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Herbivore Response to Soybean Under Differing Induction Methods

John L. Dryburgh

Louisiana State University and Agricultural and Mechanical College

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HERBIVORE RESPONSE TO SOYBEAN UNDER DIFFERING INDUCTION METHODS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Entomology

by

John L. Dryburgh

B.S. Susquehanna University, 2012

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ABSTRACT

Plants are attacked by a variety of herbivore feeding guilds and respond with specific responses to specific attacks, which may be localized or systemic. A plant's defense against one feeding guild may alter the plant's resistance to a different feeding guild. A better understanding of these interactions allows for the development of refined pest management programs. One situation in which this may occur is in interactions between chewing and piercing/sucking herbivores, such as aphids. Aphids are important crop pests due to their ability to transmit viruses, the efficacy of which can be affected by plant defenses. To determine if systemic induction has an effect on aphid feeding behaviors, three soybean varieties, (Progeny 4906RR, Davis, and Lyon) were induced by either subjecting plants to feeding by soybean looper (SBL), *Chrysodeixis includes* (Walker), larvae, or applying jasmonic acid (JA) or salicylic acid (SA) to foliage. Three days post induction, green peach aphid (GPA), *Myzus persicae* (Sulzer), apterae feeding behavior was recorded on induced and control plants using the Electrical Penetration Graph (EPG) technique. SBL feeding bioassays were used to assess the effect of previous SBL herbivory, JA or SA on SBL larval weight, and to confirm systemic induction. Both previous herbivory and JA reduced SBL larval weights when fed Progeny 4906RR tissue. Herbivory had no effect on SBL larval weights when fed Davis tissue, suggesting SBL does not induce a response in Davis. SA did not induce a response in either variety. Neither herbivory nor JA had an effect on SBL larval weight in Lyon, suggesting it does not induce a defensive response. SA increased SBL larval weights in Lyon. SBL herbivory decreased several behaviors associated with nonpersistent virus transmission in both Progeny and Davis, but had no effect on aphid feeding behavior in Lyon. JA induction increased several behaviors associated with nonpersistent virus transmission in both Progeny and Davis. Exogenous SA application also increased

behaviors associated with nonpersistent virus transmission in all three varieties. These results suggest that inducing host plant resistance with JA applications may reduce herbivore performance. However, both JA and SA may increase nonpersistent virus transmission.

INTRODUCTION

Host plant resistance (HPR) is an important aspect of pest management. However, the mechanisms that control HPR are poorly understood in many crop systems, particularly inducible mechanisms, those that require injury to be expressed. Despite this, inducible resistance traits may prove valuable in pest management strategies. Altering plant phenotype, via herbivory or artificial elicitors, changes the crop ecosystem in ways that could reduce pest numbers in plants exhibiting sub-lethal defenses (Stout et al. 2002), or through indirect defenses such as natural enemies (Degenhardt et al. 2003). These resistance mechanisms are systemic, allowing the whole plant to mount a defensive response, and are likely to be compatible with cultural and biological control tactics (Stout et al. 2002). This makes them attractive for use in sustainable pest management. Previous ecological research has shown both positive and negative effects of induced resistance on plant fitness (Cipollini and Heil, 2010). Unfortunately, the effect of induced resistance on herbivores in crop ecosystems is poorly explored.

Soybean is an economically important field crop in the United States (Funderburk et al. 1998). Additionally, it has been the subject of previous research on induced resistance in crop plants (see Accamando and Cronin, 2012; Underwood, 1998). Herbivory by the Mexican bean beetle herbivory, *Epilachna varivvestis* Mulsant is known to induce systemic resistance to future herbivory in soybean (Underwood, 1998). Herbivory also induces the production of a variety of secondary chemicals including protease inhibitors and oxidative enzymes in soybean (Kraemer et al. 1987; Felton et al. 1994).

In order to use induced HPR in pest management, its effects on the herbivore assemblage must be better understood. Crops are attacked by different types of herbivores in the field, and they respond to different feeding guilds, such as chewing herbivores and piercing-sucking

herbivores, by activating different signaling pathways (Ferry et al. 2004). Soybean is attacked by a variety of feeding guilds, and is able to tailor its induced response (Felton et al. 1994). These herbivores include a complex of lepidopteran pests, notably the soybean looper, *Chrysodeixis includes*, one of the most economically important soybean pests in the midsouth of the United States (Musser et al. 2014), as well as other economically important pests, including the three-cornered alfalfa hopper, *Spissistilus festinus* (Say), and a stink bug complex (Funderburk et al. 1998). While not economically important in the southern United States (Funderburk et al. 1998), aphids such as the soybean aphid, *Aphis glycines* Matsumura, are economically important in many soybean producing regions due to both the injury caused by feeding and by their ability to transmit plant viruses (Ng and Perry, 2004). Other aphid species also transmit viruses in soybean. For example GPA, an economically important pest in several other crop species, is a transmitter of *Soybean mosaic virus* (Halbert et al. 1981). Unfortunately, how a plant's induced response to one feeding guild affects herbivores from another is not well characterized.

Typically, plants activate the jasmonic acid pathway to respond to chewing herbivores, and the salicylic acid pathway to respond to piercing sucking herbivores and pathogens (Smith and Clements, 2012). These signaling pathways interact in a phenomenon known as crosstalk: the salicylic acid signal cascade negatively affects the jasmonic acid pathway, reducing plant defenses against insect pests, and vice versa (Van der Does et al. 2013; Vos et al. 2013). However, one study found that lepidopteran feeding induces a general defensive response effective not only against other arthropods, but also against pathogens in tomato (Stout et al. 1998). Other research suggests that damage by different feeding guilds may activate similar defensive responses (Cao et al. 2013; Ellis et al. 2002), and that it is also possible to activate

these defenses with the application of elicitors, such as the foliar application of methyl jasmonate or jasmonic acid (Thaler et al. 1996).

JA-regulated defenses may also negatively affect both aphids and pathogens. In 1998, Stout et al showed that feeding by *Helicoverpa zea* (Boddie) in tomato induces resistance against other lepidopterans, as well as aphids, which suggests broad defensive reactions, rather than herbivore specific ones. The relationship between the JA and SA signaling cascades is complicated in the case of aphids. Plants often respond to aphid attack by activating SA-dependent defenses, such as the hypersensitive response, as if they were pathogens (Smith and Boyko, 2006), yet aphids may also trigger JA-dependent responses, such as chitinases (Smith and Boyko, 2006). GPA feeding on *Arabidopsis thaliana* (L.) Heynh activates both SA- and JA-associated genes (Moran and Thompson, 2001). Other research suggests that both hormones play a role in plant defense against aphids. Several studies have shown that exogenous application of methyl jasmonate and salicylic acid affect both aphid population growth (Boughton et al. 2006) and feeding behavior (Cao et al. 2013). The exact relationship between plants and aphids depends on the specific aphid-plant systems, and thus it is difficult to generalize these interactions (Smith and Boyko, 2006). Further research is required to tease out the relationship between JA related defenses, both those induced by elicitors and those induced by herbivory, on aphids.

Induced resistance can affect aphid feeding behavior, and as aphids are transmitters of economically important plant viruses (Ng and Perry, 2004), induced resistance may prove useful in preventing the transmission of plant viruses, though this has never been tested. Aphids are excellent virus vectors due not only to their physiological adaptations, but also due to their host selection and feeding behaviors. Aphids reflexively probe surfaces (Fereses and Moreno, 2009)

and make intracellular punctures (Katis et al. 2007), behaviors required to transmit nonpersistent viruses. Nonpersistent viruses can be acquired by the aphid in minutes, and transmitted to a new plant in seconds. Due to this quick transmission, these viruses can spread rapidly (Ng and Perry, 2004). Manipulation of aphid feeding behavior may cause changes in nonpersistent virus acquisition and transmission. Several studies have shown that aphid feeding behavior varies on both constitutively resistant (Montllor and Tjallingii, 1984) and induced plants (Prado and Tjallingii, 1997; Cao et al. 2013). Aphids on resistant varieties often probe more often, though their probes are of shorter duration, and they perform fewer intracellular punctures necessary for acquiring and transmitting viruses (Prado and Tjallingii, 1997). Resistance is ideal for preventing the spread of aphid transmitted viruses. This is because while insecticides can be effective at preventing the transmission of persistent plant viruses (which require the aphid to feed on the plant for an extended period of time) due to their antifeedant properties (Mowry, 2005), nonpersistent viruses can be acquired and transmitted before the aphid expires due to the insecticide (Collar et al. 1997).

In order to utilize induced resistance in pest control and to control the transmission of plant viruses, whether through herbivory or chemical elicitors, it is necessary to understand how these stimuli affect the plant and the behavior of future herbivores in all feeding guilds. Both SA mimics and JA have been explored for use in inducing pathogen and pest resistance (Gordy, 2013) with varying degrees of success (Stout et al, 2002). There are few field studies examining the effects of herbivory-induced resistance on future herbivory (Underwood, 2002). Although a general model exists describing how different feeding guilds respond differently to the same induction methods (Fig. 1), there is evidence that different herbivores within the same guild may also respond in different manners. For example, the fall armyworm, *Spodoptera frugiperda*

(Smith), is much more sensitive to resistance induced in soybean by elicitors than is SBL (Gordy, 2013). There is also evidence that host plant plays a role in these interactions: the SA mimic benzothiadiazole (BTH) reduced GPA fecundity on tomato (Boughton et al. 2005), but had no effect on whitefly *Bemisia tabaci* (Gennadius) host preference in cotton (Inbar et al. 2001). Thus, when considering how induced resistance can be applicable in pest management, it is necessary to understand how the pest species are affected by induced resistance.

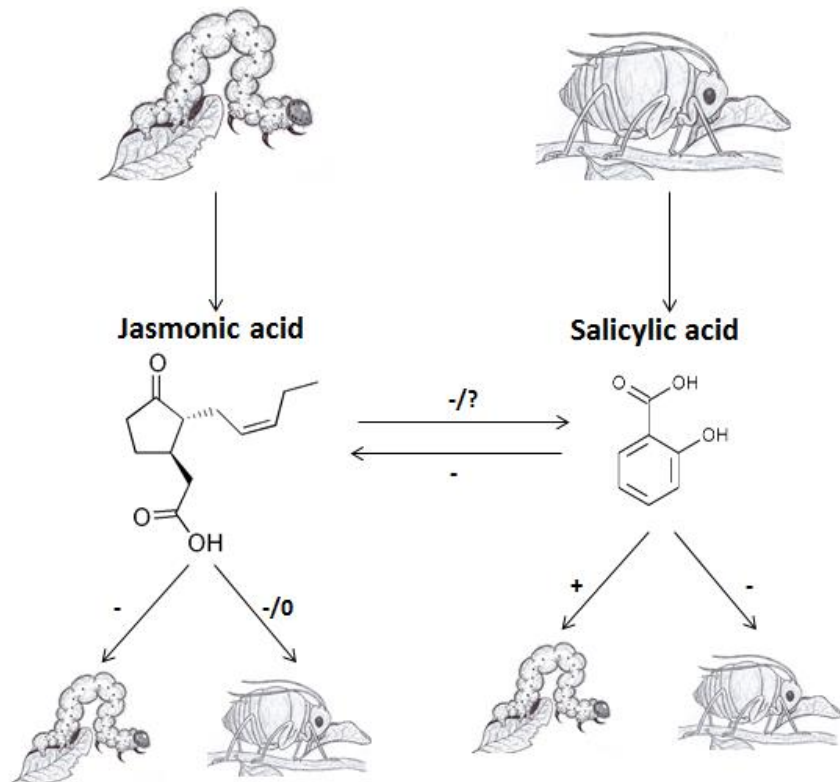


Figure 1: Presumed relationship between necrotrophic (caterpillar) and biotrophic (aphid) insect pests, their effect on plant hormonal pathways and future herbivory based on current research. Arrows and signs indicate the presumed effect on herbivores and elicitors (i.e., SA has a negative effect on JA). Chewing herbivores (lepidopteran) activate the JA pathway. This has a negative effect on further chewing herbivores, and a neutral or possibly negative effect on piercing-sucking herbivores (aphids). SA is activated by piercing-sucking herbivores: this has a negative effect on future piercing-sucking herbivores, but a positive effect on chewing herbivores.

The goal of this research is to expand understanding of the relationships between insects from different feeding guilds and two phytohormone pathways described in Fig. 1. Specifically, this research will examine the effect of three induction methods (SBL herbivory, exogenous JA or exogenous SA) on herbivory by SBL in three varieties to determine if the effect of these stimuli on soybean's induced response corresponds with the general model described above. It will also explore how these stimuli affect aphid feeding behaviors associated with virus transmission.

This will further the understanding of the role of jasmonic and salicylic acid in plant resistance to aphids. In addition, it will also examine the effect, if any, of elicitors on specific GPA feeding behaviors related to nonpersistent virus transmission, a factor that may affect their usage in pest management.

LITERATURE REVIEW

Host Plant Resistance

Plants, in both crop systems and their native environment are attacked by a wide variety of herbivores with many different feeding strategies. Plants have a wide range of traits and strategies that contribute to their ability to defend themselves from this diversity of herbivores (Arimura et al. 2005). Plants are often thought of as passive organisms, and while they are capable of tolerating a certain amount of injury, they also utilize a wide variety of defenses. These include physical defenses, such as hairs or trichomes to inhibit herbivore movement; chemical defenses such as compounds that are toxic or deterrent to herbivores; and biological defenses that use predators to attack herbivores, such as the release of volatile chemicals to repel pests or attract higher trophic levels (Chen, 2008; Baldwin, 1999). This wide variety of plant resistance mechanisms collectively form host plant resistance (HPR). HPR can, and has been exploited by humans in agricultural settings to reduce pest insect numbers and increase crop yield for thousands of years (Smith, 2005). However, it is only relatively recently that scientists began exploring and categorizing the mechanisms of HPR, and the physiological and ecological processes behind them.

HPR by itself typically does not provide enough control over pest insect populations to be used as a stand-alone tactic. There are however, exceptions, in which plant resistance is strong enough to provide almost complete control of a pest, such as the Hessian fly, *Mayetiola destructor* (Say), on winter wheat, and the grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), on grape (Smith and Clements, 2012). In both of these cases, resistant plants have antibiotic traits, resistance traits that inhibit the survival of the pest (such as toxins and toughened root tissue in the case of grapes), though resistant plants may also be more tolerant to injury by the pest

(Granett et al. 2001). Another good example of a high level of resistance to a pest is soybean resistance to the soybean aphid. Many American soybean varieties have little resistance to soybean aphid, however, there are several varieties that possess monogenic resistance, conferred by the so called *Rag* (Resistance to *Aphis glycine*) genes. These genes grant antibiotic resistance resulting in aphids colonizing these plants more slowly and feeding less readily. (Ragsdale et al. 2011).

Unfortunately, as crop systems are living systems, the use of plant resistance in pest management results in adaptation. This is clearly illustrated in the formation of biotypes, insect populations that are able to overcome plant resistance, in crops with single gene resistance, such as the aforementioned wheat, grape and soybean systems. In soybean, there are several soybean aphid biotypes, including biotype1, which colonizes susceptible soybean, biotype2, which is able to colonize plants that possess the *Rag1* gene, and biotype3, capable of colonizing soybean with the *Rag2* gene (Ragsdale et al. 2011).

Fortunately, most plant resistance mechanisms are polygenic in nature (Stout and Davis, 2009), and thus insect populations do not develop immunity to them, or at least do so over longer time periods which allows for the breeding of new resistant varieties. Plant resistance can be affected by a variety of other factors. Abiotic and environmental factors affect resistance. For example, potassium is essential to resistance mechanisms (Amtmann et al. 2008). Furthermore, breeding new crop varieties via traditional methods can be time consuming and expensive as it may be difficult to integrate multiple resistance traits into high-yielding varieties without compromising yield (Stout and Davis, 2009).

There are many advantages to using HPR in pest management strategies, particularly in integrated pest management (IPM). It is easy and inexpensive for the grower to use, as it does

not require additional work after planting. It often integrates well with other pest management strategies such as cultural control: for example, tactics such as volunteer plant destruction, crop rotation, and delayed planting can be used to augment wheat resistance against Hessian fly (Smith and Clements, 2012). When used at a large scale, HPR has the potential to reduce area wide insect populations (Stout and Davis, 2009). Finally, it can reduce the use of insecticides harmful to the environment and natural enemies: widespread adoption of resistant varieties in sorghum has both reduced insecticide application and increased the efficacy of biological control agents (Smith and Clements, 2012). Many consider HPR to be an integral part, if not the foundation of IPM (Smith and Clements, 2012). Properly utilizing HPR involves making an informed decision about which crop variety to plant, and the characteristics of this variety affect other pest management tactics.

The future development of HPR in IPM will require a better understanding of the genetic and ecological mechanisms that drive plant resistance. By better understanding the genetic basis of HPR through identifying resistance genes, it will be possible to target resistance genes to transfer into high yielding lines. Emerging technologies (for example, marker assisted selection) allow for efficient selection of plants with desired genetics for breeding (Stout and Davis, 2009). Additionally, it is already possible to insert a wide variety of foreign genes into plant genomes. Currently, this technology is used to insert genes for herbicide resistance and insecticidal proteins into plants, but as more resistance genes from crop plants are identified, it will be possible to insert these genes into other lines. Good candidates for insertion are R, or resistance genes, which provide single gene resistance to a pest. There are other candidate genes for genetic engineering, for example, genes that control terpene emission (Degenhardt, 2003) Terpenes are a class of volatile chemicals emitted by plants after herbivore to attract natural enemies. By

altering the amount and composition of these chemicals released by the plant, it may be possible to attract more or different natural enemies to crop plants (Degenhardt, 2003).

Terpene emission after herbivory is a form of induced resistance, or resistance that is only expressed after injury or other stimuli such as chemical elicitors, as opposed to constitutive resistance, which is always present (Howe and Jander, 2008). Inducible resistance is an attractive pest management strategy, as it provides resistance to pests only on plants that are injured or likely to be injured, or which the grower chooses to induce. However, there are significant challenges to using induced resistance in pest management. In order to understand the benefits and challenges of using induced resistance in pest management, it is necessary to understand some of the physiological and ecological basis behind induced resistance.

Induced Resistance

The term 'induced' refers to the method by which a resistance mechanism is activated and there are a wide variety of inducible resistance traits. Inducible resistance mechanisms include secondary metabolites such as proteinase inhibitors and glucosinolates (Howe and Jander, 2008; Chen, 2008), hypersensitive responses, anti-nutritive mechanisms (such as degradation of essential amino acids), mechanical barriers (leaf toughening, fortification of cell walls) (Chen, 2008), and the release of herbivore induced plant volatiles (HIPV) as an attractant for natural enemies (Pichersky and Gershenzon, 2002). The reason for the evolution of inducible resistance is unclear. For many years, the prevailing theory was that inducible resistance evolved to lower the fitness cost of constantly producing defensive compounds; this may not be the case. For example, many secondary metabolites that play a role in herbivore resistance have other, non-resistance related functions. For example, a defensive compound may be temporarily stored until the plant reallocates it as an intermediate in the production of a compound essential to

growth (Neilson et al. 2013). Inducible resistance mechanisms may incur ecological and metabolic costs of their own. Volatile signals emitted by a damaged plant might be picked up by another organism and utilized to the detriment of the plant. This can take the form of competing plants using volatile cues from a wounded plant, and increasing their defenses (Baldwin et al. 2006), or the hijacking of signaling systems by other trophic levels, such as when spider mites utilize volatile signals to find wounded plants (de Vos and Jander, 2010). Determining the exact costs and benefits of induced resistance is an open area of research in ecology (Karban, 2011).

Induced resistance mechanisms are generally triggered by herbivore damage, although plants possess other inducible mechanisms to respond to different stresses (Holopainen and Gershenzon, 2010), including touch, wind damage, and other environmental variables. Resistance may also be triggered by oviposition or other nonfeeding behaviors (Baldwin, 1999). While mechanical damage alone can induce resistance, plants also respond to compounds in herbivore saliva, eliciting a different response than mechanical damage alone (Lin et al. 1990; Holopainen and Gershenzon, 2010).

Induction by herbivory results from signaling cascades within the plant involving numerous plant hormones (Smith and Clement, 2012), which in turn activate the plant's defenses. This allows for plants to respond systemically to herbivory. JA is the most important plant hormone in resistance to arthropods, as evidenced by the fact that the application of exogenous methyl jasmonate induces resistance to herbivores in multiple studies (Thaler et al. 1996; Rodriguez-Saona et al. 2001; Ferry et al. 2004). JA regulates resistance against chewing herbivores, such as Lepidoptera. Other hormones, such as SA and ethylene also play major roles in resistance. SA, in particular, is vital in regulating resistance to many pathogens and to piercing-sucking herbivores such as Hemiptera, which plants treat in many ways like pathogens

(Ferry et al. 2004). SA- and JA-mediated pathways interact in a phenomenon known as crosstalk. In general, this is a negative interaction. The process by which SA suppresses JA related responses was recently elucidated (Van der Does et al. 2013). However, the effects of JA and ethylene on SA signaling are poorly understood. (Vos et al. 2013).

JA regulated defenses may also affect both aphids and pathogens. In 1998, Stout et al showed that feeding by *H. zea* in tomato induces resistance against other lepidopterans, as well as aphids, the mite *Tetranychus urticae* Koch, and the bacterial pathogen *Pseudomonas syringae*, which suggests broad defensive reactions, rather than herbivore specific ones. The relationship between the JA and SA signaling cascades is particularly muddled in the case of aphids. Aphids are adept at feeding without triggering plant defenses. *Myzus persicae* spp. *nicotianae* Blackman induced the upregulation of relatively few genes compared to lepidopteran and mirid herbivores in tobacco, and did not significantly increase either SA or JA levels (Heidel and Baldwin, 2004). They accomplish this through a variety of mechanisms including chemicals in saliva that manipulate the plant defensive response (Walling, 2008).

Despite aphids' ability to subvert plant defenses, both SA- and JA-dependent defenses can affect them. Foliar applications of both a salicylic acid mimic and methyl jasmonate have been shown to slow GPA population growth on tomato (Boughton et al. 2006). Other research suggests that both hormones play a role in plant defense against aphids. Plants often respond to aphid attack by activating SA-dependent defenses, such as the hypersensitive response, as if they were pathogens. However, they may also activate JA-dependent responses, such as chitinases, as other insect pests do (Smith and Boyko, 2006 provide a good overview of how the plant signaling pathways affect plant responses to aphids).

All of this suggests that, in spite of the crosstalk between these two hormones, it is possible for both JA- and SA-mediated defenses responses to have similar effects on herbivores. Whether this is because they activate many of the same genes (Ellis et al. 2002), or because they enact different defenses with similar but distinct consequences (Cao et al. 2013), or both in different cases, is unknown. Determining the exact relationship between JA and SA in the regulation of plant resistance is still an open area of research.

Regardless of how plants are induced, their response to injury is variable. There is a wide variance in the temporal and spatial scale of induced resistance. Resistance may be induced in as small an area as the leaf damaged, or over several plants via volatile signaling (Arimura et al. 2009). The induced response may also be tailored to the specific herbivore that caused the damage, or very broad affecting different species. A study in *Arabidopsis* showed that *Spodoptera littoralis* (Boisduval), a generalist, induced many of the same genes as *Pieris rapae* (Linnaeus), a crucifer specialist, suggesting that induced responses are not tailored to the herbivore (Reymond et al. 2004). Conversely, different herbivores may induce different combinations of resistance traits (Stout et al 1994). Different herbivores may induce the emission of different volatile chemicals (Pickett et al. 1999): the volatiles produced by the closely related species *Heliothis virescens* (Fabricus) and *H. zea* are different enough that a specialist parasitoid wasp that can distinguish between (De Moraes et al. 1998). This can make the utilization of induced resistance in pest control a challenge.

Induced Resistance in Soybean

Induced responses to herbivory in soybean are poorly characterized. Greenhouse experiments have shown that while JA induction of soybean has an antixenotic effect on SBL, it also reduces plant fitness (Accamando and Cronin, 2012). It is known that large amounts of

herbivory (~60% of total leaf area) induces a systemic antixenotic effect against the Mexican bean beetle (Underwood, 1998). This effect peaks at three days after damage, and lasts approximately 15 days before a period of induced susceptibility during which the plants become more attractive to the Mexican bean beetle. Soybean also induces a variety of secondary chemicals to combat herbivores, such as phenols, protease inhibitors, and oxidative enzymes (Felton et al. 1994). Additionally, soybean responds to different herbivores with different secondary chemicals: it induces more oxidative enzymes in response to three-cornered alfalfa hopper herbivory than bean leaf beetle herbivory (Felton et al. 1994). Soybean also induces a volatile response to herbivory. Unfortunately there is very little research on the volatile profile of soybean. Liu et al (1989) compared the volatile profiles of the susceptible variety Davis with the more resistant PI 227687 and their attractiveness to the cabbage looper and Mexican bean beetle and found that PI 227687 was less attractive to herbivores largely because of two volatile compounds, 3-tetradecene and 1-dodecene. Davis was more attractive to herbivores because it emitted several attractive volatiles such as 4-hexen-1-ol post herbivory. While Underwood found no difference in induced response between varieties, and both Felton et al (1994) and Accamando and Cronin (2012) only tested one variety, the results from Liu et al (1989) suggests that induced resistance in soybean varies by genotype.

Using Induced Resistance in Agriculture

Inducible resistance is difficult to integrate into pest management strategies. Induced responses to herbivory vary widely in scope and scale both within the plant and in its effects on herbivores. Similarly, how plants respond to herbivory is poorly understood: While a great deal is known about induced resistance at the genetic level (Smith, 2005; Ferry et al. 2004), little is known about the amount of damage required to elicit a response or the timescale over which

induced defenses are active. Moreover, while several greenhouse studies have examined the effects of inducible resistance on yield (Accamando and Cronin, 2012), the effects of induced resistance on crop yield at larger scales are as yet unknown. Work by Underwood et al. (2002) suggests that greenhouse studies may be good representatives of how induced resistance functions in the field. However, their study did not look at plant fitness. Work by Karban and Maron (2003) on resistance in wild tobacco induced by sagebrush volatiles suggests that induced resistance has a slight positive or no effect on plant fitness. Thaler (1999) and Thaler et al (2001) found that while applications of JA significantly reduced pest numbers in the field, this did not translate to increased yield. This further suggests that there is little effect of induced resistance on yield, but this has not been extensively tested on field crops. The study of induced resistance in crop systems is further complicated by any effect of genotype on induced resistance. Crop varieties are often used in the field for only a few years (Bowman, 1998). The turnover rate has greatly increased in soybean with the advent of genetically modified crops (Raymer and Grey, 2003). Thus, any analysis of the effects of induced resistance on yield could quickly become outdated.

Another problem with using induced resistance in pest management is the possibility of induced susceptibility. Work by Underwood (1998) suggests that Mexican bean beetle herbivory induces resistance to future herbivory for 3-14 days before inducing susceptibility to herbivory after around 20 days. Neonicotinoid insecticides can induce SA regulated defenses (Ford et al. 2010), so the use of these chemicals could induce susceptibility to insect damage. Unfortunately, very little research has been performed on this phenomenon.

Despite the uncertainties surrounding induced resistance, there are ways to utilize induced resistance in IPM. Currently, there is a market for volatile lures, which use synthetic

volatiles in place of naturally occurring induced and constitutive volatiles (Braasch and Kaplan, 2012). Another possible tactic is the use of chemical elicitors to induce resistance. These could be integrated into already used strategies. In crop systems that utilize intercropping, elicitors could be used to manipulate the differences between the main and trap crops. For example, elicitors could be used in a push-pull strategy as a ‘push’ component, pushing pests from the main crop into the trap crop by making crops less attractive to pests, or as a ‘pull’ component, making the trap crop more attractive, as some induced processes (such as HIPV) can be attractive to herbivores (Cook et al. 2007).

Synthetic volatiles are a promising method of attracting natural enemies to crops as well as deterring herbivores (James, 2003). Unfortunately, the use of artificial volatiles is currently problematic. Volatiles are only bioactive over a relatively small area. Field experiments have shown that naturally produced volatiles can only effect other plants that are within about 60 cm of the source (Karban et al. 2006), and artificial volatiles seem to only affect arthropods within a few square meters (Braasch and Kaplan, 2012), potentially making the number of lures required for this technique cost prohibitive.

Another factor that affects the effectiveness of volatile lures, which generally emit a high concentration of a single compound such as phenylethyl alcohol, is that the volatile blend produced by a plant, not specific volatiles, is what appears to affect host choice in herbivores and searching behavior in parasitoids (Michereff et al. 2013). Volatiles that are deterrents on their own may become attractive in a blend, and vice versa (Bruce and Pickett, 2011; Webster et al. 2010). A relatively minor chemical may be as necessary for host location as more major (or at least, more prevalent) compounds such as methyl jasmonate or methyl salicylate (Michereff et al. 2013). While this does not necessarily mean that volatile lures/deterrents are completely

ineffective, it does mean that the volatile used in the lure and the volatile profile of the crop in question should be taken into account when utilizing these strategies, as it may be a major factor in their effectiveness. Additional factors may affect the efficacy of volatile lures: some herbivores are attracted to the same volatiles that attract natural enemies (Degenhart, 2003), and some parasitoids can seemingly tell the difference between a synthetic blend of the major volatile compounds and the natural blend emitted by the plant (Turlings et al. 1991). Furthermore, luring natural enemies may cause its own problems: natural enemies have evolved to detect volatiles as they are an indicator of prey species, thus, the constant release of volatiles may alter natural enemy populations in unhealthy ways. For example, natural enemies may be lured to areas without prey, reducing their effectiveness, or they may learn to ignore the signal, as it provides false information (Degenhardt et al. 2003). A way around this problem is to genetically engineer plants that produce more or more attractive volatiles when wounded (Degenhardt et al. 2003).

One of the difficulties in using induced HPR is the inherent difficulty of measuring its effects on plant yields in field experiments. This was considered briefly above. There are many variables interacting in field crops, including pest levels, natural enemy populations, and environmental factors such as soil quality and drought (Stout et al. 2002 provides a good overview of this as it relates to the use of elicitors in the field). Additionally, it may be impossible to effectively control the amount of injury to a specific level.

Thus, other methods are used to quantify the effects of induced resistance on crops before they can be tested in the field. Molecular techniques, for example, allow the comparison of induction on gene expression. This can be limited to a few genes of interest in techniques such as RNA gel blotting, which uses gel electrophoresis to separate RNA, which is then transferred to a membrane and hybridized with DNA or RNA probes to detect specific sequences (see Ellis et al.

2002). Other techniques can analyze larger sections of the plant genome: microarrays work on much the same principle as gel blots, but at a larger scale (see Reymond et al. 2004).

Another way of measuring induced resistance is through effects on insect feeding and host selection behavior, as changes in these behaviors may greatly impact pest management tactics. This is particularly important in Hemipteran pests such as whiteflies and aphids. In these pests, feeding behaviors are an important factor affecting the transmission of damaging plant viruses. A good technique for measuring changes in feeding behaviors in hemipteran is the electrical penetration graph technique, or EPG.

Electrical Penetration Graph Technique

Damage by piercing-sucking insects such as aphids is more difficult to quantify than that from chewing insects. Because of this, and because of the difficulty involved in observing specific feeding behaviors (such as the cellular punctures required for both host selection and virus transmission) without disrupting the insect's feeding behaviors, indirect methods of observation must be used. Thus most research on aphid feeding behavior is conducted using the electrical penetration graph technique. By creating a circuit between an aphid and a plant, it is possible to monitor aphid feeding behaviors. When the aphid inserts its stylet into the plant, the circuit is closed. Resistance created by fluid composition and movement within the aphid's mouthparts, as well as voltage differentials created by the different membrane potentials of various plant tissues produce a waveform. This waveform produced can be interpreted to determine the aphid's feeding pattern (Walker, 2000).

This technique was first pioneered by McLean and Kinsey in 1964, when they attempted to elucidate aphid feeding behaviors by allowing aphids with wires attached to their dorsum to crawl upon a conductive grid suspended over plant tissue. EPG was later improved upon by

others (van Helden and Tjallingii, 2000; Walker, 2000) and adapted into the AC and DC systems, which produce different waveforms that elucidate different feeding behaviors, as well as adapted for use with other organisms such as leafhoppers and whiteflies (Reese et al. 2000). Feeding behaviors associated with aphid EPG waveforms are well described due to a combination of histological studies, artificial diet, and direct observation of stylet penetration (Walker, 2000).

The EPG technique, in addition to characterizing feeding behaviors, also allows for the comparison of behaviors under different conditions. For example, it has been used to elucidate the differences in feeding behavior between different species of insect (Calderon and Backus, 1992), on different host species (Davis and Radcliffe, 2008), on different varieties (Montllor and Tjallingii, 1989), as well as the effect of previous feeding (Prado and Tjallingii, 1997) or chemical induction (Cao et al. 2013) on behavior, and the effect of starvation on feeding behavior (Collar and Fereres, 1998). Because aphids are important virus vectors, this technique is also used to look at virus acquisition and transmission behavior. While aphids reflexively probe surfaces, they also rely on physical cues (such as epicuticular waxes and trichomes) as well as chemical cues (such as volatiles) to determine host plant suitability before probing. The time to first probe can be used as a measure of the aphid's acceptance of a plant based on these stimuli (Fereres and Moreno, 2009).

As the aphid's gustatory organs are located in the foregut, they must probe the plant, ingesting sap, in order to further determine plant suitability. The duration of the first and subsequent probes are a good indication of plant acceptance, as aphids initiate longer probes on susceptible plants compared to resistant plants (Montllor and Tjallingii, 1989).

Aphids also sample cell contents during probes. The difference in voltage between the cell and extracellular space causes a distinct potential drop (pd) when this phenomenon is recorded by EPG. Pds have three distinct phases. The second phase is essential to nonpersistent virus transmission, and is further divided into three subphases (II-1, II-2, and II-3). Phase II-1 correlates to aphid salivation into the cell, and is associated with virus transmission (Powell et al. 1995), while phase II-3 correlates to ingestion of cell contents, and virus acquisition. Archlets, which occur in phase II-3, are also associated with virus acquisition (Collar et al. 1997). Increases in the duration of these phases, as well as the number of archlets, allows for increases in the acquisition and transmission of nonpersistent viruses.

Previous research has shown that plant resistance, whether constitutive or induced, can affect aphid feeding behavior. *Melanaphis sacchari* (Zehntner) feeding on resistant sugarcane fed on phloem less compared to susceptible varieties (Ackbar et al. 2014), and GPA feeding on resistant lettuce take longer to reach the phloem and feed on the phloem less than on susceptible lettuce (Montllor and Tjallingii, 1989). Resistance also affects behaviors associated with virus transmission. In lettuce, aphids feeding on resistant plants probed more often, however the probes were shorter, and aphids performed fewer cellular punctures. Cellular punctures are required for the transmission and acquisition of nonpersistently transmitted viruses (that is, viruses which are acquired in a short [second or minutes] timespan, and only remain transmissible for a few minutes to a few hours) (Powell et al. 1995). A study using *Sitobion avenae* (Fabricius) on wheat found that the application of exogenous methyl jasmonate also caused the aphids to perform more, but fewer probes (Cao et al. 2013). While that study did not examine the effect of methyl jasmonate on virus transmission behaviors, it suggests that JA may

reduce the frequency of these behaviors. This study will examine the effect of not only elicitors, but also the effects of lepidopteran herbivory on aphid feeding behaviors.

RESEARCH OBJECTIVES

The purpose of this research was:

1. To determine the effect of systemic resistance induced by soybean looper *Chrysodeixis includens* (Walker) (SBL) herbivory, foliar jasmonic acid (JA), or foliar salicylic acid (SA) induction on SBL feeding and mortality in three different soybean varieties (Davis, Lyon, Progeny 4906RR) through leaf feeding bioassays.
2. To determine the effect of SBL herbivory, JA or SA induction on green peach aphid *Myzus persicae* (Sulzer) (GPA) feeding behaviors in three different varieties of soybean (Davis, Lyon, Progeny 4906RR) using the electrical penetration graph technique.

This research was performed to better understand the induced response in soybean to herbivory and elicitors. The intent of this research was to confirm the presence of a systemic inducible response in soybean in reaction to three stimuli; and to determine what effect variety has on this response. It accomplished this by examining the effect the induced response has on the weight and mortality of a lepidopteran herbivore and on aphid feeding behaviors associated with nonpersistent virus transmission. By better understanding how different plant genotypes respond to the same stimuli and what effect the physiological changes have on herbivores feeding on these plants, we can improve IPM in pests that differ in feeding guilds.

MATERIALS AND METHODS

Plants and Insects

Three soybean (*Glycine max* (L.) Merr.) cultivars (Lyon, Progeny 4906RR, and Davis) were used in the following experiments. Davis is a Maturity Group (MG) VI variety resistant to phytophthora rot (*Phytophthora megasperma* var. *sojae*) and to bacterial pustule (*Xanthomonas phaseoli* var. *sojense*), wildfire (*Pseudomonas tabaci*), and target spot (*Corynespora cassiicola*) (Caviness and Walter, 1966). It is susceptible to herbivory, having “no known source of insect resistance” (Hatchett, 1976). Lyon is a MG VI variety bred for SBL resistance (Hartwig et al, 1994), and is resistant to multiple pest and pathogens, including SBL, soil cyst nematode (*Heterodera glycines*), southern root-knot nematode (*Meloidogyne incognita*), bacterial pustule (*X. campestris* pv. *Glycines*), stem canker (*Diaporthe phaseolorum*), and phytophthora rot (*P. sojae*) (Hartwig et al. 1994). Progeny 4906RR (Progeny Ag Products, Wynne, AR) is a MG IV glyphosate resistant, high yielding commercial variety.

All soybean plants used in experiments were grown from seed in 13 cm (EPG experiments,) or 18cm (SBL feeding bioassays) plastic pots using Miracle Gro (Marysville, OH) potting soil and Osmocote fertilizer (Marysville, OH) (NPK 13:13:13). Plants used in soybean looper feeding bioassay were grown in the LSU Central Campus Greenhouse under ambient light, while those used for EPG experiments were grown in growth chambers (Percival Scientific, Perry, IN) at 25°C with a 14:10 (L:D) photoperiod and >50% RH.

The soybean looper colony used in this experiment, MR08, was established in 2008 with SBL collected from soybean fields at the Macon Ridge Research Station in Winnsboro, LA (Brown, 2012). Larvae were maintained on 10 ml of artificial diet (Southland Products, Lake

Village, AR) in 30-ml cups. Larvae were housed in a rearing room kept at a constant 28.5°C with 50% RH, a photoperiod of 14:10 (L:D), and a 1100 lux light level before experiments.

GPA were from a colony established from a single apterae and maintained under laboratory conditions in screened cages at room temperature (20 to 22°C) and a photoperiod of 14:10 (L:D). *M. persicae* was collected off of an unknown host in 2004, and reared on ‘Tendergreen’ mustard (*Brassica cretica* L.).

Soybean Looper Feeding Bioassays

A feeding bioassay was used to determine the effect of the three induction methods on three (Davis, Lyon, Progeny 4906RR) soybean varieties on soybean looper larval weight and mortality. All plants used were treated at the second trifoliolate stage (V2). For the experiments on soybean looper induction, a third instar SBL larva was placed on the center leaflet of the first trifoliolate leaf of the treated soybean plant, the first trifoliolate was then covered with a mesh drawstring bag to restrict movement. Larvae were allowed to feed for 24 h or until it had consumed 50% of the leaflet area, whichever came first. The control plant was left untreated.

For the experiments on jasmonic acid induction, the first trifoliolate of the treated plant was sprayed with a 2 mM solution of jasmonic acid using a Preval aerosol sprayer (CA Acquisition, Coal City, IL). The rest of the plant was shielded from accidental spray with a plastic sheet or plastic bag. The JA solution was created by mixing 42 mg of jasmonic acid with 1 mL of 95% ethanol, then diluting it into 100 mL of distilled water. The control plant was sprayed with a control solution of 1 mL of 95% ethanol in 100 mL distilled water.

For the experiments on salicylic acid, plants were induced as in the JA induction experiments, using a 2mM solution of SA created by mixing 28 mg of salicylic acid with 1 mL

of 95% ethanol, then diluting it into 100 mL of distilled water on the treated plant. A control solution of 1 mL of 95% ethanol in 100 mL distilled water was used on the control plant.

Because this experiment was designed to measure systemic resistance, tissue from the second trifoliolate was fed to SBL larva three days after induction. A single leaflet from the second trifoliolate of each of the 20 treated plants was placed into a 9 cm petri dish (VWR International, Sugar Land, TX) with moist 9 cm filter paper (410 Qualitative, VWR International, Sugar Land, TX). A single neonate SBL larva, which was fed artificial diet for the previous 24 h, was placed on the leaf in the petri dish using a paint brush. A single leaflet from one of the 20 control plants was placed in an identical setup. This was repeated with a single leaflet from the second trifoliolate of the 19 other plants in each treatment, for a total of 20 SBL neonates on 20 leaves from treated plants, 20 SBL neonates on 20 leaves from control plants. Every three days, a new leaflet from the same second trifoliolate was added to each petri dish, the old leaf was removed, and the filter paper was moistened. After seven days, SBL weight and mortality were recorded. This was repeated 6 times for the SBL, JA and SA treatments for each variety.

Aphid Feeding Behavior

EPG was used to determine how the three induction methods change aphid feeding behavior on the three different soybean varieties tested. To quantify aphid probing behavior, EPG experiments were performed in a Faraday cage using a Giga8 DC amplifier (Wageningen Agricultural University, The Netherlands) with 1 gigaohm input resistance and an AD conversion rate of 100 Hz running only the first four channels. A DI-710 (DATAQ Instruments, Inc., Akron OH) acquisition card converted the analog signals to digital signals, which were recorded using WinDaq Serial Acquisition software (DATAQ Instruments, Inc., Akron OH). Two plants were used for each test. Plants were induced as in the SBL feeding bioassays. The

experiments were performed 3 days after the plants were treated. 18- μ m gold wire (Semiconductor Packaging Material, Armonk, NY) was attached to the dorsal tergum of an apterous adult aphid with silver paint (Pelco Colloidal Silver Liquid no. 16034, Ted Pella, INC., Redding, CA). Aphids were placed on the second trifoliolate leaf in order to test for systemic resistance. Aphids feeding behavior was recorded for 30 min. Four aphids were tested at a time, two aphids on the treated plant, and two on the control plant. Each plant was used for 6 tests with 6 different sets of aphids per test. This was repeated six times for each variety, for a total of 144 aphids (72 on the experimental plants, 72 on the control) tested per variety. The percentage of aphids that probed on each treatment was calculated. Nine different behaviors were analyzed for each aphid per each 30 min recording : time to the aphid's first probe, time to the aphid's first potential drop (cellular puncture), total number of probes, number of potential drops per probe, duration (sec) of the aphid's first probe, duration of all of the aphid's probes (sec), number of archlets, duration of cellular puncture phase II-1 (sec), and the duration of cellular puncture phase II-3(sec).

Data Analysis

Soybean Looper Feeding Bioassays

The effect of treatment on the SBL mortality was analyzed using PROC TTEST (SAS 2013) on arcsin transformed data. Data were analyzed by analysis of variance (ANOVA) using PROC GLM to determine the differences among the treatments. Means were separated using Turkey's honestly significant difference (HSD) tests at $\alpha=0.05$ level. To test amongst cultivars, the difference between the average weight of the SBL larvae fed control plants and those fed treated plants (average weight of larvae fed control plants-average weight of larvae fed treated

plants) from each of the six replicates from each treatment was analyzed by ANOVA. Means were separated using Turkey's honestly significant difference (HSD) tests at $\alpha=0.05$ level.

Aphid Feeding Behavior

The effect of treatment on the percentage of aphids probing was analyzed using PROC TTEST (SAS 2013) on arcsin transformed data. Feeding behavior data was tested for normality using the Kolmogorov-Smirnov test in PROC CAPABILITY and tested for homogeneity using the Levene Test for Homogeneity of Variances in PROC GLM (SAS 2013). Feeding behavior data was not normally distributed and nonparametric statistics (The Wilcoxon Test) in PROC NPAR1WAY were used to compare feeding behaviors (SAS 2013).

RESULTS

Soybean Looper Feeding Assays

Neither previous SBL herbivory, JA treatment, nor SA treatment had any effect on SBL mortality in any variety (Table 1).

Table 1. Percentage of surviving SBL larvae by treatment and variety. (mean \pm se)

Induction Type	Soybean Variety		
	Progeny 4906RR	Davis	Lyon
SBL	86.3 \pm 3.3	88.9 \pm 1.8	70.0 \pm 9.7
UTC	88.3 \pm 2.5	96.6 \pm 1.1	68.3 \pm 6.5
<i>P</i> -value	0.9414	0.6800	0.5648
JA	96.6 \pm 1.7	81.6 \pm 5.2	56.7 \pm 12.8
UTC	96.6 \pm 1.1	82.5 \pm 10.0	52.5 \pm 14.8
<i>P</i> -value	0.7766	0.3372	0.8460
SA	94.1 \pm 2.0	92.5 \pm 2.1	85.8 \pm 6.4
UTC	87.5 \pm 2.2	90.0 \pm 5.6	83.3 \pm 4.0
<i>P</i> -value	0.6109	0.8046	0.6005

Previous SBL Herbivory

SBL larvae had significantly reduced weight after feeding on Progeny 4906RR tissue from plants induced with previous SBL herbivory ($df = 5$, $F = 24.37$, $P < 0.0001$) compared to control plants.

Previous SBL herbivory had no effect on larval weight gain in either Davis or Lyon (Table 2).

Table 2. Systemic SBL induced effects on SBL larval weights (mg) (mean \pm se)

Induction Type	Soybean Variety		
	Progeny 4906RR	Davis	Lyon
SBL	26.1 \pm 1.4 b	51.6 \pm 2.4 a	11.9 \pm 1.0 a
UTC	37.7 \pm 2.2 a	61.3 \pm 7.2 a	15.7 \pm 1.6 a
<i>P</i> -value	< 0.0001	0.5763	0.0995

JA Induction

JA induction significantly reduced the larval weight of SBL fed Progeny 4906RR ($df = 5$, $F = 32.26$, $P < 0.0001$) and Davis ($df = 5$, $F = 11.00$, $P = 0.0011$), but not Lyon (Table 3).

Table 3. Systemic JA induced effects on SBL larval weights (mg) (mean \pm se)

Induction Type	Soybean Variety		
	Progeny 4906RR	Davis	Lyon
JA	51.5 \pm 2.5 b	21.1 \pm 1.7 b	16.9 \pm 1.5 a
UTC	62.1 \pm 2.8 a	27.2 \pm 1.5 a	20.0 \pm 1.7 a
<i>P</i> -value	< 0.0001	0.0011	0.2321

SA Induction

SA induction significantly increased the larval weight of SBL fed Lyon (df = 5, F = 4.79, P = 0.0298), but had no effect on SBL larva fed Davis or Progeny 4906RR (Table 4).

Table 4. Systemic SA induced effects on SBL larval weights (mg) (mean \pm se)

Induction Type	Soybean Variety		
	Progeny 4906RR	Davis	Lyon
SA	42.6 \pm 2.0 a	29.5 \pm 2.5 a	25.6 \pm 1.4 b
UTC	40.1 \pm 2.1 a	27.9 \pm 1.9 a	21.1 \pm 1.3 a
<i>P</i> -value	0.0893	0.9433	0.0298

Varietal Effects

Amongst induction methods, varietal effects were observed only when JA was used as the inducer (Table 5), with Progeny 4906RR having the strongest induction (df = 2, F = 5.36, P = .0262).

Table 5. Comparison of induction methods by variety on the average difference between induced and control SBL larval weights (control-experimental) (mg) (mean \pm se)

Induction Type	Soybean Variety			<i>P</i> -value
	Progeny 4906RR	Davis	Lyon	
SBL	12.5 \pm 4.9 a	11.2 \pm 10.1 a	3.3 \pm 1 a	0.1809
JA	11.0 \pm 1.5 a	6.2 \pm 1.9 ab	2.0 \pm 1.9 b	0.0262
SA	-2.9 \pm 2 a	-1.4 \pm 2.9 a	-4.0 \pm 1.9 a	0.5475

Aphid Feeding Behavior

Induction had no effect on the percentage of GPA that probed plants (Table 6).

Table 6. Percentage of aphids probing at least once, by variety and treatment. (mean \pm se)

Induction Type	Soybean Variety		
	Progeny 4906RR	Davis	Lyon
SBL	63.8 \pm 1.5	39.7 \pm 5.2	31.9 \pm 6.2
UTC	54.2 \pm 7.3	57.1 \pm 9.2	30.9 \pm 4.5
<i>P</i> -value	0.3308	0.1372	0.6699
JA	72.3 \pm 1.1	63.3 \pm 9.6	21.59 \pm 6.5
UTC	59.7 \pm 3.5	46.0 \pm 7.0	34.5 \pm 6.1
<i>P</i> -value	0.1680	0.0575	0.2396
SA	51.8 \pm 9.1	46.1 \pm 7.8	27.2 \pm 5.5
UTC	37.5 \pm 8.9	41.0 \pm 4.4	26.5 \pm 7.8
<i>P</i> -value	0.0560	0.5865	0.9241

Previous SBL Herbivory

Previous herbivory reduced both the average number of archlets (df = 1, $\chi^2 = 6.36$, $P = 0.0117$), the average duration of intracellular phase II-3 (df = 1, $\chi^2 = 18.66$, $P < 0.0001$), and the number of potential drops (df = 1, $\chi^2 = 5.32$, $P = 0.0211$) in Progeny 4906RR (Table 7), and significantly reduced the average duration of phase II-3 (df = 1, $\chi^2 = 5.93$, $P = 0.0149$) in Davis (Table 8). SBL herbivory on cv. Lyon did not alter GPA feeding behavior (Table 9).

Table 7. Differences in GPA feeding behaviors on Progeny 4906RR induced with previous SBL herbivory and control plants. (mean \pm se)

Induction Type	Treatment		<i>P</i> -value
	SBL	UTC	
Average Time to First Probe (s)	298 \pm 55	211 \pm 40	0.5900
Average time to first pd (s)	360 \pm 62	273 \pm 44	0.5310
Average duration of first probe (s)	163 \pm 51	249 \pm 53	0.1620
Average duration of all probes (s)	405 \pm 61	540 \pm 55	0.0695
Average # of archlets	2.4 \pm 0.3	3.3 \pm 0.2	0.0117
Average duration of phase II-1 (s)	1.255 \pm 0.022	1.227 \pm 0.023	0.2304
Average duration of phase II-3 (s)	1.252 \pm 0.037	1.610 \pm 0.058	<0.0001
Average # of probes	3.6 \pm 0.4	3.8 \pm 0.4	0.7600
Average # pds	5.8 \pm 1.1	6.7 \pm 0.6	0.0211

Table 8. Differences in GPA feeding behaviors on Davis induced with previous SBL herbivory and control plants. (mean \pm se)

Induction Type	Treatment		<i>P</i> -value
	SBL	UTC	
Mean time to 1 st probe (s)	276 \pm 58	307 \pm 49	0.4265
Mean time to 1st pd (s)	384 \pm 74	636 \pm 48	0.6633
Mean duration of 1 st probe (s)	135 \pm 48	105 \pm 31	0.6198
Mean total probe duration (s)	389 \pm 60	344 \pm 48	0.6073
Mean # of archlets per probe	3.39 \pm 0.3	3.48 \pm 0.3	0.8501
Mean duration of phase II-1 (s)	1.305 \pm 0.025	1.394 \pm 0.025	0.1222
Mean duration of phase II-3 (s)	1.870 \pm 0.081	2.214 \pm 0.107	0.0149
Mean # of probes per 20 min	3.0 \pm 0.3	2.6 \pm 0.3	0.3836
Mean # pds per probe	2.6 \pm 1.0	3.6 \pm 0.6	0.0821

Table 9. Differences in GPA feeding behaviors on Lyon induced with previous SBL herbivory and control plants. (mean \pm se)

Induction Type	Treatment		<i>P</i> -value
	SBL	UTC	
Mean time to 1 st probe (s)	404 \pm 89	362 \pm 96	0.8428
Mean time to 1st pd (s)	491 \pm 35	536 \pm 129	0.7920
Mean duration of 1 st probe (s)	55 \pm 12	107 \pm 40	0.5332
Mean total probe duration (s)	168 \pm 41	204 \pm 45	0.5222
Mean # of archlets per probe	3.6 \pm 0.4	3.8 \pm 0.8	0.7682
Mean duration of phase II-1 (s)	1.199 \pm 0.054	1.251 \pm 0.034	0.5933
Mean duration of phase II-3 (s)	1.933 \pm 0.178	2.355 \pm 0.263	0.2941
Mean # of probes per 20 min	2.5 \pm 0.4	2.4 \pm 0.4	0.7347
Mean # pds per probe	3 \pm 0.5	3.5 \pm 0.6	0.6847

JA Induction

JA application significantly increased the duration of phase II-3 in Progeny 4906RR ($df = 1$, $\chi^2 = 21.07$, $P < 0.0001$) (Table 10) as well as the average duration of all probes ($df = 1$, $\chi^2 = 9.29$, $P = 0.0023$) and the duration of phase II-1 in cv. Davis ($df = 1$, $\chi^2 = 13.7282$, $P = 0.0002$) (Table 11), but had no effect in Lyon (Table 12).

Table 10. Differences in GPA feeding behaviors on Progeny 4906RR induced with JA and control plants. (mean \pm se)

Induction Type	Treatment		<i>P</i> -value
	JA	UTC	
Mean time to 1 st probe (s)	188 \pm 32	191 \pm 33	0.9597
Mean time to 1st pd (s)	314 \pm 45	302 \pm 45	0.7601
Mean duration of 1 st probe (s)	165 \pm 43	133 \pm 40	0.2659
Mean total probe duration (s)	483 \pm 52	408 \pm 57	0.1790
Mean # of archlets per probe	4.29 \pm 0.24	4.3 \pm 0.3	0.9196
Mean duration of phase II-1 (s)	1.431 \pm 0.020	1.397 \pm 0.017	0.3961
Mean duration of phase II-3 (s)	2.114 \pm 0.075	1.732 \pm 0.067	<0.0001
Mean # of probes per 20 min	3.6 \pm 0.3	2.8 \pm 0.2	0.0965
Mean # pds per probe	5.4 \pm 0.6	6.8 \pm 1.6	0.9948

Table 11. Differences in GPA feeding behaviors on Davis induced with JA and control plants. (mean \pm se)

Induction Type	Treatment		<i>P</i> -value
	JA	UTC	
Mean time to 1 st probe (s)	250 \pm 40	236 \pm 42	0.8299
Mean time to 1st pd (s)	469 \pm 53	363 \pm 85	0.0802
Mean duration of 1 st probe (s)	201 \pm 42	102 \pm 38	0.0718
Mean total probe duration (s)	430 \pm 51	220 \pm 41	0.0023
Mean # of archlets per probe	3.3 \pm 0.3	3.4 \pm 0.3	0.9805
Mean duration of phase II-1 (s)	1.443 \pm 0.022	1.300 \pm 0.033	0.0002
Mean duration of phase II-3 (s)	1.970 \pm 0.093	2.040 \pm 0.128	0.6593
Mean # of probes per 20 min	2.6 \pm 0.0	2.8 \pm 0.3	0.5346
Mean # pds per probe	3.5 \pm 0.7	2.3 \pm 0.6	0.0590

Table 12. Differences in GPA feeding behaviors on Lyon induced with JA and control plants. (mean \pm se)

Induction Type	Treatment		<i>P</i> -value
	JA	UTC	
Mean time to 1 st probe (s)	294 \pm 66	249 \pm 59	0.4109
Mean time to 1st pd (s)	413 \pm 94	438 \pm 104	0.8688
Mean duration of 1 st probe (s)	40 \pm 11	67 \pm 21	0.3854
Mean total probe duration (s)	103 \pm 22	274 \pm 63	0.2260
Mean # of archlets per probe	3.5 \pm 0.4	3.5 \pm 0.3	0.8878
Mean duration of phase II-1 (s)	1.307 \pm 0.045	1.320 \pm 0.046	0.6936
Mean duration of phase II-3 (s)	2.406 \pm 0.232	2.280 \pm 0.171	0.6936
Mean # of probes per 20 min	2.8 \pm 0.4	3.3 \pm 0.4	0.2592
Mean # pds per probe	2.3 \pm 0.3	3.6 \pm 0.3	0.4598

SA Induction

Exogenous SA applied to cv. Davis significantly increased the average duration of the first probe ($df = 1$, $\chi^2 = 4.34$, $P = 0.0371$) (Table 13). SA applied to Progeny 4906RR increased average probe duration ($df = 1$, $\chi^2 = 6.75$, $P = 0.0094$), average II-3 duration ($df = 1$, $\chi^2 = 8.38$, $P = 0.0038$), and the average number of probes ($df = 1$, $\chi^2 = 5.04$, $P = 0.0248$) (Table 14). SA applied to cv. Lyon only increased average phase II-1 duration ($df = 1$, $\chi^2 = 29.22$, $P < 0.0001$) (Table 15).

Table 13. Differences in GPA feeding behaviors on Progeny 4906RR induced with SA and control plants. (mean \pm se)

Induction Type	Treatment		P-value
	SA	UTC	
Mean time to 1 st probe (s)	255 \pm 50	350 \pm 78	0.5114
Mean time to 1st pd (s)	447 \pm 65	386 \pm 81	0.4542
Mean duration of 1 st probe (s)	228 \pm 43	182 \pm 66	0.1796
Mean total probe duration (s)	509 \pm 57	287 \pm 69	0.0094
Mean # of archlets per probe	3.5 \pm 0.3	3.1 \pm 0.8	0.4591
Mean duration of phase II-1 (s)	1.470 \pm 0.027	1.496 \pm 0.043	0.5110
Mean duration of phase II-3 (s)	2.794 \pm 0.141	2.061 \pm 0.159	0.0038
Mean # of probes per 20 min	2.6 \pm 0.3	1.8 \pm 0.3	0.0248
Mean # pds per probe	2.6 \pm 0.5	2.1 \pm 0.7	0.5437

Table 14. Differences in GPA feeding behaviors on Davis induced with SA and control plants. (mean \pm se)

Induction Type	Treatment		P-value
	SA	UTC	
Mean time to 1 st probe (s)	356 \pm 57	209 \pm 56	0.0371
Mean time to 1st pd (s)	580 \pm 79	424 \pm 98	0.1409
Mean duration of 1 st probe (s)	262 \pm 50	219 \pm 70	0.1439
Mean total probe duration (s)	356 \pm 53	368 \pm 70	0.8075
Mean # of archlets per probe	3.8 \pm 0.7	3.6 \pm 0.5	0.6333
Mean duration of phase II-1 (s)	1.570 \pm 0.035	1.505 \pm 0.031	0.1393
Mean duration of phase II-3 (s)	2.118 \pm 0.175	2.135 \pm 0.144	0.2670
Mean # of probes per 20 min	1.9 \pm 0.2	2.4 \pm 0.3a	0.1518
Mean # pds per probe	2 \pm 0.5	2.6 \pm 0.7	0.3502

Table 15. Differences in GPA feeding behaviors on Lyon induced with SA and control plants. (mean \pm se)

Induction Type	Treatment		<i>P</i> -value
	SA	UTC	
Mean time to 1 st probe (s)	361 \pm 76	390 \pm 106	0.9059
Mean time to 1st pd (s)	473 \pm 71	476 \pm 120	0.6931
Mean duration of 1 st probe (s)	193 \pm 71	79 \pm 30	0.6362
Mean total probe duration (s)	344 \pm 72	199 \pm 57	0.3912
Mean # of archlets per probe	4.7 \pm 0.6	4.8 \pm 0.4	0.8331
Mean duration of phase II-1 (s)	1.710 \pm 0.043	1.361 \pm 0.029	<0.0001
Mean duration of phase II-3 (s)	2.209 \pm 0.166	2.351 \pm 0.232	0.8572
Mean # of probes per 20 min	2.0 \pm 0.3	1.9 \pm 0.3	0.9211
Mean # pds per probe	3.0 \pm 0.7	2.4 \pm 0.6	0.9125

DISCUSSION

The results from the SBL feeding assays demonstrate that herbivory can induce a systemic response soybean. This supports the results of previous studies in soybean showing an induced response to herbivory in soybean (Underwood, 1998, Felton et al. 1994). It also shows that plant response to induction varies by both genotype and stimulus.

Progeny 4906RR expresses systemic inducible resistance, suggesting other commercial varieties may also do so. It may be advantageous to test commercial varieties for inducible resistance, for while varieties such as Lyon and Davis provide useful information about soybean's resistance to herbivores; they often possess undesirable traits that make them unfit for commercial production. Many cultivars have lower yields than commercial varieties (Stout and Davis, 2009). Both Davis and Lyon are MG VI, later maturing varieties that perform poorly compared to earlier maturing varieties in the southern United States due to late season defoliators and other late season pressures (Baur et al. 2000).

It is important to better understand crop responses to herbivory, as this may lead to better pest management strategies such as variety-specific economic thresholds. Alternately, induced resistance may pose a fitness cost in commercial crops (such as induced susceptibility), which must be factored into any pest management strategy. Progeny 4906RR induced with SA did not increase SBL larval weights. This suggests that there may be little crosstalk between the SA and JA pathway in this variety. This is curious, particularly compared to SA's effect on aphid feeding behavior. It also suggests that inducing plants with SA mimics, such as BTH, in the field may have little effect on SBL damage. SA may have other effects on SBL: a previous study showed that soybean induced with BTH induced has an antixenotic effect on bean leaf beetle (Srinivas

and Danielson, 2001). Further research is required to determine if this is the case, and what effect SA has on SBL in the field.

Davis has very little insect resistance (Hatchett, 1976), so it is interesting that JA induced a systemic defensive response in Davis, but SBL did not. This suggests that SBL may be able to bypass whatever resistance present in Davis. Perhaps there is a compound in SBL saliva that shuts down the defensive response. Herbivore saliva has been shown to upregulate defensives in soybean (Felton, 1994), but compounds in saliva have also been shown to shut down plant defenses. Glucose oxidase in *H. zea* saliva suppresses the production of nicotine in tobacco (Musser et al, 2005). In comparison, exogenous JA directly activated herbivore defensive pathways in soybean (Accamando and Cronin, 2012). SBL is well adapted to feed on soybean, and thus might be adapted to the defensive responses produced by Davis. Previous work (Gordy, 2013) suggests that SBL may be less responsive to soybean defenses than other lepidopterans, such as the fall armyworm. Future research is required to determine if SBL induces a defensive response in this variety, and if so, what effect this response has on other herbivores, specifically other lepidopteran pests. The lack of a response to SA is also curious. This may indicate a lack of crosstalk between SA and JA. Davis is a fungus resistant variety (Caviness and Walter, 1966), and may constitutively produce high levels of SA. Thus, the addition of further SA may not reduce its already low insect resistance. This may also explain why SA had no effect on aphid feeding behaviors.

It is unknown why neither SBL feeding nor exogenous JA had any effect in Lyon. Lyon is constitutively resistant to SBL feeding (Hartwig et al. 1994), and it is possible that it constitutively expresses high levels of JA-controlled resistance, and that it is not able to increase this level of resistance. Therefore, stimuli that increase JA levels are not capable of increasing

this level of resistance. This is supported by the increased larval weights of SBL in the SA treatment. Crosstalk from SA may lower the levels of JA in Lyon, and decreases its insect resistance. This, however, does not explain why JA application decreased aphid probing: perhaps as Lyon was bred as an SBL resistant variety, it is not capable of increasing its resistance to that herbivore, but is capable of increasing its resistance to other herbivores. Alternately, Lyon may mount a localized defensive response to SBL, but not a systemic response; however, this is not supported by SA inducing a systemic response.

The effect of variety on the strength of an induced response has been poorly explored in agriculture. Underwood et al. (2000) explored the effect of Mexican bean beetle induction on 14 soybean varieties and found significant differences in the induced response between varieties. Of the three varieties in this study, only in the JA treatment was there significant differences between the varieties in the weights of SBL fed control and treated plants. This suggests that there are varietal differences in the strength of an induced response, a result also found by Underwood et al. (2002). Neither SBL nor SA had a significant varietal effect, further suggesting that the induced response in these three varieties varies by induction method.

Previous SBL herbivory decreased the number of archlets, the duration of phase II-3, and number of potential drops in Progeny 4906RR as well as the duration of phase II-3 in Davis. This suggests that lepidopteran herbivory may have a negative effect on aphids' ability to acquire nonpersistent viruses. This agrees with previous research has indicated that defenses induced by lepidopteran pests can affect other pests (Stout et al. 1998). Thus, it may be possible that lepidopteran herbivory may reduce virus transmission in the field. However, as SBL herbivory did not affect the number or duration of GPA probes the effect of lepidopteran herbivory on aphid fitness is unknown.

Exogenous JA significantly increased the duration of phase II-3 in Progeny 4906RR and both the average duration all probes and of phase II-1 in Davis. This suggests that aphids feeding on treated Progeny plants have a greater chance to acquire nonpersistent viruses due to the longer virus acquisition phase. It also suggests that aphids feeding on treated Davis plants have a greater chance to transmit viruses both due to the increase length of the virus transmission phase of the cellular puncture and increased time probing leading to a greater chance for cellular punctures. These results indicate that exogenous JA application may increase virus transmission by aphids in some varieties. JA's effect on aphid fitness, however, is unclear. Previous research showed that JA treatments can reduce aphid fecundity on tomato (Boughton, et al. 2006). Additionally, another study found that methyl jasmonate seed treatments lead to more, shorter probes in wheat (Cao et al. 2013). While Cao et al. (2013) did not look for cellular punctures, Montllor and Tjallingii (1989) also found more but shorter probes in resistant lettuce compared to a susceptible cultivar. Aphids feeding on resistant lettuce also performed fewer cellular punctures.

It is unknown why SBL herbivory induced a systemic response that reduced nonpersistent virus transmission related behaviors in GPA, while JA increased these behaviors. This suggests that there is a component induced by herbivory that is not induced by JA application. There is precedent for this: it is already known that different feeding guilds induce different responses in soybean (Felton et al. 1994). Additionally, other research has shown that herbivore saliva induces different responses in plants (Ferry et al. 2004), and that different herbivores induce different volatiles (Pickett et al. 1999). This suggests that it is not surprising that SBL induces a different defensive response from soybean than JA does.

Exogenous SA increased the number of and average duration of probes, as well as the duration of phase II-3 in Progeny 4906RR indicating that SA may increase virus acquisition in

this variety. SA also increased the duration of the virus transmitting phase II-1 in Lyon. SA induction significantly increased the duration of the first probe on Davis. These results are surprising, as SA often regulates defenses against aphids (Ferry et al. 2004). One explanation for these results may be that JA and SA both contribute to plant defense against aphids in many plants, including tomato, wheat, and *Arabidopsis* (Stout et al. 1998, Cao et al. 2013, and Ellis et al. 2002 respectively). As aphids have a unique relationship with the plants they feed on (Smith and Boyko, 2006) perhaps changing the level of one of these hormones disrupts aphid resistance mechanisms. Alternatively, both JA and SA may change plant chemistry in a way that makes cell contents more palatable. These results suggest that it is possible that the application of JA, SA, or similar elicitors may increase behaviors associated with virus transmission and this interaction is dependent on genotype.

These results shed new light on the relationships between plants and herbivores. The effects of JA and SA on SBL herbivory are more nuanced than presented in Fig. 1. JA decreases SBL larval weight, while SA increases it or has no effect. Both JA and SA increase behaviors associated with nonpersistent virus transmission in aphids. All of these effects, however, were still influenced by variety. These updates are seen in Fig. 2, which expands on the relationships described in Fig. 1, showing the effects of plant hormones on SBL larval weight and aphid feeding behavior. These results also raise questions further questions about the role of plant hormones in induced HPR and the specific defensive responses of different varieties.

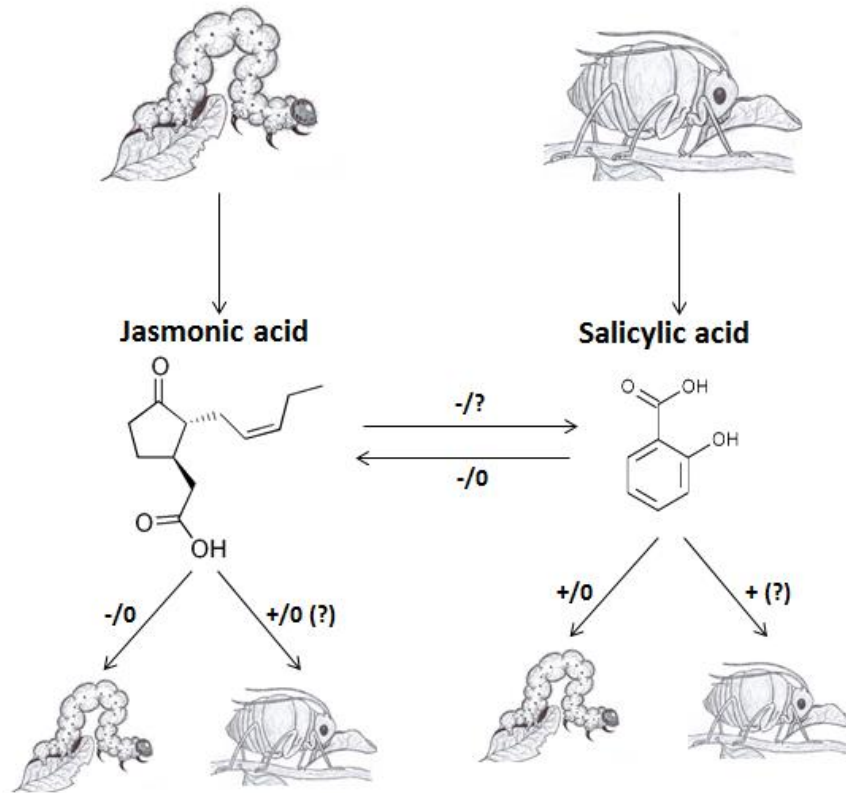


Figure 2: Relationship between necrotrophic (caterpillar) and biotrophic (aphid) insect pests, their effect on plant hormonal pathways and future herbivory based on the results of this research. Arrows and signs indicate the presumed effect on herbivores and elicitors. Arrows pointing to aphids show the effect of JA and SA on nonpersistent virus transmission related behaviors in aphids.

SUMMARY AND CONCLUSIONS

This research found that SBL herbivory, JA, and SA can induce a response in soybean, and that this response depends on plant variety. While the Progeny 4906RR invoked a defensive response to induction with SBL herbivory and exogenous JA, and Davis responded to JA, Lyon did not respond to either stimuli. However, SA decreased Lyon's resistance to SBL herbivory, while the other two varieties showed no significant response, suggesting that SA has little effect on soybean's resistance to SBL in these varieties.

Previous SBL herbivory decreased behaviors associated with virus nonpersistent transmission in GPA feeding on both Progeny P4906RR and Davis, but not those feeding on Lyon. In contrast, exogenous JA increased these behaviors in these two varieties, but not in Lyon, and SA increased these behaviors in all three varieties. This suggests that lepidopteran herbivory may induce systemic defensive responses that reduce nonpersistent virus transmission, while exogenous JA and SA may increase susceptibility to virus transmission.

Overall, this research focused on two separate poorly studied aspects of induced host plant resistance: the effect of variety on induction, and the effect of induction on virus transmission behavior in aphids. This research suggests that variety has a significant effect on a plant's response to herbivory, as well as how herbivores respond to an induced plant.

Soybean is capable of mounting an induced defensive response to lepidopteran herbivory, foliar jasmonic acid and salicylic acid application. This response is variable based on plant genotype. This underscores the importance of variety in HPR. It also suggests that experiments on induced resistance should use a wide selection of varieties and cultivars in order to understand the effect of genotype on the induced response. Future studies may also use different herbivores,

including both lepidopteran pests and those from other orders to determine what effect they have on induction.

This research also suggests that induction with elicitors may increase the likelihood of virus transmission by aphids. It is curious that both JA and SA application increase behaviors associated with virus transmission in aphids, which is unexpected as both hormones are involved in aphid resistance. Further research may uncover the physiological reason for this. These results suggest that the application of JA or SA may increase virus transmission in the field: it is also possible that insecticides that induce similar responses, such as neonicotinoids (Ford et al, 2010), may induce similar responses. By determining how elicitors affect the relationship between plants and virus transmitters it will be possible to develop better methods to spread the control of plant viruses.

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VITA

John Lawrence Dryburgh, from King of Prussia, Pennsylvania, Received his bachelor's degree in Biology from Susquehanna University in Pennsylvania in 2012. He entered graduated school in the Department of Entomology at Louisiana State University later that year. He will receive his master's degree in August 2015. He currently resides in Baton Rouge, Louisiana; after graduation he will continue his education in Entomology at Louisiana State University, pursuing a doctorate degree with research focused on sustainable management of sweetpotato pests and pathogens.