constitutively emitted VOCs may act as signals for host-seeking herbivores (Bruce & Pickett, 2011). However, in the context of plant defense, herbivore induced plant volatiles (HIPVs) may be emitted to alert distal plant parts of an ensuing attack (Holopainen & Blande, 2013; Farmer, 2001). HIPVs are also noted to play a significant role in tri-trophic interactions, by recruiting natural enemies of attacking herbivores (Dicke, 2009; Holopainen & Blande, 2013; Kessler & Heil, 2011).

Insects have complex olfactory systems, capable of detecting differences between volatile blends and doses, and can differentiate between host and non-host volatiles, as well as volatiles emitted by damaged and undamaged host-plants (Bruce & Pickett, 2011; De Bruyne & Baker, 2008; Szendrei, Malo, Stelinski, & Rodriguez-Saona, 2009). Furthermore, fractionated compounds can attract, deter, or have no effect on insect behavior when presented to herbivores individually or at concentrations that are quantitatively out of context (Bruce et al., 2008; Bruce & Pickett, 2011; Dickens, 2002). Therefore, volatile blends are often species specific and corresponding behavioral effects are likely to be context specific (Bruce et al., 2008; Bruce & Pickett, 2011).

Changes in the quality or quantity of VOCs induced by herbivory can shift insect perception of potential hosts by altering attractive or repellent signals. Consequently, HIPVs can mediate interactions between plants and other plants, pollinators, herbivores, and the natural enemies of herbivores (Dicke & Baldwin, 2010). For example, bee pollinators of wild tomato avoid VOCs emitted by plants in response to herbivore damage or application of a methyl jasmonate treatment (Kessler, Halitshke, & Poveda, 2011). Similarly, HIPVs emitted by tobacco

plants after feeding by tobacco budworm larvae were found to deter oviposition of adult conspecific females (De Moraes et al., 2001). Thus, the dynamic nature of VOCs plays a powerful role in structuring herbivore communities and mediating interactions within the broader community.

1.2 Feeding Guild Interactions

Herbivory can lead to different types of plant damage depending on the herbivore's feeding strategy or guild. Phenotypic differences between various attackers lead to differential fitness consequences and plant responses (Hlywka, Stephenson, Sears, & Yada, 1994; Mewis et al. 2006; Rodriguez-Saona, Chalmers, Raj, & Thaler, 2005; Soler et al. 2011). Herbivores are well adapted to feed on host plants, and vary in mouthpart morphology, salivary components, specificity to particular tissues or feeding sites, feeding frequency or period of day in which they are active, developmental stage, and combinations of any of these factors (Heidel & Baldwin, 2004; Novotny et al., 2010). Herbivores, such as Coleopteran and Lepidopteran species, use chewing mouthparts to remove leaf tissue or damage surface cells (Howe & Jander, 2008; Walling, 2000). Defoliators, such as these, pose a significant threat to host plants by removing photosynthetic organs, the primary energy source for plants. In contrast, many herbivores belonging to the Hemiptera order, such as aphids and cicadas, use piercing and sucking mouthparts to pierce through the epidermal cells to feed on plant fluids, such as xylem and phloem (Howe & Jander, 2008; Walling, 2000). Given the diversity in herbivore feeding approaches, plants must respond with the most effective defense strategy in order to minimize damage while ensuring fitness and reproductive success (Walling, 2000).

Plant defenses are comprised of many defensive traits that can be derived from multiple forms of the same chemical compounds, which may also interact synergistically or antagonistically (Agrawal & Fishbein, 2006). Phenotypic plasticity allows plants to adapt to dynamic pest pressures and it is expected that plants that alter their defensive traits in response to distinct herbivores will have higher fitness than plants that respond invariably (Agrawal & Karban, 1999). Adaptive responses are linked to herbivore perception and feeding guild or feeding strategy of the particular attacker (Erb, Robert, Hibbard, & Turlings, 2011; Heidel & Baldwin, 2004; Mewis et al., 2006; Rodriguez-Saona, Musser, Vogel, Hum-Musser, & Thaler, 2010; Stout, Workman, Bostock, & Duffey, 1998). However, there is still insufficient understanding of how plants allocate defenses in the face of simultaneous attacks by multiple herbivores. Few studies have addressed whether induced defenses in response to attack by individual feeding guilds are congruent with induced defenses in response to simultaneous attack from multiple guilds (Dicke, Van Loon, & Soler, 2009; Erb et al., 2011; Rodriguez-Saona et al., 2010; Stout et al., 1998).

Although interspecific competition may exist within feeding guilds through direct competition for shared resources, indirect (*e.g.* plant-mediated) competition plays a significant role in mediating interactions between herbivores from different feeding guilds (Denno, McClure, & Ott, 1995). Herbivores can induce changes in plant quality, morphology, and phytochemistry (including phytohormones and their related defensive and volatile compounds). Therefore, the co-occurrence or sequential occurrence of species from multiple feeding guilds could lead to indirect differential behavioral and performance effects (Ali & Agrawal, 2014; Kaplan, Dively, & Denno, 2009; Soler et al., 2012).

1.3 Study System

1.3.1 Solanum tuberosum Potatoes, *Solanum tuberosum* L. (Solanaceae), are one of the most agriculturally important crops grown throughout the world (Vincent et al., 2013). They are a staple in many diets and are a notably important crop in developing nations. They have been cultivated for over 8000 years and there are over 4,000 varieties grown for consumptive purposes (Vincent et al., 2013). Although potato diversity is high throughout the world, potatoes are commonly cultivated in monocultures, particularly in the United States and other industrialized countries. Such cultivation practices increase susceptibility to attacks by insecticide resistant pests (Pelletier, Horgan, & Pompon, 2013; Turnbull & Hector, 2010). There are numerous insect pests of potatoes that can attack both above- and belowground plant parts by feeding on tubers, foliage, and by transmitting pathogens (Radcliffe, 1982) leading to reduction in yield quantity and quality (Vincent et al., 2013). Potato pests are most commonly controlled using chemical methods. However, increased public attention has raised concern over the effects of insecticide use on the safety of environmental and human health. Increasing public pressure urges a shift from chemically dependent management strategies to more sustainable practices (Vincent et al., 2013).

1.3.2 Leptinotarsa decemlineata: life history and pest status Colorado Potato Beetles (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) are one of the most important agricultural pests of potato (Alyokhin, Udalov, & Benkovskaya, 2013). Originating in Mexico, *L. decemlineata* can now be found throughout many regions around the world. When first discovered in the Rocky Mountains during the early 19th century by Thomas Nuttal (then later described by Thomas Say), *L. decemlineata* were found feeding on Solanaceous weeds and had

little economic or agricultural significance (Alyokhin et al., 2013). However, as European emigrants moved west across the United States, they brought with them potatoes, which enabled host range expansion for *L. decemlineata* (Alyokhin et al., 2013). By the early 20th century, *L. decemlineata* had spread throughout most of North America, and had even reached parts of Africa, Asia, and Europe (Alyokhin et al., 2013).

Leptinotarsa decemlineata feed almost exclusively on Solanaceous plants, primarily being found in potato fields, but are also known to attack eggplant, tomato, and other plants in the nightshade family (Alyokhin et al., 2013). They are holometabolous and undergo a complete metamorphosis consisting of egg, four larval instars, pupa, and adult (Ferro, Logan, Voss, & Elkington, 1985). Adult *L. decemlineata* overwinter in field margins, emerging in the spring to mate and feed on new potato plants. Females oviposit prolifically, and can lay up to 800 eggs throughout a lifetime (Ferro et al., 1985). Once emerged, larvae can inflict considerable damage onto potato plants. *Leptinotarsa decemlineata* have chewing mouthparts and are heavy defoliators, removing up to 40 cm^2 of leaf tissue during the larval stage alone (Ferro et al., 1985; Logan, Casagrande, Faubert, & Drummond, 1985; Alyokhin et al., 2013) which can significantly reduce tuber yield if defoliation occurs during growth stages that are critical to plant development (Hare, 1980).

Visual and chemical signals, such as VOCs and feeding stimulants, play an important role in host-plant location and acceptance by *L. decemlineata* (Sablon, Dickens, Haubruge, & Verheggen, 2013; Alyokhin et al., 2013) and *L. decemlineata* orient more frequently towards volatiles emitted by Solanaceous plants over volatiles emitted by plants from other families (Visser & Nielsen, 1977). In addition, *L. decemlineata* are more attracted to plants that were

damaged by conspecific larvae compared to undamaged plants (Landolt, Tumlinson, & Alborn, 1999).

Leptinotarsa decemlineata resistance to insecticides was recorded as early as the 1950s, and insecticide resistance has continued to increase dramatically as chemical use has intensified (Alyokhin et al., 2013). This poses a serious problem for potato growers worldwide, not only because increased resistance entails increased pest pressure, but also because the development of insecticides is costly. Furthermore, the perpetuation of an increased-input/increased-resistance cycle is unsustainable and environmentally hazardous.

1.3.3 Myzus persicae: life history and pest status Another prominent pest of potatoes is the green peach aphid, *Myzus Persicae* Sulzer (Hemiptera: Aphididae). *Myzus persicae* originated in China, but can now be found on every continent and in nearly every agricultural system (Margaritopoulos, Kasprowicz, Malloch, & Fenton, 2009). *Myzus persicae* are phloem-feeding herbivores that use piercing and sucking mouthparts to reach sieve tube elements (Saguez, Giordanengo, & Vincent, 2013). Although *M. persicae* incur minimal visible damage to plants, they are common vectors for plant pathogens and can also deplete valuable nutrients, which can inhibit plant performance (Powell, Tosh, & Hardie, 2006; Radcliffe, Ragsdale, & Suranyi, 2007; Thompson & Goggin, 2006).

Myzus persicae have a unique life cycle in that they reproduce via parthenogenesis throughout a portion of their life cycle. During the spring and fall, asexual females give birth to live nymphs that immediately begin to feed on host plants (Saguez et al., 2013). Parthenogenesis and telescoping generations result in a rapid population increase of genetically identical clones (Saguez et al., 2013). Seasonal changes in temperature initiate a sexual reproduction phase in

which sexual male and female morphs mate to produce fertilized eggs that overwinter until the following spring (Saguez et al., 2013).

Another unique characteristic of the *M. persicae* life cycle is that they are heteroecious (Saguez et al., 2013). *Myzus persicae* live on a primary host during the winter, usually trees of the *Prunus* genus or other woody species, then migrate to secondary herbaceous hosts from the spring through the fall, returning to the primary host to overwinter (Margaritopoulos et al., 2009; Saguez et al., 2013). *Myzus persicae* are polyphagous and feed on hundreds of plant species across 40 different families, including Solanaceae (Saguez et al., 2013).

Myzus persicae also vary in the expression of certain phenotypic traits, such as body color, which ranges between green and red, and wing dimorphism (Blackman & Eastop, 2007; Saguez et al., 2013). Expression of these traits is dependent primarily on environmental conditions and plant quality (Van Emden, Eastop, Hughes, & Way, 1969; Saguez et al., 2013). The energetic cost associated with alate (winged morph) production is high. Therefore, when host quality is satisfactory and aphid populations are low, apterous (wingless) aphids will be produced (Powell et al., 2006; Saguez et al., 2013). However, as population density increases and host-plant resources diminish, the energetic cost associated with alate production outweighs the cost of increased conspecific competition (Dixon, Horth, & Kindlmann, 1993). With an active flight mechanism, alate aphids have increased mobility and migrate to new host plants (Saguez et al., 2013).

Several mechanisms interact in order for aphids to locate a new host plant such as visual, chemical, and tactile cues. A review by Powell et al. (2006) describes the sequence of behaviors leading to host plant selection and acceptance which includes visual cues while in flight, landing and plant contact with surface odorant cues, probing and stylet pathway activity and interactions

with gustatory cues, phloem sieve element penetration, and phloem ingestion and acceptance. However, alate morphs have increased antennal rhinaria associated with olfaction compared to apterous morphs, indicating that response to plant odors likely plays a significant role in host-plant location (Pickett, Wadhams, & Woodcock, 1992). In addition, studies have also shown that aphids can differentiate between suitable and unsuitable host plants when given a choice. For example, (*E*)-β-farnesene is a key component in the aphid alarm pheromone and *M. persicae* are repelled by tobacco plants and wild potatoes producing this compound (Gibson & Pickett, 1983; Wang, Yu, Fan, Wang, & Xia, 2015). Aphids also preferentially colonize potato plants infected by potato leaf roll virus whose volatile profiles matched those of uninfected plants, but differed quantitatively (Eigenbrode, Ding, Shiel, & Berger, 2002). *Myzus persicae* were even found to respond differentially to specific volatile blends emitted by each of four different cultivars of *S. tuberosum* further indicating their sensitivity to host-plant odors (Rajabaskar, Ding, Wu, & Eigenbrode, 2013).

The widespread dispersal of *M. persicae* across the globe has led to increased efforts to control population outbreaks. Chemical control, such as the use of systemic insecticides, has dominated control methods (Saguez et al., 2013). However, like *L. decemlineata*, *M. persicae* has developed rapid resistance to most active compounds found in chemical insecticides (Silva, Jander, Samaniego, Ramsey, & Figueroa, 2012), necessitating a shift in management approaches.

1.4 Chemical Ecology of *Solanum tuberosum*

1.4.1 Glycoalkaloids Glycoalkaloids are constitutively expressed, naturally occurring feeding deterrents that can be found in all parts of the potato plant, including foliage, tubers, roots, and sprouts **(**Chen & Miller, 2000). The two primary glycoalkaloids present in *S. tuberosum* are

solanine and chaconine (Edwards & Cobb, 1996; Hlywka et al., 1994), although other glycoalkaloids are present in various quantities and bioactivity among *Solanum* species (Pelletier et al., 2013; Tingey, Mackenzie, & Gregory, 1978). The relationship between glycoalkaloid production and herbivore damage has been observed for both *L. decemlineata* and *M. persicae*. *Myzus persicae* has no affect on glycoalkaloid levels in the wild potato species, *S. berthaultii* (Tingey, 1982). However, under heavy infestation, *M. persicae* reduced glycoalkaloid concentrations in *S. tuberosum*, cv. King Edward and Maris Piper (Fragoyiannis, McKinlay, & D'Mello, 2001). In contrast, severe defoliation by *L. decemlineata* results in significantly higher glycoalkaloid levels in *S. tuberosum*, cv. Superior (Hlywka et al., 1994).

Induction of glycoalkaloids, however, does not necessarily equate to increased plant resistance. Glycoalkaloids had no effect on *M. persicae* performance when fed an artificial diet with similar glycoalkaloid concentrations found in potato leaves. However, diets with elevated glycoalkaloid concentrations negatively affected *M. persicae* performance (Fragoyiannis, McKinlay, & D'Mello, 1998). In a preference study, *M. persicae* preferred cultivated *S. tuberosum* plants to wild *Solanum* species, with preference being linked directly to glycoalkaloid content (Altesor et al., 2014). Suppression of glycoalkaloid biosynthesis and the ability to tolerate low levels make *S. tuberosum* highly susceptible to *M. persicae* infestations. Similarly, although higher levels of glycoalkaloids can deter *L. decemlineata* larval feeding, the glycoalkaloids, solanine and chaconene, have no effect on overall *L. decemlineata* fitness when fed at levels commonly found in *S. tuberosum* foliage (Kowalski, Domek, Deahl, & Sanford, 1999).

1.4.2 Volatile organic compounds McIndoo (1926) was the first to document *L. decemlineata* response to VOCs emitted by undamaged potato plants. Since then, *trans*-2-hexen-l-ol, 1-hexanol, cis-3-hexen-l-ol, trans-2-hexenal, and linalool have been identified as being the primary volatile components emitted by *S. tuberosum* (Visser & Nielsen, 1977). However, when attacked by herbivores, potato plants emit volatile compounds that are different from those emitted by undamaged plants. HIPVs emitted by potatoes under attack from herbivores release volatile blends composed primarily of terpenes and LOX-derived volatiles (Gosset et al., 2009). However, these profiles also differ based on the type of attacker and potato species (Gosset et al., 2009). For example, while both *M. persicae* and *L. decemlineata* have been found to induce some of the same compounds, such as (*E*)-β-farnesene, *M. persicae* damage is known to induce several additional volatiles such as β-sesquiphellandrene, and β-elemene, while volatile profiles from *L. decemlineata* potato plants are quantitatively fewer and comprised of different compounds (Gosset et al., 2009).

1.5 Objectives

Cultivated potatoes have been bred for certain traits, such as increased tuber size and enhanced nutritional content (Alyokhin et al., 2013). However, they often lack sufficient levels of resistant qualities, leading to higher susceptibility to damage by herbivores and infection by pathogens (Pelletier et al., 2013; Turnbull & Hector, 2010). Both *L. decemlineata* and *M. persicae* are able to withstand certain defenses typical of the cultivated potato, *S. tuberosum* (Altesor et al., 2014; Fragoyiannis et al., 1998; Kowalski et al., 1999). However, these studies have focused on the induction of defensive traits by individual herbivore species. Yet, it is evident that multiple herbivores interact with individual plants and that damage inflicted by one

herbivore can render a defense response that differs from that of herbivores in separate feeding guilds (Dicke et al., 2009; Heidel & Baldwin, 2004; Mewis et al., 2006). Differential phytohormone responses could impart negative or positive effects on herbivore performance, and changes in the quality or quantity of plant VOC emissions can also inform herbivore behavior.

Chemical ecology and identification of inter- and intra-specific chemical signaling provides alternatives to traditional insecticide use. Chemical ecology has already significantly contributed to pest management in regards to pheromone identification and its utilization in mating disruption or as a trapping mechanism (Pickett, Wadhams, & Woodcock, 1997). Understanding how herbivores indirectly interact by affecting one another's performance or ability to locate a host can provide information useful for developing additional alternative management strategies.

The purpose of this study was to determine whether induced changes in phytochemistry can have fitness or behavioral consequences on herbivores from different feeding guilds that co-occur on potato, *S. tuberosum* cv. Atlantic. I addressed four specific questions. (1) Does a distinct response to damage by an herbivore from one feeding guild affect the performance of herbivores from a different feeding guild, and can this effect be attributed to changes in phytohormone and defensive compound quantities? (2) Can *L. decemlineata* and *M. persicae* use volatile signals to differentiate between odors from potential host plants that are either healthy and undamaged, or damaged by another feeding guild?; (3) Are these effects transferrable to the field?; and (4) What are the yield consequences of inducible defenses in response to herbivory by multiple feeding guilds?

CHAPTER 2.

RECIPROCAL EFFECT OF INSECT FEEDING GUILD ON PLANT DEFENSE AND HERBIVORE PERFORMANCE

2.1 Introduction

Herbivore damage induces defense responses in plants, which can alter plant traits and affect insect performance such as feeding, oviposition, and growth or development (Walling, 2000). Such resistant strategies can be deployed locally at the site of herbivore damage, or systemically in undamaged plant tissues (Karban & Baldwin, 1997) with immediate or persisting effects (Kaplan, Halitschke, Kessler, Sardanelli, & Denno, 2008). Therefore, herbivores that share a particular host-plant can interact indirectly even when they are spatially or temporally distant (Brunissen, Cherqui, Pelletier, Vincent, & Giordanengo, 2009).

Biosynthesis of the plant hormones jasmonic acid (JA) and salicylic acid (SA) can directly result from herbivore damage (Howe & Jander, 2008). These hormones act as signaling compounds that enable damage recognition at the site of attack and activation of both local and systemic defense responses (Thaler et al., 2012). The specificity in plant response and subsequent allocation of defenses can be linked to the recognition of insect elicitors, such as those found in oral secretions (Peiffer & Felton, 2009; Bonaventure, Van Doorn, & Baldwin, 2011; Heil et al., 2012) and oviposition fluids (Hilker, Stein, Schröder, Varama, & Mumm, 2005; Reymond, 2013). Differentiation between herbivore feeding location, patterns, and strategies (*i.e.* feeding guilds) also plays a role in the regulation of plant defenses (Stout et al., 1998; Rodriguez-Saona et al., 2005; Thaler et al., 2012). For example, sucking herbivores, such as aphids, are known to establish long-term feeding sites, using specialized piercing and sucking mouthparts to penetrate the leaf surface and feed on the phloem sap (Walling, 2000). This feeding strategy

minimizes direct damage, and induces a response similar to those activated by pathogens (Walling, 2000; Kessler & Baldwin, 2002). In general, aphid infestations are positively correlated with elevated SA production while chewing herbivores, such as Lepidopteran larvae and Coleopteran species, induce JA biosynthesis (Walling, 2000; Thaler et al., 2012). Jasmonic acid is important for the regulation of wound-response genes and plays a significant role in the overall regulation of plant resistance against many types of herbivores (Howe & Jander, 2008; Walling, 2000). Although sucking herbivores, such as aphids, are more closely associated with SA-related responses, these herbivores can also induce certain JA-related responses, and similarly, chewing herbivores are known to also induce some SA-related traits (Heidel & Baldwin, 2004; Howe & Jander, 2008; Kessler & Baldwin, 2002). Furthermore, JA and SA, and other phytohormone pathways, interact antagonistically, which adds further complexity to the overall regulation of plant defenses, where induction of one pathway suppresses another, therefore reducing the expression of defensive traits related to the suppressed pathway (Kessler & Baldwin, 2002; Thaler et al., 2012; Zarate, 2007). From a phytocentric perspective, plants can utilize this to their advantage by fine-tuning their defenses depending on the type or degree of attack (Howe & Jander, 2008). Yet, this mechanism may also be manipulated by herbivores in order to compromise plant defenses and benefit their subsequent feeding. Activation of a pathway that induces an inadequate defense could simultaneously suppress the expression of a more effective defense, therefore providing the herbivore undefended access to plant nutrients (Thompson & Goggin, 2006; Zarate, 2007). At least in the case of certain phloem-feeders, minimizing the activation of defenses by suppressing gene expression is one strategy used to counter plant resistance (Goggin, 2007; Walling, 2008; Zarate, 2007).

Multiple herbivores interact with an individual plant throughout the plant's lifetime. Therefore, in order to understand how plants communicate with herbivores and partition their defenses, it is necessary to also evaluate plant responses in the presence of multiple attackers, particularly herbivores from different feeding guilds since this is likely where differential plant responses will occur (Dicke et al., 2009; Heidel & Baldwin, 2004; Mewis et al., 2006). Remarkably, most studies to date documenting plant specificity to insects in different feeding guilds have failed to rigorously examine reciprocal interactions (Heidel & Baldwin, 2004; Mewis et al., 2006; but see: Rodriguez-Saona et al., 2010; Stout et al., 1998; Erb et al., 2011) and thorough knowledge of the reciprocal consequences of herbivore cohabitation on plant defense and this effect on herbivore populations is lacking. Understanding how varied and numerous herbivores influence the induction of resistant traits can provide fundamental information on how pest populations are assembled and distributed throughout a growing season, and subsequently how pests may be managed.

Potatoes, *Solanum tuberosum* L.*,* are the fourth most agriculturally significant crop in the world (Vincent, Alyokhin, & Giordanengo, 2013). They are essential to diets across the globe, with particular significance in developing countries. Green peach aphids, *Myzus persicae* (Sulzer) (Insecta: Hemiptera: Aphididae), and Colorado potato beetles (CPB), *Leptinotarsa decemlineata* (Say) (Insecta: Coleoptera: Chrysomelidae), are two major pests of potato and can be found throughout many parts of the world (Vincent et al., 2013). These pests can cause significant damage to potato crops by reducing plant quality and tuber yield, and in the case of *M. persicae*, by transmitting pathogens (Alyokhin et al., 2013; Saguez, 2013). Host-plant resistance can provide a source of protection from herbivore attacks, but conventional breeding has generally favored characteristics related to yield and nutrient content while often neglecting

resistant traits, like glycoalkaloid production. This can make potatoes an easy target for host-seeking herbivores that would otherwise be susceptible to these defensive compounds.

Steroidal glycoalkaloids are characteristic metabolites of Solanaceous plants. These compounds can be toxic to many organisms, including insects, bacteria, fungi, and mammals, but had no effect on *M. persicae* or *L. decemlineata* when they were fed in an artificial diet containing glycoalkaloids at similar levels found in *S. tuberosum* leaves (Kowalski, 1999; Fragoyiannis et al., 1998). However, elevated concentrations, higher than those constitutively occurring in potato leaves, can reduce *M. persicae* and *L. decemlineata* performance (Kowalksi, 1999; Fragoyiannis et al., 1998). Glycoalkaloids as resistance factors against herbivores depend on the concentrations and combinations present in the plant tissue, particularly in its effect on *L. decemlineata*, but studies evaluating the relative impact of glycoalkaloids on *L. decemlineata* development report conflicting results (Paula et al., 2014; Sablon et al., 2013). However, most have found that elevating glycoalkaloid concentrations does increase feeding deterrence (Kowalski et al., 1999; Sablon et al., 2013).

The primary objective of this study was to evaluate induction of *S. tuberosum* chemical defenses in response to a chewing herbivore, *L. decemlineata*, on the performance of a sucking herbivore, *M. persicae*, and *vice versa*. A no-choice performance assay was used to evaluate the performance of *M. persicae* and *L. decemlineata* individually, simultaneously, and sequentially in order to clarify the role of inducible chemical defenses and their differential effect on herbivores from contrasting feeding guilds. Performance was determined by counting *M. persicae* populations over time and by weighing *L. decemlineata* larval biomass at the end of the feeding trial. Plant tissue was sampled, and JA/SA and glycoalkaoid content were compared between herbivore treated and untreated plants.

2.2 Materials and Methods

2.2.1 Insect and plant material The *M. persicae* colony was established from aphids collected on potato plants (*S. tuberosum* cv. Atlantic) in a greenhouse at Michigan State University (East Lansing, MI). The colony was maintained on *S. tuberosum* plants within a growth chamber (24-25°C, 48-52% RH, L16:D8). Mixed instar (adults and nymphs) apterous aphids were randomly selected for bioassays. Second instar *L. decemlineata* larvae were used for bioassays. Larvae were collected from multiple egg clutches in a colony maintained at Michigan State University. The colony was reared on *S. tuberosum* cv. Atlantic in an insectary at 25 °C and L16:D8 photoperiod.

Experiments were carried out using four- to five-week old *S. tuberosum*, cv. Atlantic plants that were propagated from vegetative seed produced by the Montcalm Research Center (Stanton, MI). Plants were grown in 10 cm diameter plastic pots with a perlite soil mix (Suremix Perlite, Michigan Grower Products Inc., Galesburg, MI). All plants were grown in a growth chamber maintained at 25-28 °C, 55-58% RH, under a photoperiod of L16:D8 (hereafter referred to as laboratory conditions) and fertilized weekly with a water soluble 20-20-20 (N-P-K) fertilizer (J.R. Peters Inc., Allentown, PA).

Potato plants were placed in individual cages constructed of clear-acetate sheets to form cylinders (d = 11.5 cm, h = 30 cm) (Figure 1). Cages were fitted with fine mesh lids to allow ventilation and watering while preventing herbivore movement between plants. Caged plants were arranged in a completely randomized design within a single growth chamber (n = 10 for each treatment). The experiments were conducted separately between April and July 2015 under the same laboratory conditions to assess performance for each herbivore species. All

aboveground plant tissue was weighed and plant tissue was sampled at the end of each trial to evaluate phytohormone and glycoalkaloid content.

Figure 1: Performance bioassay showing exclusion cage construction and arrangement in growth chamber.

***2.2.2 Myzus persicae* performance** The bioassay to evaluate *M. persicae* performance consisted of the following five treatments: (1) *M. persicae* added to individual potato plants; (2) *M. persicae* added simultaneously with *L. decemlineata*; (3) *M. persicae* added sequentially to potato plants that were previously infested by *L. decemlineata*; (4) *M. persicae* added sequentially to potato plants that were previously damaged mechanically; and (5) undamaged plants were used as the experimental control. On the first day of the experiment, 3 *L. decemlineata* larvae were applied to all plants being treated with sequential herbivore additions (treatment (3)), and larvae were left to feed for three days. For this treatments, during the first two days, plants were checked and dead larvae were removed and replaced. The mechanically damaged plants (treatment (4)), were manipulated by removing 2 mm strips of foliar tissue from leaf margins (one leaflet from each plant being treated with mechanical damage) using a clean,

sharp blade. Leaf tissue was removed daily in the same manner to correspond with the length of time larvae remained on the plants. On the fourth day, 20 aphids were added to each plant that was previously infested with *L. decemlineata* larvae or mechanically damaged (treatments (3) & (4)). In addition, 20 aphids were applied concurrently with three *L. decemlineata* larvae to plants to evaluate performance when *M. persicae* and *L. decemlineata* fed simultaneously (treatment (2)). Twenty aphids were also applied to aphid-only control (treatment (1)). *Leptinotarsa decemlineata* larvae used throughout the duration of this experiment emerged on the same day and were obtained from the same set of egg clutches. Herbivore-free control plants were handled in the same manner as all other treatments throughout the experiment (*i.e.* removed from cage and replaced on each aphid counting day). To prevent complete defoliation, larvae were removed from the plants and mechanical damage was stopped six days after their initial application. Aphid density per plant was recorded every 4 days for 16 days.

***2.2.3 Leptinotarsa decemlineata* performance** A similar bioassay was designed to evaluate *L. decemlineata* growth in the presence and absence of *M. persicae*. Due to the feeding style of aphids, a mechanical damage treatment was not possible and only individual, simultaneous, and sequential treatments were used as follows. On the first day of the experiment, 20 aphids were placed on potato plants and were left to feed and reproduce for three days. Undamaged plants were designated as experimental controls. On the fourth day, three *L. decemlineata* larvae were applied to the plants that were previously infested with *M. persicae*, as well as to uninfested plants, and simultaneously with 20 adult aphids. *Myzus persicae* and *L. decemlienata* were left to feed for an additional 5 days after which, larvae were removed and weighed.

2.2.4 Glycoalkaloid analysis Foliar tissue (100 mg) was excised from the top third part of each plant at the end of both bioassays and prepared for analysis of α-solanine content. Tissue was frozen in liquid nitrogen and placed in cold storage (-80°C). Frozen tissue was transferred into 2 ml screw cap tubes containing 900 mg zirconia/silica beads (BioSpec, Bartelsville, OK) and 1 ml of extraction solvent (water, methanol and acetic acid, 49:49:2 v/v/v). Samples were homogenized on a FastPrep homogenizer (MP Biomedicals, Solon, OH) at 6 m s^{-1} for 45 s for two cycles. The samples were then treated in a hot water bath at 60°C for 30 m, then centrifuged at 15,000 RPM for 20 min. The supernatant was transferred to 2 ml glass vials and stored at -20°C.

All samples were analyzed at the Michigan State University Mass Spectrometry Core Facility (East Lansing, MI) using a Waters Quattro Micro triple quadrupole LC-MS device interfaced to a Shimadzu high-performance liquid chromatography apparatus. Chromatography was performed using a Supelco Ascentis Express C18 column (2.1 mm x 100 mm, 2.7-mm particle size) with column oven set to 30°C. Initial conditions were 90% solvent A (water + 0.1% formic acid, v/v) / 10% solvent B (acetonitrile) at a flow rate of 0.3 mL/min, followed by a linear gradient to 5% A:95% B at 2 min, hold at 5% A:95% B to 3 min, return to 90% A:10% B at 3.01 min, and then hold at 90% A:10% B until 5 min. Compounds were ionized by electrospray ionization in positive-ion mode, and mass spectra were acquired using multiple reaction monitoring (MRM). The capillary voltage, extractor voltage, and radiofrequency lens setting were 3.6 kV, 3 V, and 0.1 V, respectively. Cone gas and desolvation gas flow rates were 0 and 800 L/hr, and the source and desolvation temperatures were 150°C and 350°C. The source cone potentials and collision energies, respectively, for solanine were 60 and 80 V. The precursor and product ion masses used for the MRM transitions were 868.45>398.35.

2.2.5 Phytohormone analysis Jasmonic and salicylic acid were extracted from all plants at the end of each bioassay by removing 100 mg fresh tissue, as described above, with the following modification to the extraction procedure. Frozen plant tissue was transferred into 2 ml screw cap and homogenized with 1 ml extraction solvent (water, methanol and formic acid, 49:49:2 v/v/v). Samples were homogenized, heated, and centrifuged as described above. The supernatant was transferred to 2 ml glass vials and stored at -20°C and analyzed at Michigan State University Mass Spectrometry Core Facility.

Extracts containing SA, SAG, JA, and JA-Ile were analyzed using a Waters Quattro Premier triple quadrupole LC-MS device interfaced to a Waters Acquity ultra-performance liquid chromatography apparatus. Chromatography was performed using a Supelco Ascentis Express C18 column (2.1 mm x 100 mm, 2.7-mm particle size) with column oven set to 50°C. Initial conditions were 99% solvent A (water + 0.1% formic acid, v/v) / 1% solvent B (acetonitrile) at a flow rate of 0.4 mL/min for 0.5 min, followed by a linear gradient to 70% A:30% B at 1 min, then to 10% A:90% B at 3.5 min, hold at 10% A:90% B to 4.5 min, return to 99% A:1% B at 4.51 min, and then hold at 99% A:1% B until 5 min. Compounds were ionized by electrospray ionization in negative-ion mode, and mass spectra were acquired using multiple reaction monitoring (MRM). The capillary voltage, extractor voltage, and radiofrequency lens setting were 3 kV, 3 V, and 0 V, respectively. Cone gas and desolvation gas flow rates were 50 and 700 L/hr, and the source and desolvation temperatures were 120°C and 350°C. The source cone potentials and collision energies, respectively, were as follows: for SA, d4-SA, and JA, 28 and 16 V; for SAG, 15 and 15 V; for d5-JA and JA-Ile, 34 and 10 V. The precursor and product ion masses used for the MRM transitions were 137>93 (SA), 140.8>96.7 (d4-SA), 209.1>59 (JA), 214.1>62 (d5-JA), 299>137 (SAG), 322.2>130.1 (JA-Ile).

2.2.6 Statistical analyses All statistical analyses were completed using JMP (Version 12.1, SAS Institute Inc.). A repeated measures model on aphid numbers over time was used to test the effects of prior and simultaneous *L. decemlienata* feeding on *M. persicae* performance. A one-way ANOVA was used to analyze the impact of *M. persicae* feeding on *L. decemlineata* growth and to compare the effect of herbivore feeding sequence on JA, SA, JA-ile, SAG, and α-solanine. A post hoc Tukey test was used to report significant differences between groups ($p < 0.05$). Data were log- or square root-transformed as needed to meet assumptions of homogeneity of variance and normality of residuals. Differences in degrees of freedom between treatments and response variables are due to differential recovery of bioassay insects or loss of plant material.

2.3 Results

2.3.1 Myzus persicae performance Initially, *M. persicae* growth rate was not affected by mechanical damage or by *L. decemlineata* feeding. However, this effect was temporal and by 16 d, *M. persicae* growth was significantly reduced by prior mechanical damage and both prior and simultaneous *L. decemlineata* feeding (treatment: $F_{3,34} = 0.43$, $P = 0.007$; time: $F_{4,33} = 9.02$, $P < 0.001$; time-by-treatment interaction: $F = 0.49$, $P = 0.006$) (Figure2).

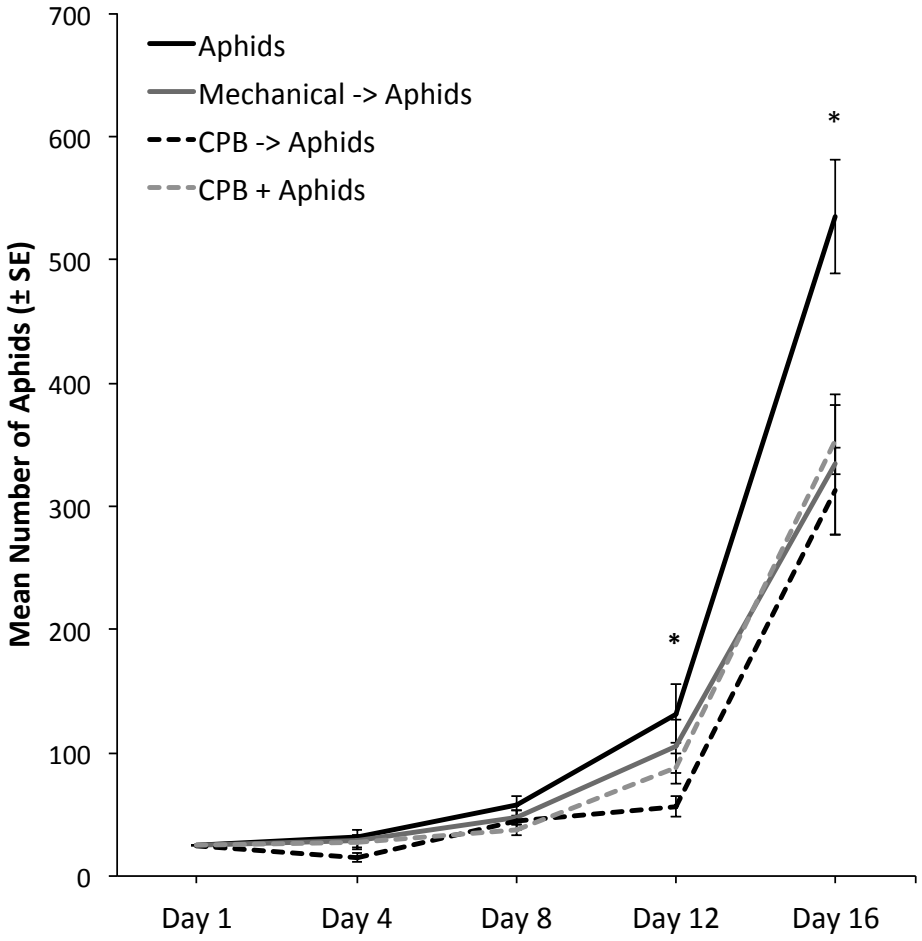

Figure 2: *M. persicae* (aphid) population growth when feeding on *S. tuberosum* alone (solid black line), with prior mechanical damage (solid grey line), with prior *L. decemlineata* (CPB) damage (dashed black line), or with simultaneous CPB damage (dashed grey line). Asterisks above error bars indicate days when aphid numbers are significantly different (Tukey HSD, $\alpha = 0.05$).

2.3.2 Leptinotarsa decemlineata performance There was no evidence that *M. persicae* feeding affected *L. decemlineata* larval growth ($F_{2,27} = 1.56$, $P = 0.228$). Larval weight was consistent across all treatment types with the mean weight for all treatments being 3.23 g (Figure 3).

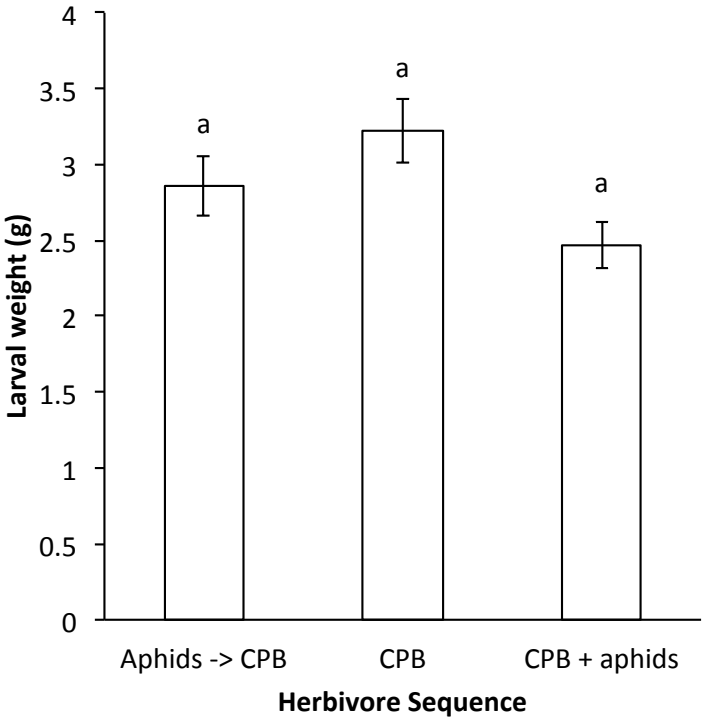

Figure 3: Mean ± SE *L. decemlineata* (CPB) larval weight when feeding on *S. tuberosum* plants after prior *M. persicae* (aphid) damage, feeding alone, or with simultaneous aphid damage. Means followed by same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

2.3.3 Glycoalkaloid content During the *M. persicae* performance trial, concentrations of the glycoalkaloid α-solanine did not differ between the control and the four treatments ($F_{4, 38} = 0.68$, $P = 0.6$) (Figure 4). However, when α-solanine was measured after the *L. decemlineata* performance trials, concentrations of were reduced when *L. decemlineata* fed after prior *M. persicae* damage compared to the controls and when *L. decemlineata* fed simultaneously with *M. persicae*, but was not significantly different from *L. decemlineata* feeding alone ($F_{3,29} = 2.97$, $P = 0.048$) (Figure 5).

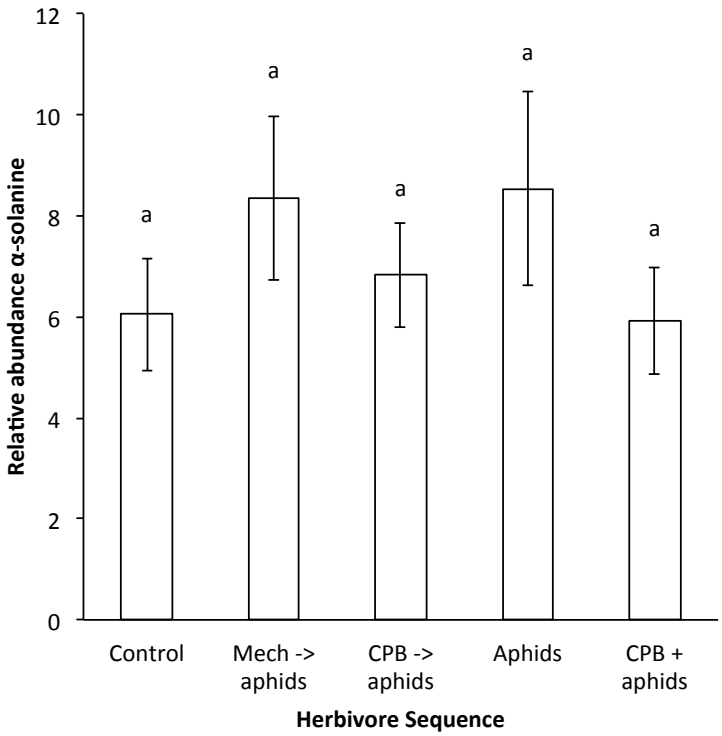

Figure 4: Impact of *M. persicae* (aphid) feeding on mean (± SE) α-solanine concentration (relative abundance in sample) from foliar extracts of *S. tuberosum* plants after feeding with prior mechanical damage, prior *L. decemlineata* (CPB) damage, alone, or simultaneously with CPB compared to undamaged controls. Means followed by same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

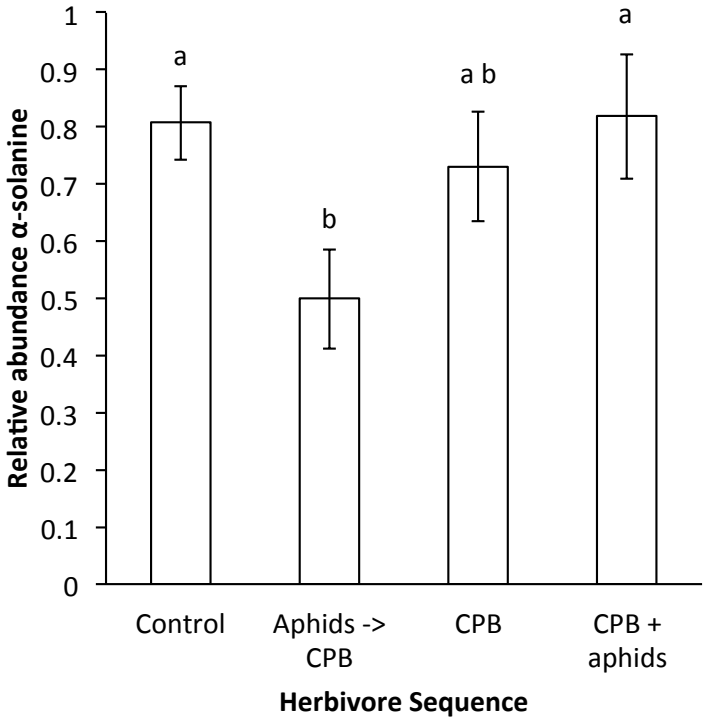

Figure 5: Impact of *L. decemlineata* (CPB) feeding on α-solanine concentration (mean ± SE) from foliar extracts of *S. tuberosum* plants after feeding with prior *M. persicae* (aphid) damage, feeding alone, or feeding simultaneously with aphids compared to undamaged controls. Means followed by same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

2.3.4 Phytohormone content During the *M. persicae* performance trial, JA increased when *L. decemlineata* damage occurred simultaneously with *M. persicae* feeding, but was lower when *L. decemlineata* were added prior to *M. persicae* and when *M. persicae* fed alone (Figure 6A) ($F_{4,26} = 3.71$, $P = 0.015$), while JA-ile increased only in response to simultaneous *L. decemlineata* and *M. persicae* feeding (Figure 6B) ($F_{4,30} = 3.74$, $P = 0.013$). Herbivore feeding sequence also affected SA production, and SA was found to be highest in response to simultaneous *L. decemlineata* and *M. persicae* feeding and lowest on controls. However, there was no significant difference between simultaneous feeding damage and the remaining three treatments or between the controls and the remaining three treatments (Figure 6C) ($F_{4,30} = 2.87$, $P = 0.04$). I found no effects of herbivore feeding sequence on SAG (Figure 6D) ($F_{4,32} = 0.969$, $P = 0.438$). In contrast,

during the *L. decemlineata* performance trial, JA was highest when *L. decemlineata* fed alone (Figure 7A) ($F_{3,20} = 4.03$, $P = 0.022$), but there was no effect on JA-ile (Figure 7B) ($F_{3,29} = 1.62$, $P = 0.207$), SA (Figure 7C) ($F_{3,24} = 0.106$, $P = 0.956$), or SAG (Figure 7D) ($F_{3,23} = 0.871$, $P = 0.47$).

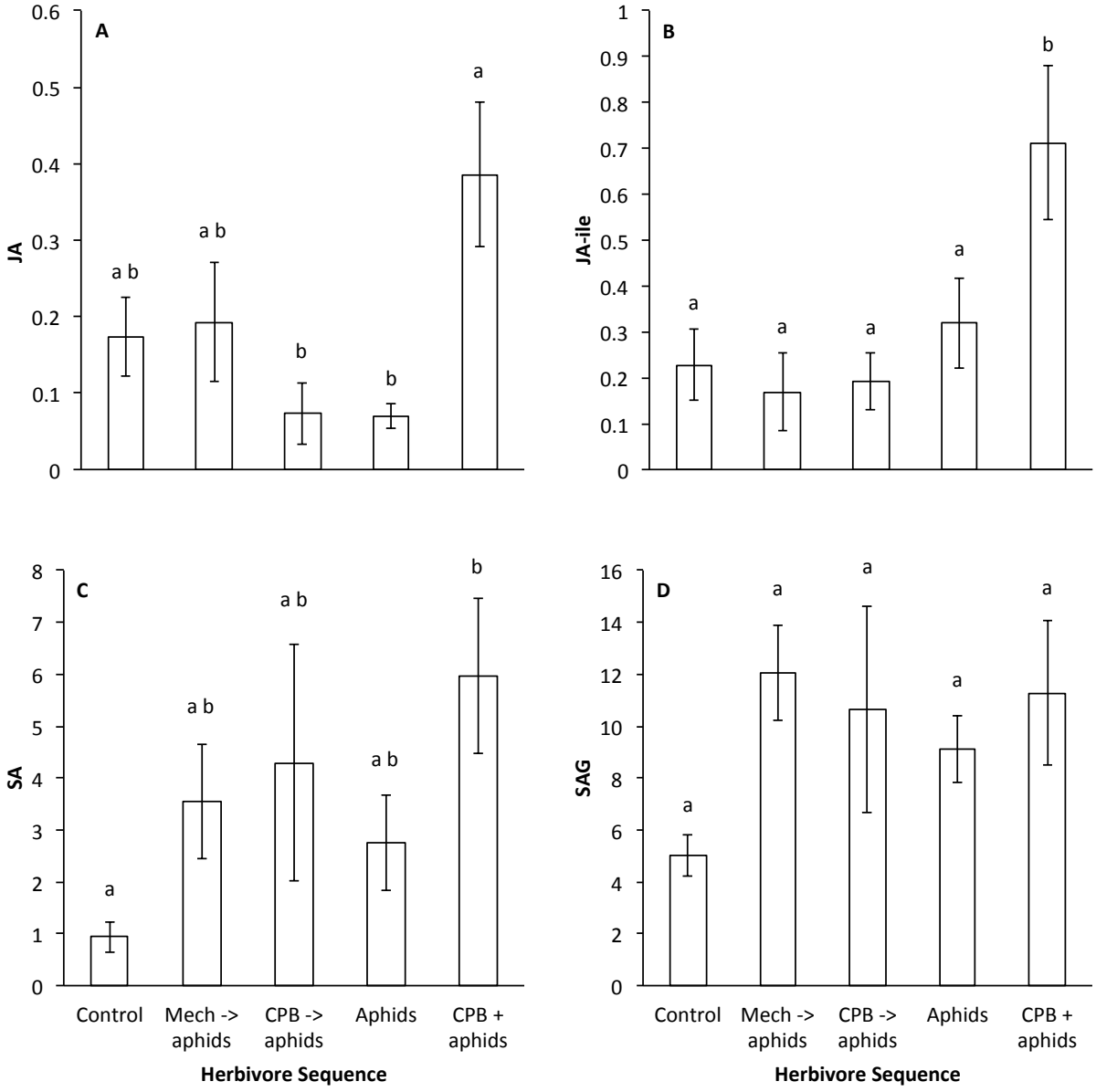

Figure 6: Impact of *M. persicae* (aphid) feeding on mean (± SE) JA (A), JA-ile (B), SA (C), and SAG (D) from foliar extracts of *S. tuberosum* plants after feeding with prior mechanical damage, prior *L. decemlineata* (CPB) damage, alone, or simultaneously with CPB, compared to undamaged controls. Means followed by same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

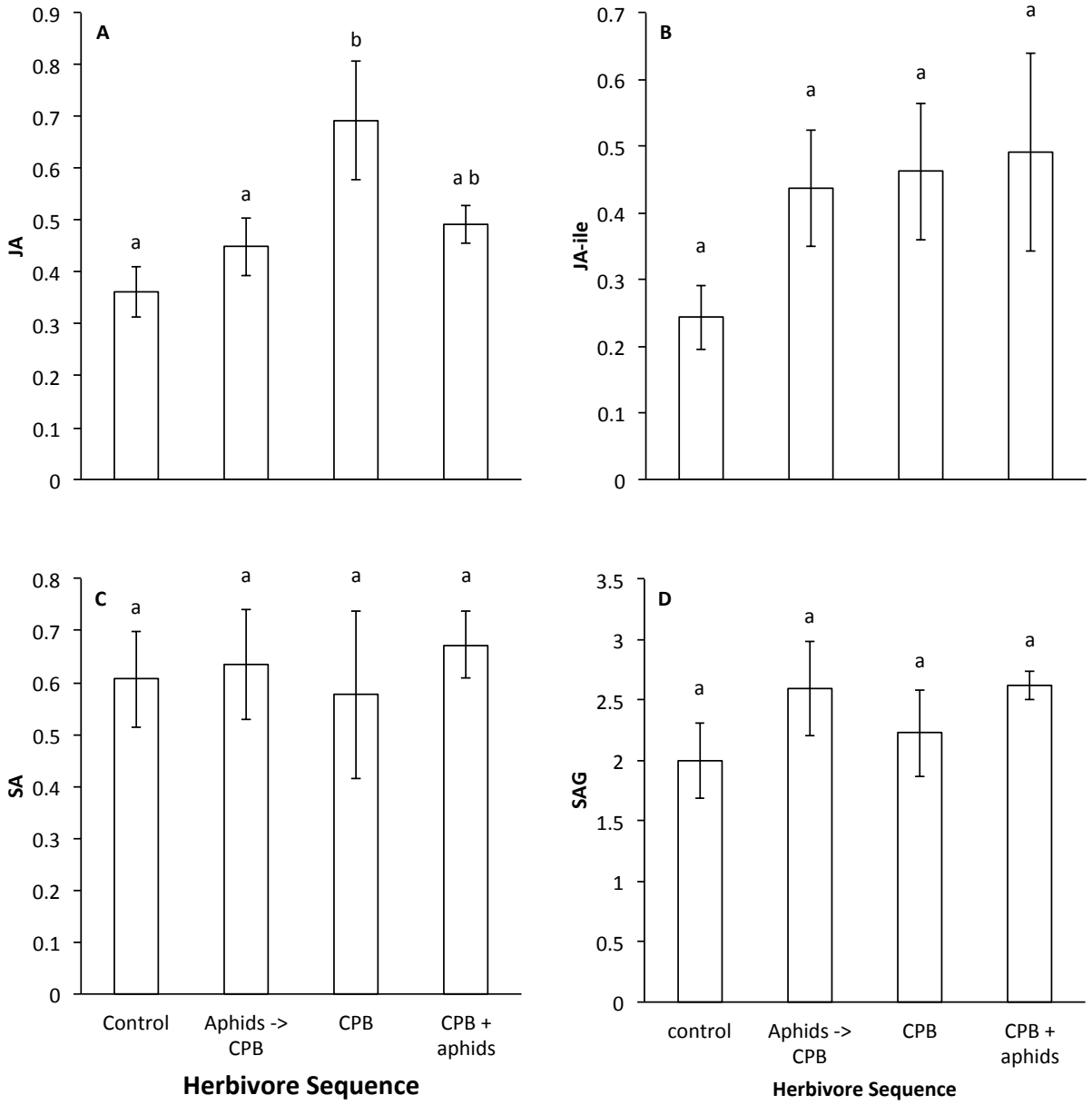

Figure 7: Impact of *L. decemlienata* (CPB) feeding on mean (± SE) JA (A), JA-ile (B), SA (C), and SAG (D) from foliar extracts of *S. tuberosum* plants after feeding with prior *M. persicae* (aphid) damage, alone, or simultaneously with aphids compared to undamaged controls. Means followed by same letter are not statistically significant (Tukey HSD, α=0.05).

2.4 Discussion

Herbivores can interact in a variety of ways, both directly (*e.g.* competition for a shared resource) and indirectly (*e.g.* plant mediated responses) (Lynch, Kaplan, Dively, & Denno,

2006). Here, I tested whether differential plant responses to herbivores from distinct feeding guilds could mediate interactions between two species. While previous studies have shown that aphid presence enhances larvae performance (at least in Lepidopteran species) (Ali & Agrawal, 2014; Rodriguez-Saona et al., 2005; Soler et al., 2012; Stout et al., 1998), this was not observed in my system. *Leptinotarsa decemlineata* larvae were not affected by herbivore damage inflicted by *M. persicae*. However, this response was not reciprocal, and *M. persicae* was negatively impacted by *L. decemlineata* presence. This has been the trend in similar studies, in which the presence of caterpillar feeding induces JA defenses and has negative fitness consequences on aphids (Agrawal, 1998; Ali & Agrawal, 2014; Cooper & Goggin, 2005; Walling, 2008). Aboveground plant biomass did not differ between treatments, so it is unlikely that these herbivore interactions were caused by a decrease in foliar availability.

During the *M. persicae* performance trial, JA levels were highest when *L. decemlineata* and *M. persicae* were added to plants simultaneously and lowest when *M. persicae* fed alone or after prior *L. decemlineata* damage. This pattern seems to fit the crosstalk model, which links induction of JA to *L. decemlineata* feeding and suppression of JA after aphid feeding. However, *M. persicae* when feeding simultaneously with *L. decemlineata* did not reduce JA. This could indicate that competition between signaling pathways exists, and the perceived risk of herbivore damage dominates the plant's response.

Although JA is important in the regulation of plant defenses, it is not free JA that triggers defensive traits. Instead, the amino acid conjugated form, Jasmonoyl-isoleucine (JA-ile) is responsible for defense activation (Farmer, 2014; Staswick & Tiryaki, 2004; Wang, Allmann, Wu, & Baldwin, 2008). Consequently, increased levels of JA-ile were observed when *L. decemlineata* and *M. persicae* were added simultaneously. However, when *L. decemlineata* were

added prior to *M. persicae*, both JA and JA-ile were suppressed. Timing and damage sequence can influence defensive outcomes (Erb et al., 2011) and in this case, plants responded differentially when *M. persicae* were added separately. This could be an artifact of JA-SA crosstalk in conjunction with the timing of herbivore applications, where the introduction of *M. persicae* post *L. decemlineata* damage induced SA, which suppressed JA. Salicylic acid accumulation was higher in all treatments containing *M. persicae* compared to controls, indicating that *M. persicae* do indeed induce SA. Glycosylated salicylic acid (SAG) functions as a storage form of SA (Klessig, 1994) but may also serve to prolong gradual release of SA after herbivore attack, therefore playing a prominent role in systemic acquired resistance (SAR) against pathogens (Kawano, Tanaka, Kadono, & Muto, 2004). Glycosylated salicylic acid was the same across all treatments, and given the slow-acting nature of SAG activity, measurements may have been taken too early to see demonstrable changes. In comparison, it took 28 d to observe SAG increases in the xylem sap of pathogen-infected *Brassica napus* (Ratzinger, Riediger, von Tiedemann, & Karlovsky, 2009), nearly twice as long as the duration of this experiment (16 d from initial *M. persicae* infestation).

Previous studies have shown that the presence of aphids decrease plant resistance to chewing herbivores (Ali & Agrawal, 2014; Rodriguez-Saona et al., 2005; Stout et al., 1998). However, there was no evidence that *M. persicae* induced a change in the measured phytohormones or defensive compounds drastically enough to affect *L. decemlineata* performance. *Leptinotarsa decemlineata* feeding alone induced the highest amount of JA, but in all treatments where *M. persicae* were present, *M. persicae* reduced JA to levels similar to those of the controls. Although JA was lower when *M. persicae* were present, this effect was not large enough to influence beetle feeding and biomass. Additionally, there was no difference between

the controls and treatments in JA-ile, SA, or SAG content, indicating that there may be additional interactions at play. For example, symbiotic bacteria are known to assist *L. decemlineata* in manipulating plant defenses by interfering with the JA-signaling pathway through activation of the SA-pathway, and subsequently, suppressing JA-inducible defenses (Chung et al., 2013). Therefore, although JA accumulation may occur, interference with JA signal transduction may transpire somewhere between the induction of free JA and the expression of JA-inducible traits (Doares, Narvaez-Vasquez, Conconi, & Ryan, 1995). However, even if *L. decemlineata* were activating the SA-pathway, it would still be expected that SA levels would increase in herbivore treatments compared to the controls. It is still unclear what role crosstalk plays in mediating interactions between *L. decemlineata* and *M. persicae*.

Leptinotarsa decemlineata and *M. persicae* have contrasting effects on glycoalkaloid production, where damage by *L. decemlineata* elevates glycoalkaloid concentration and damage by *M. persicae* hinders glycoalkaloid levels (Fragoyiannis et al., 2001; Hlywka et al., 1994). Jasmonic acid stimulates glycoalkaloid production (Chen, Flickinger, & Miller, 1998), and it is therefore expected that changes in JA concentration should correlate to glycoalkaloid levels. However, this relationship was not observed in this study. In this study, only one glycoalkaloid was measured (α-solanine). Although α-solanine is an important feeding deterrent in *S. tuberosum*, other glycoalkaloids, such as α-chaconine, are also present, and the various combinations and concentrations determine the degree of insect resistance (Kowalski et al., 1999).

These measurements provide only a glimpse of the many mechanisms that can be functioning when plant-herbivore or herbivore-herbivore interactions occur. In this case, the reciprocal effects on herbivore performance due to plant response to each feeding guild were

asymmetrical, where *L. decemlineata* negatively affected *M. persicae* growth, while *M. persicae* had no effect on *L. decemlineata* performance. Asymmetry of plant-mediated interactions between herbivores from different feeding guilds has been demonstrated in other systems, most notably in aphid-caterpillar interactions, where aphids positively affect caterpillar growth and performance, with either negative or temporal effects on aphid performance (Ali & Agrawal, 2014; Soler et al., 2012). Both *M. persicae* and *L. decemlineata* are regulated by JA defenses. However, aphids are commonly known to induce SA, which may be a feeding strategy used by aphids and other sucking herbivores to manipulate plant defenses (Ali & Agrawal, 2014; Zarate et al., 2007). Induction of SA and crosstalk between JA and SA signaling pathways would potentially lead to reduction in JA, allowing aphids to be less apparent to plant detection. Additionally, with lowered JA defenses, the plant could then become susceptible to damage by other herbivores that are also regulated by JA related defenses. However, other plant hormones, such as abscisic acid (ABA) and ethylene (ET), are also known to regulate plant functions and can interact with other signaling pathways (Erb, Meldau, & Howe, 2012; Thaler et al., 2012; Walling, 2000). Performing similar experiments while taking additional measurements, such as ABA, ET, and α-chaconine could improve our understanding of how *S. tuberosum* handles herbivore attacks by these two species.

CHAPTER 3.

RECIPROCAL EFFECT OF FEEDING GUILD DAMAGE ON PLANT VOLATILES AND HERBIVORE HOST-PLANT PREFERENCE

3.1 Introduction

Host-plant location for phytophagous insects is vital for successful reproduction and nutrient acquisition. Herbivores distinguish between cues among host plants and non-host plants, and plant volatiles play a significant role in guiding insects to their hosts (Bruce, 2011). Volatile organic compounds (VOCs) are primarily composed of terpenes, benzenoids, and green leaf volatiles with most plant species constitutively emitting between 20 and 60 of these various compounds (Dudareva, Negre, Nagegowda, & Orlova, 2006). VOCs can inform host-seeking herbivores of plant identity, quality, and location (Bruce, 2011). However, volatile blends and proportions can change over time and in response to biotic and abiotic changes (Bruce, Wadhams, & Woodcock, 2005).

It is well documented that volatile blends emitted by plants under herbivore attack differ from those constitutively emitted by intact plants (Karban & Baldwin, 1997), and furthermore, the feeding guild of the attacking herbivore influences the defense response, which subsequently impacts the quality and quantity of the volatiles emitted, leading to changes in herbivore behavior and performance (De Moraes, Mescher, & Tumlinson, 2001; Rodriguez-Saona et al., 2005). Changes in volatile profiles can impact host-plant location, either by making the plant unattractive (De Moraes et al., 2001), more attractive (Ngumbi, Eigenbrode, Bosque-Perez, Ding, & Rodriguez, 2007), or cryptic (Thiery & Visser, 1987). Therefore, host-plant recognition by one herbivore could be affected by the plant's response to herbivores from another feeding guild.

Herbivore damage can induce volatiles, such as green leaf volatiles and terpenes, through the JA signaling pathway (Dicke et al., 2009; Matsui, 2006; Schmelz, Alborn, Banchio, & Tumlinson, 2003) but less is known about the induction of volatiles through the SA pathway (Dicke et al., 2009). Activation of JA and SA related responses are in part regulated by herbivore feeding guild, and in the previous chapter I found that plant response can mediate reciprocal interactions between herbivores from separate guilds. Although studies have shown that plant induced responses to different feeding guilds can affect reciprocal herbivore performance or behavior, linking these two traits in a single study can improve our understanding of herbivore host-plant selection and the consequences of cohabitation on a single host plant (Erb et al., 2011; Lynch et al., 2006).

This study explores the induction of plant volatiles in response to feeding by contrasting feeding guilds and evaluates the influence of feeding guild related plant response on herbivore host-plant location. First, I evaluated whether herbivores from different feeding guilds, aphids (*Myzus persicae*) and Colorado potato beetles (*Leptinotarsa decemlineata*) differentiate between potato plants (*Solanum tuberosum)* induced by herbivory from the contrasting guild. I then determined the composition of volatiles induced by these distinct herbivores on potato in order to better understand what chemical cues might play a role in host-plant location. Understanding how multiple herbivores affect defense responses within an individual plant and how herbivores locate host plants can provide information relative to the development of alternative control strategies via the manipulation of host-locating cues and disruption of host-seeking behavior.

3.2 Materials and Methods

3.2.1 Insect and plant material The *M. persicae* colony was established from aphids provided by Cornell University (Ithaca, NY). The colony was maintained on *S. tuberosum* cv. Atlantic plants within a growth chamber (24°-25°C, 48-52%RH, L16:D8). *Leptinotarsa decemlineata* individuals were obtained from a colony reared on *S. tuberosum* cv. Atlantic in an insectary at 25 °C and L16:D8 photoperiod at Michigan State University.

Experiments were carried out using four- to five-week old *S. tuberosum* cv. Atlantic plants that were propagated from vegetative seed produced at the Montcalm Potato Research Farm (Stanton, MI). Plants were grown in 10 cm plastic pots with a perlite soil mix (Suremix Perlite, Michigan Grower Products Inc., Galesburg, MI). All plants were grown in a growth chamber maintained at 25-28 °C, 55-58% RH, under a photoperiod of L16:D8 and fertilized weekly with a water soluble 20-20-20 (N-P-K) fertilizer (J.R. Peters Inc., Allentown, PA).

3.2.2 Y-tube assay A glass y-tube olfactometer (Figure 8) was used to assess the behavioral responses of each herbivore species to infested and uninfested plants. The olfactometer consisted of an 11 cm long glass tube that branched into two 7.5 cm arms (Michigan State University, East Lansing, MI). The internal diameter of the tube and arms was 1.5 cm. Each arm of the olfactometer was connected with Teflon tubing to a 35 cm tall x 15 cm wide closed glass chamber (Michigan State University, East Lansing, MI). Each chamber contained either an infested or uninfested potato plant, allowing each test herbivore to make a choice between the two host-plant odors. Charcoal purified and humidified air was pushed through the glass chambers and into both arms of the olfactometer at a constant airflow of 0.1 L/min regulated by flow meters. The bioassays were carried out in a temperature controlled room maintained at

25±1°C and 58-70% RH. The olfactometer was positioned horizontally with a single light source fixed 30 cm in front of the olfactometer at a height of 20 cm. At the beginning of the assays, an individual herbivore was placed in the base of the olfactometer and was observed until a choice had been made, or for a maximum of 15 min. A choice was recorded when the herbivore moved at least halfway into one of the arms connecting to an odor source. Non-responding herbivores were recorded as such, but excluded from statistical analyses. The odor sources were rotated after every two replications in order to exclude positional biases and plants were replaced after six replications. The y-tube was washed with Alconox® Powdered Precision Cleaner (New York, NY) and then rinsed with acetone. The glassware was then heated in a drying oven at 60°C and left to cool at room temperature prior to use in proceeding assays.

Figure 8: Y-tube olfactometer used for two-choice preference test. Each arm of the olfactometer is connected to an odor source. A four-sided box (not shown) is fitted over the y-tube so that the only light source comes in from the front of the apparatus.

3.2.3 Myzus persicae response to Leptinotarsa decemlineata infested and uninfested plants

Prior to the start of each assay, two potato plants were individually isolated in separate cages. Three second-instar *L. decemlineata* larvae were added to one plant and left to feed freely for three days while the other plant remained uninfested. After three days, alates were randomly selected from the *M. persicae* colony and individually placed into 2 oz plastic cups with lids for one hour prior to running the assays. Potato plants were then added to the glass volatile collection chambers so that each arm of the olfactometer was connected to either an herbivore-damaged or undamaged host-plant odor source. Bioassays were carried out as described above.

All bioassays were conducted between 2 March 2015 and 23 March 2015 using a total of 45 individuals.

3.2.4 Leptinotarsa decemlineata response to Myzus persicae infested and uninfested plants To induce a plant response due to *M. persicae* feeding, two potato plants were individually isolated in separate cages five days prior to the start of each assay. Twenty mixed instar apterous aphids were placed onto one potato plant and left to feed freely. On the fifth day, gravid females were randomly selected from the *L. decemlineata* colony and individually placed into 2 oz. plastic cups with lids for one hour prior to the start of each assay. Bioassays were carried out as previously described. All bioassays were conducted between 15 April 2015 and 11 August 2015 using a total of 53 individuals.

3.2.5 Collection and analysis of plant volatiles from herbivore infested and uninfested plants Plants were infested by either *L. decemlineata* or *M. persicae* as described above, or damaged mechanically using a sharp, clean blade by removing 2 mm foliar tissue from the leaf margin of a fully expanded primary leaflet each day for three days. Five or six plants were used during each headspace collection. Collections were repeated 10 times per treatment yet differences in collection efficiency resulted in a total of 8 valid *L. decemlineata* infested samples, 3 valid *M. persicae* infested samples, and 9 valid uninfested control samples. Infested and uninfested plants were confined in separate glass volatile collection chambers and a push-pull system was used to collect headspace. Charcoal filtered air was pushed through a valve at the base of the collection chamber and pulled through a HayeSep Q (Agilent, Santa Clara, CA, USA) adsorbent trap near the top of the chamber for 3 h. Adsorbent traps were eluted with 200 µl of dichloromethane and

tetradecane (200 ng) was added to each sample as an internal standard. Volatile extracts were analyzed using a gas chromatograph (Agilent 7890A) equipped with a DB-5 capillary column coupled with a Mass Selective Detector (Agilent 5975C). Compounds were separated by injecting 1.0 µl aliquots into the GC/MSD. Compounds were identified by comparing mass spectra with the NIST library. The compounds with spectral fit values equal to or greater than 90 and appropriate LRI values were considered positive identifications. Compounds were quantified as equivalents of the total amount of tetradecane within each analyzed volatile collection sample.

3.2.6 Statistical analyses A chi-square (χ^2) test of goodness-of-fit was used to compare the observed number of herbivores entering either the treatment or control arm for each species, with the null hypothesis being that each species would choose both arms of the olfactometer equally. Analyses were conducted using R software version 3.1.2 (R Core Team, 2014).

The characteristic set of variables in a volatile profile that defined a particular group (*e.g.* damaged versus non-damaged plants) was found using the 'varSelRFBoot' function of the package 'varSelRF' for the 'randomForest' analysis (R software version 3.2.4, R Development Core Team 2016). A *varSelRF* algorithm with Random Forests was used to select the minimum set of VOCs that were characteristic of differences between infected and non-infected plants. The tree-based Random Forests algorithm performs hierarchical clustering via multi-scale and combinatorial bootstrap resampling and is most appropriate for data where the variables (VOCs in this case) outnumber the samples, and where the variables are auto correlated, which is a typical problem of conventional multivariate analysis of such data. Two-hundred bootstrapping iterations of the Random Forest algorithm were employed to arrive at a minimal set of VOCs that could differentiate between damaged and non-damaged plants. The mean decrease in accuracy

(MDA) was also calculated when individual VOCs are removed from the analysis. MDA values indicate the importance value of particular VOCs for the discrimination between treatments. A one-way ANOVA was then used to compare the effect of herbivore feeding on each compound within the identified characteristic set of VOCs from all treatments and Tukey tests were used to evaluate significance between each group ($p < 0.05$).

3.3 Results

3.3.1 Response of Myzus persicae to Leptinotarsa decemlineata infested and uninfested plants

Myzus persicae differentiated between the volatile blends emitted by *L. decemlineata* infested plants and uninfested plants (χ^2 (1, n = 30) = 8.53, P <0.01) (Figure 9). Including the number of 'no choices' (n = 15) in the analysis did not impact the statistical significance of choices between *L. decemlineata* infested and uninfested plants, and were therefore removed from the analysis. Of the 30 aphids that responded in the olfactometer, 77% oriented towards *L. decemlineata* infested plants.

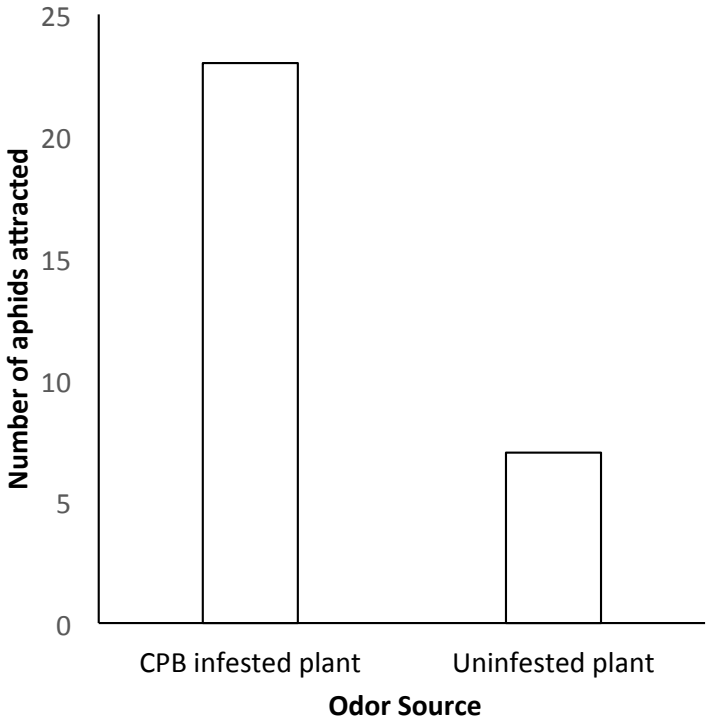

Figure 9: Total number of *M. persicae* (aphids) responding to volatiles emitted by *L. decemlineata* (CPB) damaged plants and undamaged plants (n = 30).

3.3.2 Response of Leptinotarsa decemlineata to Myzus persicae infested and uninfested plants

Leptinotarsa decemlineata were also able to differentiate plant volatiles from damaged and undamaged plants (χ^2 (1, n = 49) = 9, p < 0.01) (Figure 10). However, in contrast to *M. persicae* preference, *L. decemlineata* females were more attracted to the volatile blends emitted by uninfested plants compared to *M. persicae* infested plants. Including the number of 'no choices' (n = 4) did not impact the significance of choices between *M. persicae* infested and uninfested plants and these observations were removed from the analysis. Of the 49 beetles that responded in the olfactometer, 71% oriented towards uninfested plants over *M. persicae* infested plants.

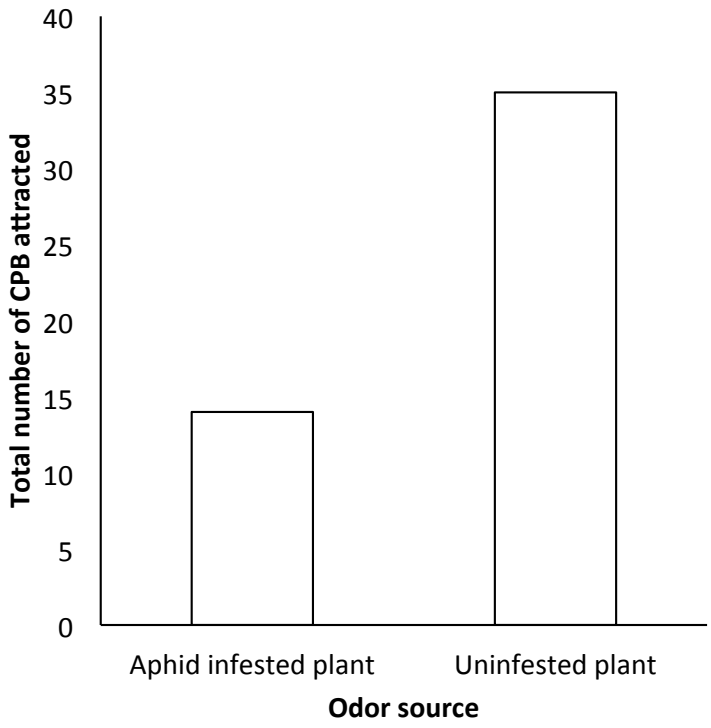

Figure 10: Total number of *L. decemlineata* (CPB) responding to volatiles emitted by *M. persicae* (aphid) damaged plants and undamaged plants (n = 49).

3.3.3 Volatile collection and headspace analysis In total, 21 compounds were identified between headspace collections from *S. tuberosum* plants under herbivore attack by *M. persicae* or *L. decemlineata*, mechanical damage, and no damage. Undamaged and *L. decemlineata* damaged plants emitted the most variety in their volatile profiles (20 and 19 compounds, respectively), while only 11 compounds were identified in the headspace collected from *M. persicae* damaged or mechanically damaged plants (Table 1, Figure 11). Nine compounds were found across all plant treatments, but in varying quantities.

Using a Random Forest algorithm, a minimum of three compounds, 3-ethyl-3-methylheptane, 4,8-dimethyl-1,3(E), 7-nonatriene, and methyl salicylate were identified that discriminated between the VOC signatures of these plant treatments, with an estimate prediction error of 0.532 and a 'leave-one-out' bootstrap error of 0.581. I found that 3-ethyl-3-

methylheptane was highest in plants that were damaged by *L. decemlineata* and lowest in mechanically damaged plants ($F_{3,19} = 10.1$, $P < 0.001$); 4,8-dimethyl-1,3(E), 7-nonatriene was highest in plants that were damaged by *L. decemlienata*, but was not found in either *M. persicae* or mechanically damaged treatments ($F_{3,19} = 5.41$, $P = 0.007$); methyl salicylate was not apparent in either *M. persicae* or mechanically damaged treatments ($F_{3,19} = 6.46$, $P = 0.003$) (Figure 12).

Table 1: Mean percent contribution of individual compounds to total headspace of *M. persicae* (aphid) damaged, *L. decemlineata* (CPB), mechanically (Mech) damaged, and undamaged *S. tuberosum* plants.

Compound name	***Aphid damage***	***CPB damage***	***Mech. damage***	***No damage***
2,4 Dimethyl-1-heptene	0.00	2.16	3.87	2.94
Hexanoic acid	0.00	1.26	0.00	3.76
Octanal	8.66	0.00	11.67	1.51
1-Hexanol, 2-ethyl-	4.39	4.77	8.04	5.89
Benzyl alcohol	0.00	9.96	0.00	8.62
3-Ethyl-3-methylheptane	4.08	3.24	3.63	2.69
1,2 Cyclohexanediol	4.60	3.41	4.98	6.75
Acetophenone	3.16	1.95	13.32	2.45
Nonanal	24.03	2.80	22.44	4.42
4,8-Dimethyl-1,3(E), 7-Nonatriene	0.00	4.49	0.00	2.93
Octanoic acid	0.00	2.21	0.00	7.17
Methyl salicylate	0.00	4.00	0.00	3.99
Decanal	9.70	5.84	8.60	2.64
Nonanoic acid	0.00	4.93	0.00	13.34
Decanoic acid	0.00	18.46	0.00	10.05
α-Copaene	7.71	0.00	0.00	2.16
Unknown terpene	2.38	2.11	11.00	4.30
β-Caryophyllene	20.50	17.19	4.13	8.81
α-Humulene	0.00	2.49	0.00	1.36
Curcumene	0.00	2.31	0.00	0.00
Germacrene D	10.80	6.43	8.32	4.24
Number of compounds represented	**11**	**19**	**11**	**20**

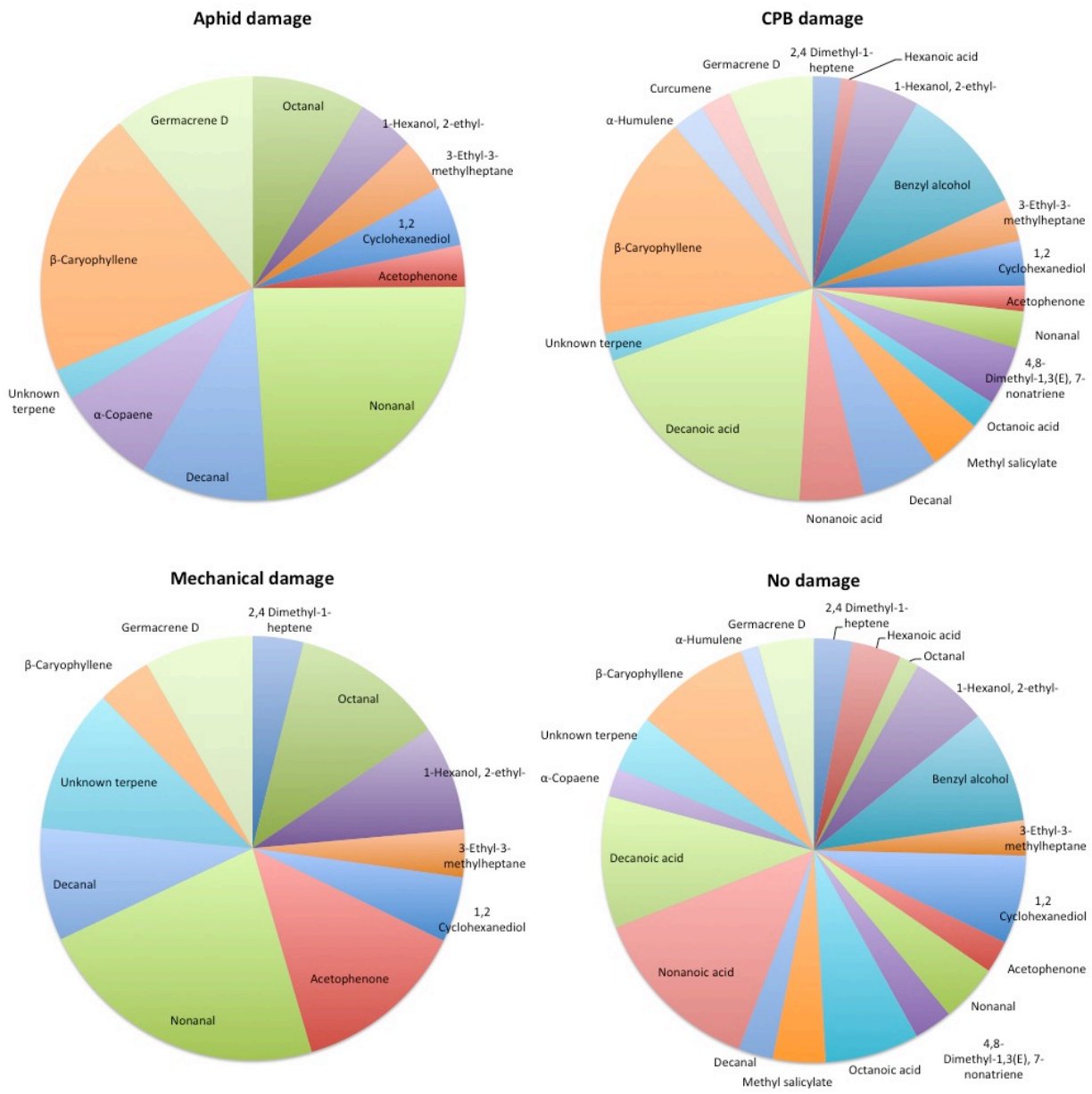

Figure 11: Mean proportions of individual compounds to volatiles blends emitted by *M. persicae* (aphid) damaged (n = 3), *L. decemlineata* (CPB) damaged (n = 8), mechanically damaged (n = 3), and undamaged (n = 9) *S. tuberosum* plants.

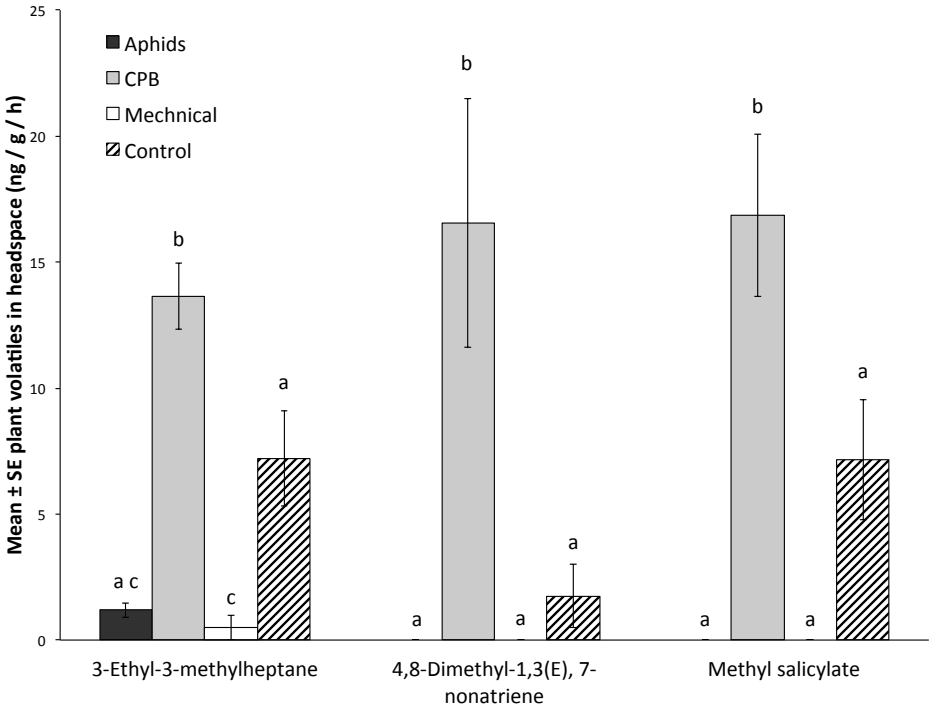

Figure 12: Comparison of the three major compounds, 3-ethyl-3-methylheptane, 4,8-dimethyl-1,3(E), 7-nonatriene, and methyl salicylate, that were identified as distinguishing among all treatments via the random forest algorithm. Means followed by same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

3.4 Discussion

Plants produce a range of VOCs characteristic to the plant species and the plant's association with biotic and abiotic conditions (Bruce, 2005). Attack by herbivores can induce a change in the chemical composition of volatile bouquets, both qualitatively and quantitatively (Dicke, van Poecke, & De Boer, 2003; Paré & Tumlinson, 1997). In this study I demonstrated that damage inflicted mechanically and damage inflicted by herbivores from different feeding guilds uniquely altered the chemical profiles of the volatile emissions of *S. tuberosum* under laboratory conditions. Herbivores utilize VOCs to locate host plants (Bruce, 2005) and here I have shown that both *L. decemlineata* and *M. persicae* can differentiate between odors emitted by plants damaged by herbivores from another feeding guild compared to those emitted by undamaged plants. Both *L. decemlineata* and *M. persicae* demonstrated a distinct preference

towards a particular plant type. However, the response was not parallel and *M. persicae* oriented more frequently towards plants that were damaged by *L. decemlineata* while *L. decemlineata* oriented more frequently towards undamaged plants.

In contrast with previous studies investigating the effect of feeding guild on volatile release, *M. persicae* damage induced fewer VOCs than *L. decemlineata* (Gosset et al., 2009). Additionally, the general consensus has been that aphids prefer healthy plants over mechanically or herbivore damaged ones (Bernasconi, Turlings, Ambrosetti, Bassetti, & Dorn, 1998; Nottingham et al., 1991), yet this was not the case in my study, where *M. persicae* preferred plants that were previously damaged by *L. decemlineata*. Although the profiles differ qualitatively, comparatively speaking, the headspace sampled from *L. decemlineata* damaged and undamaged plants contained nearly the same quantity of volatiles. *Myzus persicae* is a generalist herbivore, so its response to VOCs might also be based on more general cues. β-Caryophyllene is widespread throughout many plant species (Knudsen, Tollsten, & Bergström, 1993) and has been identified as a candidate attractant for *M. persicae* (Eigenbrode et al., 2002) and β-caryophyllene was over twice as high in *L. decemlineata* damaged plants compared to controls. However, it is difficult to attribute attraction to a single compound because it is the blend of volatiles, as opposed to individual compounds, that influence herbivore behavior (Ngumbi et al., 2007). Additional studies focusing on the compounds and doses present as well as their combinatorial effect on *M. persicae* are necessary to better understand host-plant attraction.

Also contrary to previous studies that have shown that *L. decemlineata* are more attracted to damaged plants than undamaged plants (Bolter, Dicke, Van Loon, Visser, Posthumus, 1997; Landolt et al., 1999; Schütz, Weißbecker, Klein, & Hummel, 1997), this study concluded that *L.*

decemlineata were more attracted to undamaged plants. However, the treatments used in these previous studies only compared wound induced responses, such as response to mechanical damage, conspecifics, other chewing herbivores, and applications of compounds associated with chewing herbivores. Previous studies also did not include damage induced by herbivores from different feeding guilds, although it is known that feeding guild can influence defensive outcomes (Dicke, 2009). Nonanal, one of the dominant leaf volatiles released by potato plants after *L. decemlineata* damage (Gosset et al., 2009) was previously found to be unattractive to *L. decemlineata* (Schultz et al., 1997). In my samples, nonanal was most present in *M. persicae* damaged plants and could be a contributing factor in *L. decemlineata* host choice.

The differences in the volatile profiles observed in this study compared to previous studies in potato could be linked to species or cultivar variation as well as timing of induction and herbivore density. For example, the volatile profiles that were identified by Gosset et al. (2009) were taken between 8 and 72 hr following infestation by either 60 *M. persicae* or two *L. decemlineata* larvae. In contrast, I used 25 *M. persicae* and collected volatiles after 5 d, and three *L. decemlineata* larvae and collected volatiles after 3 d. Additionally, the cultivar used in that study was *S. tuberosum*, cv. Désirée. The blend of volatiles emitted by a particular cultivar can differ from other varieties and influence herbivore response (Rajabaskar et al., 2013). As domestication favors desirable characteristics, resistant traits may be lost, leading to diverging phenotypic responses. For example, recent domestication for high-yielding cranberry varieties negatively impacted certain defensive traits, such as lowered JA induction and induced volatile emissions, subsequently leading to increased pest performance (Rodriguez-Saona et al., 2011). Similarly, the domestication of North American maize lines has resulted in the loss of (E)-β-caryophyllene production, a natural enemy attractant (Köllner et al., 2008). Therefore, future

studies should also take into account cultivar variation when interpreting herbivore responses to host volatiles.

It was expected that herbivores would seek host plants that would maximize their performance. However, in this study it was found that herbivore host-plant preference was not necessarily correlated to herbivore performance. For example, in the previous chapter, I demonstrated that *M. persicae* performed best when they were feeding on previously undamaged plants. Plants that were mechanically damaged or damaged by *L. decemlineata* negatively impacted *M. persicae* growth, while *L. decemlineata* performed equally well on undamaged and *M. persicae* damaged plants. Yet both herbivores were able to discriminate between damaged and undamaged plants. Surprisingly, *M. persicae* preferred *L. decemlineata* damaged plants, despite the fitness consequence, while *L. decemlineata* preferred undamaged plants. It is unknown which volatiles these herbivores are responding to and additional studies focusing on the combinations and concentrations of compounds present in the volatile profiles are necessary in order to determine which compounds activate an herbivore response.

VOCs not only act as signals for host seeking herbivores, but also mediate interactions between plants and other neighboring organisms, such as nearby conspecific and heterospecific plants, pollinators, and herbivore natural enemies (Dicke & Baldwin, 2010; Dicke et al., 2003; Dudareva et al., 2006; Poelman, van Loon, & Dicke, 2008). Changes in volatile composition, such as those induced by herbivory, can alter the interactions between plants and their associated organisms, thus changing community composition (Poelman, 2015). Although we are only beginning to gain understanding on the effects of plant defense and resistance to multiple herbivores, it is important to take into consideration the full range of community interactions, particularly when making management decisions in agroecosystems. Understanding how

changes in a single component within a landscape can facilitate community-wide effects can help predict the outcomes of management decisions and whether these decisions will be beneficial to the community, the plant, and ultimately, the grower and consumers.

CHAPTER 4.

FIELD STUDY: RECIPROCAL EFFECT OF INSECT FEEDING GUILD ON PLANT DEFENSE, HERBIVORE PERFORMANCE, AND YIELD

4.1 Introduction

Observing plant-insect interactions in a laboratory setting is a useful way to scrutinize behavioral and biological processes that occur under controlled conditions. However, we cannot always rely on laboratory results to extrapolate how interactions occur in natural settings. Therefore, rigorous field tests are often necessary to validate the reliability of laboratory results. I previously found that *L. decemlineata* and *M. persicae* can indirectly interact through plant-mediated responses. *Myzus persicae* was negatively affected by *L. decemlineata* larval feeding, but *M. persicae* feeding had no effect on *L. decemlineata* performance. However, these results are contingent on controlled laboratory conditions and it is unknown whether the same effect would be observed in the field.

Investigating the impact of feeding guild on plant defensive traits and herbivore performance in a field setting can address both fundamental and applied questions. Phenotypic plasticity allows plants to respond to herbivore attacks; however, knowing what the trade-off is between investment in reproduction or growth and investment in plant defenses (Agrawal, 1999; Zangerl & Bazzaz, 1992) could be useful for the manipulation of crop protection and optimization of yield (Kaplan et al., 2009). It is expected that under low pest pressure, the cost of defense is greater than the fitness benefit, therefore, to maximize fitness, plants should only respond defensively when the risk of herbivore damage to fitness is greater than the cost associated with defense (Agrawal, 1999). Understanding the causal mechanisms and the consequences of defense can lead to improvement of crop quality. Additionally, as demonstrated

in the previous chapters, herbivores from different feeding guilds can interact on the plant interface with variable consequences. Although it may seem intuitive that as the variety of herbivores increase, the more negative the cumulative effect will be on plant fitness, there is evidence that the co-occurrence of herbivores from different feeding guilds could also lead to a positive outcome. For example, the presence of the mirid bug (*Helopeltis sulawesi*), a sucking herbivore, reduces oviposition and feeding of the pod-boring moth (*Conopomorpha cramerella*) on cacao, leading to a 51% yield increase (Wielgoss, Clough, Fiala, Rumede, & Tscharntke, 2012). Evaluating the yield loss or increase associated with plant-mediated interactions between herbivores from different feeding guilds could lead to more precise pest management recommendations.

This study mimics the initial performance assays conducted under laboratory conditions in which I addressed whether the plant response to reciprocal damage by herbivores from distinct feeding guilds could impact plant response and herbivore performance. For this study, I also investigated whether the cost of defense associated with the sequence of herbivore damage affects plant performance (*i.e.* yield) shortly after damage is inflicted as well as at the end of the season harvest.

4.2 Materials and Methods

4.2.1 Field site and insect material All experiments were carried out at the Montcalm Research Farm (Stanton, MI) during the 2015 growing season (May-Sep). Potatoes (*Solanum tuberosum* cv. Atlantic) were planted in mounded rows at the beginning of the season. On Jun 19 I deployed 70 exclusion cages randomly throughout the field. Cages were constructed using four 1 m segments of 2.54 cm PVC pipe and flagging wire covered with fine mesh to create a tent with a

drawstring opening at the top. Loop stakes were used to secure the cages in place and the sides of the cages were buried 15 cm below the soil surface (Figure 13). Neighboring plants were removed to ensure that only one plant was contained within each cage. Cages were monitored weekly and unwanted insects were manually removed when necessary.

Mixed instar apterous *M. persicae* were obtained from a colony established from aphids collected on potato plants (*S. tuberosum* cv. Atlantic) in a greenhouse at Michigan State University (East Lansing, MI). *Leptinotarsa decemlineata* neonates were collected from multiple egg clutches in a colony maintained at Michigan State University.

Figure 13: Exclusion cage construction for field study. Each cage contained one *S. tuberosum* plant.

4.2.2 Myzus persicae performance In order to evaluate *M. persicae* performance in the field, the following three treatments were applied (n = 10 for each treatment): (1) *M. persicae* added to

individual plants; (2) *M. persicae* added simultaneously with *L. decemlineata* larvae; (3) *M. persicae* added to plants that were previously infested with *L. decemlineata* larvae. Undamaged plants were used as the experimental control (n = 7). Five days after the cages were deployed in the field, 20 *L. decemlineata* larvae were applied to plants being treated with sequential herbivore damage (treatment (3)). Dead larvae were replaced 2 d later so that each replicate had a minimum of 15 neonates (max = 17). After 5 d, 50 aphids were added to each plant receiving sequential herbivore damage (treatment (3)), simultaneous herbivore damage (treatment (2)), and to the aphid only controls (treatment (1)). Additionally, 20 *L. decemlineata* larvae were added to each plant receiving simultaneous herbivore damage (treatment (2)). Aphid densities were counted approximately every 5 d, for a total of five counts. Two to three observations were made for each replicate on each counting date. The highest number recorded was used for statistical analyses because it was more likely to miss an aphid than it was to count it twice. On the fifth counting date, foliar tissue of 5 plants from each treatment and 3 control plants were sampled for glycoalkaloid and phytohormone analysis and then the whole plant was harvested to evaluate yield. The remaining plants were left caged in the field for the end of season yield evaluation (n = 5 for all herbivore treatments, n = 4 for undamaged controls).

4.2.3 Leptinotarsa decemlineata performance Three treatments (n = 10 for each treatment) plus undamaged controls (n = 7) were used to evaluate *L. decemlineata* performance in the field. *Leptinotarsa decemlineata* larvae were added either (1) to individual potato plants; (2) simultaneously with *M. persicae*; or (3) to plants previously infested by *M. persicae*. Five days after the cages were deployed in the field, 50 aphids were added to plants being treated with sequential herbivore damage (treatment (3)). Aphids were left to feed for 5 d, after which 15 *L.*

decemlineata larvae were added to each plant receiving only *L. decemlineata* damage (treatment (1)), each plant receiving simultaneous damage (treatment (2)), and each plant receiving sequential damage (treatment (3)). An additional 50 aphids were added to each simultaneously damaged treatment plant (treatment (2)). Dead larvae were replaced 2 d later so that each plant had a minimum of 15 larvae (max = 16). Herbivores were left to feed for an additional 5 d. Larvae were then removed and weight was recorded. Foliar tissue was sampled for glycoalkaloid and phytohormone analysis from a subset of five plants of each treatment plus 3 control plants, and then the whole plant was harvested to evaluate yield. Larvae were removed from the remaining plants 7 d later and weighed in the lab to determine whether the initial *L.* decemlineata performance results were consistent over time. The remaining plants were left in the field until the end of season for additional yield comparisons (n = 5 for all herbivore treatments, n = 4 for undamaged controls).

4.2.4 Glycoalkaloid and phytohormone analysis Foliar tissue was sampled in the field by removing an upper lateral stem that was then sealed in a plastic storage bag and placed in a cooler with dry ice. Once a sample was collected from each plant, the coolers were transported back to the laboratory. Samples were removed individually from the cooler and 100 mg of tissue were excised and then immediately flash frozen in liquid nitrogen and placed in cold storage (-80°C). Frozen tissue was processed and analyzed for glycoalkaloid and phytohormone content as previously described for the laboratory performance assays.

4.2.5 Yield Yield was measured both mid-season (*i.e.* at the end of each performance trial) and at the end of season. For mid-season yield, half of the plants from each treatment were sampled (n

= 5 for each treatment, n = 3 for controls). All remaining plants in the field (n = 5 for each treatment, n = 4 for controls) were harvested on Sep 20. Above- and below-ground plant material was bagged separately and transported back to the lab to be weighed. Whole plant weight was recorded for mid-season yield calculations, but not for end of season calculations because aboveground biomass dies off prior to harvest. Total tuber weight and average tuber weight per plant were recorded for both mid-season and end of season yield calculations. The simultaneous treatment (*L. decemlineata* and *M. persicae*) treatment was removed from final yield analysis for the aphid performance trial due to cage contamination by field larvae.

4.2.6 Statistical analyses All analyses were carried out using JMP (Version 12.1, SAS Institute Inc.). A repeated measures model on aphid number over time was used to test the effects of prior and simultaneous *L. decemlineata* feeding on *M. persicae* performance. One-way ANOVAs were used to analyze the impact of *M. persicae* feeding on *L. decemlineata* larval growth (mean larval weight per plant) and to compare the effect of herbivore feeding sequence on JA, SA, JA-ile, α-solanine, and mid-season and end-of-season yield. Tukey tests were used to identify significant differences between each group ($p < 0.05$). Data were log transformed as necessary to meet assumptions of homogeneity of variance and normality of residuals. Differences in degrees of freedom between treatments and response variables are due to differential recovery of insects, infestations by non-target herbivores in field cages, or loss of plant material.

4.3 Results

4.3.1 Myzus persicae performance *Myzus persicae* population growth was not initially impacted by the presence of *L. decemlineata*. However, after eight days, aphid numbers were on average

over two times higher on plants without *L. decemlineata* than aphid numbers on plants with simultaneous damage, and over six times higher compared to plants with prior *L. decemlineata* damage (Figure 14) (treatment: $F_{2,7} = 6.32$, $P = 0.027$, time: $F_{5,3} = 18.93$, $P = 0.018$, treatment-by-time interaction: $F_{6,10} = 1.95$, $P = 0.213$).

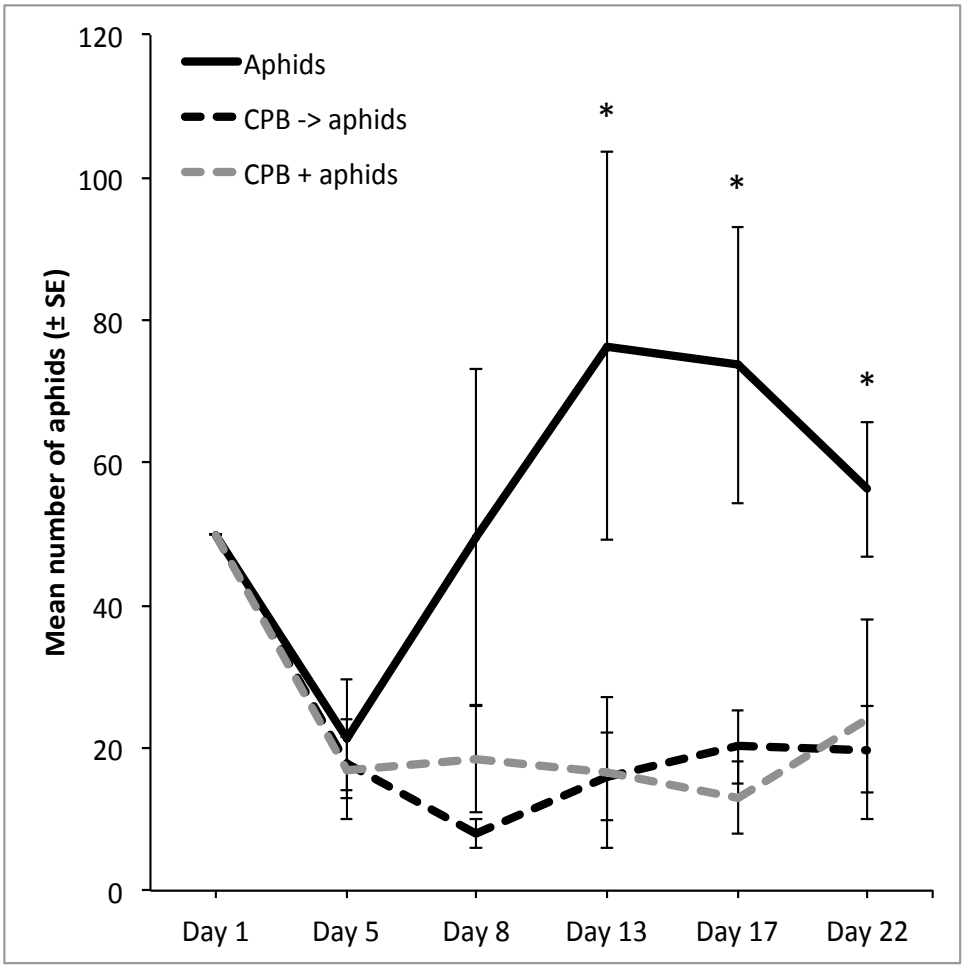

Figure 14: *M. persicae* (aphid) population growth when feeding on field grown *S. tuberosum* alone (solid black line), with prior *L. decemlineata* (CPB) damage (dashed black line), or with simultaneous CPB damage (dashed grey line). Asterisks above error bars indicate days when aphid numbers are significantly different (Tukey HSD, $\alpha = 0.05$).

4.3.2 *Leptinotarsa decemlineata* performance Initially, *L. decemlineata* performed better when feeding alone. On the first sampling date, larval weight was over 1.5 times higher when *L. decemlineata* fed alone compared to *L. decemlineata* feeding after prior *M. persicae* damage, but

there was no difference in larval weight between *L. decemlineata* feeding alone and *L. decemlineata* feeding with *M. persicae* simultaneously (Figure 15A) ($F_{2,8} = 5.41$, $P = 0.03$). However, there were no differences in larval weight detected on the second sampling date, one week later (Figure 15B) ($F_{2,12} = 2.11$, $P = 0.16$).

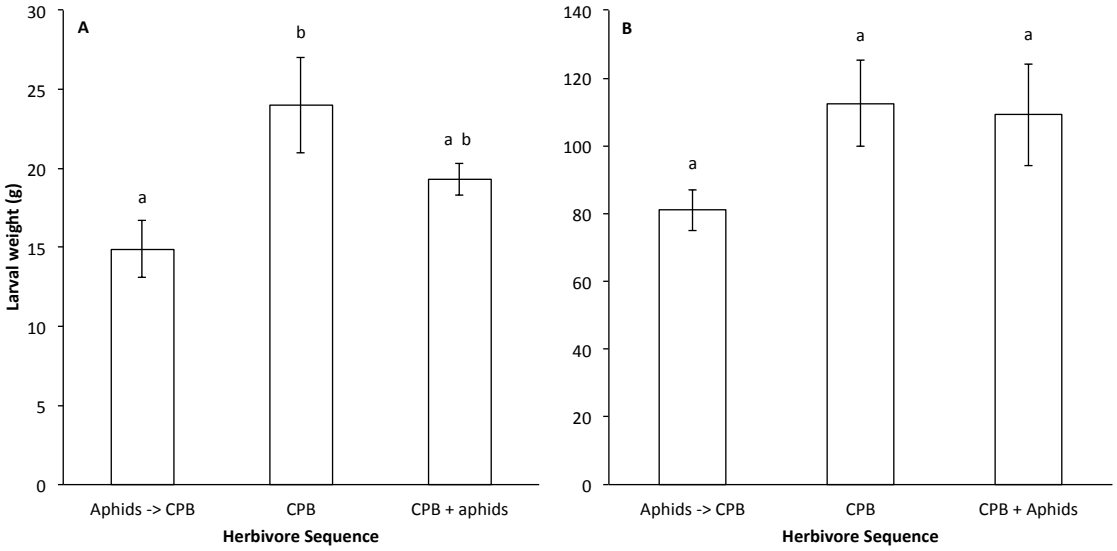

Figure 15: Impact of herbivore feeding sequence on mean *L. decemlineata* (CPB) larval weight ± SE for first (A) and second (B) sampling dates when CPB fed on *S. tuberosum* plants after prior *M. persicae* (aphid) damage, feeding alone, or feeding with simultaneous aphid damage. Means followed by same letters are not statistically significant (Tukey HSD, $\alpha = 0.05$).

4.3.3 Glycoalkaloid content Feeding sequence had no significant effect on α-solanine during the *M. persicae* performance trial (Figure 16) ($F_{3,9} = 2.52$, $P = 0.124$). There was also no evidence that feeding sequence affected α-solanine during the *L. decemlineata* performance evaluations (Figure 17) ($F_{3,9} = 0.837$, $P = 0.507$).

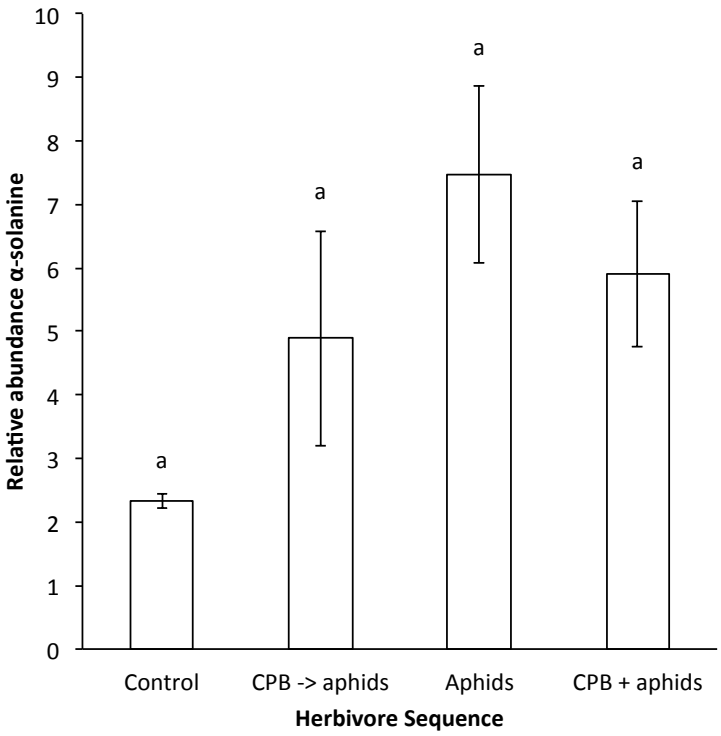

Figure 16: Impact of *M. persicae* (aphid) feeding on α-solanine concentration (mean ± SE) from foliar extracts of field grown *S. tuberosum* plants after feeding with prior *L. decemlineata* (CPB) damage, feeding alone, or feeding simultaneously with CPB compared to undamaged controls. Means followed by same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

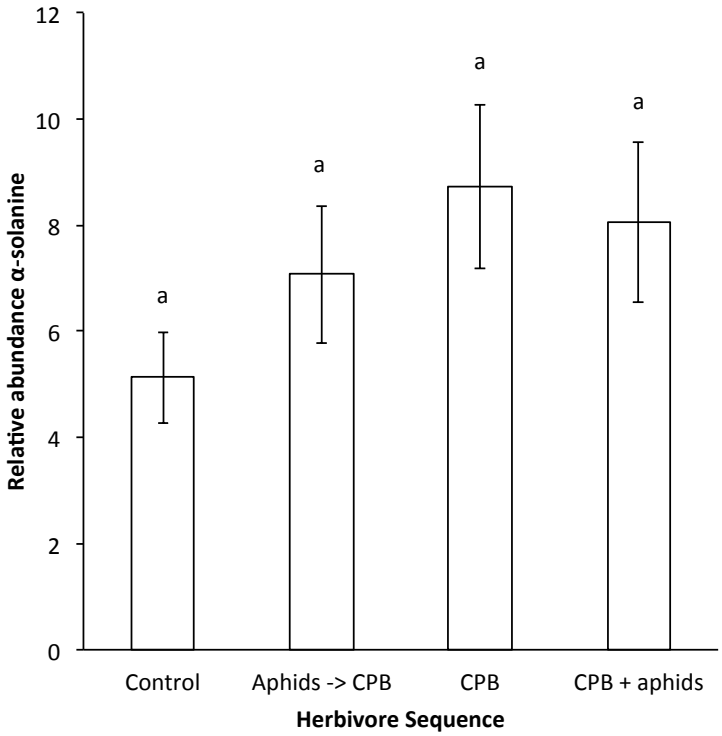

Figure 17: Impact of *L. decemlineata* (CPB) feeding on α-solanine concentration (mean ± SE) from foliar extracts of field grown *S. tuberosum* plants after feeding with prior *M. persicae* (aphid) damage, feeding alone, or feeding simultaneously with aphids compared to undamaged controls. Means followed by same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

4.3.4 Phytohormone content During the *M. persicae* performance trial, feeding sequence had no significant effect on JA (Figure 18A) ($F_{3,9} = 1.58$, $P = 0.262$), JA-ile (Figure 18B) ($F_{3,9} = 0.226$, $P = 0.876$), SA (Figure 18C) ($F_{3,11} = 0.872$, $P = 0.485$), or SAG (Figure 18D) ($F_{3,9} = 1.15$, $P = 0.382$). Similarly, there was no evidence that feeding sequence affected JA (Figure 19A) ($F_{3,10} = 1.14$, $P = 0.379$), JA-ile (Figure 19B) ($F_{3,9} = 0.343$, $P = 0.795$), SA (Figure 19C) ($F_{3,10} = 1.97$, $P = 0.183$), or SAG (Figure 19D) ($F_{3,10} = 0.55$, $P = 0.66$) during the *L. decemlineata* performance assays.

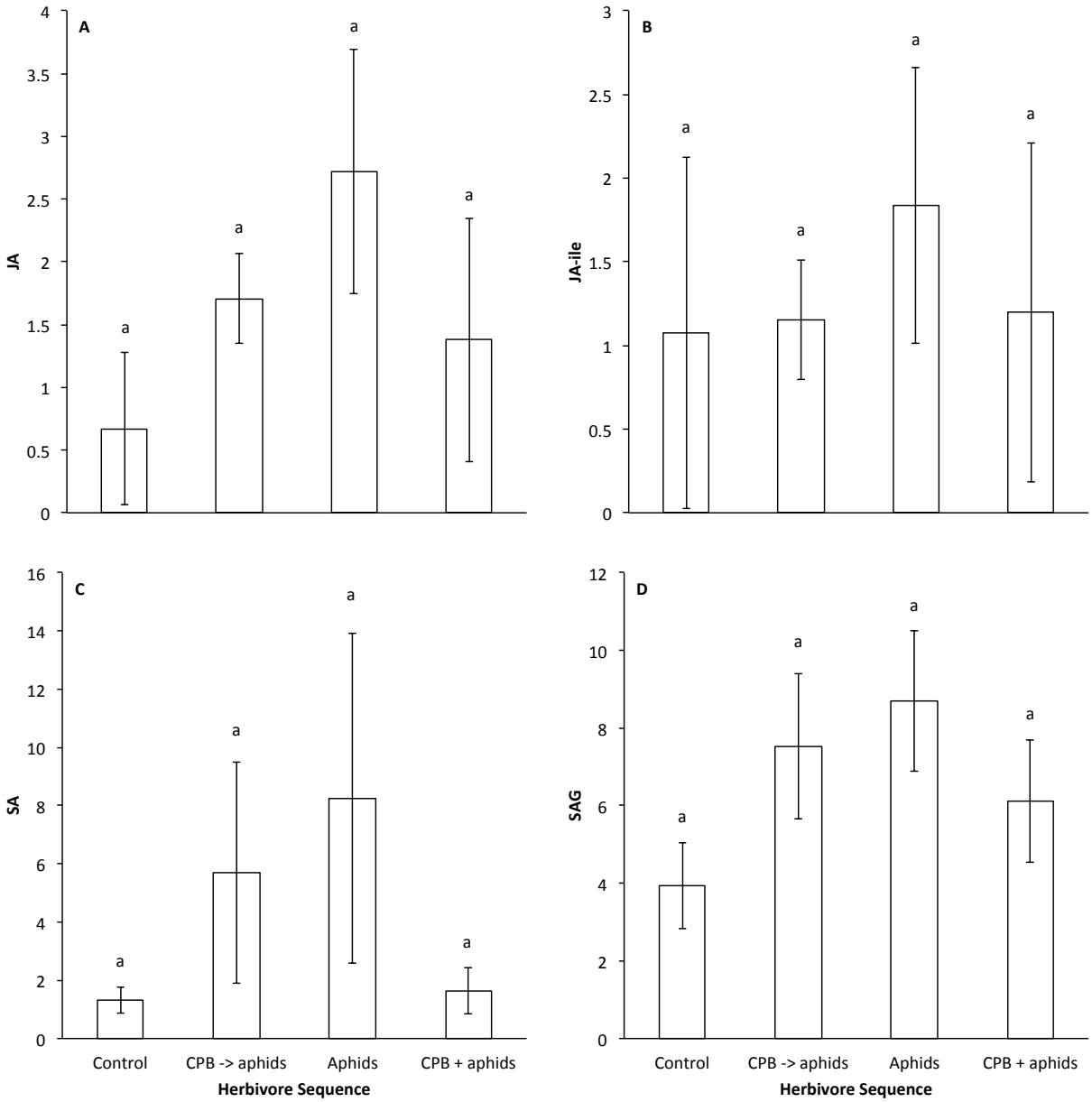

Figure 18: Impact of *M. persicae* (aphid) feeding on mean (± SE) JA (A), JA-ile (B), SA (C), and SAG (D) from foliar extracts of field grown *S. tuberosum* plants after feeding with prior *L. decemlineata* (CPB) damage, alone, or simultaneously with CPCB compared to undamaged controls. Means followed by same letter are not statistically significant (Tukey HSD, α=0.05).

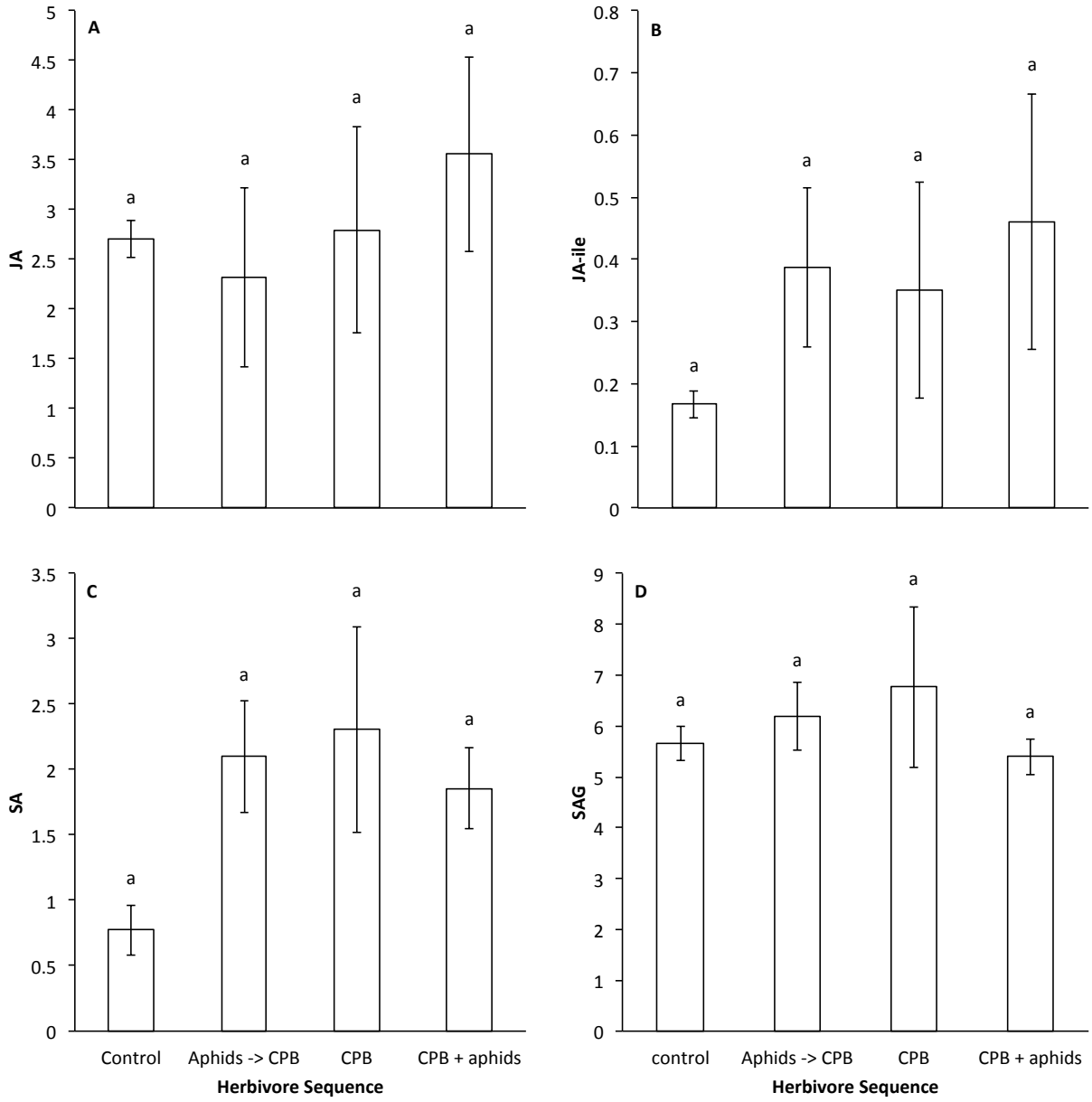

Figure 19: Impact of *L. decemlineata* (CPB) feeding on mean (± SE) JA (A), JA-ile (B), SA (C), and SAG (D) from foliar extracts of field grown *S. tuberosum* plants after feeding with prior *M. persicae* (aphid) damage, alone, or simultaneously with aphids compared to undamaged controls. Means followed by same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

4.3.5 Yield

4.3.5.1 Midseason yield

The midseason mean tuber weight did not vary between treatments within the *M. persicae* performance trial (Figure 20) ($F_{3,9} = 3.72$, $P = 0.055$). Individual tuber weight can vary

within a single plant, with the greatest difference among *M. persicae* performance treatments being observed as 181 g. Although this variation exists, there was no difference between the number of tubers ($F_{3,14} = 0.486$, $P = 0.698$) or the maximum tuber weight ($F_{3,14} = 0.98$, $P = 0.431$) for each plant between treatments. Only cumulative tuber biomass for each plant was recorded at the midseason sampling for *L. decemlineata* performance and no differences were detected between mean tuber mass between herbivore damaged treatments and undamaged controls (Figure 21) ($F_{3,10} = 2.53$, $P = 0.116$).

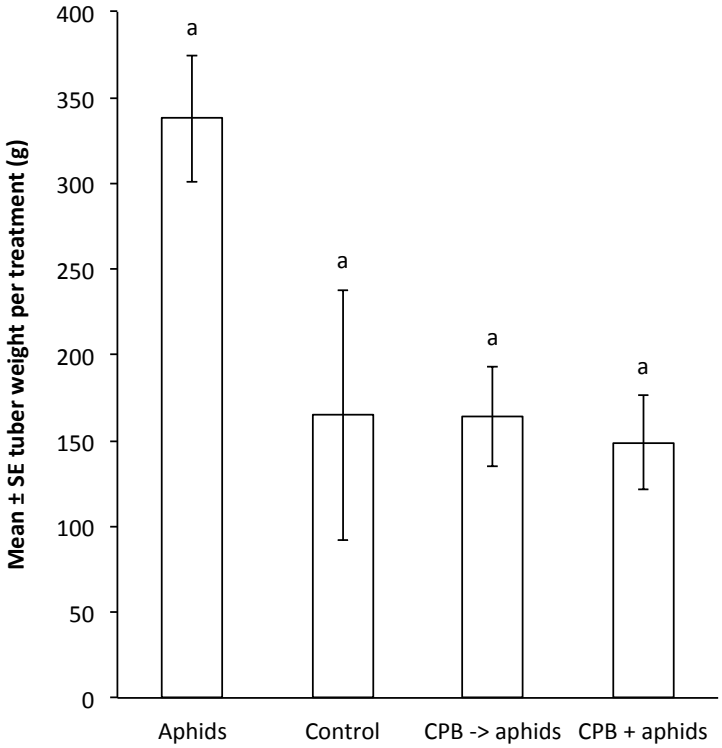

Figure 20: Midseason mean tuber weight ± SE from *S. tuberosum* plants damaged by *M. persicae* (aphid) with prior *L. decemlineata* (CPB) damage, with aphids feeding alone, with aphids and CPB feeding simultaneously, or with no herbivore damage. Means followed by the same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

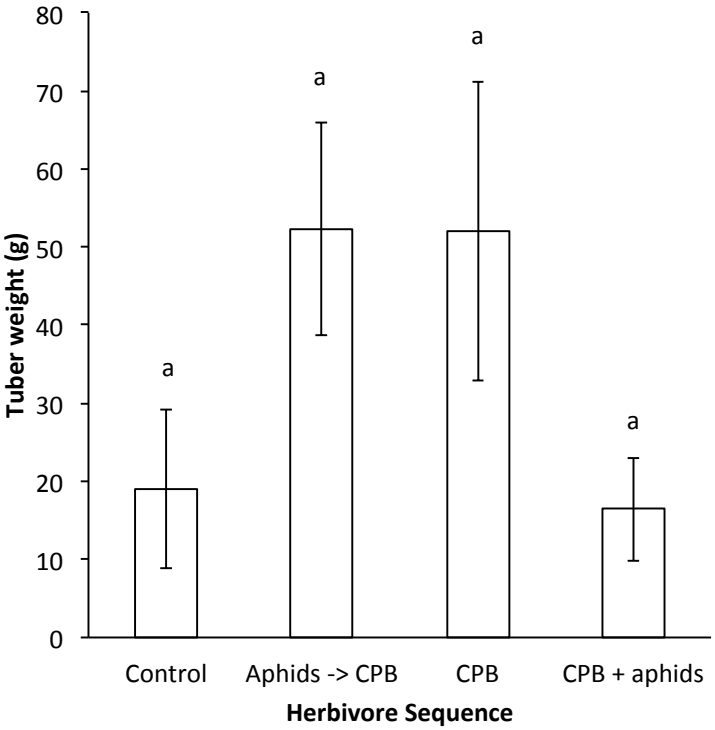

Figure 21: Midseason mean tuber weight ± SE from *S. tuberosum* plants damaged by *L. decemlineata* (CPB) with prior *M. persicae* (aphid) damage, with CPB feeding alone, with CPB and aphids feeding simultaneously, or with no herbivore damage. Means followed by the same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

4.3.5.2 End of season yield

After plants were harvested, tuber weight was summed for each plant and then averaged for each treatment. Mean tuber weight was consistent across all treatments at the end of the *M. persicae* trial ($\mu = 859.4$ g), indicating that herbivore damage had no effect on end of season yield ($F_{2,13} = 1.15$, $P = 0.344$) (Figure 22). The greatest difference between individual tuber weights within a single plant was 440 g. Although this variation exists, there was no difference between the number of tubers ($F_{3,16} = 0.9$, $P = 0.463$) or the maximum tuber weight ($F_{3,16} = 0.434$, $P = 0.732$) for each plant between treatments. Similarly, mean tuber weight did not differ between *L. decemlineata* performance treatments (Figure 23) ($F_{3,14} = 1.18$, $P = 0.352$). Mean tuber weight for all treatments was 1110.5 g. The greatest difference between individual tuber

weights within a single plant was 660 g. Still, there was no difference between the number of tubers ($F_{3,14} = 1.24$, $P = 0.332$) or the maximum tuber weight ($F_{3,14} = 1.46$, $P = 0.269$) for each plant between treatments.

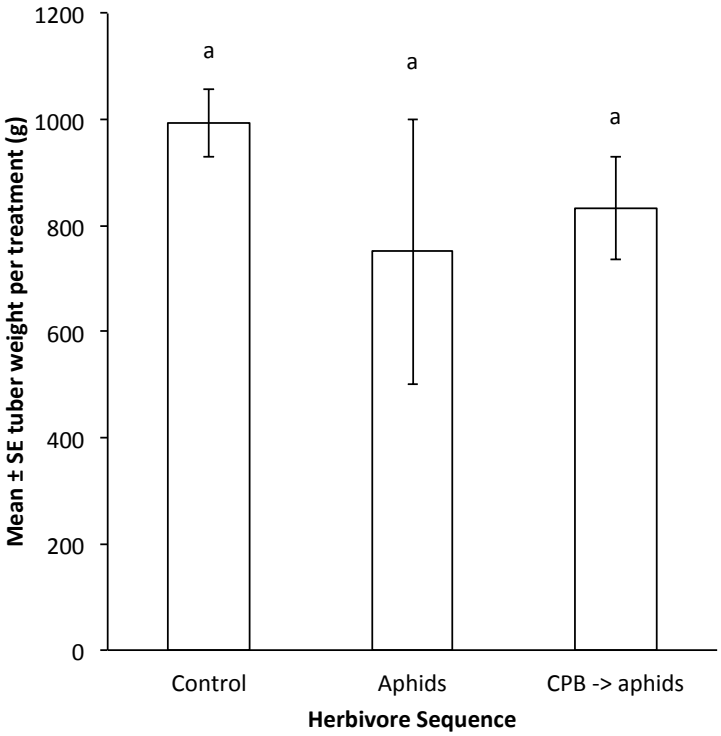

Figure 22: Mean end of season tuber weight ± SE of *S. tuberosum* plants without herbivore damage, with *M. persicae* (aphids) feeding alone, or with aphids feeding after prior *L. decemlineata* (CPB) damage. Means followed by the same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

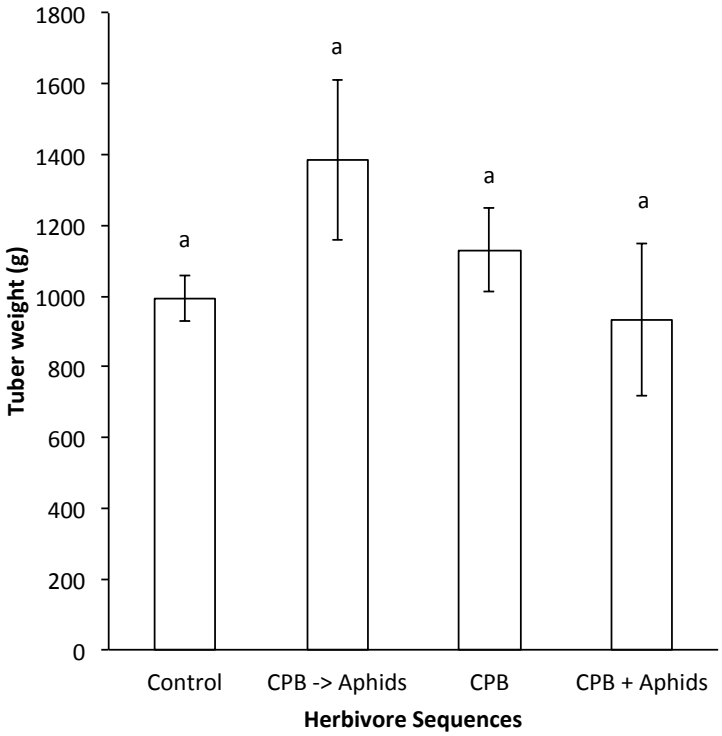

Figure 23: Mean end of season mean tuber weight ± SE from *S. tuberosum* plants damaged by *L. decemlineata* (CPB) with prior *M. persicae* (aphid) damage, with CPB feeding alone, with CPB and aphids feeding simultaneously, or with no herbivore damage. Means followed by the same letter are not statistically significant (Tukey HSD, $\alpha = 0.05$).

4.4 Discussion

This study corroborates some of my previous laboratory findings, but, given the vulnerability of field studies to confounding variables, there was some variation between laboratory and field results. In both the laboratory and in the field, *M. persicae* growth was negatively impacted by prior and simultaneous *L. decemlineata* damage. Although in the field *M. persicae* growth was highest when feeding alone, these populations began to decrease after 13 days, while in the laboratory there was an exponential increase throughout the length of the bioassay. However, the decrease observed in the field was not large enough to impact statistical significance, and given the difference in the length of time allotted for the lab and field trials, it is difficult to compare these results equivocally.

Although there was no difference in larval weight during the lab study I did observe differences in *L. decemlineata* larval weight during the first field sampling. Larval weight was highest when *L. decemlineata* were feeding alone, and lowest when *L. decemlineata* fed after prior *M. persicae* damage, although previous work has shown that the presence of aphids can weaken JA related defenses by inducing the SA pathway, with subsequent fitness benefits to feeding guilds that are susceptible to those defenses (Ali & Agrawal, 2014; Rodriguez-Saona et al., 2005; Soler et al., 2012; Stout et al., 1998). Additionally, there was no evidence of SA induction in field grown *S. tuberosum* when *M. persicae* were present and the difference in larval weight was not detected one week later during the second sampling date.

I was unable to detect a difference in phytohormone or glycoalkaloid content for both *M. persicae* and *L. decemlineata* field performance trials, but this may be attributed to inadequate sampling methods. Due to the location of the field site, it was not possible to flash freeze freshly excised plant material, as would be the normal sampling procedure. Instead, plant tissue was removed and transported back to the lab in coolers containing dry ice. It is possible that phytochemical changes occurred within the foliar tissue between the time the tissue was initially sampled in the field and when the tissue was processed for phytohormone and glycoalkaloid analysis in the lab, which could distort the results.

Potatoes are targets for numerous insect pests that if left uncontrolled, can inflict direct and indirect damage that significantly reduce harvestable yield (Vincent et al., 2013). In this study, I examined the effects of sequential and simultaneous feeding by herbivores from two different feeding guilds on potato yield, but found no significant differences either during midseason sampling or at the end of season harvest. The potato plants thrived throughout the duration of the experiment and there was minimal visible herbivore damage inflicted. It is

therefore possible that pest pressure was not sufficient to induce a significant change in yield or defensive traits. However, it should be noted that the loss of plants or insects and overall low replication in this study reduced the chances of detecting a true effect. Reproducing this study with larger replication would likely resolve some differences that were not observed as significant and increase the likelihood of finding statistically significant results.

CHAPTER 5.

DISCUSSION AND FUTURE DIRECTIONS

5.1 Key Findings

The goal of this project was to investigate the reciprocal effects of feeding guild induced plant responses on herbivore performance and preference. In Chapter 2, plant response to herbivore damage by *Myzus persicae* and *Leptinotarsa decemlineata* and its effect on herbivore performance was evaluated. *Myzus persicae* performance was negatively impacted by prior and simultaneous feeding damage by *L. decemlineata* and by mechanical damage. However, *L. decemlineata* was unaffected by *M. persicae feeding* damage. In Chapter 3, herbivore host-plant preference in response to plant volatiles induced by specific feeding guilds was evaluated. Surprisingly, despite that *M. persicae* performed poorly when *L. decemlineata* was present, when given a choice, *M. persicae* preferred *L. decemlineata* infested host plants. Although *M. persicae* did not affect *L. decemlineata* performance, *L. decemlineata* still determinedly oriented towards uninfested host plants. Variation in the volatile profiles emitted by plants under attack from these different feeding guilds could play a role in host plant choice. Finally, in Chapter 4, a field study was conducted to assess plant-herbivore performance in an agroecological setting. As was found in the lab, *M. persicae* performed better when feeding alone, and although there was an initial increase in larval weight when *L. decemlineata* fed alone, this effect was temporal and one week later, there was no difference detected in larval weight. Yield evaluations showed that *S. tuberosum* plants were tolerant to the levels of infestation by each feeding guild and in each combination, and total tuber biomass was not affected.

The response of plants to herbivore attack is regulated by phytohormone signaling cascades (Thaler et al. 2012). Here, I measured the induction of two phytohormones, jasmonic and salicylic acid, as well as the expression of the feeding deterrent, α-solanine in response to individual, sequential, and simultaneous feeding by herbivores from distinct feeding guilds. Plants responded differently to the various feeding combinations of *M. persicae* and *L. decemlineata*. It is difficult, and furthermore, unreasonable, to attribute a single explanation for these responses as several mechanisms may be interacting.

5.2 Explaining Plant-Mediated Feeding Guild Interactions

5.2.1 Sequence of herbivory Sequence of herbivore damage can play a role in regulating herbivore communities, where the induction of defenses determined by an initial attacker can affect plant response to subsequent herbivores (Erb et al., 2011; Kaplan & Denno, 2007; Poelman, Broekgaarden, Van Loon, & Dicke, 2008; Thaler et al., 2012). Aboveground feeding, for example, on teosinte (*Zea mays mexicana)* and cultivated maize (*Zea mays mays*) by *Spodoptera frugiperda* does not affect the fitness of *Diabrotica virgifera* larvae on the roots if *D. virgifera* colonizes the plant first. However, when *S. frugiperda* feeds on leaves prior to *D. virgifera* arrival, *D. virgifera* fitness decreases (Erb et al., 2011). Additionally, there is also evidence that sequence of herbivore damage can have long lasting effects on subsequent herbivore populations. In *Brassica oleracea*, early season herbivory by *Pieris rapae* differentially influences late season colonization by *Mamestra brassicae* and *Plutella xylostella* (Poelman et al., 2008). In this study phytohormone and glycoalkaloid levels differed when insects were added sequentially, compared to feeding alone or simultaneously. *Myzus persicae* and *L. decemlineata* produce multiple generations that can colonize and move within a single

field or to new field plots throughout the growing season. As a result, the sequence of herbivore arrival on a host-plant can shape fluctuating community populations based on plant responses to herbivore damage throughout the season.

5.2.2 Competing signaling pathways The strength of regulation by either JA or SA could also factor into relative defense expression (Thaler et al., 2012). The severity of damage due to herbivory significantly affects the degree of induced resistance (Baldwin & Schmelz, 1994; Underwood, 2000). Additionally, exogenous applications of herbivore elicitors show that plants respond in a dose dependent manner and increasing elicitor concentration can affect pathway interactions (Leon-Reyes et al., 2009; Mur, Kenton, Atzorn, Miersch, & Wasternack, 2006; Thaler et al., 2002). Therefore plant response and signal transduction could be linked to the perceived severity of damage imposed by each herbivore species.

Leptinotarsa decemlineata are heavy defoliators of potato (Ferro et al., 1985; Hare, 1980; Logan et al.,1985) while *M. persicae* inflict minimal physical damage (Powell et al., 2006; Radcliffe et al., 2007; Thompson & Goggin, 2006). As such, *L. decemlineata* could be perceived as a strong, immediate threat to plant fitness, whereas the consequences of aphid feeding may not be seen as an immediate threat. If plants prioritize defenses based on the risk of damage, *L. decemlineata* would impart a stronger defense response due to the higher risk of damage, which could take precedence over the plant's response to aphid feeding. This was observed when herbivores were added simultaneously, where *L. decemlineata* generally dominated the defense response. Although, this effect could be temporal with less strength in explaining plant responses to sequential damage.

5.2.3 Specialist-generalist paradigm Herbivore specialization can also elicit specific plant responses. *Myzus persicae* and *L. decemlineata* have different relationships with *S. tuberosum*, not only in their feeding strategies, but also in their utilization of *S. tuberosum* as a host plant. *Myzus persicae* are highly polyphagous, generalist feeders, while *L. decemlineata* are oligophagous and feed primarily on Solanaceous plants (Alyokhin et al., 2013; Saguez et al., 2013). Although *L. decemlineata* have broader feeding habits than classically defined specialists and their feeding association with cultivated potato is more recent (Jermy, 2012), they fit into the specialist-generalist continuum, where herbivores that are more specialized or monophagous, are typically more resistant or tolerant of host-plant toxins. In contrast, herbivores that are less specialized, or polyphagous, are more susceptible to general plant toxins (Ali & Agrawal, 2012; Cornell & Hawkins, 2003). Subsequently, the specialist-generalist paradigm predicts that due to higher susceptibility to plant toxins, generalists will suppress more defense related plant traits than specialists (Agrawal, 2000; Ali & Agrawal, 2012; Bowers & Stamp, 1993; Cornell & Hawkins, 2003; Poelman et al., 2008). Indeed, VOCs were suppressed when *M. persicae* fed on *S. tuberosum*, but not when *S. tuberosum* was fed on by *L. decemlineata*. Previous work suggests that *L. decemlineata* are able to detoxify allelochemicals, such as certain glycoalkaloids, while *M. persicae* suppresses these defenses (Fragoyiannis et al., 2001; Hlywka et al, 1994). Although this fits into the specialist-generalist scheme, this pattern was not observed within this study. *Myzus persicae* feeding alone, simultaneously, or after *L. decemlineaata* had no effect on α-solanine levels. Instead, α-solanine was only suppressed when *M. persicae* fed prior to *L. decemlineata* and this amount was not significantly different from when *L. decemlineata* fed alone. Drawing comparisons between specialists and generalists and their relationship with plant

defense is risky in this scenario where feeding guild potentially confounds such conclusions (Ali & Agrawal, 2012).

5.3 Future Outlook

Myzus persicae and *L. decemlineata* are cohabitants of potato and can infest potato fields simultaneously. Therefore, understanding how they reciprocally interact through plant-mediated responses can help predict their distribution in the field. Furthermore, understanding biochemical qualities and specificity of defense induction in response to these herbivores may allow us to exploit inducible defenses and utilize them in integrated pest management programs. Induced resistance has potential as an alternative pest management strategy with lower environmental impacts than traditional chemical pesticides (Bostock, 1999). For example, VOCs elicit behavioral changes in herbivores, and studies investigating the influence of VOCs on herbivore behavior indicate the growing potential for application in managed agricultural systems for increased biological control, such as through the attraction of natural enemies or deterrence of herbivorous pests (Heil, 2008; Hermann & Thaler, 2014; Hiltpold & Turlings, 2012; Kaplan, 2012; Ninkovic, Feng, Olsson, & Pettersson, 2013). However, site specificity and local ecology ultimately affect the outcome and effectiveness of pest suppression, and instances have been observed where herbivore attraction was inadvertently increased in response to HIPVs (Carroll, Schmelz, Meagher, & Teal, 2006).

Plants and herbivores interact in diverse landscapes with fluctuating community members. Plants are the primary producers in agroecosystems and changes in plant quality can have community wide implications (Poelman, 2015). Numerous organisms rely on plants for information and resources, including neighboring plants, pollinators, herbivores, and herbivore

natural enemies (Dicke & Baldwin, 2010). However, the role that induced defense plays in facilitating community structure is less studied (Poelman, 2015). Understanding the effect of plant response to herbivory, including phytohormone crosstalk and VOCs, on basal interactions as well as cumulative community-wide reactions can help disentangle the positive and negative consequences of incorporating inducible defenses into synergistic pest management programs.

In this study feeding guild and sequence of feeding guild infestation differentially altered plant response with reciprocal effects on herbivore performance and preference. However, phytohormone response did not necessarily correlate to improved or decreased fitness for the sequentially arriving herbivore, indicating that there may be other unknown inducible compounds involved in *M. persicae* - *L. decemlineata* interactions on *S. tuberosum*. Additionally, it was expected that herbivore performance would correlate with host-plant preference (*i.e.* herbivores would avoid plants with negative fitness consequences). However, this was not observed in this study, yet host-plant preference was still likely influenced by HIPVs. This study contributes to the knowledge base of plant defense responses to multiple feeding guilds. Future studies that specifically focus on the timing and sequence of induction, as well as the expression of defensive traits, are necessary to clarify these interactions. A community-based approach should also be taken to further understand the role of plant responses to multiple attackers and their impact on other community members.

REFERENCES

REFERENCES

Agrawal, A. A. (1998). Induced responses to herbivory and increased plant performance. *Science*, *279*(5354), 1201–1202.

Agrawal, A. A. (1999). Induced plant defense: evolution of induction and adaptive phenotypic plasticity. In A. A. Agrawal, S. Tuzun, & E. Bent (Eds.), *Inducible plant defenses against pathogens and herbivores: Biochemistry, ecology, and agriculture* (pp. 251–268). St. Paul, MN: Phytopathological Society Press.

Agrawal, A. A., & Karban, R. (1999). Why induced defenses may be favored over constitutive strategies in plants. In R. Tollrian & C. D. Harvell (Eds.), *The ecology and evolution of inducible plant defenses* (pp. 45–61). Princeton University Press, Princeton.

Agrawal, A. A. (2011). Current trends in the evolutionary ecology of plant defence. *Functional Ecology*, *25*(2), 420–432.

Agrawal, A. A. (2000). Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. *Oikos*, *89*(3), 493–500.

Agrawal, A. A., & Fishbein, M. (2006). Plant defense syndromes. *Ecological Society of America*, *87*(7), S132–S166.

Ali, J. G., & Agrawal, A. A. (2014). Asymmetry of plant-mediated interactions between specialist aphids and caterpillars on two milkweeds. *Functional Ecology*, *28*(6), 1404–1412.

Ali, J. G., & Agrawal, A. A. (2012). Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, *17*(5), 293–302.

Altesor, P., García, Á., Font, E., Rodríguez-Haralambides, A., Vilaró, F., Oesterheld, M., … González, A. (2014). Glycoalkaloids of wild and cultivated Solanum: effects on specialist and generalist insect herbivores. *Journal of Chemical Ecology*, *40*(6), 599–608.

Alyokhin, A., Udalov, M., & Benkovskaya, G. (2013). The Colorado potato beetle. In P. Giordanengo, C. Vincent, & A. Alyokhin (Eds.), *Insect Pests of Potato* (pp. 11–29). Elsevier.

Baldwin, I. T., & Schmelz, E. A. (1994). Constraints on an induced defense: the role of leaf area. *Oecologia*, *97*(3), 424–430.

Bandoly, M., Hilker, M., & Steppuhn, A. (2015). Oviposition by *Spodoptera exigua* on *Nicotiana attenuata* primes induced plant defence against larval herbivory. *The Plant Journal*, *83*(4), 661–672.

Beckers, G. J. M., & Spoel, S. H. (2008). Fine-tuning plant defence signalling: salicylate versus jasmonate. *Plant Biology*, *8*(1), 1–10.

Bernasconi, M. L., Turlings, T. C. J., Ambrosetti, L., Bassetti, P., & Dorn, S. (1998). Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. *Entomologia Experimentalis et Applicata*, *87*(2), 133–142.

Blackman, R. L., & Eastop, V. F. (2007). Taxonomic issues. In H. F. van Emden & R. Harrington (Eds.), *Aphids as crop pests* (pp. 1–30). Harpenden: CABI.

Bolter, C. J., Dicke, M., Van Loon, J. J. A., Visser, J. H., & Posthumus, M. A. (1997). Attraction of Colorado potato beetle to Herbivore-damaged plants during herbivory and after its termination. *Journal of Chemical Ecology*, *23*(4), 1003–1023.

Bonaventure, G., Van Doorn, A., & Baldwin, I. T. (2011). Herbivore-associated elicitors: FAC signaling and metabolism. *Trends in Plant Science*, *16*(6), 294–9.

Bostock, R. M. (1999). Signal conflicts and synergies in induced resistance to multiple attackers. *Physiological and Molecular Plant Pathology*, *55*(2), 99–109.

Bowers, M. D., & Stamp, N. E. (1993). Effects of plant age, genotype, and herbivory on Plantago performance and chemistry. *Ecology*, *74*(6), 1778–1792.

Bruce, T. J. A., Matthes, M. C., Chamberlain, K., Woodcock, C. M., Mohib, A., Webster, B., … Napier, J. A. (2008). cis-Jasmone induces Arabidopsis genes that affect the chemical ecology of multitrophic interactions with aphids and their parasitoids. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(12), 4553–8.

Bruce, T. J. A., & Pickett, J. A. (2011). Perception of plant volatile blends by herbivorous insects--finding the right mix. *Phytochemistry*, *72*(13), 1605–11.

Bruce, T. J. A., Wadhams, L. J., & Woodcock, C. M. (2005). Insect host location: a volatile situation. *Trends in Plant Science*, *10*(6), 269–74.

Bruinsma, M., & Dicke, M. (2008). Herbivore-induced indirect defense: from induction mechanisms to community ecology. In A. Schaller (Ed.), *Induced Plant Resistance to Herbivory* (pp. 31–60). Dordrecht: Springer Netherlands.

Brunissen, L., Cherqui, A., Pelletier, Y., Vincent, C., & Giordanengo, P. (2009). Host-plant mediated interactions between two aphid species. *Entomologia Experimentalis et Applicata*, *132*(1), 30–38.

Carroll, M. J., Schmelz, E. A., Meagher, R. L., & Teal, P. E. A. (2006). Attraction of *Spodoptera frugiperda* larvae to volatiles from herbivore-damaged maize seedlings. *Journal of Chemical Ecology*, *32*(9), 1911–1924.

Chen, Z., Flickinger, N. J., & Miller, A. R. (1998). Jasmonic acid and environments increase steroidal glycoalkaloid biosynthesis in *Solanum chacoense*. *HortScience*, *33*(3), 456–a–.

Chen, Z., & Miller, R. A. (2000). Steroidal alkaloids in Solanaceous vegetable crops. In J. Janick (Ed.), *Horticultural Reviews* (pp. 171–196). Oxford, UK: John Wiley & Sons, Inc.

Chung, S. H., Rosa, C., Scully, E. D., Peiffer, M., Tooker, J. F., Hoover, K., … Felton, G. W. (2013). Herbivore exploits orally secreted bacteria to suppress plant defenses. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(39), 15728–33.

Cornell, H. V., & Hawkins, B. A. (2003). Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. *The American Naturalist*, *161*(4), 507–522.

Cui, J., Bahrami, A. K., Pringle, E. G., Hernandez-Guzman, G., Bender, C. L., Pierce, N. E., & Ausubel, F. M. (2005). *Pseudomonas syringae* manipulates systemic plant defenses against pathogens and herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(5), 1791–6.

Dangash, A., Neeta, P., Bharillya, A., Jhala, A., & Jain, D. (2014). Impact of exogenous elicitors on artemisinin production and trichome density in *Artemisia annua* L. under subtropical conditions. *Notulae Scientia Biologicae*, *6*(3), 349–353.

De Bruyne, M., & Baker, T. C. (2008). Odor detection in insects: volatile codes. *Journal of Chemical Ecology*, *34*(7), 882–897.

De Moraes, C., Mescher, M. C., & Tumlinson, J. H. (2001). Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature*, *410*, 577–579.

De Vos, M., & Jander, G. (2009). *Myzus persicae* (green peach aphid) salivary components induce defence responses in *Arabidopsis thaliana*. *Plant Cell and Environment*, *32*, 1548–1560.

Denno, R. F., McClure, M. S., & Ott, J. R. (1995). Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Annual Review of Entomology*, *40*, 297–331.

Dicke, M. (2009). Behavioural and community ecology of plants that cry for help. *Plant, Cell & Environment*, *32*(6), 654–665.

Dicke, M. (1999). Specificity of herbivore-induced plant defences. *Novartis Foundation Symposium*, *223*, 43–54; discussion 54–59, 160–165.

Dicke, M., & Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the "cry for help." *Trends in Plant Science*, *15*(3), 167–175.

Dicke, M., van Poecke, R. M. P., & De Boer, J. G. (2003). Inducible indirect defence of plants: from mechanisms to ecological functions. *Basic and Applied Ecology*, *4*(MAY), 27–42.

Dicke, M., van Loon, J. J. A., & Soler, R. (2009). Chemical complexity of volatiles from plants induced by multiple attack. *Nature Chemical Biology*, *5*(5), 317–24.

Dickens, J. (2002). Behavioural responses of larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), to host plant volatile blends attractive to adults. *Agricultural and Forest Entomology*, *4*, 309–314.

Diezel, C., von Dahl, C. C., Gaquerel, E., & Baldwin, I. T. (2009). Different Lepidopteran elicitors account for cross-talk in herbivory-induced phytohormone signaling. *Plant Physiology*, *150*(3), 1576–1586.

Dixon, A. F. G., Horth, S., & Kindlmann, P. (1993). Migration in insects: cost and strategies. *Journal of Animal Ecology*, *62*(1), 182–190.

Doares, S. H., Narvaez-Vasquez, J., Conconi, A., & Ryan, C. A. (1995). Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. *Plant Physiology*, *108*(4), 1741–1746.

Dudareva, N., Negre, F., Nagegowda, D. A., & Orlova, I. (2006). Plant volatiles: recent advances and future perspectives. *Critical Reviews in Plant Sciences*, *25*(5), 417–440.

Dudareva, N., Pichersky, E., & Gershenzon, J. (2004). Biochemistry of plant volatiles. *Plant Physiology*, *135*(4), 1893–902.

Durrant, W. E., & Dong, X. (2004). Systemic acquired resistance. *Annual Review of Phytopathology*, *42*, 185–209.

Edwards, E. J., & Cobb, A. H. (1996). Improved high-performance liquid chromatographic method for the analysis of potato (*Solanum tuberosum*) glycoalkaloids. *Journal of Agricultural and Food Chemistry*, *44*(9), 2705–2709.

Eigenbrode, S. D., Ding, H., Shiel, P., & Berger, P. H. (2002). Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). *Proceedings. Biological Sciences / The Royal Society*, *269*(1490), 455–60.

Erb, M., Meldau, S., & Howe, G. A. (2012). Role of phytohormones in insect-specific plant reactions. *Trends in Plant Science*, *17*(5), 250–259.

Erb, M., Robert, C. A. M., Hibbard, B. E., & Turlings, T. C. J. (2011). Sequence of arrival determines plant-mediated interactions between herbivores. *Journal of Ecology*, *99*(1), 7–15.

Farmer, E. E. (2001). Surface-to-air signals. *Nature*, *411(6839)*, 854–856.

Farmer, E. E. (2014). *Leaf Defence*. Oxford: Oxford University Press.

Ferro, D. N., Logan, J. A., Voss, R. H., & Elkinton, J. S. (1985). Colorado potato beetle (Coleoptera: Chrysomelidae) temperature-dependent growth and feeding rates. *Environmental Entomology*, *14*(3), 343–348.

Fragoyiannis, D. A., McKinlay, R. G., & D'Mello, J. P. F. (2001). Interactions of Aphid Herbivory and Nitrogen Availability on the Total Foliar Glycoalkaloid Content of Potato Plants. *Journal of Chemical Ecology*, *27*(9), 1749–1762.

Fragoyiannis, D. A., McKinlay, R. G., & D'Mello, J. P. F. (1998). Studies of the growth, development and reproductive performance of the aphid *Myzus persicae* on artificial diets containing potato glycoalkaloids. *Entomologia Experimentalis et Applicata*, *88*, 59–66.

Gibson, R. W., & Pickett, J. A. (1983). Wild potato repels aphids by release of aphid alarm pheromone. *Nature*, *302*(5909), 608–609.

Goggin, F. L. (2007). Plant-aphid interactions: molecular and ecological perspectives. *Current Opinion in Plant Biology*, *10*(4), 399–408.

Gosset, V., Harmel, N., Göbel, C., Francis, F., Haubruge, E., Wathelet, J.-P., … Fauconnier, M.-L. (2009). Attacks by a piercing-sucking insect (*Myzus persicae* Sultzer) or a chewing insect (*Leptinotarsa decemlineata* Say) on potato plants (*Solanum tuberosum* L.) induce differential changes in volatile compound release and oxylipin synthesis. *Journal of Experimental Botany*, *60*(4), 1231–40.

Hare, D. J. (1980). Impact of defoliation by the Colorado potato beetle on potato yields. *Journal of Economic Entomology*, *73*, 369–373.

Heidel, A. J., & Baldwin, I. T. (2004). Microarray analysis of salicylic acid- and jasmonic acid-signalling in responses of *Nicotiana attenuata* to attack by insects from multiple feeding guilds. *Plant Cell and Environment*, *27*(11), 1362–1373.

Heil, M. (2008). Indirect defence via tritrophic interactions. *The New Phytologist*, *178*(1), 41–61.

Heil, M., Ibarra-Laclette, E., Adame-Alvarez, R. M., Martinez, O., Ramirez-Chavez, E., Molina-Torres, J., & Herrera-Estrella, L. (2012). How plants sense wounds: damage-self recognition is based on plant-derived elicitors and induces octadecanoid signaling. *PloS One*, *7*(2), e30537.

Hermann, S. L., & Thaler, J. S. (2014). Prey perception of predation risk: volatile chemical cues mediate non-consumptive effects of a predator on a herbivorous insect. *Oecologia*, *176*, 669–676.

Hilker, M., Stein, C., Schröder, R., Varama, M., & Mumm, R. (2005). Insect egg deposition induces defence responses in *Pinus sylvestris*: characterisation of the elicitor. *The Journal of Experimental Biology*, *208*(Pt 10), 1849–54.

Hiltpold, I., & Turlings, T. C. J. (2012). Manipulation of chemically mediated interactions in agricultural soils to enhance the control of crop pests and to improve crop yield. *Journal of Chemical Ecology*, *38*(6), 641–650.

Hlywka, J. J., Stephenson, G. R., Sears, M. K., & Yada, R. Y. (1994). Effects of insect damage on glycoalkaloid content in potatoes (*Solanum tuberosum*). *Journal of Agricultural and Food Chemistry*, *42*(11), 2545–2550.

Holopainen, J. K., & Blande, J. D. (2013). Where do herbivore-induced plant volatiles go? *Frontiers in Plant Science*, *4*(June), 185.

Howe, G. A., & Jander, G. (2008). Plant immunity to insect herbivores. *Annual Review of Plant Biology*, *59*, 41–66.

Howe, G. A., & Schaller, A. (2008). Direct defenses in plants and their induction by wounding and insect herbivores. In A. Schaller (Ed.), *Induced Plant Resistance to Herbivory* (pp. 7–29). Dordrecht: Springer Netherlands.

Jermy, T. (2012). Hypotheses on oligophagy: how far the case of the Colorado potato beetle supports them. In P. H. Jolivet, M. L. Cox, & E. Petitpierre (Eds.), *Novel aspects of the biology of Chrysomelidae* (pp. 127–137). Springer Science & Business Media.

Kaplan, I. (2012). Attracting carnivorous arthropods with plant volatiles: the future of biocontrol or playing with fire? *Biological Control*, *60*(2), 77–89.

Kaplan, I., & Denno, R. F. (2007). Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters*, *10*(10), 977–94.

Kaplan, I., Dively, G. P., & Denno, R. F. (2009). The costs of anti-herbivore defense traits in agricultural crop plants: a case study involving leafhoppers and trichomes. *Ecological Applications*, *19*(4), 864–872.

Kaplan, I., Halitschke, R., Kessler, A., Sardanelli, S., & Denno, R. F. (2008). Constitutive and induced defenses to herbivory in above- and belowground plant tissues. *The Ecological Society of America*, *89*(2), 392–406.

Karban, R., & Baldwin, I. T. (1997). *Induced Responses to Herbivory*. University of Chicago Press.

Karban, R., & Meyers, J. H. (1989). Induced plant responses to herbivory. *Annual Review of Ecology and Systematics*, *20*, 331–348.

Kawano, T., Tanaka, S., Kadono, T., & Muto, S. (2004). Salicylic acid glucoside acts as a slow inducer of oxidative burst in tobacco suspension culture. *Zeitschrift Für Naturforschung C*, *59*(9-10), 684–692.

Kessler, A., & Baldwin, I. T. (2002). Plant responses to insect herbivory: the emerging molecular analysis. *Annual Review of Plant Biology*, *53*, 299–328.

Kessler, A., Halitshke, R., & Poveda, K. (2011). Herbivory-mediated pollinator limitation: negative impacts of induced volatiles on plant—pollinator interactions. *Ecology*, *2*(9), 1769–1780.

Kessler, A., & Heil, M. (2011). The multiple faces of indirect defences and their agents of natural selection. *Functional Ecology*, *25*(2), 348–357.

Klessig, D. F. (1994). The salicylic acid signal in plants. *Plant Molecular Biology*, *26*(5), 1439–1458.

Knudsen, J. T., Tollsten, L., & Bergström, L. G. (1993). Floral scents—a checklist of volatile compounds isolated by head-space techniques. *Phytochemistry*, *33*(2), 253–280.

Kobayashi, H., Yanaka, M., & Ikeda, T. M. (2010). Exogenous methyl jasmonate alters trichome density on leaf surfaces of Rhodes grass (*Chloris gayana* Kunth). *Journal of Plant Growth Regulation*, *29*(4), 506–511.

Köllner, T. G., Held, M., Lenk, C., Hiltpold, I., Turlings, T. C. J., Gershenzon, J., & Degenhardt, J. (2008). A maize (E)-beta-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. *The Plant Cell*, *20*(2), 482–94.

Kowalski, S. P., Domek, J. M., Deahl, K. L., & Sanford, L. L. (1999). Performance of Colorado potato beetle larvae, *Leptinotarsa decemlineata* (Say), reared on synthetic diets supplemented with Solanum glycoalkaloids. *American Journal of Potato Research*, *76*(5), 305–312.

Landolt, P. J., Tumlinson, J. H., & Alborn, D. H. (1999). Attraction of Colorado potato beetle (Coleoptera: Chrysomelidae) to damaged and chemically induced potato plants. *Environmental Entomology*, *28*(6), 973–978.

Leon-Reyes, A., Du, Y., Koornneef, A., Proietti, S., Körbes, A. P., Memelink, J., … Ritsema, T. (2010). Ethylene signaling renders the jasmonate response of Arabidopsis insensitive to future suppression by salicylic acid. *Molecular Plant-Microbe Interactions : MPMI*, *23*(2), 187–97.

Loake, G., & Grant, M. (2007). Salicylic acid in plant defence--the players and protagonists. *Current Opinion in Plant Biology*, *10*(5), 466–72.

Logan, P. A., Casagrande, R. A., Faubert, H. H., & Drummond, F. A. (1985). Temperature-dependent development and feeding of Immature Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *Environmental Entomology*, *14*(3), 275–283.

Lynch, M. E., Kaplan, I., Dively, G. P., & Denno, R. F. (2006). Host-plant-mediated competition via induced resistance: interactions between pest herbivores on potatoes. *Ecological Applications*, *16*(3), 855–864.

Margaritopoulos, J., Kasprowicz, L., Malloch, G., & Fenton, B. (2009). Tracking the global dispersal of a cosmopolitan insect pest, the peach potato aphid. *BMC Ecology*, *9*(1), 13.

Matsui, K. (2006). Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. *Current Opinion in Plant Biology*, *9*(3), 274–80.

Mewis, I., Tokuhisa, J. G., Schultz, J. C., Appel, H. M., Ulrichs, C., & Gershenzon, J. (2006). Gene expression and glucosinolate accumulation in *Arabidopsis thaliana* in response to generalist and specialist herbivores of different feeding guilds and the role of defense signaling pathways. *Phytochemistry*, *67*(22), 2450–62.

Mur, L. A. J., Kenton, P., Atzorn, R., Miersch, O., & Wasternack, C. (2006). The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiology*, *140*(1), 249–62. doi:10.1104/pp.105.072348

Ngumbi, E., Eigenbrode, S. D., Bosque-Pérez, N. A., Ding, H., & Rodriguez, A. (2007). *Myzus persicae* is arrested more by blends than by individual compounds elevated in headspace of PLRV-infected potato. *Journal of Chemical Ecology*, *33*(9), 1733–47.

Ninkovic, V., Feng, Y., Olsson, U., & Pettersson, J. (2013). Ladybird footprints induce aphid avoidance behavior. *Biological Control*, *65*(1), 63–71.

Nottingham, S. F., Hardie, J., Dawson, G. W., Hick, A. J., Pickett, J. A., Wadhams, L. J., & Woodcock, C. M. (1991). Behavioral and electrophysiological responses of aphids to host and nonhost plant volatiles. *Journal of Chemical Ecology*, *17*(6), 1231–42.

Novotny, V., Miller, S. E., Baje, L., Balagawi, S., Basset, Y., Cizek, L., … Weiblen, G. D. (2010). Guild-specific patterns of species richness and host specialization in plant–herbivore food webs from a tropical forest. *Journal of Animal Ecology*, *79*, 1193–1203.

Paré, P. W., & Tumlinson, J. H. (1997). Induced synthesis of plant volatiles. *Nature*, *385*(6611), 30–31.

Paula, A., Álvaro, G., Elizabeth, F., Alejandra, R.-H., Francisco, V., Martín, O., … González, A. (2014). Glycoalkaloids of wild and cultivated Solanum : effects on specialist and generalist insect herbivores. *Journal of Chemical Ecology*, *40*(6), 599–608.

Peiffer, M., & Felton, G. W. (2009). Do caterpillars secrete "oral secretions"? *Journal of Chemical Ecology*, *35*(3), 326–35.

Pelletier, Y., Horgan, F. G., & Pompon, J. (2013). Potato resistance against insect herbivores: resources and opportunities. In *Insect Pests of Potato* (pp. 439–462). Elsevier.

Pickett, J. A., Wadhams, L. J., & Woodcock, C. M. (1992). The chemical ecology of aphids. *Annual Review of Entomology*, *37*, 67–90.

Pickett, J. A., Wadhams, L. J., & Woodcock, C. M. (1997). Developing sustainable pest control from chemical ecology. *Agriculture, Ecosystems & Environment*, *64*(2), 149–156.

Pieterse, C. M. ., & Van Loon, L. C. (1999). Salicylic acid-independent plant defence pathways. *Trends in Plant Science*, *4*(2), 52–58.

Poelman, E. H., Broekgaarden, C., Van Loon, J. J. A., & Dicke, M. (2008). Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. *Molecular Ecology*, *17*(14), 3352–65. d

Poelman, E. H., van Loon, J. J. A., & Dicke, M. (2008). Consequences of variation in plant defense for biodiversity at higher trophic levels. *Trends in Plant Science*, *13*(10), 534–41.

Poelman, E. H. (2015). From induced resistance to defence in plant-insect interactions. *Entomologia Experimentalis et Applicata*, *157*(1), 11–17.

Powell, G., Tosh, C. R., & Hardie, J. (2006). Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*, *51*(1), 309–330.

Radcliffe, E. B. (1982). Insect pests of potato. *Annual Review of Entomology*, *27*, 173–204.

Radcliffe, E. B., Ragsdale, D. W., & Suranyi, R. A. (2007). IPM case studies: seed potato. In *Aphids as crop pests* (pp. 613–717). CAB International.

Rajabaskar, D., Ding, H., Wu, Y., & Eigenbrode, S. D. (2013). Behavioral responses of green peach aphid, *Myzus persicae* (Sulzer), to the volatile organic compound emissions from four potato varieties. *American Journal of Potato Research*, *90.2*, 171–178.

Ratzinger, A., Riediger, N., von Tiedemann, A., & Karlovsky, P. (2009). Salicylic acid and salicylic acid glucoside in xylem sap of *Brassica napus* infected with *Verticillium longisporum*. *Journal of Plant Research*, *122*(571-579).

Reymond, P. (2013). Perception, signaling and molecular basis of oviposition-mediated plant responses. *Planta*, *238*(2), 247–58.

Rodriguez-Saona, C. R., Musser, R. O., Vogel, H., Hum-Musser, S. M., & Thaler, J. S. (2010). Molecular, biochemical, and organismal analyses of tomato plants simultaneously

attacked by herbivores from two feeding guilds. *Journal of Chemical Ecology*, *36*(10), 1043–57.

Rodriguez-Saona, C., Chalmers, J. A., Raj, S., & Thaler, J. S. (2005). Induced plant responses to multiple damagers: differential effects on an herbivore and its parasitoid. *Oecologia*, *143*(4), 566–577.

Rodriguez-Saona, C., Vorsa, N., Singh, A. P., Johnson-Cicalese, J., Szendrei, Z., Mescher, M. C., & Frost, C. J. (2011). Tracing the history of plant traits under domestication in cranberries: potential consequences on anti-herbivore defences. *Journal of Experimental Botany*, *62*(8), 2633–44.

Sablon, L., Dickens, J., Haubruge, É., & Verheggen, F. (2013). Chemical ecology of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), and potential for alternative control methods. *Insects*, *4*(1), 31–54.

Saguez, J., Giordanengo, P., & Vincent, C. (2013). Aphids as major potato pests. In P. Giordanengo, C. Vincent, & A. Alyokhin (Eds.), *Insect Pests of Potato* (pp. 31–63). Elsevier.

Schmelz, E. A., Alborn, H. T., Banchio, E., & Tumlinson, J. H. (2003). Quantitative relationships between induced jasmonic acid levels and volatile emission in *Zea mays* during *Spodoptera exigua* herbivory. *Planta*, *216*(4), 665–73.

Schütz, S., Weißbecker, B., Klein, A., & Hummel, H. E. (1997). Host plant selection of the Colorado potato beetle as influenced by damage induced volatiles of the potato plant. *Naturwissenschaften*, *84*(5), 212–217.

Silva, A. X., Jander, G., Samaniego, H., Ramsey, J. S., & Figueroa, C. C. (2012). Insecticide resistance mechanisms in the green peach aphid *Myzus persicae* (Hemiptera: Aphididae): a transcriptomic survey. *PloS One*, *7*(6), e36366.

Soler, R., Badenes-Pérez, F. R., Broekgaarden, C., Zheng, S.-J., David, A., Boland, W., & Dicke, M. (2012). Plant-mediated facilitation between a leaf-feeding and a phloem-feeding insect in a brassicaceous plant: from insect performance to gene transcription. *Functional Ecology*, *26*(1), 156–166.

Staswick, P. E., & Tiryaki, I. (2004). The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. *The Plant Cell*, *16*(8), 2117–27.

Stout, M. J., Workman, K. V., Bostock, R. M., & Duffey, S. S. (1998). Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. *Oecologia*, *113*(1), 74–81.

Szendrei, Z., Malo, E., Stelinski, L., & Rodriguez-Saona, C. (2009). Response of cranberry weevil (Coleoptera: Curculionidae) to host plant volatiles. *Environmental Entomology*, *38*(3), 861–869.

Thaler, J. S., Humphrey, P. T., & Whiteman, N. K. (2012). Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Science*, *17*(5), 260–270.

Thaler, J. S., Fidantsef, A. L., & Bostock, R. M. (2002). Antagonism between jasmonate- and salicylate-mediated induced plant resistance: effects of concentration and timing of elicitation on defense-related proteins, herbivore, and pathogen performance in tomato. *Journal of Chemical Ecology*, *28*(6), 1131–1159.

Thiery, D., & Visser, J. H. (1987). Misleading the Colorado potato beetle with an odor blend. *Journal of Chemical Ecology*, *13*(5), 1139–46.

Thompson, G. A., & Goggin, F. L. (2006). Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. *Journal of Experimental Botany*, *57*(4), 755–766.

Tingey, W. M. (1982). Glandular pubescence, glycoalkaloid composition, and resistance to the green peach aphid, potato leafhopper, and potato fleabeetle in *Solanum berthaultii*. *American Potato Journal*, *49*(3), 95–106.

Tingey, W. M., Mackenzie, D. J., & Gregory, P. (1978). Total foliar glycoalkaloids and resistance of wild potato species to *Empoasca fabae* (Harris). *American Potato Journal*, *55*(10), 577–585.

Traw, B. M., & Bergelson, J. (2003). Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*. *Plant Physiology*, *133*(3), 1367–1375.

Turnbull, L. A., & Hector, A. (2010). How to get even with pests: organic farming supports higher biodiversity. Research involving the Colorado potato beetle shows that this increased diversity can deliver a better ecosystem service in the form of more effective pest control. *Nature*, *466*(7302), 36–38.

Turner, J. G., Ellis, C., & Devoto, A. (2002). The jasmonate signal pathway. *Plant Cell*, *14*(suppl_1), S153–164.

Underwood, N. (2000). Density dependence in induced plant resistance to herbivore damage: threshold, strength and genetic variation. *Oikos*, *89*(2), 295–300.

Van Emden, H. F., Eastop, V. F., Hughes, R. D., & Way, M. J. (1969). The ecology of *Myzus persicae*. *Annual Review of Entomology*, *14*(1), 197–270.

Vincent, C., Alyokhin, A., & Giordanengo, P. (2013). Potatoes and their pests - setting the stage. In *Insect Pests of Potato* (pp. 3–8). Elsevier.

Visser, J. H. (1979). Electroantennogram responses of the Colorado beetle, *Leptinotarsa decemlineata*, to plant volatiles. *The Netherlands Entomological Society*, *25*, 86–97.

Visser, J. H., & Nielsen, J. K. (1977). Specificity in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata. Entomologia Experimentalis et Applicata*, *21*(1), 14–22.

Walling, L. L. (2000). The myriad plant responses to herbivores. *Journal Of Plant Growth Regulation*, *19*(2), 195–216.

Walling, L. L. (2008). Avoiding effective defenses: strategies employed by phloem-feeding insects. *Plant Physiology*, *146*(3), 859–866.

Wang, G. P., Yu, X. D., Fan, G., Wang, C. S., & Xia, L. Q. (2015). Expressing an (E)-b-farnesene synthase in the chloroplast of tobacco affects the preference of green peach aphid and its parasitoid. *Journal of Integrative Plant Biology*, *57*(9), 770–782.

Wang, L., Allmann, S., Wu, J., & Baldwin, I. T. (2008). Comparisons of lipoxygenase3- and jasmonate-resistant 4/6-silenced plants reveal that jasmonic acid and jasmonic acid-amino acid conjugates play different roles in herbivore resistance of *Nicotiana attenuata*. *Plant Physiology*, *146*(3), 904–15.

Weech, M.H., Chapleau, M., Pan, L., Ide, C., & Bede, J. C. (2008). Caterpillar saliva interferes with induced *Arabidopsis thaliana* defence responses via the systemic acquired resistance pathway. *Journal of Experimental Botany*, *59*(9), 2437–48.

Wielgoss, A., Clough, Y., Fiala, B., Rumede, A., & Tscharntke, T. (2012). A minor pest reduces yield losses by a major pest: plant-mediated herbivore interactions in Indonesian cacao. *Journal of Applied Ecology*, *49*(2), 465–473.

Wiesner, M., Hanschen, F. S., Schreiner, M., Glatt, H., & Zrenner, R. (2013). Induced production of 1-Methoxy-indol-3-ylmethyl glucosinolate by jasmonic acid and methyl jasmonate in sprouts and leaves of pak choi (*Brassica rapa* ssp. chinensis). *International Journal of Molecular Sciences*, *14.7*, 14996–15016.

Wu, Ji., & Baldwin, I. T. (2009). Herbivory-induced signalling in plants: perception and action. *Plant, Cell & Environment*, *32*(9), 1161–1174.

Yoshida, Y., Sano, R., Wada, T., Takabayashi, J., & Okada, K. (2009). Jasmonic acid control of GLABRA3 links inducible defense and trichome patterning in *Arabidopsis*. *Development*, *136*, 1039–1048.

Zangerl, A. R., & Bazzaz, F. A. (1992). Theory and pattern in plant defense allocation. In R. S. Fritz & E. L. Simms (Eds.), *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics* (pp. 363–391). University of Chicago Press.

Zarate, S. I., Kempema, L. A., & Walling, L. L. (2007). Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiology*, *143*(2), 866–75.