Investigation of soybean susceptibility to soybean aphids and how aphids affect plant-mediated pest interactions

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Investigation of soybean susceptibility to soybean aphids and how aphids affect plant-mediated pest interactions

by

Jessica Dawn Hohenstein

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Genetics and Genomics

Program of Study Committee:
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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University
Ames, Iowa
2017

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DEDICATION

This dissertation is dedicated to my husband, Matthew Hohenstein. Without his unwavering love, support, and patience I would not have been able to complete the research that lies within this document. I also dedicate this work to my parents Gordy and Debbie Nelson, from whom my passion for plant science “stems”. 
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>viii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ix</td>
</tr>
<tr>
<td>CHAPTER 1. GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Literature Review</td>
<td>1</td>
</tr>
<tr>
<td>Soybean</td>
<td>1</td>
</tr>
<tr>
<td>Soybean aphids</td>
<td>2</td>
</tr>
<tr>
<td>Soybean cyst nematodes</td>
<td>3</td>
</tr>
<tr>
<td>Phytohormones in local plant-aphid interactions</td>
<td>4</td>
</tr>
<tr>
<td>Herbivory-triggered plant-mediated systemic changes and interactions</td>
<td>9</td>
</tr>
<tr>
<td>Project Objectives</td>
<td>14</td>
</tr>
<tr>
<td>Dissertation Organization</td>
<td>15</td>
</tr>
<tr>
<td>Secondary Publications</td>
<td>17</td>
</tr>
<tr>
<td>References</td>
<td>17</td>
</tr>
<tr>
<td>CHAPTER 2. SOYBEAN APHIDS EXPLOIT SOYBEAN ABSCISIC ACID SIGNALING TO PROMOTE SUSCEPTIBILITY</td>
<td>30</td>
</tr>
<tr>
<td>Summary</td>
<td>30</td>
</tr>
<tr>
<td>Introduction</td>
<td>31</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>34</td>
</tr>
<tr>
<td>Plant material</td>
<td>34</td>
</tr>
<tr>
<td>Hormone treatment experiments</td>
<td>35</td>
</tr>
<tr>
<td>Gene silencing experiments</td>
<td>36</td>
</tr>
<tr>
<td>Vector construction</td>
<td>36</td>
</tr>
<tr>
<td>Plant maintenance and viral inoculation</td>
<td>36</td>
</tr>
<tr>
<td>Aphid population quantification experiments</td>
<td>37</td>
</tr>
<tr>
<td>Wounding/gene expression experiments</td>
<td>38</td>
</tr>
<tr>
<td>Sample processing and gene expression quantification</td>
<td>38</td>
</tr>
<tr>
<td>ABA quantification</td>
<td>39</td>
</tr>
<tr>
<td>ABA extraction</td>
<td>39</td>
</tr>
<tr>
<td>Direct infusion mass experiments</td>
<td>39</td>
</tr>
<tr>
<td>LC-MS methods</td>
<td>40</td>
</tr>
<tr>
<td>Statistical analyses</td>
<td>41</td>
</tr>
</tbody>
</table>
CHAPTER 3. DISSECTING PLANT-MEDIATED PEST INTERACTIONS IN SOYBEAN: LOCAL AND SYSTEMIC EFFECTS OF SOYBEAN APHID INFESTATION

Abstract........................................................................................................... 67

Introduction..................................................................................................... 68

Results............................................................................................................. 72

Analysis of differentially expressed genes...................................................... 72

Transcripts common to leaf and root responses.............................................. 74

Hierarchical clustering of soybean response to foliar soybean aphid feeding................................................................. 76

Transcription factor analysis......................................................................... 79

Transcription factor binding site analysis...................................................... 83

Functional analysis........................................................................................ 84

Discussion......................................................................................................... 86

Early whole-plant response to aphid feeding................................................. 87

Late whole-plant response to aphid feeding................................................... 90

Conclusions..................................................................................................... 94

Materials and Methods ................................................................................ 95

Plant material and growth conditions ............................................................ 95

Insect material and aphid infestation .............................................................. 96

Experimental design and tissue collection ...................................................... 96

RNA isolation and RNA-seq ........................................................................ 97
CHAPTER 4. GENERAL SUMMARY AND CONCLUSIONS 

References................................................................. 129
# LIST OF FIGURES

## CHAPTER 2

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soybean aphid feeding induces ABA accumulation</td>
<td>59</td>
</tr>
<tr>
<td>2</td>
<td>Exogenous abscisic acid treatment blocks wound-induced JA signaling.</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>Gene knockdown confirmation</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>Soybean aphids have lower performance on ABA-knockdown plants</td>
<td>62</td>
</tr>
<tr>
<td>5</td>
<td>Basal defense gene expression is higher in ABA-knockdown plants</td>
<td>63</td>
</tr>
<tr>
<td>6</td>
<td>Aphid-triggered suppression of JA signaling is mediated through the ABA pathway</td>
<td>64</td>
</tr>
</tbody>
</table>

**Supplementary Figure 1.** Soybean aphid feeding induces ABA accumulation ..... 65

## CHAPTER 3

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Response to foliar soybean aphid feeding is dynamic</td>
<td>115</td>
</tr>
<tr>
<td>2</td>
<td>Fold Change hierarchical clustering of gene expression responding to soybean aphid feeding</td>
<td>116</td>
</tr>
<tr>
<td>3</td>
<td>Gene ontology associated with response to soybean aphid feeding</td>
<td>118</td>
</tr>
<tr>
<td>4</td>
<td>Transcription factor expression patterns of the response to foliar soybean aphid feeding</td>
<td>120</td>
</tr>
<tr>
<td>5</td>
<td>Nematode performance is altered in transgenic knockdown hairy roots</td>
<td>121</td>
</tr>
</tbody>
</table>

**Supplementary Figure 1.** Transcription factor binding site analysis reveals similarly regulated gene clusters 122
LIST OF TABLES

CHAPTER 2

Supplementary Table 1. Primers used to generate RNAi constructs and quantify gene expression (qRT-PCR) .................................................. 66

CHAPTER 3

Table 1. Number and direction of differentially expressed genes triggered by soybean aphid feeding (FDR<0.05) .................................................. 114
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ABSTRACT

The soybean aphid (*Aphis glycines*) is an important insect pest of soybean (*Glycine max*). Previously, jasmonic acid (JA) was shown to elicit effective plant defense responses against soybean aphids. However, aphids were able to attenuate wound- or JA-induced responses in infested leaves in the compatible interaction and the mechanism of suppression remained uncharacterized. We hypothesized that aphids induce a decoy pathway to suppress plant defense responses and showed that aphids exploit the abscisic acid (ABA) pathway to suppress JA-mediated signaling. Both endogenous and exogenous ABA suppressed the wound-induced JA response. Furthermore, aphid populations were significantly reduced in the absence of a functional ABA biosynthetic (*aba2* RNAi) or signaling (*scof-1* RNAi) pathway and attenuation of JA responses by aphids was abolished in these mutants.

Suppression of defenses by aphids could result in susceptibility toward other pests infesting the plant. Previously, soybean aphid feeding was shown to facilitate the performance of other soybean aphid biotypes or soybean cyst nematodes (*Heterodera glycines*) on uninfected systemic leaves or roots of soybean, respectively. Therefore, it is critical to understand the impact of soybean aphids on a whole-plant level, including the plant-mediated response triggered on uninfested plant tissues. We quantified aphid-induced transcriptome changes in locally infested leaves and systemic roots during an early (12 hours) and late (7 days) aphid infestation. Our results suggest that leaves and roots have distinct responses to foliar soybean aphid feeding and the plant response is highly variable across time. Aphids caused delayed onset of defenses and a growth-
defense tradeoff in locally infested leaves. Most interestingly, foliar feeding by soybean aphids triggered the transient repression of widespread defense responses in roots.

This report provides the first genetic evidence of aphid counter-defense mechanisms in soybean and begins to unravel the systemic plant response to soybean aphids. Aphids use host ABA signaling to suppress plant defense responses in locally infested tissues but this does not seem to be the case in the transient suppression of root defense transcripts. Thus, suppression of defenses in uninfected tissues caused by soybean aphids likely occurs via different mechanisms.
CHAPTER 1. GENERAL INTRODUCTION

Literature Review

Soybean

Soybean (Glycine max L. Merr) is one of the most important and versatile crops worldwide. High seed oil and protein content enables this crop to be a high quality nutritive component in human and livestock diets. Soybean seeds are composed of about 18% oil and 38% protein, account for 61% of the world’s oilseed production, and are likely the most important source of plant protein meal in the world for both direct and indirect human consumption (Hartman et al., 2011; Soystats, 2016). Additionally, soybean is a key source for biodiesel fuel production. The United States is the global leader in soybean production, and in 2015 US soybean harvests from 82.7 million acres produced 3.93 billion bushels equaling an economic value of $34.5 billion (Soystats, 2016). Nearly 43% of US-produced soybeans in 2015 were exported, making the crop an important part of the global economy (Soystats, 2016).

Despite the economic importance of the crop, diseases and pests are still major limitations for soybean production. Soybean aphids (Aphis glycines) and soybean cyst nematodes (Heterodera glycines, SCN) are some of the most important and devastating soybean insect pests and pathogens, respectively. While aphid- and nematode- resistant varieties are commercially available, currently commercialized resistance sources are not effective against all soybean aphid biotypes or SCN HG types. Uncontrolled growth of pests can have significant yield penalties, thus insecticides and nematicides are often used, even though increased production costs are associated with these technologies. The soybean genome has been sequenced and consists of 20 chromosomes with 56,044
predicted protein-coding genes (Schmutz et al., 2010; Goodstein et al., 2012). This, along with high-throughput genome and transcriptome sequencing technologies, has greatly aided in the discovery and study of the genetic architecture of agronomically important traits such as stress tolerance and disease resistance.

**Soybean aphids**

The soybean aphid is an invasive insect pest that was first reported in the United States in 2000 and have since spread to most major soybean growing regions (Ragsdale et al., 2011). The insects have a complex lifecycle (Ragsdale et al., 2004). During the fall season, soybean aphids migrate to their primary host, European buckthorn (*Rhamnus* spp.) to complete the sexual phase of their lifecycle. During the summer, soybean aphids colonize soybean, their secondary host, and undergo rapid reproduction by parthenogenesis which can yield 15-18 generations in a single soybean growing season. These insects possess piercing-sucking mouthparts called a stylet which are used to ingest phloem sap and water from the plant vasculature on leaves, stems, and pods (Ragsdale et al., 2004). On its journey to reach the phloem, the stylet follows an intercellular path with frequent probing of cells until the phloem sieve element is reached (Tjallingii, 1988; Tjallingii, 2006). Probing-induced damage on individual cells occurs, but aphids produce gelling saliva to seal these holes and thereby minimize plant damage signals compared to chewing insects. While in the phloem, watery saliva is secreted from the stylet and ingestion of photoassimilates, amino acids, and other phloem components occurs (Prado & Tjallingii, 1994). Aphids manipulate source-sink relationships to divert nutrients toward their feeding site and alter amino acid production by the host plant (Girousse et al., 2005; Chiozza et al., 2010). Furthermore, aphids secrete sugar-rich honeydew, thus
promoting sooty mold fungal growth on leaves, which can interfere with photosynthesis. Due to these factors, uncontrolled aphid populations on susceptible plants can cause yield losses up to 40% (Ragsdale et al., 2007). Symptoms of aphid infestation include stunted plants, leaf yellowing, reduced pod set and number of seeds (Ragsdale et al., 2011). Aphids can also vector plant viruses (Hill et al., 2001). To combat soybean aphid population growth, soybean varieties containing aphid resistance genes became commercially available in 2010 although biotypes that readily overcome plant resistance have been found in North America (Kim, KS et al., 2008; Hill et al., 2010; Ragsdale et al., 2011; Alt & Ryan-Mahmutagic, 2013). Insecticides are widely used by farmers to control aphids, but this management strategy increases production costs and continued use of similar insecticide classes has contributed to evolution of insecticide resistance (Wang et al., 2011; Koch & Potter, 2016). Therefore, more research is needed to understand mechanisms of soybean resistance and susceptibility to these insects to develop new measures to control soybean aphid populations.

**Soybean cyst nematodes**

The soybean cyst nematode (SCN) is the most economically important pathogen of soybean (Koenning & Wrather, 2010). The life cycle of this sedentary parasite is approximately 4 weeks and consists of four juvenile stages (J1-J4) and an adult stage (Niblack et al., 2006). The juvenile stages 1 and 2 develop within the egg and the nematode hatches as a J2, which migrates to and penetrates soybean roots. Once the root vascular tissue is reached, nematodes induce the formation of a nutrient sink feeding cell called a syncytium which results from the fusion of hundreds of cells via the dissolution of cell walls (Mitchum, 2016). At this point, the nematode becomes sedentary and molt
three more times before reaching adulthood (Niblack et al., 2006). Males exit the roots while females remain sedentary within the root and the body swells so that the posterior end protrudes outside of the root. Males mate with the protruding females and several hundred eggs are produced by the female, which swells to a lemon-shape. After the female dies, the body is referred to as the cyst and this protects eggs from damage by environmental stresses and serves as a shelter structure in the soil for overwintering and long-term survival of the eggs. During a growing season, SCN can complete up to 3 to 4 generations. Infections are very difficult to eradicate as eggs can be viable many years. Symptoms of SCN infection on soybean include reduced root and shoot growth, root necrosis, reduced nodulation, reduced yield, and under some conditions, leaf chlorosis. An estimated $1.2 billion are lost annually in the US due to infection from this pathogen (Koenning & Wrather, 2010). Most effective management of SCN involves rotating soybeans varieties with different sources of SCN resistance and non-host crops such as maize (Niblack et al., 2006; Mitchum, 2016).

**Phytohormones in local plant-aphid interactions**

Induced plant responses triggered by pathogens and pests are often mediated by blends of phytohormone signaling through jasmonic acid (JA), ethylene (ET), or salicylic acid (SA) yet other phytohormonal signals such as abscisic acid (ABA), cytokinins, gibberellins, and auxin also play roles in plant resistance or susceptibility responses to herbivory to modulate the composition, quantity, and temporal molecular signatures to tailor defense responses to specific attackers (De Vos et al., 2005; Smith & Boyko, 2007; Asselbergh et al., 2008; Pieterse et al., 2009; Robert-Seilaniantz et al., 2011). While SA and JA often exhibit antagonistic crosstalk, these pathways may also coordinately
activate expression of genes in response to specific stresses (Salzman et al., 2005; Mur et al., 2006; Selig et al., 2016). Generally, biotrophic pathogens are controlled by SA-mediated responses whereas JA and ET pathways have been shown extensively to be involved in resistance against necrotrophic pathogens and herbivorous insects (Glazebrook, 2005; Howe & Jander, 2008; Koornneef & Pieterse, 2008; Bari & Jones, 2009; Morkunas et al., 2011).

Several studies on plant-aphid interactions reported the differential regulation of SA- and/or JA-mediated signaling pathways in response to aphid feeding. SA-related responses were induced by aphids in Arabidopsis, Nicotiana attenuata, Medicago truncatula, wheat, tomato, sorghum, and barley [Reviewed in (Thompson & Goggin, 2006; Goggin, 2007; Bari & Jones, 2009; Giordanengo et al., 2010; Morkunas et al., 2011; Kamphuis et al., 2013; Jaouannet et al., 2014)]. However, there does not seem to be a consensus on its overall impact on phloem-feeding insects. For example, Myzus persicae populations on Arabidopsis SA-signaling mutants (npr1) and SA-deficient transgenic plants (NahG) were either not significantly different than on wild-type plants or there was a decrease in aphid numbers, suggesting SA-mediated responses may have no effect to a small positive effect on the performance of M. persicae (Moran & Thompson, 2001; Mewis et al., 2005; Pegadaraju et al., 2005; Mewis et al., 2006; Louis et al., 2012). Conversely, a functional SA pathway is required for gene-for-gene resistance mediated by Mi-1.2 against the potato aphid (Macrosiphum euphorbiae) in tomato (Li et al., 2006). In soybean, SA signaling is induced by soybean aphids in both susceptible and resistant plants but earlier and in higher quantities in resistant plants (Li et al., 2008; Studham & MacIntosh, 2013). Treatment with exogenous SA negatively
impacted soybean aphid performance in resistant plants but not susceptible plants suggesting that SA signaling acts as a defense against soybean aphids but the insects may be able to block or detoxify SA-mediated defenses in susceptible plants (Studham & MacIntosh, 2013; Selig et al., 2016).

Strong evidence indicates that JA-mediated defenses are effective against several aphid species, even though JA responses are suppressed or only modestly induced in the compatible response to phloem-feeding insects [Reviewed in (Thompson & Goggin, 2006; Goggin, 2007; Howe & Jander, 2008; Bari & Jones, 2009; Giordanengo et al., 2010; Kamphuis et al., 2013; Jaouannet et al., 2014)]. JA-mediated induction of anti-nutritive and anti-digestive proteins such as proteinase inhibitors or polyphenol oxidase negatively affect chewing insects [reviewed in (Howe & Jander, 2008)]. Proteinase inhibitors were also shown to decrease weight or fecundity and increase mortality of aphids such as *M. persicae*, greenbug (*Schizaphis graminum*), bird cherry oat aphid (*Rhopalosiphum padi*), and Russian wheat aphids (*Diuraphis noxia*) (Tran et al., 1997; Rahbe et al., 2003). In addition, application of exogenous jasmonates to *Arabidopsis*, *Medicago truncatula*, cotton, tomato, wheat, and sorghum reduced aphid preference for or performance on JA-treated plants (Omer et al., 2001; Slesak et al., 2001; Thaler et al., 2001; Ellis et al., 2002; Bruce et al., 2003; Cooper et al., 2004; Zhu-Salzman et al., 2004; Cooper & Goggin, 2005; Boughton et al., 2006; Thompson & Goggin, 2006; Gao et al., 2007). *Arabidopsis* mutant plants with constitutive activation of JA biosynthesis (*fou2*) or signaling (*cev1*) displayed reduced aphid performance compared to wild-type plants, whereas *coil* mutants which have impaired JA signaling, had higher populations of both generalist and specialist aphids than wild-type plants (Ellis et al., 2002; Mewis et al.,
2006; Kuśnierczyk et al., 2011). In soybean, treatment of plants with exogenous jasmonates reduced aphid populations (Kanobe, 2012; Selig et al., 2016) revealing its involvement in resistance against soybean aphids. Interestingly, Studham and MacIntosh (2013) found upregulation of transcripts associated with JA and ET biosynthesis 7 days after soybean aphid infestation but marker genes associated with JA or ET signaling were repressed, leading the authors to propose that soybean aphids are able to block response to these hormones. Moreover, levels of the JA precursor linolenic acid were reduced by soybean aphid infestation, suggesting that aphids could block the biosynthesis of JA (Kanobe et al., 2015). Furthermore, plants previously infested with soybean aphids accumulated lower levels of the wound- and JA-inducible cysteine proteinase inhibitor N2, PinN2 (Botella et al., 1996) compared to uninfested controls upon wounding or treatment with exogenous JA (Kanobe, 2012). These data suggest that soybean aphids block both JA biosynthesis and signaling; however, the underlying mechanism triggered by aphids to achieve this defense suppression is currently unknown.

Phloem-feeders exploit phytohormone antagonism by eliciting a decoy response to suppress effective defenses (Walling, 2008). The silverleaf whitefly (SLWF, Bemisia tabaci) induces SA responses to suppress effectual JA defenses in Arabidopsis (Zarate et al., 2007). However in soybean, Selig et al. (2016) found no difference in aphid population size on susceptible soybeans treated with MeJA versus a combination of SA and MeJA indicating that there is no SA-JA antagonism in this plant regarding induced defenses against soybean aphids. On the other hand, a number of transcripts involved in abscisic acid (ABA) biosynthesis and signaling were highly induced in the late response to soybean aphid feeding, which led Studham and MacIntosh (2013) to propose that the
induction of ABA signaling acts as a decoy response to block effective defenses. ABA controls abiotic stress responses to cold, drought, and high salinity and is also an important modulator of SA and JA pathways during plant defense responses (Asselbergh et al., 2008; Ton et al., 2009; Morkunas et al., 2011). Many studies report negative crosstalk between SA and ABA, whereby ABA promoted susceptibility toward a variety of pests or pathogens including *Phytophthora sojae* in soybean (Ward et al., 1989; McDonald & Cahill, 1999; Mohr & Cahill, 2001), *Pseudomonas syringae* in *Arabidopsis* (de Torres-Zabala et al., 2007), as well as rice blast fungus (*Magnaporthe grisea*) and migratory nematodes (*Hirschmanniella oryzae*) in rice (Jiang et al., 2010). The interaction between ABA and JA is complex and depends on cellular hormone context. In *Arabidopsis*, two distinct antagonistic branches of JA signaling exist: the ABA-promoted MYC2 branch and the ethylene-promoted ERF1/ORA59 branch (Anderson et al., 2004; Kazan & Manners, 2013). The MYC2 branch activates transcription of an anti-insect vegetative storage protein *AtVSP1* while the ERF1/ORA59 branch activates transcription of a plant defensin *AtPDF1.2*, which is involved in defenses against *Fusarium oxysporum* in *Arabidopsis* (Anderson et al., 2004; Kazan & Manners, 2013).

ABA biosynthesis or signaling transcripts were also induced by aphids in *Arabidopsis*, sorghum, and wheat (Zhu-Salzman et al., 2004; Boyko et al., 2006; Park et al., 2006; Smith et al., 2010; Hillwig et al., 2016). While the ABA response may be induced in part due to the removal of water and nutrients from the vasculature, Hillwig et al. (2016) reported differential regulation of ABA responses in *Arabidopsis* in response to *M. persicae* saliva, indicating ABA responses can be induced by salivary elicitors independent of water and nutrient stress. Recent reports revealed that a functional ABA
pathway was important in optimal aphid population growth. For example, *Arabidopsis aba1-1, aba2, abi1* and *Medicago truncatula sta-1* mutants all supported significantly fewer aphids compared to wild-type plants (Kerchev *et al.*, 2013; Guo *et al.*, 2016; Hillwig *et al.*, 2016). *Arabidopsis aba2* mutants had elevated basal and inducible JA/ET signaling (Anderson *et al.*, 2004) and this mutant, along with *aba1-1* mutants, was less attractive to *M. persicae*. Furthermore, *aba1-1* mutants and had higher levels of indole glucosinolates indol-3-ylmethylglucosinolate (I3M) and 4-methoxyindol-3-ylmethylglucosinolate (4MI3M) which have antixenotic and antibiotic activities against the aphids (Kim & Jander, 2007; Kim, JH *et al.*, 2008; Kerchev *et al.*, 2013; Hillwig *et al.*, 2016). Conversely, *Arabidopsis abi4* mutants supported more aphids than wild-type plants (Kerchev *et al.*, 2013), and this highlights the complexity of the ABA pathway in the plant-aphid interaction. Taken together, these results suggest that the ABA pathway is important in plant-aphid interactions yet its role in the compatible soybean-soybean aphid interaction has not yet been characterized and needs further investigation.

**Herbivory-triggered plant-mediated systemic changes and interactions**

Plant responses triggered by pathogens or herbivores are rarely confined to locally infested tissues. Phytohormones SA, JA, ET, and ABA or their amino acid-, methyl ester- or glucose ester conjugates act as or induce the production of mobile defense signals that are translocated to uninfected systemic tissues through plant vasculature or as volatiles [reviewed in (Baluška, 2013; Shah & Zeier, 2013; Lacombe & Achard, 2016)]. Hydraulic or electrical signals and other molecules such as peptides, small RNAs, oxylipins, calcium, reactive oxygen species (ROS), and nutrients mediate systemic signal transduction both up- and downstream of phytohormone signaling (Johnson *et al.*, 2009;
Vicente et al., 2012; Gilroy et al., 2016). Perception of mobile signal(s) triggers changes in systemic leaf and root transcriptomes, phytohormone signaling, primary or defensive metabolites, resource allocation, tissue biomass and morphology, or root exudate content or concentration [Reviewed in (Bezemer & van Dam, 2005; Erb et al., 2008; Shah & Zeier, 2013; Wondafrash et al., 2013; Pieterse et al., 2014; Biere & Goverse, 2016; Papadopoulou & van Dam, 2017)]. Systemic changes are likely induced to increase host resistance to subsequent assault by the attacker or other pathogens and thus these molecular, biochemical, and structural systemic changes underlie plant-mediated indirect interactions between spatially separated pests.

Two well-characterized phenomena of systemic induced defenses are systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Conrath et al., 2015). Both SAR and ISR confer priming (i.e. faster activation of defense transcripts upon attack with another pest or pathogen) of systemic defense responses and broad-spectrum resistance toward many pathogens but differs in mechanism and type of defenses activated. In SAR, pathogens or oviposition by herbivores triggers the systemic induction of defenses via the SA-mediated signaling pathway and induce pathogenesis-related (PR) transcripts (Shah & Zeier, 2013; Hilfiker et al., 2014; Conrath et al., 2015). In ISR, beneficial plant growth-promoting rhizobacteria prime systemic plant defense responses via JA and ET signaling (Pieterse et al., 2014).

The majority of studies conducted on herbivore-induced changes of systemic phenotypes have focused on herbivores that cause extensive tissue damage (i.e. chewers or their saliva/regurgitant). Foliar chewing herbivory and application of jasmonates triggers reallocation of carbon or amino acids away from the attacked site, likely as a
mechanism of tolerance to the herbivore (Bast et al., 2005; Schwachtje et al., 2006; Gomez et al., 2010). Chewing herbivores also activate systemic ET or ABA phytohormone signaling (Ankala et al., 2013; Vos et al., 2013) and accumulation of secondary metabolites such as nicotine, glucosinolates, pyrrolizidine alkaloids, terpenes, and anti-digestive proteinase inhibitors [reviewed in (Bezemer & van Dam, 2005; Erb et al., 2008)]. In addition to changing internal chemistry, foliar herbivory by chewing insects alters root exudate compositions or concentrations (Bardgett et al., 1998). Root exudates are composed of volatile and non-volatile organic compounds including carbohydrates, amino acids, fatty acids, organic acids, and plant defensive compounds and are involved in attraction of beneficial and detrimental microorganisms, insects, and nematodes (Bais et al., 2006; Badri & Vivanco, 2009; Farnier et al., 2012).

Compared to chewing herbivores, relatively fewer studies have identified systemic molecular or biochemical changes triggered by phloem-feeding insects and even fewer have focused on molecular effects that connect aboveground and belowground tissues. Phloem-feeders induce carbon and nitrogen assimilation and mobilization transcripts such as sugar transporters, glutamine synthetase, or nitrate transporters in systemic leaves or phloem, likely to increase host nutritional status of the infested tissue (Divol et al., 2005; Kerchev et al., 2013; Petrova & Smith, 2015). This may result in altered C and N contents in local and systemic tissues, including free amino acid levels (Sandstrom et al., 2000; Girousse et al., 2005; Chiozza et al., 2010; Rodriguez-Saona et al., 2010) or a change in biomass of the systemic tissue (Yang et al., 2011; Park & Ryu, 2014). Changes in nutritional status of systemic tissues may have implications in plant-mediated pest interactions. In barley, foliar feeding by bird cherry oat aphids increased
concentrations of root minerals including N, calcium, potassium, and sulfur and potentially contributed toward facilitation of root-dwelling wireworm performance (Johnson et al., 2009). Additionally, nitrogen availability was important in determining the outcome of the interaction between foliar-feeding Brevicoryne brassicae aphids and belowground Heterodera schachtii cyst nematodes in Arabidopsis (Kutyniok & Muller, 2013). Other studies reported aphid-triggered induction of transcripts involved in cell wall biogenesis and modification such as cellulose synthases, pectinesterases, xyloglucan endoglucosyltransferase/hydrolases (XTH) and polygalacturonases in systemic leaves or phloem in Arabidopsis, tobacco, or celery (Voelckel et al., 2004; Divol et al., 2005; Kerchev et al., 2013). These changes could be related to a change in cell or organ structure or as part of a defense response, as cell wall modifications act to reinforce extracellular barriers or trigger the release of cell wall fragments that activate defense signaling (Vorwerk et al., 2004; Malinovsky et al., 2014).

Alteration of systemic defense signaling by phloem-feeding insects has been reported more recently. In Arabidopsis, Myzus persicae feeding caused local induction of SA, ET, ABA, and redox-related transcripts whereas in systemic leaves, aphid feeding triggered strong induction of JA-induced anti-insect vegetative storage proteins AtVSP1 and AtVSP2, some ABA-related transcripts, and the repression of a cytochrome P450 involved in glucosinolate metabolism (Kerchev et al., 2013). Interestingly, there was virtually no overlap of differentially expressed genes between the locally and systemic Arabidopsis leaf tissues. Likewise in Solanum stoloniferum, both M. persicae and Macrosiphum euphorbiae induced local SA and ET signaling while few JA-responsive genes changed expression (Alvarez et al., 2013). In this study, the systemic leaf response
to both aphids was weak and there were no overlapping transcripts between the infested and uninfested leaves. Previous infestation of *M. persicae* or *M. euphorbiae* in potato (*Solanum tuberosum*) facilitated the feeding behavior of *M. persicae* on previously infested leaves but induced phloem-based resistance toward aphids on systemic leaves (Dugravot *et al.*, 2007).

Foliar feeding by phloem-feeders also triggers systemic changes in root chemistry, phytohormone signaling, and performance of rhizosphere microorganisms. In pepper (*Capsicum annuum*), feeding by *M. persicae* or whiteflies (*Bemisia tabaci*) triggered local upregulation of SA and JA signaling and similar root defense responses (Lee *et al.*, 2012; Park & Ryu, 2014). Additionally, feeding by *M. persicae* altered root exudation which recruited beneficial rhizosphere microorganisms *Bacillus subtilis* and *Paenibacillus spp.* This recruitment triggered induced susceptibility toward the aphids but increased resistance toward pathogenic root and leaf bacteria *Ralstonia solanacearum* (Lee *et al.*, 2012; Kim *et al.*, 2016). In *Arabidopsis*, foliar feeding by the specialist aphid *Brevicoryne brassicae* reduced root concentrations of three short-chain aliphatic glucosinolates and reduced the number of *Heterodera schachtii* cyst nematodes (Kutyniok & Muller, 2012). In tomato, infection with *Meloidogyne incognita* root knot nematodes decreased root SA concentrations but previous infestation with *M. persicae* abolished this effect and induced resistance against the nematodes (Kafle *et al.*, 2017).

Many of these studies report induced resistance in systemic tissues, whether it was related to altered nutritional content or induction of defense responses. However, soybean aphids both induce susceptibility and hinder resistance against toward different soybean aphid biotypes in uninfested systemic leaves (Varenhorst *et al.*, 2015).
Additionally, studies have reported evidence of plant-mediated interaction between soybean aphid and soybean cyst nematodes (Hong et al., 2010; Hong et al., 2011; McCarville et al., 2014). When considering the effect of foliar soybean aphid feeding on cyst nematodes, the presence of soybean aphids had a generally positive (McCarville et al., 2012; McCarville et al., 2014) effect on the number of eggs and adult female SCN unless pest populations were high (McCarville et al., 2014), when aphids negatively affected SCN numbers likely due to resource competition, or very low (Heeren et al., 2012) when aphid presence on leaves had no effect on SCN populations. To date, molecular studies characterizing the compatible and incompatible interaction of soybean with soybean aphids have been conducted on locally infested leaf tissues. No studies have investigated the molecular effect of soybean aphids on a whole-plant level or uncovered plant-mediated mechanisms underlying the interactions between the two economically important pests. Therefore, it is important to investigate the molecular effect of soybean aphids both on the local and systemic level and understand how these changes may affect performance of spatially or temporally separated pests.

**Project Objectives**

Specific aims of this research were to:

A) Determine and characterize molecular mechanisms of soybean defense suppression in a compatible interaction with soybean aphids

B) Understand the molecular effect of foliar soybean aphid feeding on a whole-plant level (local leaf and systemic root) and identify potential aphid-triggered molecular mechanisms that could affect soybean cyst nematode performance
Dissertation Organization

This dissertation is written following the journal paper format with a total of four chapters and covers my work exploring the plant molecular phenotype of the compatible (susceptible) soybean-soybean aphid interaction at the local and systemic level.

Chapter 1 serves as a general introduction that provides a brief background on plant-aphid interactions, how plants respond to insect feeding in locally infested and systemic uninfested tissues, and how phloem-feeding insects may take advantage of host hormone signaling to promote susceptibility. Additionally, the general introduction identifies gaps in scientific knowledge that my research aims to cover and describes the project objectives of the studies that lie within this document.

Chapter 2 is entitled “Soybean aphids exploit soybean abscisic acid signaling to promote susceptibility” and is a hypothesis-driven investigation of aphid-triggered susceptibility through induction of host phytohormonal antagonistic crosstalk between abscisic acid (ABA) and jasmonic acid (JA)-mediated signaling. Previous research had shown that soybean aphids attenuate the expression of JA-mediated defense transcripts but the mechanism by which this occurred was unknown. We found evidence that both exogenous and endogenous ABA acts antagonistically to the JA-mediated defense induction in soybean. Furthermore, we found that plants with impaired abilities to produce (aba2 RNAi) or respond (scof-1 RNAi) to ABA had lower soybean aphid populations and aphids were not able to weaken JA signaling in these plants. From this research, we concluded that aphids exploit host ABA signaling to attenuate JA-mediated defense responses. I generated VIGS constructs and conducted all plant and aphid experiments, qRT-PCR, and statistical analyses. Additionally, this research drew on the
expertise of scientists from West Virginia University (Dr. Nik Kovinich, Genetics and Developmental Biology, Division of Plant and Soil Sciences) and Penn State University (Dr. Anjel M. Helms under the supervision of Dr. John F. Tooker, Department of Entomology) who measured ABA hormone levels. I wrote all sections of the paper apart from the ABA quantification section in the Materials and Methods, which was provided by Dr. Nik Kovinich.

Chapter 3 is entitled “Dissecting plant-mediated pest interactions in soybean: local and systemic effects of soybean aphid infestation” and is a primarily discovery-based exploration of the local leaf and systemic root transcriptome response of foliar feeding by soybean aphids after 12 hours and 7 days. Our objectives for this study were to understand the molecular effect of soybean aphid feeding on a whole-plant level and to identify potential aphid-triggered molecular mechanisms that could affect SCN performance. Our results revealed that aphid feeding on leaves triggered a highly dynamic transcriptomic response between the leaf and root tissues within a given time point as well as across time. Early (12 hours) leaf responses had mixed expression of defense and cell wall-related transcripts while widespread defense and stress responsive genes including AP2-EREBP and WRKY transcription factors and genes involved in SA- and JA-mediated signaling pathways were transiently repressed in the root response. During the late (7 days) response, leaves responded to aphid feeding by the induction of thousands of defense and stress transcripts while growth, morphogenesis, and photosynthesis transcripts were repressed. In roots, few transcriptional changes occurred at the late time point. Functional knockdown analysis of transcripts identified by the transcriptome analysis was conducted using transgenic hairy roots to test soybean cyst
nematode performance. The results revealed these genes to be potentially important for soybean cyst nematode development. All whole-plant and aphid experiments were conducted in a teamwork effort between me and Mrs. Martha Ibore Natukunda, who works under the supervision of Dr. Gustavo MacIntosh and Dr. Asheesh Singh. I conducted bioinformatic analysis with the help of Dr. Michelle A. Graham. I generated the constructs and knockdown transgenic hairy roots while Thomas R. Maier, who works with Dr. Thomas J. Baum, conducted the nematode infection assay and counted the number of females on each root system. I conducted all statistical analyses on the hairy root gene expression levels and number of females. I wrote all sections of the paper.

Chapter 4 consists of a summary of my major findings of the previous chapters, general conclusions of the research, and lays out future directions that could be pursued based on the findings of my work.

Secondary Publications

Other publications to which I made a contribution during the course of my research include the following:


References


Kanobe C. 2012. Fatty acid changes in soybean (Glycine max) under soybean aphid (Aphis glycines) infestation and their implications on plant defense against insects. *Graduate Theses and Dissertations Paper 12840*.


CHAPTER 2. SOYBEAN APHIDS EXPLOIT SOYBEAN ABScisic ACID SIGNALING TO PROMOTE SUSCEPTIBILITY

Modified from a manuscript to be submitted to New Phytologist

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Summary

- The soybean aphid (*Aphis glycines*) is an important insect pest of soybean (*Glycine max*) and uncontrolled populations can severely reduce yields. Jasmonic acid (JA)-mediated defenses are effective against soybean aphids yet aphids can block JA responses. We test the hypothesis that soybean aphids block JA-mediated responses by induction of an antagonistic abscisic acid (ABA) response.

- Chemical elicitor treatment and knockdown of ABA biosynthesis (*ABA2*) and signaling (*SCOF-1*) genes show that ABA blocks wound-induced JA responses in soybean and that a functional ABA signaling pathway is necessary for normal aphid population growth.

- Elevated basal JA and SA responses are likely causes of reduced soybean aphid population in knockdown plants. Additionally, lose the ability to block JA responses in the knockdown plants indicating that the aphid-regulated repression of JA responses is mediated by soybean’s endogenous ABA pathway.

- Our data demonstrate a positive impact of ABA signaling on aphid performance and supports the hypothesis that aphids induce ABA responses to block effective JA-mediated defense responses in soybean.

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Key Words: soybean aphid, abscisic acid, jasmonic acid, hormone antagonism, plant defense

**Introduction**

Soybean aphids (*Aphis glycines* Matsumura) are phloem-feeding insect pests native to Asia that were first reported in the US in the year 2000. Since their introduction, this invasive insect pest has spread to most major soybean (*Glycine max*) producing states (Ragsdale *et al.*, 2011). Soybean aphids produce several generations on soybean crops during the growing season (Ragsdale *et al.*, 2004). They ingest phloem sap and water from the plant vasculature, and can vector plant viruses (Hill *et al.*, 2001; Thompson & Goggin, 2006). Even without viral infection, uncontrolled aphid populations can reduce yield by up to 40% (Ragsdale *et al.*, 2007). Unlike chewing insects that produce large amounts of plant damage, phloem-feeding insects minimize tissue damage using their piercing-sucking mouthparts (Thompson & Goggin, 2006). Perception of aphids by plants is thought to include detection of saliva, chitin fragments from the insect exoskeleton, or physiological changes in sieve elements (Libault *et al.*, 2007; Smith & Boyko, 2007; Hogenhout & Bos, 2011).

Plant responses triggered by pests and pathogenes are largely mediated by phytohormone signaling through salicylic acid (SA)-, jasmonic acid (JA)-, and ethylene (ET)-mediated pathways (Howe & Jander, 2008; Bari & Jones, 2009; Morkunas *et al.*, 2011). Extensive crosstalk exists between these pathways and they can act separately or in concert while exhibiting synergistic or antagonistic relationships. For example, JA and ET often exhibit coordinated efforts in activating defense gene expression whereas SA and JA are often antagonistically related [Reviewed in (Koornneef & Pieterse, 2008; Pieterse *et al.*, 2009)]. These major players frequently interact with other phytohormonal
signals such as abscisic acid (ABA), cytokinins, gibberellins, and auxin to modulate the composition, quantity, and temporal signatures of defense to tailor responses to specific attackers (De Vos et al., 2005; Smith & Boyko, 2007; Asselbergh et al., 2008; Pieterse et al., 2009).

SA is an important mediator of the defense response to biotrophic pathogens and aphid feeding induces SA-related responses in Arabidopsis, Medicago truncatula, Nicotiana attenuata, tomato, wheat, barley, sorghum, and soybean [Reviewed in (Thompson & Goggin, 2006; Goggin, 2007; Bari & Jones, 2009; Giordanengo et al., 2010; Morkunas et al., 2011; Kamphuis et al., 2013; Jaouannet et al., 2014)]. JA-mediated signaling is crucial for defense responses against necrotrophic pathogens and chewing insects. Growing evidence indicates that JA-mediated defenses are effective against several aphid species even though JA responses are suppressed or only modestly induced in compatible interactions in response to phloem-feeding insects [Reviewed in (Thompson & Goggin, 2006; Goggin, 2007; Howe & Jander, 2008; Bari & Jones, 2009; Giordanengo et al., 2010; Kamphuis et al., 2013; Jaouannet et al., 2014)]. In soybean, JA-mediated signaling induces an effective defense response against soybean aphids (Kanobe, 2012; Selig et al., 2016). Studham and MacIntosh (2013) found upregulation of transcripts associated with JA and ET biosynthesis 7 days after infestation but the plants lacked a JA response and the authors proposed that soybean aphids are able to block JA responses. Consistent with this, it was demonstrated that soybean aphids may block JA biosynthesis by reducing the JA precursor linolenic acid (Kanobe et al., 2015). Moreover, plants previously infested with soybean aphids accumulated significantly lower levels of the wound- and JA-inducible cysteine proteinase inhibitor N2, PinN2 (Botella et al.,
1996), compared to uninfested controls upon wounding or treatment with exogenous JA (Kanobe, 2012). These data suggest that soybean aphids block both JA biosynthesis and signaling; however, the underlying mechanism is currently unknown.

It has been proposed that aphids may exploit phytohormone antagonism by eliciting a decoy response to suppress effective defenses (Walling, 2008; Studham & MacIntosh, 2013). The silverleaf whitefly, also a phloem-feeding insect pest, induces SA responses to suppress effectual JA defenses in Arabidopsis (Zarate et al., 2007). Several studies show the induction of SA responses and only slight induction or repression of JA in response to aphid feeding, yet there is no general consensus on the impact of SA in plant-aphid interactions (Kamphuis et al., 2013; Jaouannet et al., 2014).

Several studies reveal differential regulation of the ABA pathway in response to aphid feeding or aphid saliva in Arabidopsis, Medicago truncatula, sorghum, wheat, and soybean (Zhu-Salzman et al., 2004; Park et al., 2006; Smith et al., 2010; Kerchev et al., 2013; Studham & MacIntosh, 2013; Guo et al., 2016; Hillwig et al., 2016). ABA is involved in regulating physiological processes such as seed maturation and dormancy as well as the response to environmental stress factors such as temperature, drought, and salinity (Dong et al., 2015). However, ABA is also an important modulator in plant-pathogen and plant-pest interactions and its effect on plant defense responses seems to be pathosystem-dependent [Reviewed in (Mauch-Mani & Mauch, 2005; Robert-Seilaniantz et al., 2007; Asselbergh et al., 2008; Flors et al., 2009; Ton et al., 2009; Morkunas et al., 2011)]. ABA-mediated susceptibility is often described to occur by acting antagonistically with other phytohormonally-mediated pathways such as SA or JA/ET or by inhibiting the production of defensive secondary metabolites and has been reported in
Arabidopsis (Mohr & Cahill, 2003; Anderson et al., 2004; de Torres-Zabala et al., 2007; Zabala et al., 2009; Kerchev et al., 2013; Hillwig et al., 2016), tomato (Audenaert et al., 2002; Thaler & Bostock, 2004), rice (Jiang et al., 2010; Nahar et al., 2012; Xu et al., 2013), tobacco (Kusajima et al., 2010), and soybean (Ward et al., 1989; McDonald & Cahill, 1999; Mohr & Cahill, 2001).

In soybean, Studham and MacIntosh (2013) reported that aphids induced accumulation of transcripts associated with ABA biosynthesis and signaling in response to aphid feeding after 7 days. Based on this and the fact that soybean aphids simultaneously block JA responses, we hypothesized that aphids induce a decoy ABA response to suppress JA-mediated defense responses in susceptible soybean plants. We also hypothesized that ABA-related responses are important for normal soybean aphid population growth. We tested these hypotheses and demonstrated that knockdown of ABA biosynthesis (ABA2) and signaling (SCOF-1) genes significantly reduced aphid population growth, which was likely due to elevated basal JA and SA signaling in the knockdown plants. Moreover, we provide genetic evidence that the aphid-regulated block in JA responses is mediated by soybean’s endogenous ABA pathway.

Materials and Methods

Plant material

For all experiments, aphid-susceptible soybean (Glycine max (L.) Merr.) seeds cv. SD01-76R were used. Seeds were sterilized overnight using chlorine gas as previously described (Paz et al., 2006). Plants were grown in a growth chamber in steam sterilized Metro-Mix® 900 soil (Sun Gro Horticulture, Vancouver, BC, Canada) at a constant temperature of 25°C with a 16h light: 8h dark photoperiod unless stated otherwise.
**Hormone treatment experiments**

To test the hypothesis that ABA treatment negatively impacted the wound response of soybean, a hydroponic setup was used. Unifoliate stage seedlings were gently removed from soil, roots rinsed in water, and plants placed on platforms with the roots submerged in modified Hoagland’s hydroponic media containing 1.25 mM KNO₃, 2.16 mM Ca(NO₃)₂·4H₂O, 0.75 mM MgSO₄·7H₂O, 0.3 mM KH₂PO₄, 50 µM KCl, 50 µM H₃BO₃, 10 µM MnSO₄·H₂O, 2 µM ZnSO₄·7H₂O, 2.4µM CuSO₄·5H₂O, 100 µM EDTA-Na₂, 100 µM FeSO₄·7H₂O. Three plants were included in each container. Media was fully replaced once weekly and the volume of media was maintained by adding deionized water as needed. A 100mM ABA stock was dissolved in 100% methanol. When plants reached the V3 stage, hydroponic media was replaced with media supplemented with control (0µM, 0.1% methanol) solution or 100 µM ± ABA-supplemented solution (Sigma-Aldrich, St. Louis, MO). Treatments were administered at the V3 growth stage (Fehr & Caviness, 1977). Four treatments were administered: 0µM ABA, 0µM ABA+wounded, 100µM ABA, 100µM ABA+wounded. Each treatment had 5 replicates for a total of 20 containers. After 24h hours of ABA pre-treatment, half of the V1 leaves were wounded with a tweezers and half were left unwounded within each ABA level. Six hours after wounding, V1 leaves of three plants in the same container were pooled for a total of 5 replicates per treatment. Samples were immediately frozen with liquid nitrogen and stored at -80°C until further sample processing.
Gene silencing experiments

Vector construction

We used virus-induced gene silencing to test whether mutation of ABA biosynthesis or signaling affected aphid population or the plant wound response. *Bean Pod Mottle Virus* (BPMV) RNA components as well as *Soybean Mosaic Virus* (SMV) helper component were a kind gift from Dr. Steven Whitham (Iowa State University, Ames, IA) and Dr. Michelle Graham (USDA-ARS CICGR, Ames, IA). To generate *aba2* RNAi and *scof-1* RNAi constructs, approximately 300bp fragments of *ABA2* and *SCOF-1* were amplified from soybean cDNA by PCR (primers listed in supplementary table 1), then digested with *BamHI* and *XhoI* and ligated into the RNA2 vector. After construct sequences were confirmed, plants were bombarded, grown, tested for BPMV presence, and then tissue was collected, lyophilized, and stored according to Whitham *et al.* (2016). This tissue was used as the stock source for viral inoculation.

Plant maintenance and viral inoculation

Ten to fifteen seeds were planted in one pot (Poly-tainer™ #2, Nursery Supplies Inc., Orange, CA) and after one week seedlings were thinned to 4 per pot. For the duration of each experiment, plants were watered with 1 L of water per pot as needed and additionally fertilized once per week with 1 L of a 1:1 mixture of 6% All-Purpose Scott’s Miracle-Gro Excel (21-5-20, The Scott’s Company LLC, Marysville, Ohio, USA) and 6% Cal-Mag Miracle-Gro Professional (15-5-15, The Scott’s Co.) applied at a rate of 12.5 mL L⁻¹ water. At the unifoliate stage, plants were dark-treated for approximately 24 hours and the chamber temperature was lowered to 21°C day/18°C night to facilitate viral infection and spreading. A slurry was made by grinding 30 mg dried stock tissue with
2mL of 50mM phosphate buffer pH 7.0 with a mortar and pestle. The slurry was transferred to a microcentrifuge tube and was briefly centrifuged. Carborundum (320-grit) was sprinkled onto the unifoliate leaves of 7-8 day old soybean seedlings. Then, 15µL of the supernatant was pipetted onto each unifoliate leaf and rubbed gently taking care to avoid tearing large holes in leaves. Plants grew for approximately 3 weeks to allow for viral spread and gene silencing.

**Aphid population quantification experiments**

Aphid population quantification experiments were set up in a randomized complete block design with each pot being a block. Each BPMV construct treatment was represented once in each pot with a total of 4 BPMV construct treatments (mock, virus vector control, *aba2* RNAi, and *scof-1* RNAi). Each experiment consisted of 13-15 pots. Soybean aphids (biotype 1) were obtained from a lab colony at Iowa State University. Ten five-to-six day old age-synchronized aphids were placed on one leaflet of the V3 or V4 trifoliate using a fine tip paintbrush and were confined using a clip cage (BioQuip products, Rancho Dominguez, CA, USA). Aphids were allowed to feed and reproduce for 7 days then populations were counted. Additional mock-inoculated plants were left uninfested to compare ABA levels in control and aphid-infested plants. Both aphid-infested and control leaflets had clip cages and were gently brushed to remove aphids or mimic any mechanical stimulation caused by the cage or brushing. Leaf tissue from 2-4 plants with or without aphids was pooled and collected into liquid nitrogen and all samples were stored at -80°C until further processing.
**Wounding/gene expression experiments**

Gene expression experiments were set up in a randomized complete block design with each pot acting as one block. Each pot contained four plants of the same BPMV construct treatment. Each plant in the pot received a different wounding treatment: control (C), aphid-infested for 7 days (A), wounded for 6 hours (W), or aphid-infested for 7 days and wounded for 6 hours (AW). Each experiment consisted of 6 pots per construct and samples were collected by pooling two leaflets of the same wounding treatment from two pots to generate 3 replicates for gene expression analysis. Thirty aphids were used per plant and plants were infested as described previously, with both aphid-infested and aphid-free leaflets having clip cages. Aphids fed for 7 days then leaflets of all treatments were gently brushed to remove aphids. One aphid-treated plant per pot and one untreated plant per pot were wounded by crushing the leaflet with a small tweezers. After 6 hours, leaflets from the same wounding treatment from two pots were collected by trimming the leaf size to the size of the clip cage, pooled, and immediately collected into liquid nitrogen. All samples were stored at -80°C until further processing.

**Sample processing and gene expression quantification**

All samples were ground in liquid nitrogen using a mortar and pestle. Total RNA was extracted from leaves using TriReagent (Ambion®, Life Technologies, NY, USA). Genomic DNA contamination was removed using TURBO DNA-free™ kit (Ambion®, Life Technologies, NY, USA) and PCR was done to check for any undigested contaminating genomic DNA. One microgram of cDNA was synthesized using qScript™ Flex cDNA Synthesis Kit (Quanta Biosciences, Beverly, MA, USA) using Oligo dT primers. Quantitative PCR (qRT-PCR) was performed using PerfeCTa® SYBR® Green
FastMix®, Low ROX (Quanta Biosciences Beverly, MA, USA) in an Mx4000 (Stratagene, Agilent, Technologies, Santa Clara, CA, USA). Cycle threshold values were quantified and analyzed according to a standard curve and then normalized to internal control gene Glyma20g27950.1. All primers used in this study can be found in supplemental table 1.

**ABA quantification**

**ABA extraction**

ABA was extracted and quantified using a method adapted from (Forcat et al., 2008). Standards and anhydrous acetic acid were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO). Solvents were LC-MS grade (Fisher). Briefly, lyophilized soybean leaves were pulverized with 5 mm stainless steel grinding beads in a Mixer Mill MM400 (Retsch) equipped with an Adapterrack PTFE pre-frozen at -20 °C. The fine powder (5 mg) was extracted on ice for 30 min in 400 µl of methanol:acetic acid:water (10:1:89) that contained 5µM (+)-catechin as an internal standard because of its ionization potential and absence from soybean (Kovinich et al., 2011). The supernatant was removed by centrifugation and the extraction repeated once as indicated above. The pooled supernatants were flash frozen in liquid nitrogen and lyophilized to dryness. The residue was resuspended in 100 µl of methanol:acetic acid:water (10:1:89), filtered by centrifugation through 2 µm PTFE for analysis by LC-MS.

**Direct infusion mass experiments**

A Q-Exactive Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA, USA) was used for exact mass measurements and parameter optimization prior to UHPLC-MS analysis. On each day the mass analyzer was calibrated to ensure mass
accuracy and efficient ion transmission for maximizing ion signal. Briefly, the hormone standards were prepared at 20 µM in 1:1 water:acetonitrile. Standards were introduced individually by direct infusion through a heated electrospray source inlet (HESI) using a 4.00 kV spray voltage bias relative to the entrance orifice of the mass spectrometer. The capillary and Aux gas temperatures were programmed at 250 °C and 50 °C, respectively. The generated ions passed through the S-lens ion guide (60.0 V) and were subsequently transferred into an Orbitrap mass analyzer. The Orbitrap was scanned from m/z 80.0 to 600.0 with a resolving power of 70,000. A target AGC of 1.0x10^6 with a 200 ms injection time was used for the analysis. These parameters were found to give maximum signal across all standard infusion experiments. The exact m/z values of each standard were recorded and used to generate an inclusion list for LC-MS experiments (see below).

**LC-MS methods**

A Q-Exactive Orbitrap mass spectrometer interfaced to an Accela UHPLC (Thermo Fisher Scientific, San Jose, CA) was used for hormone identification and quantification. Standards were analyzed in order to record retention times and to generate calibration curves prior to sample analysis. Standards were injected onto the system via the PAL autosampler (5µL) at concentrations 5, 2.5, 1, 0.5, and 0.1 µM. The UPLC was programmed for gradient delivery of water (solvent A) and acetonitrile (solvent B) each containing 0.1% acetic acid at 300 µL min⁻¹. The gradient for solvent B was from 10-25% 0-2 min, 25-35% 2-7 min, 35-100% 7-9 min, 100% 9-11 min, 10% 11-12 min, held at 10% until 15 min. Separations were achieved using an Acquity UPLC BEH Shield RP18 analytical column (Waters) with a pore size of1.7 µm. The column held at 35°C using a column heater (Thermo Fisher Scientific, San Jose, CA). Analytes separated by
the column were directed to the HESI source using the parameters discussed above. The Q-Exactive was operated in Selected Ion Monitoring mode, via the inclusion list generated from direct infusion experiments (see above). That is, the mass analyzer was programmed to scan each elution window and set to record ion intensity for the defined standard \( m/z \) values. For sample analysis, injection volumes were normalized to the amount of internal standard using Xcalibur software and then to the amount of dry tissue weight using Excel. ABA extraction and quantification was also confirmed using the protocol described in Schmelz et al. (2004).

**Statistical analyses**

Experiments were set up using either completely randomized design where t-tests (t-test assuming unequal variance using Microsoft Excel) were used to determine significance or a randomized complete block design (RCBD) was used and data was analyzed by ANOVA analysis, as indicated in each figure. If an overall significant difference was detected, pair-wise comparisons were made using least significant difference (LSD) multiple comparison correction. All RCBD statistical analyses were done using Statistix9 Student Edition.

**Results**

**Soybean aphid feeding induces ABA accumulation**

To determine whether ABA levels increase due to aphid feeding, we quantified the hormone in leaves of aphid-treated and untreated plants 7 days after infestation using a new method adapted from Forcat et al., (2008). We found that aphid feeding causes a significant increase in ABA (Fig. 1). Confirmation of this analysis was done using another established method (Schmelz et al., 2004) (Supplementary Fig. 1). Our results are
consistent with the previous report that soybean aphids induce the expression of ABA biosynthesis and signaling transcripts in susceptible soybean plants after 7 days of feeding Studham and MacIntosh (2013). On average, aphids feeding for 7 days increase ABA levels by approximately 43% when compared to uninfested control plants.

**Exogenous ABA pre-treatment antagonizes wound-induced JA signaling in soybean**

The ABA signaling pathway exhibits a complex antagonistic relationship with the JA phytohormonal pathway in rice salt responses and in Arabidopsis (Moons et al., 1997; Anderson et al., 2004; Kazan & Manners, 2013). To test whether ABA has a negative effect on JA signaling in soybean, we pre-treated plants for 24 hours by supplementing hydroponic media with either 0µM or 100µM ABA followed by wounding treatment of leaves to generate a JA response. In unwounded plants, the addition of ABA had no effect on transcript levels of the JA- and wound-inducible marker gene PinN2 (Fig. 2). As expected, wounding highly induced (63-fold) PinN2 transcript levels in plants that had not been pre-treated with ABA. However, in plants previously exposed to ABA, PinN2 transcripts accumulated to significantly lower levels after wounding. These results indicate that ABA has the ability to block JA-regulated responses in soybean and are consistent with a role for ABA as antagonist of effective defenses against aphids.

**Soybean aphids have compromised performance on ABA biosynthesis- and signaling-knockdown plants**

To test whether ABA is necessary for successful aphid population growth in the compatible interaction, we knocked down the expression of genes involved in ABA biosynthesis and signaling through virus-induced gene silencing (VIGS) using a bean pod mottle virus (BPMV) system optimized for soybean (Zhang et al., 2010; Whitham et al.,
The chosen VIGS targets were ABA2 and SCO-1. ABA2 encodes XANTHOXIN DEHYDROGENASE, a short-chain dehydrogenase that is involved in the conversion of xanthoxin to abscisic aldehyde during the penultimate step in ABA biosynthesis (Gonzalez-Guzman et al., 2002). To determine if ABA downstream signaling is important for aphid performance, we knocked down SCO-1, a cold- and ABA-regulated transcriptional activator for ABA-responsive element (ABRE)-binding transcription factors (Kim et al., 2001). SCO-1 expression is upregulated highly by aphid feeding at 1 and 7 days after infestation (Studham & MacIntosh, 2013), suggesting that this transcription factor may be a key regulator of the ABA response observed during aphid infestation in soybean. Quantitative RT-PCR (qRT-PCR) was employed to verify knockdown of these genes; on average, the transcript level of ABA2 was reduced 91% (P<0.05) in aba2 RNAi plants while SCO-1 was reduced 67% (P<0.05) in scof-1 RNAi plants relative to the vector control plants (Fig. 3). Knockdown of ABA2 and SCO-1 resulted in 24.7% and 25.2% lower aphid populations, respectively, compared to vector controls, and 31.1% and 31.5%, respectively, compared to mock-inoculated plants (P<0.05) (Fig. 4). These results indicate that functional ABA biosynthesis and signaling through SCO-1 are important for successful aphid population growth. It is also interesting to note that the viral vector control plants have fewer aphids (P<0.05) compared to mock-inoculated plants.

ABA biosynthesis and signaling knockdown plants have an enhanced basal level of JA- and SA-mediated signaling

To investigate the underlying mechanism of decreased aphid populations in ABA-deficient plants, we used qRT-PCR to analyze basal levels of PinN2 and the SA-
responsive marker gene PR1a in control (without aphids or wounding) plants. PinN2 was expressed at a higher basal level in both aba2 RNAi plants and scof-1 RNAi plants compared to mock-treated and vector control plants, which were not significantly different from each other (Fig. 5A). The basal PinN2 levels in aba2 RNAi and scof-1 RNAi plants were similar to levels of PinN2 in wounded mock and vector control plants (compare expression levels to those in Fig. 6). Levels of PR1a were higher in all virus-treated plants compared to mock-inoculated plants, indicating that BPMV inoculation of plants causes induction of the SA signaling pathway (Fig 5B). Additionally, aba2 RNAi but not scof-1 RNAi plants had elevated PR1a levels compared to the vector control. Taken together, these results suggest that SA signaling negatively impacts soybean aphids, as vector control plants have 8.5% fewer aphids than mock plants (see Fig. 4) and the difference in JA response accumulation could be responsible for the additional reduction in aphid populations seen in aba2 RNAi and scof-1 RNAi plants compared to the vector control. Therefore, our data suggests that a blend of both JA- and SA-mediated defenses are important in controlling soybean aphid population growth.

**Soybean aphids block JA responses using soybean ABA-mediated signaling**

Soybean aphids block JA- and wound-induced accumulation of PinN2 (Kanobe, 2012). To investigate whether aphids use soybean ABA signaling to block JA responses, we conducted a series of wounding experiments on control and VIGS plants without (wounded only; W) or with aphid pre-treatment (aphids+wounded, AW) and measured PinN2 transcript levels. We confirmed that previous exposure to aphid feeding was able to reduce the wound induction of transcription of PinN2 compared to plants without aphids on both mock- and vector control-inoculated plants (Fig. 6). On mock-inoculated
plants, aphids suppressed $PinN2$ levels 2.03-fold ($P<0.05$) compared to uninfested wounded plants while aphids on vector control plants reduced $PinN2$ levels 1.46-fold yet the aphid-triggered reduction in wound responses was not statistically significant at $P<0.05$. However, in $aba2$ RNAi plants, aphid pre-treatment was not able to block accumulation of JA-responsive $PinN2$ ($P>0.05$) in response to wounding. Furthermore aphid presence on $scof-1$ RNAi wounded plants resulted in accumulation of $PinN2$ to 1.93-fold higher than wounded plants without aphid pre-treatment, although this difference was not statistically significant ($P>0.05$). These data support the hypothesis that the aphid-regulated block in JA signaling is mediated by the plant’s endogenous ABA signaling pathway.

**Discussion**

In this work, we showed that soybean aphids induce the accumulation of ABA and that aphids perform better on plants with intact ABA biosynthesis and signaling, suggesting this pathway is necessary for normal aphid population growth. We show that ABA mediates the block of JA responses. In $aba2$ RNAi and $scof-1$ RNAi plants, where ABA biosynthesis and signaling genes are knocked down, respectively, JA responses are constitutively higher than in control plants. Finally, our results provide evidence that the aphid-regulated block of JA responses is mediated through the soybean ABA pathway. Taken together, these data strongly support the hypothesis that soybean aphids induce an ABA-dependent decoy response to suppress effective JA-mediated defenses.

JA is considered the most important phytohormone in the response to insect herbivore feeding (Howe & Jander, 2008). JA-mediated responses reduce insect herbivory by inducing expression of anti-nutritive and anti-digestive proteins such as
polyphenol oxidase and proteinase inhibitors that affect chewing and some phloem-feeding insects (Cooper & Goggin, 2005; Howe & Jander, 2008). Exogenous application of jasmonates to Arabidopsis, Medicago truncatula, cotton, wheat, tomato, sorghum, and soybean reduce aphid performance on, or preference for, JA-treated plants (Omer et al., 2001; Slesak et al., 2001; Thaler et al., 2001; Ellis et al., 2002; Bruce et al., 2003; Cooper et al., 2004; Zhu-Salzman et al., 2004; Cooper & Goggin, 2005; Boughton et al., 2006; Thompson & Goggin, 2006; Gao et al., 2007; Kanobe, 2012; Selig et al., 2016). Additionally, Arabidopsis fou2 and cev1 mutants which have constitutively high JA biosynthesis or signaling, respectively, have reduced aphid performance whereas coi1 mutants, compromised in JA signaling, accumulate higher populations of both generalist and specialist aphids than wild type plants (Ellis et al., 2002; Mewis et al., 2006; Kuśnierczyk et al., 2011). JA/methyl JA (MeJA)-mediated defenses are effective against soybean aphids (Kanobe, 2012; Selig et al., 2016) yet JA responses are suppressed by aphids after 7 days of infestation (Kanobe, 2012; Studham & MacIntosh, 2013). A concomitant increase in ABA biosynthesis and signaling transcripts led Studham and MacIntosh (2013) to propose that the induction of ABA responses may act as a decoy response that blocks JA signaling.

Growing evidence indicates the ABA pathway plays a role in suppressing defense responses (Flors et al., 2009). For instance, Pseudomonas syringae, Botrytis cinerea, Xanthomonas oryzae, and Phytophthora sojae exploit the ABA pathway to promote susceptibility by suppressing SA-mediated defenses in Arabidopsis, tomato, rice, and soybean (Ward et al., 1989; McDonald & Cahill, 1999; Mohr & Cahill, 2001; Audenaert et al., 2002; Mohr & Cahill, 2003; Thaler & Bostock, 2004; de Torres-Zabala et al.,
2007; Zabala et al., 2009; Xu et al., 2013). In *Arabidopsis*, ABA blocks JA/ET-mediated signaling and plant defenses against *Fusarium oxysporum* (Anderson et al., 2004). In plant-aphid interactions, recent reports show reduced susceptibility toward aphids on ABA mutants in *Arabidopsis* and *Medicago truncatula* indicating phloem-feeding insects perform better on plants with a functional ABA pathway (Kerchev et al., 2013; Guo et al., 2016; Hillwig et al., 2016). *Arabidopsis aba2* and *aba1-1* mutants were less attractive to *M. persicae* and had higher levels of indol-3-ylmethylglucosinolate (I3M) and 4-methoxyindol-3-ylmethylglucosinolate (4MI3M), indole glucosinolates which have antixenotic and antibiotic activities against the aphids (Kim & Jander, 2007; Kim et al., 2008; Kerchev et al., 2013; Hillwig et al., 2016). Similarly, we found that soybean aphids performed worse on ABA biosynthesis and signaling knockdown mutants.

The interaction between ABA and JA signaling pathways is complex and depends on the hormonal context. In *Arabidopsis*, ABA activates the MYC2-branch of JA signaling (with *VSP1* as a marker gene) but represses the ethylene-modulated (ERF/OR59) branch of JA signaling (with *PDF1.2* as a marker gene) (Kazan & Manners, 2013). Also in *Arabidopsis*, ABA treatment suppressed basal and induced transcription of four JA/ET responsive defense genes including *PDF1.2*, as well as GUS expression under the *PDF1.2* promoter (Anderson et al., 2004). Additionally, in the presence of ABA, neither exogenous MeJA nor ethylene could activate expression of *PDF1.2* in wild-type plants. Furthermore, *aba2-1* and *jin1-9/myc2* *Arabidopsis* mutants deficient in ABA biosynthesis or signaling, respectively, had elevated levels of basal and inducible *PDF1.2* and other JA/ET-responsive genes, while overexpression of *AtMYC2* resulted in suppression of *PDF1.2* (Anderson et al., 2004). Thus, we can speculate that
ABA induction by soybean aphids may repress the ERF branch of JA signaling. In soybean, *PinN2* is responsive to wounding only in the presence of a functional ethylene signaling pathway (Botella *et al.*, 1996). Studham and MacIntosh (2013) found transcription of both JA and ET marker genes was repressed after 7 days of aphid feeding. We confirmed the ABA-JA antagonism as plants pre-treated with ABA were unable to respond to wounding to the degree of plants without ABA pre-treatment using *PinN2* as marker (Fig. 2). Additionally, *aba2* RNAi and *scof-1* RNAi plants had higher basal *PinN2* levels compared to mock and vector control plants (Fig. 5A). Our results support the hypothesis that soybean aphids induce a decoy ABA response to suppress JA-mediated defense responses in susceptible soybean plants. Our mock and vector control plants corroborate the results of Kanobe (2012) in that plants with previous exposure to aphids had reduced *PinN2* transcript levels compared to plants without aphids (Fig. 6). However, in *aba2* RNAi and *scof-1* RNAi plants, aphids were not able to reduce accumulation of *PinN2*. Taken together, these results indicate that exogenous and endogenous ABA blocks JA-mediated responses in soybean, likely the JA/ET branch, and that soybean aphids mediate the attenuation of JA signaling via host ABA signaling.

Water stress also induces the ABA signaling pathway and in *Arabidopsis*, short-term water stress decreased the accumulation of *PDF1.2* compared to well-watered control plants (Anderson *et al.*, 2004). In soybean, researchers have noticed that slight drought stress exacerbates soybean aphid populations in the field (Rice *et al.*, 2004) and Nachappa *et al.* (2016) reported higher soybean aphid populations on drought-stressed soybean plants than on plants maintained in overwatered soil conditions. The authors argued that in drought-stressed plants, ABA signaling is induced with simultaneous
repression of JA and SA responses whereas in plants maintained in saturated conditions, ABA signaling is suppressed and the JA and SA pathways are induced. These results suggest that the increase in JA or JA/ET signaling is likely the major mechanism underlying the aphid population decrease in aba2 RNAi and scof-1 RNAi plants. Although our data is consistent with the role of ABA as a decoy response induced by soybean aphids to suppress defense responses, it is important to consider that manipulation of the ABA pathway may result in changes in the plant water status. Previous studies have suggested the reduction in aphid populations on ABA-deficient plants could be due to decreased water potential which reduces aphid xylem absorption and osmoregulation, as the up-regulation of the ABA signaling pathway facilitates water uptake from the xylem by aphids under drought stress (Guo et al., 2016). The effect of water stress on different aphid populations varies which suggests that the magnitude of stress as well as the identity of both plant and herbivore (as well as virulent versus avirulent biotypes) are important in determining the role of ABA in plant responses to insects (Huberty & Denno, 2004; Mody et al., 2009). Thus, the effect of mutating ABA processes on plant water status cannot be discarded in explaining the reduced populations in our mutants; the resulting aphid phenotype observed in the current study could be due to a balance between plant water status and defense response suppression.

The decrease in aphid populations on all virus-inoculated plants compared to mock plants is consistent with a previous study that found a negative impact of BPMV on aphid populations (Donaldson & Gratton, 2007) and may be partly explained by increased PR1a levels (Fig. 5B). Reports vary on whether SA-related responses are effective against aphids or if SA plays a decoy role to suppress JA-mediated defenses
[reviewed in (Thompson & Goggin, 2006; Goggin, 2007; Giordanengo et al., 2010; Kamphuis et al., 2013; Jaouannet et al., 2014)]. In soybean, SA signaling is induced in both resistant and susceptible soybean plants but earlier and to a higher degree in resistant plants (Li et al., 2008). Additionally, exogenous SA treatment had a negative impact on soybean aphid populations in resistant plants but not susceptible plants suggesting that SA signaling acts defensively against soybean aphids but the insects may be able to block or avoid SA-mediated defenses in susceptible plants (Studham & MacIntosh, 2013; Selig et al., 2016). Furthermore, Selig et al. (2016) found that susceptible soybeans treated with both SA and MeJA had aphid populations similar to MeJA-only treated soybeans, indicating that SA and JA do not have an antagonistic interaction regarding induced defenses against soybean aphids. Thus, it is evident that SA-mediated signaling also has a negative impact on soybean aphid performance. The difference in JA response may account for the further decrease in soybean aphid performance seen in aba2 RNAi and scof-1 RNAi plants compared to the vector control. Therefore, coordination of both JA- and SA-mediated signaling are important in controlling soybean aphid population growth, and ABA is involved in regulating both pathways, although likely through different regulatory components as *PR1a* levels were higher in *aba2* RNAi plants but not *scof-1* RNAi plants.

In conclusion, this study has provided insight into the compatible interaction between soybean and soybean aphids and our results highlight the importance of phytohormone crosstalk in plant-aphid interactions. It is evident that ABA plays an important role in regulating plant defense responses (Mauch-Mani & Mauch, 2005; Robert-Seilaniantz et al., 2007; Asselbergh et al., 2008; Flors et al., 2009; Ton et al.,
Our results suggest that ABA accumulation is beneficial to aphids and support the hypothesis that soybean aphids induce a decoy ABA response to suppress effective defenses that likely include a blend of both JA/ET- and SA-mediated signaling. The mechanism by which aphids induce ABA remains unclear but could be mediated by salivary effectors. Therefore, characterizing aphid effectors and the mechanism by which ABA interferes with defense responses will be crucial to decreasing plant susceptibility to these invasive insect pests.

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**Author Contributions**

GCM and JDH conceived the study and experiments; JDH, AMH, JFT, and NK executed experiments, GCM and JDH analyzed and interpreted all data, JDH wrote the manuscript.

**References**


**Fehr WR, Caviness CE.** 1977. Stages of soybean development. *Iowa Agricultural and Home Economics Experiment Station Special Report* (80): 3-11.


Kanobe C. 2012. Fatty acid changes in soybean (Glycine max) under soybean aphid (Aphis glycines) infestation and their implications on plant defense against insects. *Graduate Theses and Dissertations Paper 12840*.


Ward EWB, Cahill DM, Bhattacharyya MK. 1989. Abscisic-Acid Suppression of Phenylalanine Ammonia-Lyase Activity and Messenger RNA, and Resistance of


Figure 1. Soybean aphid feeding induces ABA accumulation. Plants with or without soybean aphids for 7 days were assayed for ABA concentration using LC-MS method adapted from Forcat et al. (2008). Values were normalized to dry tissue weight. Graph shows average ABA concentration ± standard error. Different letters indicate significance at P<0.05 using t-test: Two-Sample Assuming Unequal Variances in Microsoft Excel.
Figure 2. Exogenous abscisic acid treatment blocks wound-induced JA signaling. Plants were placed in 0µM or 100µM (±ABA)-supplemented media for 24 hours then wounded with a pair of small tweezers. Six hours after wounding, samples were collected. Relative expression of PinN2 was assayed using quantitative PCR. Graph shows average PinN2 expression ± standard error. Different letters indicate significance at P<0.05 with LSD multiple comparisons test.
**Figure 3. Gene knockdown confirmation.** Basal expression levels of *ABA2* (A) or *SCOF-1* (B) were assayed via quantitative PCR in untreated vector and *aba2* RNAi or *scof-1* RNAi plants, respectively. Graphs show average expression level ± standard error. Different letters indicate significance at P<0.05, LSD multiple comparisons test.
Figure 4. Soybean aphids have lower performance on ABA-knockdown plants. Ten age-synchronized apterous aphids were placed on the abaxial surface of the leaf and confined within clip cages. Aphid population was quantified seven days after infestation. Graph shows average number of aphids per plant ± standard error. Different letters indicate significance at P<0.05, LSD multiple comparisons test.
Figure 5. Basal defense gene expression is higher in ABA-knockdown plants. Basal expression levels of *PinN2* (A) or *PR1a* (B) were assayed in untreated plants using quantitative PCR. Graph shows average expression level ± standard error. Different letters indicate significance at P<0.05, LSD multiple comparisons test.
Figure 6. Aphid-triggered suppression of JA signaling is mediated through the ABA pathway. Expression level of PinN2 was measured using quantitative PCR in wounded plants (W) or wounded plants that had been exposed to aphid feeding for 7 days (AW). Graph shows average PinN2 expression ± standard error for each treatment. Different letters within the same BPMV construct treatment indicate significance at P<0.05, LSD multiple comparisons test.
Supplementary Figure 1. Soybean aphid feeding induces ABA accumulation. Plants with or without soybean aphids for 7 days were assayed for ABA concentration using the method described in Schmelz et al. (2004). Graph shows average ABA concentration ± standard error normalized to control plant average. Different letters indicate significance at P<0.05; t-Test: Two-Sample Assuming Unequal Variances in Excel.
Supplementary Table 1. Primers used to generate RNAi constructs and quantify gene expression (qRT-PCR).

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<th>Template</th>
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Quantitative RT-PCR

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CHAPTER 3. DISSECTING PLANT-MEDIATED PEST INTERACTIONS IN SOYBEAN: LOCAL AND SYSTEMIC EFFECTS OF SOYBEAN APHID INFESTATION

Modified from a manuscript to be submitted to BMC Genomics

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Abstract

Soybean aphids are specialized phloem-feeding insects that cause significant crop damage and yield reduction. Recent studies show that soybean aphid feeding systemically impacts performance of both intra- and interspecific pests such as root-dwelling parasitic soybean cyst nematodes. To date, the few molecular studies of soybean aphid infestation have focused on locally infested tissues; no molecular data exist that investigate soybean aphid-triggered systemic molecular changes. To explore the whole-plant compatible response to soybean aphids, we compared transcriptome changes in leaves and roots during an early (12h) and late (7d) foliar soybean aphid infestation. Divergent gene expression patterns across time and tissue suggest that foliar aphid feeding triggered a highly dynamic plant response and that local and systemic tissues had distinct responses to herbivory by soybean aphids within a given time point. Analysis of differentially expressed genes revealed strong transient repression of systemic root defenses and the delayed induction of local leaf defenses, both involving salicylate- and jasmonate-related signaling. The induction of defense transcripts in the late local leaf response was also associated with strong repression of transcripts involved in plant growth, development,

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and photosynthesis, suggesting that late aphid infestation triggers a tradeoff between defense and growth responses. This study is the first to investigate the systemic molecular response to soybean aphids and is the first to report transient repression of systemic innate immune responses by a phloem-feeding insect at the transcript level.

Key Words: soybean aphid, transcriptome, defense, suppression, systemic, soybean cyst nematode

Introduction

Soybean aphids (*Aphis glycines* Matsumura) are invasive insect pests that were first reported in the United States in 2000 and have since spread to most major soybean (*Glycine max*) growing regions (Ragsdale et al., 2011). These insects possess piercing-sucking mouthparts (stylet) which are used to ingest phloem sap and water from the plant vasculature on leaves, stems, and pods (Ragsdale et al., 2004). Uncontrolled aphid populations can cause major yield losses (Ragsdale et al., 2007). Unlike chewing insects that produce large amounts of plant damage, phloem-feeding insects minimize tissue damage using their piercing-sucking mouthparts (Thompson & Goggin, 2006). Thus, perception of aphids by locally infested plant tissues is thought to include detection of herbivore-associated molecular patterns (HAMPs) such as chitin within the exoskeleton, salivary elicitors, or physiological changes in sieve elements (Hogenhout & Bos, 2011; Bonaventure, 2012).

Plant defense responses triggered by pathogens and pests are largely mediated by salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) phytohormone signaling pathways that crosstalk with each other and other phytohormonal signaling pathways such as abscisic acid (ABA), auxins, cytokinins, and gibberellins to produce finely-tuned
defense responses (Bari & Jones, 2009; Pieterse et al., 2009; Robert-Seilaniantz et al., 2011). In general, SA signal transduction is triggered by biotrophic pathogens and phloem-feeding insects whereas JA and ET pathways are mostly induced by necrotrophic pathogens and chewing insects (Howe & Jander, 2008; Bari & Jones, 2009; Morkunas et al., 2011). In the soybean-soybean aphid system, transcriptome analyses revealed that plants resistant to aphids (carrying the Rag1 gene) involved induction of SA-mediated defenses and the response peaked early while defense responses were delayed in plants that were susceptible to aphids (Li et al., 2008; Studham & MacIntosh, 2013). Exogenous application of SA decreased aphid populations in resistant plants and JA or MeJA repressed aphid populations in both resistant and susceptible plants indicating both hormones are important regulators of defenses against soybean aphids (Kanobe, 2012; Studham & MacIntosh, 2013; Selig et al., 2016). Studham and MacIntosh (2013) reported strong induction of JA and ET biosynthesis transcripts in the compatible interaction after 7 days of aphid feeding. However, associated induction of marker gene expression was not seen and they proposed that aphids block JA-mediated defenses via suppression of host defense responses. Consistent with this, we previously found that soybean aphids block expression of the wound- and JA-inducible cysteine proteinase inhibitor N2 (PinN2) by exploiting host antagonistic ABA signaling (see Chapter 2). Thus far, all studies conducted on the plant molecular response of soybean aphid feeding have been aimed at characterizing the susceptible or resistant response in the locally infested leaf tissue.

A multitude of studies have demonstrated that pathogens and insect herbivores have significant effects on systemic molecular responses [reviewed in (Biere & Goverse,
2016; Papadopoulou & van Dam, 2017)]. Attack by pathogens, herbivores, or application of exogenous hormones triggers the production of mobile defense signals that are translocated to systemic tissues through the plant vasculature or as volatiles (Baluška, 2013; Gilroy et al., 2016). When the signal is perceived in the systemic tissue, this triggers changes in systemic tissue transcriptomes, phytohormone signaling, defensive metabolite content, root exudate composition, resource allocation, or tissue biomass. (Bardgett et al., 1998; Vestergard et al., 2004; Bezem & van Dam, 2005; Divol et al., 2005; Erb et al., 2008; Frost & Hunter, 2008; Kutyniok & Muller, 2012; Kerchev et al., 2013; Wondafrash et al., 2013; Kim et al., 2016). Systemic changes are often induced in plants to enhance host plant resistance to subsequent assault by the attacker or other pathogens. Thus, these molecular, biochemical, and structural systemic changes play a major role in plant-mediated indirect interactions between spatially separated pests.

Most studies on systemic molecular responses triggered by herbivores used chewing insects [reviewed in (Bezem & van Dam, 2005; Erb et al., 2008)]. However, relatively few studies have characterized molecular systemic changes triggered by phloem feeding insects. Phloem-feeders are known to alter carbon (C) and nitrogen (N) contents in local and systemic tissues and enhance nutritional status of the infested tissue (Sandstrom et al., 2000; Girousse et al., 2005; Chiozza et al., 2010). Aphid feeding triggers induction of C and N assimilation and mobilization transcripts in uninfected systemic leaf or phloem tissues, likely to enhance nutritional status of the infested leaf (Divol et al., 2005; Petrova & Smith, 2015).

Phloem-feeders also induce changes in local and systemic defense signaling. Combinations of SA, ET, or JA defense signaling pathways were activated in systemic

These systemic changes in nutrient content, cell wall structure, hormonal defense signaling, as well as defensive or exudate metabolites have the potential to alter the performance and behavior of other pests and root microbiota. For example, aboveground feeding by *Rhopalosiphum padi* aphids increased concentrations of barley root nitrogen, calcium, potassium, and sulfur and this potentially contributed toward facilitation of belowground wireworm performance (Johnson *et al.*, 2009). In potato, *M. persicae* feeding was inhibited on systemic leaves following previous aphid feeding on a different leaf (Dugravot *et al.*, 2007). Furthermore, presence of *M. persicae* significantly lowered *Heterodera schachtii* (beet cyst nematode, BCN) numbers on *Arabidopsis* roots (Kutyniok & Muller, 2012). Additionally, *M. persicae* altered root exudation in pepper, thereby modulating rhizosphere bacteria populations which led to induced resistance against pathogenic bacteria but caused the plants to be more susceptible toward both the aphids and beneficial root-associated bacteria (Lee *et al.*, 2012; Kim *et al.*, 2016).
However, nothing is known about the systemic effects brought about by foliar soybean aphid infestation at the molecular level.

We evaluated the transcriptome of locally infested leaves and systemic roots during an early (12h) and late (7d) soybean aphid infestation in aphid-susceptible plants using RNA sequencing to understand the molecular effect of soybean aphid feeding on a whole-plant level. Recent studies have identified that feeding by soybean aphids systemically facilitates the performance of both virulent and avirulent soybean aphid biotypes (Varenhorst et al., 2015) and also alters the performance of the economically important root-dwelling sedentary endoparasitic soybean cyst nematodes (Heterodera glycines, SCN) (Hong et al., 2011; McCarville et al., 2012; McCarville et al., 2014). In these cases, aphid presence improved host quality for SCN but at high pest population densities, competition between herbivores offset the facilitation of SCN performance, likely due to a decrease in host resources (McCarville et al., 2014). Thus, another objective of this study was to identify potential molecular mechanisms that could affect SCN performance. Our results indicate that soybean aphids trigger a dynamic response across tissue and time. At the early time point, widespread repression of SA- and JA-mediated defenses in roots was evident. In the late time point, evidence of a growth-defense tradeoff is prominent in locally infested leaves with the induction of defense responses and repression of growth and development.

Results

Analysis of differentially expressed genes

To identify aboveground and belowground transcriptomic changes that occur in response to foliar aphid feeding, V3 leaves (Fehr & Caviness, 1977) of soybean were
exposed to soybean aphids confined within clip cages for 12 hours or 7 days. Leaves without aphids (mock) were also treated with clip cages to mimic effects caused by the cage. Leaf and root tissues from 9 infested and 9 mock plants for each time point were individually collected and RNA was extracted and purified. Equal amounts of RNA from 3 plants within the same time and tissue combination treatment was pooled, resulting in a total of 24 samples (3 replicates with aphids, 3 replicates without aphids for each tissue within each time point). These samples were subjected to high-throughput RNA sequencing (Illumina RNA-seq). During initial analysis of biological variance, two root samples (one aphid replicate, one mock replicate) from the 12 hour collection showed highly divergent expression patterns compared to the two other replicates and were not included in the analysis. In total from 22 samples there were 586,061,831 raw 100 base pair (bp) single-end reads: 292,281,287 from 12 leaf samples and 293,780,544 from 10 root samples. Overall, nearly 88% of raw input reads mapped uniquely to the Williams 82 soybean reference genome version 2 (Schmutz et al., 2010). Using a False Discovery Rate (FDR) < 0.05, we identified a total of 13,080 genes that were differentially expressed (DE) in response to early and late foliar soybean aphid feeding in local leaf and systemic root tissues (Supplementary File 1). Regardless of time point, the locally infested leaf had a larger transcriptomic response than the systemic root. At the 12h time point, 1130 and 852 genes were differentially expressed in leaf and root tissue, respectively, and the majority of DE genes were repressed (Table 1). After 7 days of aphid feeding, 12,378 and 158 genes were differentially expressed in leaf and root tissue, respectively, with several of the genes repressed in the roots but leaves having a more balanced expression pattern. Locally infested leaves after 7 days of aphid feeding (L7D)
had the strongest change in transcriptional response both in terms of number of DE genes and fold change magnitude relative to all other time and tissue combinations. Overall, 11,713 (89.5%) genes were unique to one time and tissue combination (Fig. 1). When considering the overlap between tissues at a given time, 66 genes were common between leaf and root responses at 12 hours whereas 62 were common between tissues at 7 days. Across time, 731 genes were common in leaves whereas only 5 genes were common in roots. Only one transcript—Glyma.20G162300, which encodes a soybean homolog of a Medicago truncatula protein kinase involved in resistance to Pseudomonas syringae—was common to all times and tissues but the direction and magnitude of expression greatly varies between time and tissue. The limited transcript overlap and change in expression patterns indicates that foliar feeding by soybean aphids causes a dynamic response across tissue and time.

**Transcripts common to leaf and root responses**

To understand if specific genes and processes were regulated similarly by aphids on a whole-plant level, we examined the DE genes that were modified by aphid feeding in both tissues at both time points. For the early response, 66 DE transcripts overlapped between leaves and roots and the direction of regulation was different for only 4 of these transcripts (Supplementary File 2). In the late response, 62 transcripts overlapped between the tissues and half of the transcripts had opposing direction of regulation (Supplementary File 3). At 12h, most overlapping transcripts were repressed in both tissues. Notable genes included several soybean homologs of Arabidopsis AtEXO and AtXTH23 as well as one soybean homolog of each AtXTH22, AtXT2, and AtCSLC04 which are involved in cell expansion and cell wall biogenesis and remodeling (Rose et
al., 2002; Cocuron et al., 2007; Schroder et al., 2009). Four AP2-EREBP transcription factors which mediate abiotic and biotic stress responses (Licausi et al., 2013) were repressed in both tissues. A few soybean homologs of Arabidopsis transcription factors AtWRKY40, AtWRKY70, and AtJAZ8 which regulate basal defense responses (Xu et al., 2006; Knoth et al., 2007; Chini et al., 2016) overlapped between tissues and all but one soybean homolog of AtWRKY70 in leaves were repressed in both tissues suggesting that defense responses may have mixed regulation in leaves but were repressed in roots. Additionally, many NBS-LRR disease resistance proteins overlapped between leaves and roots and all were repressed in both tissues. These results, while representing only approximately 6 and 8% of leaf and root responses, respectively, reveal that a subset of cell wall metabolism and defense responses are differentially regulated in both leaf and root tissues in the early response to soybean aphid feeding.

Of the 62 transcripts overlapping between tissues at 7 days, 50% of the overlapping transcripts were induced in leaves while only 21% were induced in roots. Two soybean homologs of Arabidopsis CYP707A1 which encodes an abscisic acid 8’-hydroxylase and is responsible for ABA catabolism (Dong et al., 2015) were differentially regulated in both tissues. Soybean homologs of AtNIA1 (nitrate reductase 1), AtNIR1 (nitrite reductase 1), AtNRT1.5 (nitrate transporter 1.5), and AtAMT1;2 (ammonium transporter 1;2) were all repressed in both leaf and root tissues, suggesting the repression of nitrogen uptake, assimilation, and transport transcripts (Wang et al., 2012). Several soybean homologs of AtLOX1 which encodes a 9-lipoxygenase involved in plant development and defense responses (Howe & Schilmiller, 2002; Porta & Rocha-Sosa, 2002) were repressed in both tissues. Five NBS-LRR disease resistance proteins
had mixed expression between leaves and roots. Of these, Glyma.01G046900, a TIR-NBS-LRR class disease resistance protein, was one of the most highly repressed transcripts in both locally infested leaves and systemic roots. The function of this gene in plant-insect interactions is not known but the transcript is constitutively upregulated in plants resistant to soybean cyst nematodes, suggesting it may be involved in defense responses toward the endoparasitic pathogen (Wan et al., 2015). Overall, our results suggest that while some transcripts were similarly differentially regulated between the two tissues, the limited overlap and change in expression direction suggests that leaf and root tissues have distinct responses to aphid feeding within each time point.

**Hierarchical clustering of soybean response to foliar soybean aphid feeding**

To visualize global trends in gene expression due to aphid feeding, we analyzed our results using hierarchical clustering (Fig. 2A). Fold changes of all 13,080 unique genes were represented in each column, whether or not the expression difference (Aphid/Mock) was significant at FDR<0.05 for a certain time/tissue combination. Hierarchical clustering revealed that gene expression in leaves and roots were more similar to each other within a given time point than within a given tissue across time. Additionally, clustering identified six groups of genes (clusters) with similar expression patterns that reflect overall trends in response to foliar aphid feeding. Clusters C1-C6 contained 4101, 704, 3487, 837, 2478, and 1473 genes, respectively. DE genes in each cluster are listed in Supplementary File 1 and the number of DE genes within each cluster and each time/tissue combination in Fig. 2B. Transcripts comprising C1 show mixed expression in leaves after 12 hours of aphid feeding with the repressed transcripts showing strong repression. Furthermore, foliar aphid feeding causes strong repression of
more of these transcripts in roots at the same time point. In the late response, these transcripts were strongly induced in leaves but weakly repressed in roots. Thus, over time, leaf responses in this cluster shift from mixed expression early to strong induction late while in roots, these genes were largely repressed at both time points with early root responses showing a larger magnitude of repression than late roots. Transcripts in C2 were weakly induced in leaves while a majority of the transcripts were strongly repressed in roots at the early time point. These genes show an induction pattern in both leaves and roots at the later time point. C3 transcripts were weakly upregulated in early leaf responses and show mixed expression in roots at the same time point. In the late response to aphid feeding, leaf transcripts in C3 were strongly repressed while roots show a weak mixed transcriptional response. In the early response to aphid feeding, the genes that comprise C4 show repression in leaves but were weakly induced in roots. Later, these genes were repressed in both leaves and roots although to a higher degree in the locally infested leaf tissue. Genes in C5 were strongly repressed in early leaf responses and a majority shows some degree of repression in roots. After 7 days of aphid feeding, these genes were strongly induced in leaves and weakly in roots, indicating that over time, both above- and belowground tissues shift expression of these transcripts from repression early to induction late. Finally, leaves initially respond to aphid feeding by repressing the genes in C6 whereas a majority of these genes were weakly induced in roots. Later, leaves intensify the repression of these genes while expression remains weakly induced in roots.

To elucidate the biological function of the groups of genes identified in the clusters, we conducted gene ontology (GO) analysis to identify significantly
overrepresented (P<0.05) biological processes within each cluster. Terms that had more than 100 genes are presented in Fig. 3 and the full list of significant terms can be found in Supplementary File 4. Clusters C1-C6 contained 69, 10, 24, 23, 27, and 33 significant GO terms, respectively, with C1, C2, and C5 having similar biological processes and C3, C4, and C6 having similar processes. GO analysis of transcripts in C1, C2, and C5 indicates these genes are largely associated with defense (31 terms among C1, C2, and C5) and stress (14 terms) responses, phytohormone-related processes (17 terms including jasmonic acid, ethylene, salicylic acid, and abscisic acid), and signal transduction (11 terms). Coupled with the expression pattern of these transcripts, this suggests that in leaves, defense and stress responses, probably mediated by phytohormones, were mixed or repressed early whereas these processes were strongly induced late. In roots, our data showed strong repression of defense-, stress- and, phytohormone-related transcripts early and weak mixed expression of these transcripts late. Additional overrepresented GO terms in C1 and C5 include transcripts involved in cell wall metabolism (3 terms) and growth processes (6 terms). Photosynthesis-related processes (3 terms) were also overrepresented in C2.

GO enrichment analysis of C3, C4, and C6 revealed overrepresentation of transcripts involved in growth processes such as organ formation or morphogenesis (16 terms among C3, C4, and C6), photosynthesis and chloroplast-related metabolism (29 terms), sugar and starch metabolism (9 terms), cell wall metabolism (6 terms), cell membrane-related processes (6 terms), and RNA metabolism (7 terms). Additional overrepresented GO terms in C3 included ammonium transport (2 terms) and response to abscisic acid stimulus (GO:0009737). When coupled with expression patterns of
transcripts in these clusters, early leaf responses show induction of these transcripts in C3 but repression of transcripts in C4, C6 while in late leaf responses, transcripts in all clusters were strongly repressed. These results suggest that while some transcripts associated with these GO terms were induced early, aphid feeding generally causes the repression of growth-related processes at the 7 day time point.

**Transcription factor analysis**

To identify transcription factors that play a role in regulating responses to foliar soybean aphid feeding, we utilized the SoyDB transcription factor database (Wang *et al.*, 2010). A total of 1447 unique transcription factors belonging to 53 different families were differentially expressed across all tissues and times (Supplementary File 5). Not surprisingly, the expression pattern of transcription factors was generally similar to the overall expression pattern for each cluster in the hierarchical clustering heat map. L7D had the most differential regulation of transcription factors both in terms of magnitude and variety while R7D had the least. By plotting the transcription factors from each time and tissue (Fig. 4), we identified transcription factor families that were important in the response to soybean aphids. The top 10 transcription factor families with the most DE genes included AP2-EREBP, WRKY, NAC, GRAS, C2H2(Zn), TPR, PHD, MYB/HD-like, bHLH, and Homeodomain families. Expression of these and several other transcription factor families drastically varied with time and even between aboveground and belowground tissues within a given time point.

To relate the transcription factor families to the gene sets they may regulate, we analyzed the transcription factors by cluster (for the distribution of transcription factors within clusters, see Supplementary File 5) and found that several of the transcription
factors identified in the clusters are related to the biological processes assigned to the cluster. As previously discussed, GO analysis revealed genes in C1, C2, and C5 were associated with phytohormone signaling as well as defense and stress responses. Analysis of the transcription factors in these clusters revealed families known to regulate abiotic and biotic stress responses such as the AP2-EREBP, WRKY, ZIM, and GRAS families [Reviewed in (Howe & Jander, 2008; Rushton et al., 2010; Sun et al., 2012; Licausi et al., 2013; Nuruzzaman et al., 2013; Chini et al., 2016; Singh et al., 2016)]. Within these clusters during the early time point, members of the AP2-EREBP family were mostly repressed in both tissues with roots having greater number of representatives than leaves. In roots, 29 AP2-EREBP transcription factors were repressed and several of these contain a ERF-associated amphiphilic repression (EAR) motif which function as transcriptional repressors of ethylene responses (Licausi et al., 2013). During the late response to aphid feeding, 71 AP2-EREBP family transcription factors from each of the CBF/DREB, ERF, AP2, and RAV subfamilies were highly upregulated whereas this family had few representatives in the late root response.

The WRKY transcription factor family displayed mixed expression in the early leaf response but 26 members were repressed in roots. Furthermore, 72 members were induced in late leaf responses but this family was not differentially regulated in the late root response. WRKY-domain proteins are important in positively or negatively regulating basal defenses in response to aphids (Bhattarai et al., 2010; Kloth et al., 2016), nematodes (Grunewald et al., 2008; Bhattarai et al., 2010; Ali et al., 2014), pathogens (Dong et al., 2003), and tolerance to abiotic stresses (Song et al., 2016; Yan et al., 2016).
Few transcripts encoding soybean homologs of Jasmonate ZIM-domain (JAZ) proteins were repressed in leaf and root tissues during the early susceptible response to foliar aphid feeding. However, after seven days of aphid feeding, 17 were induced in leaves ranging from 1.4- to 129-fold in aphid-treated plants compared to mock plants. These proteins play a crucial role in repression of JA-activated transcription (Shyu et al., 2012; Chini et al., 2016). JAZ proteins interact with GRAS-family transcription factors to regulate balances between growth and defense (Pieterse et al., 2014). Interestingly, transcription factors in the GRAS family exhibited similar regulation to the JAZ family in that few repressed homologs were represented in the early interaction whereas several members were induced during the late time point. Of the induced GRAS transcription factors, there were 34 soybean homologs of SCARECROW-LIKE (AtSCL1, AtSCL14), AtSCL13 and AtPAT1, as well as DELLA-encoding genes (AtGAI), which are involved in stress and defense responses, light signaling, and repression of gibberellin (GA)-signaling, respectively [Reviewed in (Sun et al., 2012)]. Homologs of several positive regulators of GA responses (AtSCL3) and some involved in shoot meristem maintenance (AtHAM3) (Sun et al., 2012) were repressed (primarily Cluster 3). Neither JAZ nor GRAS families were differentially regulated in the late root response. Of interesting note, GmSCOF-1 (Glyma.17G236200) is repressed in early roots and upregulated in the late leaf response. This gene is involved in cold and ABA responses (Kim et al., 2001), negatively regulates JA-mediated responses, and is necessary for successful aphid colonization (See Chapter 2).

As previously discussed, GO analysis revealed genes in C3, C4, and C6 were primarily associated with growth, development, and photosynthesis. Transcription factor
analysis for these three clusters revealed families known to regulate these and other processes such as GRAS, C_{2}C_{2}(Zn) CONSTANS-like, MYB/Homeodomain (HD)-like and several other subfamilies of HD-containing transcription factors, (Carre & Kim, 2002; Mukherjee et al., 2009; Bou-Torrent et al., 2012; Sun et al., 2012; Ambawat et al., 2013; Belamkar et al., 2014; Simon et al., 2015; So et al., 2015). Within these clusters, two soybean homologs of \textit{AtCOL4} in the C_{2}C_{2}(Zn) CONSTANS-like transcription factor family were slightly upregulated in leaves at the early time point but 19 members were repressed in late leaves. Neither early nor late roots had differential regulation of this transcription factor family which is involved in photoperiodic flowering response and circadian regulation (Simon et al., 2015). The HD and multifunctional MYB/HD-like families had mixed expression in the early leaf response while several members (HD, 49 members; MYB/HD-like, 77 members) were repressed in late leaves. No regulation of these families was seen in the early root response but in the late roots, some transcription factors were differentially regulated. Of the 18 differentially expressed transcription factors throughout the 6 expression clusters in the overall R7D response, 9 belonged to these HD and MYB/HD-like transcription factor families and included two repressed soybean homologs of \textit{AtMYB79}, four induced homologs of \textit{AtLHY1}, one induced homolog of \textit{AtRVE1}, and two induced homologs \textit{AtHB40} which are regulated by auxin and/or circadian rhythm (Carre & Kim, 2002; Son et al., 2005; Rawat et al., 2009). Additionally, one soybean homolog of \textit{AtCIB1}, was repressed more than 10-fold. \textit{AtCIB1} positively regulates \textit{FLOWERING TIME} mRNA expression and promotes floral initiation and acts as a node in plant growth-defense tradeoff by partially negatively regulating basal defense responses (Liu et al., 2008; Malinovsky, F. G. \textit{et al.}, 2014).
Taken together, our results suggest that in leaves, defense- and stress-related transcription factors have mixed regulation at early time points but are greatly induced at late time points whereas transcription factors regulating growth have mixed expression early while they are strongly repressed in late leaf responses. In early root responses, transcriptional regulation of defense is overwhelmingly repressed. Additionally, little regulation at the transcriptional level occurs in late roots.

**Transcription factor binding site analysis**

To understand regulatory components controlling the expression of DE genes in our dataset, we analyzed the promoter region of the genes within each cluster to identify significantly (t<0.01) over-represented transcription factor binding sites using Clover (cis element overrepresentation) (Frith *et al*., 2004) and the JASPAR transcription factor binding database (Mathelier *et al*., 2016). We found significant overrepresentation of 131 unique transcription factor binding motifs distributed across the 6 expression clusters. The motif descriptions and number of gene promoters containing the significant motifs within each cluster can be found in Supplementary File 6. In C1-6, we found 111, 95, 30, 9, 80, and 16 significantly overrepresented TF binding motifs, respectively. We compared the transcriptional binding motifs between all six clusters and found that while several motifs were unique to a single cluster, the majority of motifs were shared between clusters (Supplementary Fig. 1). Specifically, several motifs were shared between C1, C2, and C5. As discussed previously, genes in these clusters are involved in defense and stress responses. Accordingly, promoters of DE transcripts in these clusters have overrepresentation of binding sites for a number of WRKY (13 motifs), AP2-EREBP (9 motifs), and MYC (3 motifs) transcription factors which are involved in innate immunity.
and stress responses (Rushton et al., 2010; Kazan & Manners, 2013; Licausi et al., 2013). Conversely, clusters C3, C4, and C6, which have gene ontology terms associated with growth and morphology, photosynthesis, and cell wall metabolism had very little overlap between the clusters.

**Functional analysis**

Recently, evidence of plant-mediated interactions between soybean aphid presence and soybean cyst nematode (SCN) performance was found in field and greenhouse studies. McCarville et al. (2012) reported that simultaneous infection of soybean aphids and *Phialophora gregata*, the causal fungal agent of brown stem rot (BSR) increased SCN performance on SCN-resistant (containing *Resistance to Heterodera glycines* (Rhg) genes) or SCN-susceptible plants (rhg) more than 5 times compared to uninfested plants. Additional experiments using only soybean aphids and SCN confirmed that aphid presence affected nematode performance. McCarville et al. (2014) reported a 33% increase in number of SCN females and eggs on SCN-resistant cultivars after one SCN generation. Conversely, they reported that soybean aphid presence negatively affected the number of SCN females and eggs on SCN-susceptible cultivars. The authors suggested aphid presence improves the host quality for SCN at low but detectable pest densities but at high herbivore population densities, competition between herbivores becomes a factor and the facilitation of SCN performance by soybean aphids was counteracted by a decrease in host resources (Soler et al., 2013; McCarville et al., 2014).

To identify potential molecular mechanisms mediating the soybean aphid-SCN interaction, we selected potential candidate genes from our 7 day dataset based on
expression pattern and the literature. A potential candidate gene involved in defense
responses to nematodes was Glyma.01G046900 and was repressed 2050-fold in leaves
and 46-fold in roots. This gene encodes a TIR-NBS-LRR protein that was constitutively
upregulated in SCN-resistant varieties compared to a susceptible variety (Wan et al.,
2015). Another set of candidate genes common between leaves and roots were six
repressed transcripts Glyma.15G026500, Glyma.04G105500, Glyma.04G105900,
Glyma.05G098600, Glyma.14G173500, and Glyma.16G096200, which encode soybean
homologs of Arabidopsis 9-lipoxygenase (9-LOX) AtLOX1 (At1g55020). 9-LOXs
catalyze the incorporation of molecular oxygen into position 9 of free linoleic (18:2) and
linolenic (18:3) fatty acids (Porta & Rocha-Sosa, 2002). In maize, ZmLOX3 9-LOX was
involved in phenylpropanoid-mediated defense responses against Meloidogyne incognita
root knot nematodes (RKN) (Gao et al., 2008). Roots of the lox3-4 loss-of-function
mutants displayed increased attraction to RKN and an increased number of juveniles and
eggs. Of these six soybean genes, Glyma.15G026500 encodes a full length 9-LOX
according to the V2 genome assembly (Schmutz et al., 2010). Using transgenic hairy
roots, knock-down lines were generated to mimic root repression of these genes after
foliar aphid treatment. We hypothesized that knocking down these genes would lead to an
increase in nematode performance if their function was related to defense against SCN.
Compared to empty vector controls, both Glyma.15G026500-RNAi roots and
Glyma.01G046900-RNAi roots had significantly lower transcript levels of
Glyma.15G026500 and Glyma.01G046900, respectively (P<0.05; Fig. 5A,B). However,
the number of mature female cyst nematodes showed a small decrease in both
Glyma.01G046900-RNAi (P<0.05) and Glyma.15G026500-RNAi (P=0.085) roots (Fig. 5C).

**Discussion**

Aphid feeding triggers extensive transcriptional and metabolic reprogramming of local responses in several plant species [reviewed in (Thompson & Goggin, 2006; Giordanengo et al., 2010; Morkunas et al., 2011; Heidel-Fischer et al., 2014; Jaouannet et al., 2014; Foyer et al., 2015)]. More recently, a few studies have focused on molecular changes triggered by phloem-feeding insects in uninfested systemic leaf, stem, or root tissues. We conducted a transcriptome analysis of soybean leaf (local) and root (systemic) tissues to understand changes caused by early (12 hours) or late (7 days) soybean aphid feeding on a whole-plant level during a compatible interaction. To our knowledge, this is the first genome-wide transcriptome report of foliar aphid feeding on root transcriptome changes.

Our results revealed that different tissues had distinct responses to soybean aphid feeding. Within a given time point there was little overlap in DE transcripts between local and systemic tissues. Likewise, in *Arabidopsis* and *Solanum stoloniferum*, feeding by *Myzus persicae* or *Macrosiphum euphorbiae* aphids triggered vastly different expression profiles in locally infested leaves versus systemic uninfested leaves (Alvarez et al., 2013; Kerchev et al., 2013), suggesting gene expression patterns are likely tissue-specific. Furthermore, we found that the soybean response to aphids was highly variable over time. In leaves, the number and magnitude of expression changes increased greatly as the infestation persisted (nearly 11 times more DE genes were found 7 days after infestation than at 12 hours). There was also a large set of overlapping genes between these datasets.
but direction of expression was opposite for many of the genes. Many studies conducted on compatible local responses to aphid feeding reported that longer infestations lead to higher transcriptional changes (De Vos et al., 2005; Couldridge et al., 2007; Kuśnierzcyk et al., 2008; Kerchev et al., 2013; Studham & MacIntosh, 2013), perhaps due to the delayed perception of insect infestation and subsequent deployment of defense responses compared to an incompatible response, which typically occurs more quickly (Li et al., 2008; Studham & MacIntosh, 2013; Foyer et al., 2015). Conversely, in roots, most transcriptional changes were detected early and tapered off over time (over 5 times more DE genes were found in roots at 12 hours compared to 7 days) and very few DE transcripts were common in roots between the early and late infestation. Similar to our results, a greater response was seen earlier in systemic leaves after Myzus persicae feeding in Arabidopsis compared to a later response (Kerchev et al., 2013). Also, the DE genes in locally infested tissues exhibited a greater amount of temporal overlap than DE genes in systemic tissues (Kerchev et al., 2013). Taken together, these results suggest that aphids induce highly dynamic plant responses across both time and tissue.

**Early whole-plant response to aphid feeding**

In the early response (12 hours) to foliar soybean aphid feeding, a majority of DE genes were repressed in local and systemic tissues. In leaves, many transcripts involved in cell wall biogenesis or modification processes were differentially expressed. Many transcripts were downregulated, including xyloglucan endotransglucosylases/hydrolases (XTHs), expansins, and pectin-lyases while others are upregulated including pectinmethylesterases, cellulose synthases, fasciclin-like arabinogalactan-proteins and laccases. The differential regulation of cell wall metabolism appears to be a common
phenomenon in response to aphid feeding, as transcripts encoding these and other cell wall modifying proteins were differentially regulated in both local and systemic tissues in many plants (Moran et al., 2002; Voelckel et al., 2004; Divol et al., 2005; Giordanengo et al., 2010; Kerchev et al., 2013). Induction of genes involved in cell wall modification may act to reinforce extracellular barriers at the site of pest or pathogen penetration or trigger the release of cell wall fragments that can act as signaling molecules in defense responses (Vorwerk et al., 2004; Malinovsky, F.G. et al., 2014; Foyer et al., 2015).

Myzus persicae preferred mutants of XTH33 over wild type Arabidopsis plants suggesting the involvement of XTHs in defense responses against aphids (Divol et al., 2007). Thus, the soybean aphid-triggered repression of XTHs may be related suppression of cell wall reinforcement that could facilitate stylet progression and feeding, thus enhancing aphid performance. Alternatively, the modifications may be induced in order to strengthen cell wall barriers against aphids. Several stress and defense transcripts were differentially expressed in locally infested leaves. Six WRKY transcription factors and a few pathogenesis-related (PR) genes had mixed expression patterns whereas a JAZ transcriptional repressors as well as many leucine-rich receptor kinases and NBS-LRR genes were repressed in leaves. Taken together, these results suggest that defense responses in locally infested leaves had mixed expression.

Interestingly, foliar aphid feeding caused transient repression of a broad range of defense responses in systemic root tissues including a number of SA- and JA- responsive genes and many WRKY transcription factors. The SA pathway plays an important role in plant defenses against nematodes [reviewed in (Li, RJ et al., 2015)]. In tomato, SA was required for Mi-1.2-mediated resistance to potato aphids (Martinez de Ilarduya et al.,
2003; Cooper *et al.*, 2004; Li *et al.*, 2006). In *Arabidopsis*, the SA pathway was important for defense against beet cyst nematodes (*Heterodera schachtii*) (Wubben *et al.*, 2008). In soybean, SA pathway genes were induced in the resistant response to SCN but suppressed in susceptible plants (Kandoth *et al.*, 2011; Mazarei *et al.*, 2011). Overexpression of soybean salicylic acid methyltransferase or *Arabidopsis* genes involved in SA signaling such as *AtPAD4, AtNPR1, AtTGA2*, or *AtPR-5* in soybean led to increased resistance of plants to SCN compared to control plants. (Lin *et al.*, 2013; Youssef *et al.*, 2013; Matthews *et al.*, 2014; Lin *et al.*, 2016) This highlights the importance of the SA pathway in the soybean defense response to SCN.

Additionally, a number of transcription factors from the WRKY family were repressed. In *Arabidopsis*, repression of WRKY transcription factors *AtWRKY6, AtWRKY11, AtWRKY17* and *AtWRKY33* by beet cyst nematodes (*Heterodera schachtii*) was important for nematode development, likely by interfering with defense signaling and biosynthesis of the phytoalexin camalexin (Ali *et al.*, 2014). We observed that the closest soybean homologs for these transcription factors, with exception of the soybean homolog for *AtWRKY17*, were repressed in roots in response to foliar soybean aphid feeding. Thus, the repression of the SA pathway and WRKY transcription factors triggered by aboveground soybean aphid feeding may increase host suitability for SCN and could explain facilitated SCN reproduction by soybean aphid presence (McCarville *et al.*, 2012; McCarville *et al.*, 2014). The identity or signature of the aphid-induced signaling molecule that triggers the widespread repression of root defense and stress transcripts is still unclear.
Late whole-plant response to aphid feeding

Locally infested leaf tissues produced a large transcriptional response to late soybean aphid feeding, as nearly 27% of the predicted protein-coding genes in soybean were differentially regulated. Overall, defense and stress responsive genes were highly upregulated while those involved in growth, morphogenesis, and photosynthesis processes were strongly repressed. This dichotomy indicates that in the late local response, resources are preferentially allocated to defense rather than growth and development processes. Induced defenses are often resource-costly and the tradeoff between growth and defense is prevalent in many systems [reviewed in (Vos et al., 2013)]. For example, in rice, expression of OsWRKY70 prioritized defense responses over growth via antagonism of gibberellin (GA) signaling (Li, R et al., 2015). Following this, soybean homologs corresponding to OsWRKY70 and DELLA proteins were highly upregulated in leaves by soybean aphid feeding while positive regulators of GA signaling were strongly repressed.

Several other transcription factors were differentially expressed in leaves including several members from the AP2-EREBP and WRKY families. Studham and MacIntosh (2013) also found several AP2-EREBP family transcription factors to be differentially regulated in the compatible soybean-soybean aphid interaction after 7 days of aphid feeding. Furthermore, in a systematic meta-analysis of plant transcriptome responses to phloem-feeding insects in Arabidopsis, these transcription factor families were overrepresented in several plant-phloem-feeder interactions (Foyer et al., 2015) suggesting some overall similar responses to phloem-feeding insects although specific transcription factors varied depending on the plant and aphid species. WRKY
transcription factors play roles in both positive and negative regulation of basal defense responses to pests and pathogens and often control or are controlled by hormonal signaling pathways (Dong et al., 2003; Rushton et al., 2010; Kloth et al., 2016).

In agreement with Studham and MacIntosh (2013), phytohormone signaling through SA, JA, ET, and ABA pathways was prominent in the leaf response. Crosstalk between these pathways modulates the quantity, composition, and timing of phytohormone signaling that are important in tailoring an effective defense response to a specific attacker (De Vos et al., 2005; Foyer et al., 2015). In the promoters of the upregulated defense- and stress-related genes (i.e. C1, C2, C5), we found significant overrepresentation of binding sites for transcription factors from WRKY and ERF families, suggesting some degree of family self-regulation. Indeed, many WRKY gene promoters contain the W-box sequence for binding of other WRKY family transcription factors, which often forming homo- or heterocomplexes with other WRKY transcription factors to regulate transcription of target genes with high specificity (Dong et al., 2003; Eulgem & Somssich, 2007). Thus, while the defense- and stress-related DE genes share several common regulatory motifs, these gene sets are likely regulated in a combinatorial manner which promotes the fine-tuning of phytohormone signaling and other plant responses to a specific attacker (Eulgem & Somssich, 2007; Deb & Kundu, 2015).

Some phloem-feeding insects exploit antagonistic crosstalk between the hormonal signaling pathways suppress effective defenses against the attacker [(Walling, 2008), see Chapter 2]. Interestingly, we found the upregulation of several jasmonate ZIM-domain proteins (JAZs) which play a crucial role in repression of JA-mediated defense responses (Chini et al., 2016). In the compatible interaction between soybean and soybean aphids,
JA-mediated defense signaling is effective against aphids but responses to the hormone are repressed or only slightly induced (Studham & MacIntosh, 2013; Selig et al., 2016). We previously reported that antagonism between the ABA and JA pathways is responsible for aphid-triggered attenuation of JA responses through the aphid- and ABA-induced GmSCOF-1 (see Chapter 2). Thus, the regulation of genes involved in the ABA-mediated signaling pathway including GmSCOF-1 and the strong induction of JAZ proteins in our dataset could point toward this hormone antagonism. In addition, the induction of WRKY transcription factors with putative positive and negative regulatory roles in basal defenses suggest that aphids may trigger competition between defense and susceptibility signaling in the late leaf response.

In contrast to leaf tissues, foliar feeding by aphids had a relatively small effect on the systemic root transcriptome and a majority of the DE genes were repressed. Nearly 40% of root responses overlap with the leaf response, while the overlap accounts for only 0.5% of the leaf response. However, the large disparity in response size and the fact that half of the overlapping transcripts are regulated in opposite directions between the two tissues suggests the root response is not merely a belowground extension of the leaf response. Plant response to herbivory can be relatively uniform throughout the plant or vastly different in terms of size and type of response between spatially separated plant parts and varies between the plant and phloem feeder as well as time after infection. For example, Park and Ryu (2014) saw similar induction of SA- and JA-related transcripts in leaves and roots of pepper (Capsicum annum L.) infested with whiteflies (Bemisia tabaci Genn.) whereas Kerchev et al. (2013) and Alvarez et al. (2013) found very little overlap between local and systemic leaf in response to Myzus persicae feeding on
Arabidopsis or Solanum stoloniferum, respectively. Furthermore, feeding by the specialist aphid Brevicoryne brassicae on Arabidopsis shoots changed levels of shoot aliphatic glucosinolate (GS) and several other metabolites but only caused a significant change in levels of three short-chain aliphatic GS in the systemic root tissue (Kutyniok & Muller, 2012).

Several soybean homologs of AtLOX1 were repressed in both leaves and roots. 9-lipoxygenases (9-LOX) catalyze the oxidation of free linolenic acid at position 9 to produce 9-oxylipins that are important in root development and defense responses (Gao et al., 2008). Nalam et al. (2012) demonstrated that Myzus persicae feeding on Arabidopsis shoots induced root expression of the 9-LOX AtLOX5 in Arabidopsis and that root-derived 9-oxylipins are important for promoting susceptibility to Myzus persicae on shoots. However, these same LOX5-derived oxylipins were also involved in inducing PAD4-mediated shoot defense responses against the insects suggesting a complex involvement of oxylipins in the M. persicae-Arabidopsis interaction (Nalam et al., 2013). Furthermore, in maize, ZmLOX3 encodes a 9-LOX involved in phenylpropanoid-mediated defense responses against Meloidogyne incognita root knot nematodes (Gao et al., 2008). The loss-of-function insertional mutant lox3-4 displayed increased nematode attraction and number of juveniles and eggs but had increased SA, JA, and ET content and signaling in roots. When we knocked down Glyma.15G026500 which encodes a soybean homolog of AtLOX1, population of female SCN slightly decreased, although not significantly, suggesting this 9-LOX is likely not involved in soybean defenses toward nematodes. Additionally, knockdown of Glyma.01G046900, a TIR-NBS-LRR structure protein constitutively upregulated in SCN-resistant varieties (Wan et al., 2015) had a
small negative impact on number of SCN females. Therefore, the observation that soybean aphid presence induces susceptibility to SCN (McCarville et al., 2012; McCarville et al., 2014) is likely not linked to the repression of these individual genes. Rather, the overall balance of differential gene expression and the confined local defense response at the later time point or more likely, the transient widespread repression of general defense responses seen at early time point may partially contribute to increased SCN reproduction. These results are consistent with the hypothesis from McCarville et al. (2014) that the increase in SCN female number caused by soybean aphids is likely due to a suppression of a broad-based, general plant defense to nematodes that is not mediated by the Resistance to Heterodera glycines (Rhg) genes, as populations increased on both SCN-resistant (Rhg) and SCN-susceptible (rhg) cultivars in the field (McCarville et al., 2012). Alternatively, increased host quality via improvement of root nutrient content may be responsible for increased SCN females, but this was not evaluated in our study. Johnson et al. (2009) suggested that increase in mass of barley root-feeding wireworms was potentially due to the bird cherry-oat aphid (Rhopalosiphum padi)-induced increase in root mineral concentrations. Thus, further studies on plant nutrient status could be conducted to further elucidate the effect of soybean aphid effects on roots.

**Conclusions**

This study is the first to quantify whole-plant molecular changes triggered by soybean aphids. Our results suggest that different tissues (local and systemic) respond distinctly to foliar soybean aphid feeding and the response is highly dynamic across time. The early whole-plant response consists of mixed expression of defenses in leaves with the repression of wide-ranging root defenses. The latter is consistent with the beneficial
impact of soybean aphids on soybean cyst nematode development (McCarville et al., 2012; McCarville et al., 2014). A number of researchers reported induced defenses in systemic tissues after exposure to phloem feeding insects in other plant species (Martinez de Ilarduya et al., 2003; Voelckel et al., 2004; Divol et al., 2005; Dugravot et al., 2007; Yang et al., 2011; Lee et al., 2012; Kerchev et al., 2013; Park & Ryu, 2014; Kim et al., 2016). The induction of defenses is likely a mechanism to increase host resistance against subsequent pest attack. However, we show that instead, soybean aphids suppress systemic defenses in previously colonized plants and likely do not trigger the induction of many systemic defenses, as evidenced by the relatively small transcriptome at the late time point. Additionally, the late induction of defense responses found in our study supports other reports that show deferred defense responses toward aphids in susceptible plants, possibly due to delayed perception of insect infestation and the ensuing deployment of defense responses.

Materials and Methods

Plant material and growth conditions

Aphid-susceptible soybean (Glycine max (L.) Merr.) plants cv. IA3027 (developed at Iowa State University) were grown in growth chambers set at a constant temperature of 25°C with a 16:8 light:dark photoperiod. Seeds were sterilized overnight using chlorine gas as previously described (Paz et al., 2006). In each pot, three seeds were planted in steam sterilized Metro-Mix® 900 soil (Sun Gro Horticulture, Vancouver, BC, Canada) and after one week, seedlings were thinned to one per pot. For the duration of the experiment, plants were watered twice weekly and additionally fertilized once per week with a 1:1 mixture of 6% All-Purpose Scott’s Miracle-Gro Excel (21-5-20, The
Scott’s Company LLC, Marysville, Ohio, USA) and 6% Cal-Mag Miracle-Gro Professional (15-5-15, The Scott’s Co.) applied at a rate of 12.5 mL L⁻¹ water. Five chambers were used in the experiment; chamber environments were monitored using Track-It™ temperature and humidity data loggers (Monarch Instruments, Amherst, NH, USA) to ensure similar environments. Average PAR was measured in each chamber using a LightScout Solar/Electric Quantum Meter (Spectrum Technologies, Inc., Bridgend, Wales, UK) and light levels were adjusted to an overall average of 375 µmol m⁻² s⁻¹. Plants used in these experiments were at the V3 growth stage (Fehr & Caviness, 1977).

Insect material and aphid infestation

Soybean aphids (*Aphis glycines* Matsumura (Hemiptera: Aphididae); biotype 1) were obtained from a laboratory colony maintained on aphid-susceptible IA3027 plants at Iowa State University. Experimental plants were infested by transferring 30 mixed-age apterous aphids to the abaxial side of the center V3 leaflet using a small paintbrush. To prevent movement, aphids were confined using clip cages (BioQuip products, Rancho Dominguez, CA, USA). Clip cages were fastened on both experimental and mock plants to mimic any environmental changes caused by the cage. Aphids were allowed to feed and reproduce for 12 hours or 7 days.

Experimental design and tissue collection

Five chambers were randomly assigned to be “mock” or “aphid” treatments to eliminate potential priming effects (Studham & MacIntosh, 2013). Three chambers were used for aphid treatment and two chambers were used for mock treatment. Within chambers, treatments were set up in a completely randomized design and each treatment
consisted of 9 biological replicates (due to space limitations, one of the two mock chambers consisted of 18 biological replicates for each treatment). In total, there were 36 aphid-susceptible plants. Leaf and root samples were collected after 12 hours and 7 days of aphid feeding. During collection, aphids were gently brushed off of the leaflet and mock leaves were also brushed to mimic any mechanical stimulus caused by removing aphids. Roots were collected by gently loosening soil, washed twice in water, and were patted dry with paper towels. Roots were trimmed and the sample contained approximately the lower half of the entire root system. Individual leaflet and root samples were collected into foil packets and immediately flash frozen in liquid nitrogen. All samples were stored in -80°C until processed.

**RNA isolation and RNA-seq**

Each individual sample was ground in liquid nitrogen using a mortar and pestle. RNA was extracted using Qiagen® RNeasy® Plant Mini Kit (Qiagen®, Hilden, Germany). The manufacturer’s protocol was followed with modifications. For roots, ~300 mg ground tissue was used. Additionally, all leaf and root samples were incubated at 56°C for two minutes with intermittent vortexing to aid in tissue disruption. Lastly for leaf samples, at least three rounds of RPE buffer washes were used instead of two. Genomic DNA was degraded using Ambion® Turbo DNA-free™ kit (Ambion®, Austin, TX) and samples were cleaned using Qiagen® RNeasy® MinElute™ Column (Qiagen®, Hilden, Germany), according to the manufacturer’s instructions. RNA integrity was measured on each sample using the Agilent® 2100 Bioanalyzer™ (Agilent®, Santa Clara, CA, USA). RIN scores above 6.8 were considered high quality and were used for further analysis. Equal amounts of RNA from three plants within same treatment and chamber were
pooled and the concentration was measured using a NanoDrop™ ND-1000 Spectrophotometer (Thermo Fisher Scientific®, Waltham, MA, USA). A total of 24 samples (2 aphid levels: mock and aphid x 2 time levels: 12 hour and 7 day x 2 tissue levels: leaflet and root x 3 pooled replicates) were submitted to the Iowa State University DNA facility for multiplex library preparation and subsequent single-end sequencing using Illumina HiSeq2500 high output mode with a read length of 100 base pairs (bp).

**Bioinformatic and statistical analyses**

In total, 24 libraries were sequenced, 12 from leaf samples and 12 from root samples. The resulting 100bp RNA-sequencing reads were trimmed using Scythe (https://github.com/vsbuffalo/scythe), FastX trimmer (http://hannonlab.cshl.edu/fastx_toolkit/), and Sickle (https://github.com/najoshi/sickle) to remove sequencing adaptors, sequencing artifacts, and short or low quality sequences, respectively. Read alignment to the *Glycine max* Williams 82 reference genome version 2 (Wm82.a2.v2, available at phytozome.net) (Schmutz et al., 2010) was done using default settings in TopHat version 2.0.3 (Trapnell et al., 2009). Samtools (Li et al., 2009) was used to eliminate reads that unreliably mapped to the reference genome. The Bioconductor package Rsamtools (Morgan et al., 2016) was used to import the resulting mapping BAM files into the statistical program R (https://www.r-project.org/). The package rtracklayer (Lawrence et al., 2009) was used to import gene features corresponding to *G. max* version 2 (Schmutz et al., 2010) and GenomicAlignments (Lawrence et al., 2013) was used to count the number of reads aligning to a specific gene and generate a matrix containing gene counts for each sample. Genes with log counts per million (cpm) > 1 in at least two replicates were used in further analyses. The
Bioconductor package edgeR (Robinson et al., 2010) was used to carry out data normalization by tissue type using the Trimmed Mean of M (TMM) values method (Robinson & Oshlack, 2010). To compare sample replicates, the R graphics program ggplot2 (Wickham, 2009) was used to generate principal component plots and biological coefficient of variance plots. Two 12 hour root samples (one mock and one aphid) were eliminated from further analysis because the samples were drastically dissimilar from other samples of the same tissue type; separate analyses with or without these samples verified their inclusion affected the tagwise dispersion estimate. All 12 leaf samples were analyzed using edgeR to identify differentially expressed (DE) genes and for roots, 10 samples were used. Genes were considered significant with a false discovery rate (FDR) <0.05.

Hierarchical clustering heat maps

To visualize global trends in gene expression and identify groups of genes with similar expression patterns in response to foliar soybean aphid feeding, hierarchical clustering analysis based on DE gene expression from each time and tissue combination was performed using log cpm for each sample. The R hclust function was used with the default complete linkage method to generate dendograms of DE genes. Clustering was based on z-score and the clustering order was used to produce heatmaps based on fold change data.

Annotation and analysis of DE genes

Annotations for the DE genes were obtained using the SoyBase (Grant et al., 2010) Genome Annotation tool (www.soybase.org/genomeannotation/). Gene identity and function were ascertained from the UniRef100 (Apweiler et al., 2004) hit, the best
Arabidopsis thaliana homolog hit, and gene ontology (GO) information (The Arabidopsis Information Resource [TAIR] version 10, www.arabidopsis.org). Using Fisher’s Exact Test (Fisher, 1960) with Bonferroni multiple comparison correction (Bonferroni, 1935), we identified significantly (P-value <0.05) overrepresented biological process GO terms within each cluster.

Identification of DE transcription factors

To identify DE transcription factors that play a role in regulating responses to foliar soybean aphid feeding, the SoyDB transcription factor database (Wang et al., 2010) was used. Gene identifiers in the database were converted to the Williams 82 version 2 genome assembly and annotation (Schmutz et al., 2010) using the Soybase (Grant et al., 2010) gene identifier conversion tool (https://soybase.org/correspondence/).

Identification of overrepresented transcription factor binding sites in DE gene promoters

To understand regulatory factors controlling the expression of DE genes in our dataset, the promoter sequence of the genes within each cluster was analyzed to identify significantly (t<0.01) over-represented transcription factor binding sites using Clover (cis element overrepresentation) (Frith et al., 2004) and the JASPAR transcription factor binding database (Mathelier et al., 2016). Promoter size was limited to 500 bases upstream of the start methionine and if a promoter contained gaps, had ambiguous bases, or were less than 500 bases in length they were excluded from further analysis.

Functional analysis

Directional sense and antisense fragments of Glyma.15G026500 (~560bp) or Glyma.01G046900 (~670bp) were generated using primers listed in Supplementary File 7. PCR products were digested with AscI and SwaI (Thermo Fisher Scientific) for the
sense fragment and AvrII and BamHI (Thermo Fisher Scientific) for the antisense fragment and cloned into the respective sites of pG2RNAi2 (GenBank: KT954097). After sequences were confirmed, transgenic hairy roots were generated and nematode infection assays were performed as in (Noon et al., 2016). RNA from uninfected roots was extracted and DNA digestions were performed as described above. One microgram of cDNA was synthesized using qScript™ Flex cDNA Synthesis Kit (Quanta Biosciences, Beverly, MA, USA) using Oligo dT primers. Relative quantitative PCR (qRT-PCR) was done using PerfeCTa® SYBR® Green FastMix®, Low ROX (Quanta Biosciences Beverly, MA, USA) using the Mx4000 (Stratagene, Agilent, Technologies, Santa Clara, CA, USA). Cycle threshold values were quantified according to the standard curve method (Applied Biosystems), normalized to internal control gene Glyma20g27950 and log transformed. Primers used in qRT-PCR can be found in Supplemental table Y. Statistical significance of nematode numbers and log transformed gene expression was determined using Student’s t-test in Microsoft Excel.

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Author Contribution Statement

GCM, AKS, and JDH conceived the study. JDH, MIN, and TRM conducted the experiments. MAG, JDH, and GCM analyzed all data. JDH wrote the manuscript. These
experiments were funded in part by grants from the Iowa Soybean Association to GCM, AKS, and TJB.

References


Baluška F. 2013. Long-Distance Systemic Signaling and Communication in Plants: Springer-Verlag Berlin Heidelberg.


Bhattarai KK, Atamian HS, Kaloshian I, Eulgem T. 2010. WRKY72-type transcription factors contribute to basal immunity in tomato and Arabidopsis as well as gene-for-gene resistance mediated by the tomato R gene Mi-1. *Plant J* 63.


**Fehr WR, Caviness CE. 1977.** Stages of soybean development. *Iowa Agricultural and Home Economics Experiment Station Special Report* (80): 3-11.


Kanobe C. 2012. Fatty acid changes in soybean (Glycine max) under soybean aphid (Aphis glycines) infestation and their implications on plant defense against insects. *Graduate Theses and Dissertations Paper* 12840.


Tables

Table 1. Number and direction of differentially expressed genes triggered by soybean aphid feeding (FDR<0.05)

<table>
<thead>
<tr>
<th>Tissue+Time</th>
<th>Total DEGs</th>
<th>Induced (% of total)</th>
<th>Repressed (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L12H</td>
<td>1,130</td>
<td>237 (21%)</td>
<td>893 (79%)</td>
</tr>
<tr>
<td>R12H</td>
<td>852</td>
<td>19 (2.2%)</td>
<td>833 (97.8%)</td>
</tr>
<tr>
<td>L7D</td>
<td>12,378</td>
<td>6784 (54.8%)</td>
<td>5594 (45.2%)</td>
</tr>
<tr>
<td>R7D</td>
<td>158</td>
<td>47 (29.7%)</td>
<td>111 (70.3%)</td>
</tr>
</tbody>
</table>
Figures 1. Response to foliar soybean aphid feeding is dynamic. Significant (FDR<0.05) DE genes within each time + tissue combination were compared across tissue and time using the VENNY tool http://bioinfogp.cnb.csic.es/tools/venny/index.html. DE genes responding to soybean aphid feeding are listed in Supplementary File 1.
Figure 2. Fold Change hierarchical clustering of gene expression responding to soybean aphid feeding. (A) The hierarchical clustering heat map was generated using all 13,080 significant (FDR<0.05) unique differentially expressed genes responding to foliar soybean aphid feeding in different tissues and across time. Hclust was used to cluster genes with similar expression patterns based on the Z-score. Fold change was calculated for each gene in each time + tissue combination and was superimposed onto the heat map. The columns differentiate the responses...
Figure 2, continued. of each tissue (local leaf and systemic root) at each time point (12 hours after aphid infestation, 7 days after aphid infestation). The rows group genes by similar expression pattern, resulting in six expression clusters (colored bars numbered 1-6). Within the heat map, yellow indicates a gene was induced whereas blue indicates a gene was repressed; intensity of the color corresponds with weaker or stronger expression fold change. DE genes responding to soybean aphid feeding are listed in Supplementary File 1. (B) The number of significant DE genes within each cluster for each time and tissue combination.
Figure 3. Gene ontology associated with response to soybean aphid feeding. Subset of significantly (P-value <0.05) overrepresented gene ontology (GO) biological process terms within the DE genes responding foliar soybean aphid feeding were identified within each of the six treatment clusters. GO biological processes with
**Figure 3. continued:** more than 100 genes are shown. Number of genes within each cluster are given. Text within parentheses indicates the cluster in which the GO term was significant. A complete list of GO terms are listed in Supplementary File 4. Resp, response; Reg, regulation; JA, jasmonic acid; SAR, systemic acquired resistance; SA, salicylic acid; ABA, abscisic acid; ER, endoplasmic reticulum; ET, ethylene; isopent. diphos. biosynt., mev-indep, isopentenyl diphosphate biosynthetic process, mevalonate-independent pathway.
Figure 4. Transcription factor expression patterns of the response to foliar soybean aphid feeding. Transcription factors were identified in each of the six clusters. Expression of transcription factors was observed across genotypes and tissues in both leaves and roots in response to foliar soybean aphid feeding. Absolute fold change is plotted on the x-axis, transcription factor families are plotted on the y-axis. For visualization purposes, transcription factors with an absolute fold change greater than 19.9 were plotted as 19.9. The full list of transcription factors is presented in Supplementary File 5.
Figure 5. Nematode performance is altered in transgenic knockdown hairy roots. Results show compiled data for two independent experiments. Asterisk indicates significance from control at P<0.05 using t-test: Two-Sample Assuming Unequal Variances in Excel. A. Expression of Glyma.01G046900 (TIR-NBS-LRR) and B. Glyma.15G026500 (9-LOX) in vector control and RNAi-knockdown lines. Relative expression was assayed using quantitative PCR and normalized to UBQ. Relative expression values were log transformed and value of 3 added to make all values positive. C. Nematodes performance on vector control and RNAi-knockdown root lines.
Supplementary materials

Supplementary Figure 1. Transcription factor binding site analysis reveals similarly regulated gene clusters. Significant (t<0.01) transcription factor binding motifs within each cluster were compared using the diagram (Bardou et al., 2014). The full list of significant motifs is presented in Supplementary File 6.

**Supplementary File 1.** 13,080 genes significantly differentially expressed in response to foliar soybean aphid feeding.

**Supplementary File 2.** 66 Differentially expressed genes overlapping between leaf and root tissues at 12 hours.

**Supplementary File 3.** 62 Differentially expressed genes overlapping between leaf and root tissues at 7 days.

**Supplementary File 4.** Gene Ontology (GO) terms significantly (P<0.05) overrepresented within clusters in response to foliar soybean aphid feeding.
**Supplementary File 5.** Differentially expressed transcription factors responding foliar soybean aphid feeding.

**Supplementary File 6.** Identification of overrepresented transcription factor binding sites within clusters.

**Supplementary File 7.** Primers used to generate RNAi constructs and quantify gene expression.
CHAPTER 4. GENERAL SUMMARY AND CONCLUSIONS

Soybean aphids are invasive insect pests that have the potential to cause major yield losses. Several studies have attempted to elucidate the mechanisms of resistance or tolerance to soybean aphids either using transcriptome data, genome-wide association studies or quantitative trait loci studies. However, very few studies have functionally characterized resistance mechanisms aside from (Kanobe, 2012) and Selig et al. (2016) who used exogenous hormone application to show that jasmonic acid (JA) or methyl jasmonate (MeJA)-mediated signaling decreased aphid populations in both resistant and susceptible soybean plants. Additionally, Selig et al. (2016) and Studham and MacIntosh (2013) exogenously applied salicylic acid (SA) and found this hormone to be important in the response to soybean aphids.

Previous studies showed the ability of soybean aphids to attenuate the expression of wound- and JA-inducible transcripts such as PinN2 and GH3 (Kanobe, 2012), and some evidence suggests that this suppression could be mediated in part by a reduction in JA biosynthesis due to aphid-induced changes in fatty acid levels (Kanobe et al., 2015). However, the mechanisms by which aphids block JA-signaling downstream of JA production that results in a reduction in effective JA-mediated defense remained uncharacterized. We tested the hypothesis that the induction of abscisic acid (ABA) biosynthesis and signaling by soybean aphids (Studham & MacIntosh, 2013) acts as a decoy response. Our study investigated hormone crosstalk in soybean. We found that soybean aphids induced ABA accumulation and that the hormone was capable of attenuating wound-induced expression of PinN2. In addition to this, we showed that aphid populations were stunted on ABA biosynthetic (aba2 RNAi) and signaling (scof-1
RNAi) plants suggesting a functional ABA pathway is required for successful aphid population growth. Biosynthetic knockdown plants also had higher basal JA (PinN2) and SA (PR1a) signaling, suggesting that endogenous ABA acts to suppress both JA and SA-mediated defense responses in soybean, although through different mechanisms, as ABA signaling (scof-1 RNAi) knockdown plants did not differ from vector controls in PR1a expression but ABA biosynthesis (aba2 RNAi) knockdown plants had significantly higher PR1a levels than vector control plants. Moreover, in the absence of a functional ABA biosynthetic or signaling pathway, aphids were no longer able to attenuate the wound-induced expression of PinN2 providing genetic evidence that soybean aphids exploit soybean ABA signaling to suppress defense responses.

Our research did not address the mechanism of ABA induction by aphids. As the removal of nutrients and water from the vasculature likely causes the induction of stress-related ABA-mediated signaling, the induction of ABA by soybean aphids may be related to a stress response. However, the induction of ABA responses was found to be partially independent of the physical withdrawal of plant sap in Arabidopsis as Myzus persicae saliva was able to induce ABA responses (Hillwig et al., 2016). Therefore, transcriptional profiling of plant response to soybean aphid saliva could be conducted to determine if ABA responses are actively induced and JA defenses repressed. If this is the case, biotechnological approaches could be used to generate plants resistant to these effector molecules once the effectors are identified and characterized. Additionally, the mechanism by which ABA suppresses JA responses in soybean is still unknown. In Arabidopsis, ABA positively regulates the MYC2 branch of JA signaling to activate expression of AtVSP1 while it antagonizes the JA-ethylene signaling pathway (marked
by PDF1.2 expression) (Anderson et al., 2004; Kazan & Manners, 2013). Since PinN2 transcripts can only be induced by JA or wounding in the presence of a functional ethylene pathway (Botella et al., 1996), it is plausible that aphids suppress the JA/ET branch in soybean via the antagonistic ABA-regulated MYC2 branch of JA signaling, although it is not known whether this avenue of hormone crosstalk is similar between Arabidopsis and soybean. The soybean homolog of AtMYC2 or the homolog of the associated marker gene AtVSP1 was not differentially expressed in the dataset reported by Studham and MacIntosh (2013). However, in the transcriptome of locally infested leaves presented in this research (Chapter 3), five soybean homologs of AtMYC2 (AT1G32640) as well as SCOF-1 were upregulated in the late leaf response. Additionally, several transcripts encoding jasmonate ZIM proteins (JAZ) were also highly upregulated in our late leaf (7 days) transcriptome results. JAZ proteins play a crucial role in repression of JA-activated transcription and can be positively or negatively regulated by ABA (Shyu et al., 2012; Chini et al., 2016).

Despite induction of ABA responses in the transcriptome study presented in this research, many genes associated with defense pathways were also upregulated in the late leaf response. This included transcripts associated with SA, JA, and ET pathways and defense-related transcription factor families such as AP2-EREBPs and WRKYs. This increase in defense transcripts was accompanied by the strong repression of plant growth, development, and photosynthesis genes, suggesting that late aphid infestation triggers a tradeoff between defense and growth responses. When our results are directly compared to the 7 day susceptible response reported by Studham and MacIntosh (2013), 66% of their reported genes were present in our dataset yet this overlap accounts for only 1.5% of
the local response in our dataset. Differences in results could be due to the use of different genotype, as IA3027 (the genotype used in our transcriptome study) was shown to possess mild antixenosis (Hanson et al., 2016) whereas this has not been shown for the genotype SD01-76R used by Studham and MacIntosh (2013). Differences in design (i.e. confined caged aphids in our study versus free-roaming aphids in Studham’s paper), quantification (i.e. RNA-seq in our study versus Studham’s microarray) or analysis method (i.e. FDR and fold-change cutoff restrictions) may underlie qualitative and quantitative differences between studies.

When examining the systemic response, we found that foliar feeding by soybean aphids triggered distinct responses in locally infested leaves versus systemic roots. The late (7d) root response to soybean aphid feeding was relatively small but included the repression of genes thought to be involved in defenses against nematodes including, 9-lipoxygenases (Gao et al., 2008) and a TIR-NBS-LRR gene (Wan et al., 2015). However, knocking down these genes had a slight negative effect on soybean cyst nematode performance, suggesting these genes may not be involved in cyst nematode resistance. Perhaps the most interesting result was that foliar aphid feeding transiently repressed a broad range of defense responses in systemic root tissues including a number of SA- and JA-responsive genes as well as many WRKY transcription factors. Our study is the first to report such repression of defense gene expression by phloem-feeders. Rather, a number of researchers reported induced defenses in systemic tissues after exposure to phloem feeding insects in other plant species, likely as a mechanism to increase host resistance against subsequent pest attack. While local suppression of defenses is mediated via ABA, this does not seem to be the case for systemic repression of defenses. In the
root response, ABA signaling components *GmSCOF*-1 and soybean homologs of *AtATAF1* and *AtMYC2* were repressed along with other ABA-responsive genes at the early time point (see Chapter 3). Thus, the mechanism of defense suppression is still currently unknown.

The repression of systemic defenses by soybean aphid feeding has important implications in plant-mediated pest interactions, specifically with parasitic soybean cyst nematodes (SCN). Previous reports showed that soybean aphids had a generally positive (McCarville *et al.*, 2012; McCarville *et al.*, 2014) effect on the number of SCN females and eggs unless pest populations were very high (McCarville *et al.*, 2014), when aphids negatively affected SCN numbers probably due to resource competition, or very low (less than 10 aphids per plant) (Heeren *et al.*, 2012) when aphids had no effect on SCN. The SA pathway plays a role in plant defenses against cyst nematodes [reviewed in (Li *et al.*, 2015)]. In *Arabidopsis*, the SA pathway was important for defense against beet cyst nematodes (*Heterodera schachtii*) (Wubben *et al.*, 2008). In soybean, several genes in the SA pathway were upregulated in the resistant response to SCN (Kandoth *et al.*, 2011; Mazarei *et al.*, 2011) Overexpression of soybean salicylic acid methyltransferase (Lin *et al.*, 2013; Lin *et al.*, 2016) or SA pathway genes from *Arabidopsis* such as *AtPAD4*, *AtNPR1*, *AtTGA2*, or *AtPR-5* (Youssef *et al.*, 2013; Matthews *et al.*, 2014) in soybean led to reduced susceptibility of plants to SCN compared to control plants. This highlights the importance of the SA pathway in the soybean defense response to SCN. Also, repression of WRKY transcription factors by *H. schachtii* was crucial for nematode development in *Arabidopsis*, likely by interfering with defense signaling and biosynthesis of the phytoalexins (Ali *et al.*, 2014). Thus, the transient repression of the SA pathway or
WRKY transcription factors could contribute toward aphid-induced systemic facilitation of SCN performance seen previously (McCarville et al., 2012; McCarville et al., 2014).

In addition to suppression of defense responses, systemic changes in primary metabolism or root exudates may underlie the interaction between soybean aphids and SCN. Aphids are known to alter local and systemic carbon and nitrogen content (Sandstrom et al., 2000; Girousse et al., 2005) as well as root exudation, which is important in attraction of rhizosphere organisms (Bais et al., 2006; Badri & Vivanco, 2009; Lee et al., 2012; Kim et al., 2016). We did not quantify these changes through our study. Thus, further investigation of these systemic metabolic changes could be done to more fully understand the whole-plant effect of soybean aphid feeding.

In conclusion, we used a combination of functional genetics and genomics technology to identify mechanisms of host susceptibility to aphids and how aphid feeding may affect other pests. Our study is the first to provide genetic evidence of local repression of defenses by soybean aphids and is also the first to quantify whole-plant molecular changes triggered by soybean aphids. Furthermore, we related these expression changes to the performance of another herbivore, soybean cyst nematodes. Completion of these studies has resulted in the characterization of crosstalk between hormonal pathways in soybean and has started work discovering mechanisms that may explain plant-mediated pest interactions.

References


Kanobe C. 2012. Fatty acid changes in soybean (Glycine max) under soybean aphid (Aphis glycines) infestation and their implications on plant defense against insects. *Graduate Theses and Dissertations Paper 12840*.


