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ORIGINAL PAPER

Determining the duration of *Aphis glycines* (Hemiptera: Aphididae) induced susceptibility effect in soybean

Adam J. Varenhorst[1] · Michael T. McCarville[1] · Matthew E. O'Neal[1]

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Abstract Insect herbivores can increase the suitability of host plants for conspecifics by inducing susceptibility. Induced susceptibility can be separated into feeding facilitation, whereby herbivore feeding increases performance of conspecifics regardless of the genotype of the herbivore or plant, and obviation of resistance, whereby feeding by a virulent herbivore increases performance of avirulent conspecifics on resistant plants. Both forms occur between *Aphis glycines* (Hemiptera: Aphididae) and soybean. In natural and agricultural settings, *A. glycines* populations can colonize plants for brief periods before emigrating or being removed due to predation or insecticides. It is unclear if induced susceptibility lasts beyond the period when *A. glycines* are present on the plant. We measured the duration of induced susceptibility in the *A. glycines*-soybean system within a growth chamber by removing inducer populations after 24 h. We used an *A. glycines*-resistant soybean infested with an inducer population of either virulent, avirulent, or no aphids. Response populations of either virulent or avirulent aphids were added at three post-infestation times (24, 120, 216 h) and their densities measured 11 days after infestation. Feeding facilitation was lost within 24 h of the removal of avirulent inducer populations, and obviation of resistance diminished over time and was completely lost within 216 h of the removal of the virulent inducer populations. We discuss how these results support a hypothesis that virulence in *A. glycines* is due to effector proteins secreted by feeding aphids. We suggest that the duration of induced susceptibility may impact the durability of *A. glycines* resistance in soybean.

Keywords Soybean aphid · Induced susceptibility · Feeding facilitation · Obviation of resistance

Introduction

Insect herbivores can directly or indirectly alter the suitability of a host plant for both conspecifics and heterospecifics (Karban and Myers 1989). Such alterations of host plants can be categorized as either negative (e.g., induced resistance) or positive (e.g., induced susceptibility) for subsequent herbivores (Karban and Myers 1989; Price et al. 2011). These herbivore-induced effects in plants may affect initial herbivore survival, fecundity, and/or preference for the host plant, and they may also affect subsequent conspecific or heterospecific herbivore populations (Karban and Baldwin 1997; Price et al. 2011). The duration of either induced resistance or susceptibility vary, classified as either short- or long-term effects, depending upon the plant–insect system studied (Karban and Myers 1989; Karban and Baldwin 1997). In general, short-term responses are elicited by and affect the initial herbivore, while long-term responses are elicited by an initial herbivore (i.e., an inducer population) and affect the survival of herbivores that arrive after the initial herbivore (i.e., a response population) (Karban and Myers 1989; Karban and Baldwin 1997). Some potential causes of induced effects include physical contact, chemical cues, plant viruses, insect endosymbionts, or insect proteins (Schoonhoven

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✉ Adam J. Varenhorst
ajv@iastate.edu

Matthew E. O'Neal
oneal@iastate.edu

[1] Department of Entomology, Iowa State University, 117 Insectary, Ames, IA 50011, USA

et al. 2005; Oliver et al. 2010; Casteel and Jander 2013; Pitino and Hogenhout 2013).

There are several examples of induced susceptibility benefiting aphids (Hemiptera: Aphididae) on a shared host plant. Initial *Acyrthosiphum pisum* Harris (Hemiptera: Aphididae) feeding led to improved suitability of *Vicia faba* (Fabales: Fabaceae) for subsequent *A. pisum* populations (Takemoto et al. 2013). Similar results have been observed regarding *Myzus periscae* (Hemiptera: Aphididae) on *Prunus persica* (Rosales: Rosaceae) (Sauge et al. 2006). At least six other aphid species are recognized as inducing susceptibility in their host plants (Karban and Baldwin 1997). Of these, all are reported as affecting the same generation of aphids, but there are also two for which the induced susceptibility also altered the plant for the next generation of aphids on perennial plants (Fisher 1987; Messina et al. 1993). It is unknown whether induced susceptibility is a general phenomenon observed across all aphid–plant systems or is the result of specialization. Adding to the growing list of cases, in which induced susceptibility occurs, especially for aphids that are economic pests of crops, may lead to better pest management. Shedding light on this phenomenon may also improve our understanding of aphid resistance in crop plants, and the nature of virulence in biotypes that can survive on resistant crop varieties.

For example, *Aphis glycines* is an invasive pest of soybean in North America that can greatly reduce yield (Ragsdale et al. 2011). Soon after the discovery of *A. glycines* in the USA, soybean breeders discovered several genes that confer resistance to *A. glycines* (i.e., *Rag* genes) in the soybean germplasm (reviewed in Ragsdale et al. 2011). In a laboratory setting, the response of resistant and susceptible soybean to *A. glycines* infestation differed, with resistant plants responding more rapidly to infestations than susceptible plants (Studham and MacIntosh 2013). When tested in the field, *Rag*-containing plants consistently have fewer *A. glycines* than aphid-susceptible cultivars, but resistant cultivars are rarely free of aphids and sometimes support large populations (Hesler et al. 2013) that exceed an economic threshold (McCarville et al. 2014). Despite the genetic bottleneck associated with *A. glycines* arrival in North America (Michel et al. 2011) and the limited commercial use of *Rag* genes in North America (Hesler et al. 2013), several virulent biotypes have been found in the USA (Kim et al. 2008; Hill et al. 2010; Alt and Ryan-Mahmutagic 2013). These virulent biotypes are defined by the specific *Rag* genes on which they can survive. To date, for every *Rag* gene that has been incorporated into a soybean cultivar either alone or in a combination, a virulent biotype has been found in the USA (Kim et al. 2008; Hill et al. 2010; Alt and Ryan-Mahmutagic 2013).

Feeding by *A. glycines* induces susceptibility for subsequent *A. glycines* on both susceptible and resistant soybean varieties (Varenhorst et al. 2015). This induced susceptibility was observed with both avirulent and virulent biotypes and could be divided into two different mechanisms: feeding facilitation (Denno and Benrey 1997) and obviation of resistance (Baluch et al. 2012). For *A. glycines* and soybean, both mechanisms were observed (Varenhorst et al. 2015). For example, feeding facilitation was observed when *A. glycines* populations on aphid-resistant soybean had larger populations after initial herbivory by an inducer population of *A. glycines* when compared to a control lacking an inducer population, regardless of its biotype that resulted in a 714 % increase in population density. Obviation of resistance occurs when a virulent biotype overcomes a plant's resistance such that avirulent biotypes survive as if the plant is susceptible. Obviation of resistance was confirmed by significant increases in avirulent *A. glycines* populations on resistant soybean after initial herbivory by an inducer population of virulent *A. glycines* when compared to controls lacking a virulent inducer population. There was a 2078 % increase in population density between the avirulent response population with no inducer treatment and the avirulent response population with a virulent inducer treatment. Obviation of *Rag* resistance by virulent *A. glycines* resulted in response populations of avirulent populations that were equivalent to that of a virulent response population with a virulent inducer (Varenhorst et al. 2015). The consequences of both of these mechanisms are relevant in light of recent findings by Wenger et al. (2014), which suggest an improvement in fitness for avirulent *A. glycines* on aphid-resistant soybean decreases the relative frequency of virulent biotypes. These results align with the goal of an insect resistance management (IRM) plan to reduce/slow the spread of virulence. The extent that induced susceptibility can support a IRM plan is unknown, though initial modeling suggests that this phenomenon will reduce the frequency of virulent biotypes when a refuge of aphid-susceptible soybean is planted with the aphid-resistant cultivar (Varenhorst et al. 2015).

In the previous experiments (Varenhorst et al. 2015) with *A. glycines*, plants were co-infested with both an inducer population and a subsequent response population. In natural and agricultural settings, aphids form colonies on plants for brief periods before leaving due to emigration, predation, or an insecticide application. Subsequent re-colonization can occur, often with populations that exceed the densities of the previous colony. This is typically referred to as pest resurgence, and in the case where insecticides are used, a function of the removal of aphid predators (Pedigo and Rice 2009). It is not known if the

impact of an inducer population on a soybean plant will persist if it is removed from the plant, potentially contributing to a pest's resurgence. Our objective was to determine whether induced susceptibility persists in the absence of an inducer population on soybean. The duration of induced susceptibility was tested using the methods developed by Varenhorst et al. (2015). Furthermore, we explored if the two components of induce susceptibility (feeding facilitation and obviation of resistance) differed in their persistence after the inducer population was removed. The design was amended with both a virulent and avirulent biotype of *A. glycines* and a *Rag*-containing soybean variety to explore the persistence and duration of both feeding facilitation and obviation of resistance.

Materials and methods

Aphid colonies and soybean cultivars

Two populations of *A. glycines* from The Ohio State University were used for this experiment. The populations are defined by their response to *Rag1*, an avirulent population (biotype-1) and a virulent population (biotype-2) (Kim et al. 2008). Individuals used to create these populations were initially collected and identified in Illinois (Kim et al. 2008). The avirulent population was raised on aphid-susceptible soybean (IA3027), while the virulent population was raised on a near-isogenic, aphid-resistant soybean containing the *Rag1* gene (IA3027RA1). These two cultivars are near isogenic, sharing approximately 93.75 % genetic identity (Wiarda et al. 2012).

Duration of induced susceptibility effects

We hypothesized that both feeding facilitation and obviation of resistance would persist in soybean after the removal of the initial *A. glycines* populations. We measured the duration of these effects by infesting *Rag1* containing soybean (IA3027RA1) with an initial population of *A. glycines*, termed an inducer population, and allow them to feed for a period of 24 h. After 24 h, the inducer population was removed using a fine tip paintbrush, and a subsequent population of *A. glycines*, termed response populations, was infested. The response populations were defined by the time between the removal of the inducer population and their infestation (post-infestation interval, or PII). The response population densities were measured 11 days after being added to plants, a time span that allows for the production of two generations of *A. glycines* (McCornack et al. 2004). Table 1 outlines the timing of these events. During the 11-day period, alates of *A. glycines* were not observed.

Table 1 Sequence of events for legacy effect experiment

Event	24 h	120 h	216 h
Planting	Day 1	Day 1	Day 1
Infestation of inducer[a]	Day 17	Day 17	Day 17
Removal of inducer	Day 18	Day 18	Day 18
Infestation of response[b]	Day 18	Day 22	Day 26
Counting of response	Day 29	Day 33	Day 37

[a] Inducer populations consisted of 50 avirulent, 50 virulent, or no *A. glycines*

[b] Response populations consisted of 5 avirulent *A. glycines*

To test our hypothesis, we used nine treatments. Each treatment was a combination of two factors, inducer populations and response population infestation time. The three inducer populations used were: no inducer (none), 50 avirulent *A. glycines* (avirulent), and 50 virulent *A. glycines* (virulent). Three response infestation times used: 24, 120, and 216 h PII. Inducer populations of either no aphids, biotype-1, or biotype-2 *A. glycines* nymphs were applied to the first full trifoliate of individual potted plants when the plants reached the second trifoliate growth stage. Each individual potted plant was enclosed within a mesh net to prevent plant-to-plant movement of either the inducer or the subsequent response population. After 24 h the inducer populations were removed from all of the previously infested plants using a fine tip paintbrush. Inducer populations remained on the first full trifoliate for the 24-h period, although they were not caged onto the trifoliate. Varenhorst et al. (2015) determined that the maximum effect of induced susceptibility occurred with an inducer population of 50 *A. glycines*. Therefore, to determine the duration of induced susceptibility, inducer populations of 50 avirulent and 50 virulent *A. glycines* were used. Both inducer population and response population were compromised of mixed aged *A. glycines* nymphs.

Infestations of the response population were applied at three intervals, defined by the time between the removal of the inducer population and the infestation of the response population. These treatments occurred at 24, 120, and 216 h PII. Response populations were added to the second full trifoliate of each plant and consisted of five avirulent *A. glycines* that were allowed to move freely about the plant. The total number of *A. glycines* present in each response population was counted 11 days after the response population was infested. We measured both the presence and length of induced susceptibility by adding response populations at various times after the removal of the inducer population (Table 1).

Each experimental unit (i.e., potted plant) was grown in 16-cm diameter pots in a Percival E41L2C9 growth

chamber (Percival Scientific, Incorporated, Perry, IA) using a 14:10 light/dark cycle and a constant temperature of 27 °C with a relative humidity of 60 %. Each of the experimental units received one of the nine treatment combinations. This experiment was repeated twice in a growth chamber using a randomized complete block design with three blocks per repetition (six total experimental units per treatment).

Statistical Analysis

To address our a priori hypotheses, we analyzed the number of *A. glycines* per plant in the response population at 11 days after plants were infested with response populations. Data were analyzed separately for each PII time point. To reduce heteroscedasticity, the *A. glycines* per plant data were log transformed. All data were analyzed using the PROC MIXED procedure with SAS statistical software version 9.3 (SAS Institute, Cary, NC). The impact of each treatment factor was determined using an analysis of variance (ANOVA). The statistical model used to analyze data for each of the PII included the fixed effect of inducer population treatment. The random effects included repetition, inducer population treatment × repetition, and block(repetition). We tested for the significance of all random effects using a log-likelihood ratio statistic (−2RES Log Likelihood). The log-likelihood statistic follows an approximate χ^2 distribution with one degree of freedom, and was used to determine whether inclusion of each random effect significantly improved model fit over the null model (Littell et al. 2002).

The duration of the induced susceptibility effects was determined by comparing the effect of the inducer population treatment factor on the response population abundance at each PII. If we determined that induced susceptibility was present, we next tested whether it was due to the effect of feeding facilitation, or obviation of resistance. We tested for these effects using contrast statements within PROC MIXED using the same model as previously described with a significance level of $P < 0.05$. At each PII time point, the inducer population treatments were simultaneously compared. Feeding facilitation was confirmed if the response population on the avirulent inducer treatment was significantly greater than the response population of the no inducer control (Denno and Benrey 1997; Varenhorst et al. 2015). Obviation of resistance was confirmed if the response population of the virulent inducer treatment was significantly greater than the response population of the avirulent inducer treatment (Baluch et al. 2012; Varenhorst et al. 2015).

Results

Duration of induced susceptibility

We observed significant differences in the abundance of a response population with variation in the inducer population at the 24 and 120 h, but not the 216 h PII (Table 2). This was observed by analyzing data for the significance of a fixed effect of inducer population treatment for each of the PII levels. In the 24 h PII experiment, we observed a significant interaction between repetition and inducer treatment. This interaction was apparent when we compared the averages from the two repetitions from the 24 h PII experiment for the treatments receiving an inducer population compared to the no inducer treatment. There was little variation between the two repetitions for the no inducer treatment (an average difference of 2 aphids per plant). For the avirulent and virulent inducer treatments, we observed an average difference of 17 and 75 aphids per plant, respectively, between the two repetitions. Despite this interaction, we consistently observed more aphids on plants that received avirulent aphids as an inducer population than no inducer in both repetitions, and plants that were assigned virulent aphids as an inducer consistently had more aphids than either of the other two treatments.

Because the inducer population significantly affected the response populations at the 24 and 120 h PII, we compared the impact of the various inducer population treatments at each PII. The response population on plants receiving an avirulent inducer population (i.e., avirulent treatment) was significantly greater than on plants that did not have an inducer population (i.e., none treatment) at 24 h PII ($F = 128.63$; $df = 1, 2$; $P < 0.0077$) (Fig. 1; 24 h PII), but not at 120 h PII or 216 h PII. The response population for the virulent treatment was significantly greater than that of the response population for the avirulent treatment at 24 h PII ($F = 843.04$; $df = 1, 2$; $P < 0.0012$) (Fig. 1; 24 h PII) and 120 h PII ($F = 42.92$; $df = 1, 2$; $P < 0.0225$) (Fig. 1; 120 h PII). At 216 h PII there was no significant differences among the treatments. Therefore, induced susceptibility effects were observed for the avirulent treatment at 24 h PII, and for the virulent treatment at 24 h PII and 120 h PII.

Discussion

Our results demonstrate that *A. glycines* feeding alters resistant soybean such that it is more susceptible to future infestations of conspecifics. The length of time this effect

Table 2 Analysis of variance tables of treatment effects

Effect	Fixed/Random	*df*	*F* statistic[a]/χ^2
24 h PII[b]			
Repetition	R	1	0.20
Block (repetition)	R	1	1.00
Inducer population	F	2, 2	867.31**
Repetition × inducer population	R	1	4.30*
120 h PII			
Repetition	R	1	0.40
Block (repetition)	R	1	5.10*
Inducer population	F	2, 2	50.65*
Repetition × inducer population	R	1	1.20
216 h PII			
Repetition	R	1	0.20
Block (repetition)	R	1	0.00
Inducer population	F	2, 2	4.83
Repetition × inducer population	R	1	0.50

[a] An F statistic was used to test for the significance of fixed effects, while a χ^2 test was used for random effects

[b] Post-inducer population infestation

* Significant effect at $P < 0.05$, **$P < 0.01$, ***$P < 0.0001$

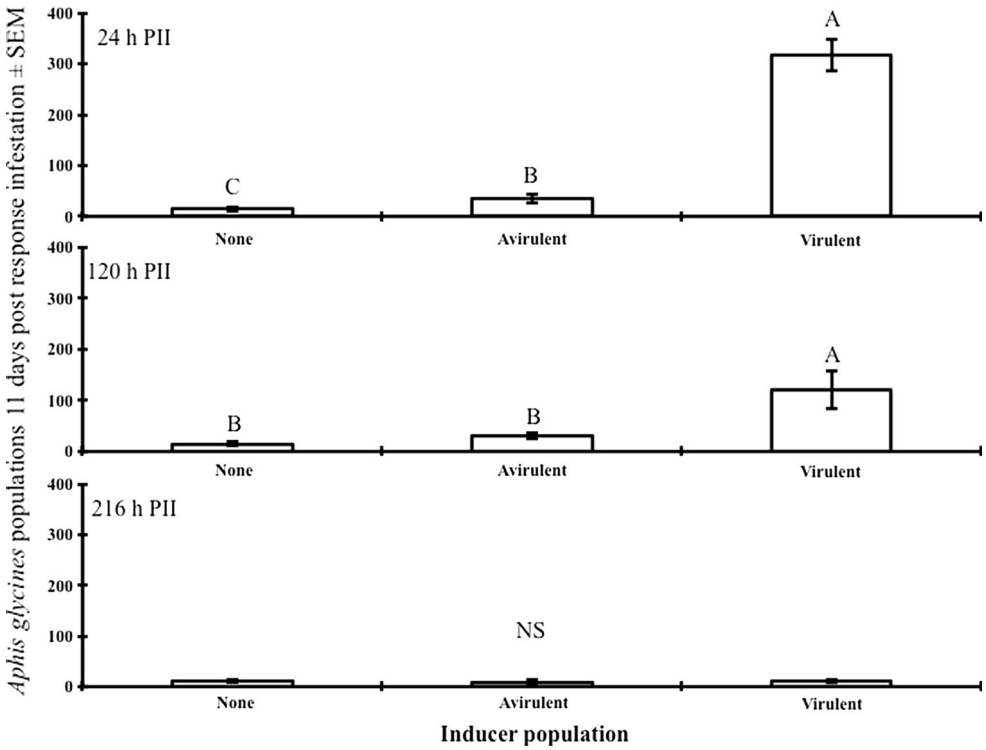

Fig. 1 The duration of induced susceptibility effects was measured in a growth chamber experiment. Aphid-resistant plants were infested with an inducer population of either virulent, avirulent, or no aphids for 24 h and then removed from plants. Response populations were then added at three post-inducer infestation times (24 h PII, 120 h PII, and 216 h PII). *Capital letters* indicate significance among treatments ($P < 0.05$)

lasts after the inducer population of *A. glycines* is removed varies by aphid biotype (i.e., virulence). Increases in *A. glycines* populations due to an inducer population (i.e., induced susceptibility) occurred in two ways, by feeding facilitation (Denno and Benrey 1997; Price et al. 2011) or obviation or resistance (Baluch et al. 2012). Feeding facilitation was observed when the response population increased after the plant experienced herbivory from an avirulent inducer treatment compared to the control where no inducer was present. Feeding facilitation was only

observed at 24 h PII. Obviation of resistance was observed when the response population increased on the virulent inducer treatment compared to the avirulent inducer treatment, which occurred at 24 and at 120 h PII but with diminished impact. There was no evidence of either feeding facilitation or obviation of resistance at 216 h PII for any of the treatments. Therefore, we conclude that in the absence of the inducer population the effect of feeding facilitation persists for 24 h and the effect of obviation resistance persists for at least 120 h. Based on these results, the observed feeding facilitation effect is best described as a short-term induced effect. The obviation of *Rag* resistance persisted for at least 120 h and would possibly affect subsequent *A. glycines* and therefore is best described as a long-term induced effect (Karban and Baldwin 1997; Price et al. 2011).

Our experiment was not designed to compare the impact of induced susceptibility at different time points. For example we cannot compare the treatment across PII levels. However, we observed an interesting change in the difference between treatments receiving no inducer versus an avirulent inducer between the 24 and 120 PII levels: this difference was significant at the 24 h PII but not 120 PII. This difference suggests that feeding facilitation did not occur at 120 h PII level. But between the two PII levels, there was only a difference of 4 aphids per plant between the avirulent treatments at 24 and 120 PII. It may be that we were unable to observe feeding facilitation at the 120 h PII level because of insufficient statistical power. Regardless of whether feeding facilitation still occurs 120 h after an inducer population was removed, we note that our experimental design was sufficient to observe a difference between the virulent and avirulent inducer treatments. We suggest that this reflects a greater impact of obviation of resistance on the plants' physiology compared to feeding facilitation.

Based on the duration of the obviation of resistance, we hypothesize a mechanism responsible for this effect. There are several factors that can explain how the physiology of a plant can be altered by aphids, including endosymbionts (Oliver et al. 2010), viruses (Mauck et al. 2012; Casteel and Jander 2013), and effector proteins found in salivary excretions (Rodriguez and Bos 2013). These factors may help explain how an avirulent aphid could survive on a resistant plant that is co-infested with a virulent biotype. For example, virulence could be due to the presence of specialized endosymbionts (Oliver et al. 2010). However, we did not observe evidence of the horizontal transmission of endosymbionts between the virulent and avirulent populations (i.e., virulence was not observed at each PII time point). In addition, endosymbiotic bacteria are unlikely to be the cause of obviation of resistance as horizontal transmission of bacteria is rare. Also our inducer and response populations were temporally and spatially separated on the soybean plant making horizontal transmission even less probable (Oliver et al. 2010).

In a review, Mauck et al. (2012) describe the potential for plant viruses to affect aphid settling and feeding preferences resulting in greater attraction to a host plant. In contrast to non-persistently transmitted viruses, persistently transmitted plant viruses have the potential to make the host plant more suitable and promote long-term feeding. Persistently transmitted viruses generally are acquired through extended feeding bouts and benefit from vector settling. Plant virus infection is unlikely to be the cause of the observed obviation of resistance as the only persistently transmitted soybean virus in North America is *Soybean dwarf virus* (Hartman 1999), which is rarely vectored by *A. glycines* (Harrison et al. 2005; Wang et al. 2006; Damsteegt et al. 2011). Additional evidence that a plant virus is unlikely responsible for the obviation of resistance is the reduction in the response populations at 120 and 216 h for the virulent inducer population treatment. This observation is not consistent with results from other studies of plant virus infection on aphid populations in which the virus infection improved aphid populations for up to one week post-infection (Casteel et al. 2014). Due to the asymptomatic nature of our plants and the reduction of the effect over time, we conclude that a plant virus was not the cause of the obviation of resistance.

We suggest that effector proteins are the most probable explanation of obviation of resistance. Previous research has indicated that aphid effector proteins are capable of suppressing host plant defense pathways and modulating a range of host cell processes (Hogenhout and Bos 2011; Pitino and Hogenhout 2013; Rodriguez and Bos 2013). Pitino and Hogenhout (2013) demonstrated that the impact of aphid effector proteins vary by aphid species. On *Arabidopsis* (Brassicaceae), homologs of effector proteins from *A. pisum,* a specialist of plants in the Fabaceae family, did not improve reproduction of *M. periscae,* a generalist capable of utilizing plants from multiple families. In contrast, expression of *M. periscae* effector homologs did result in increased *M. periscae* reproduction. Both enzymes and binding proteins are present in the saliva of aphids and are potential explanations for how aphids influence the host plant's defense response to herbivory (Will et al. 2007; Harmel et al. 2008; Hogenhout and Bos 2011). Aphid effector proteins may possibly explain the differences in aphid specialization (i.e., diet breadth) and also biotypic variation within an aphid species in the form of virulence to aphid-resistant traits (Rodriguez and Bos 2013). Our hypothesis is further supported by Bansal et al. (2014)'s discovery of 47 protein transcripts present in *A. glycines* that matched effectors present in *A. pisum* with known functions. Finally, the ultimate source of the effector

proteins may not be limited to the aphid. Proteins produced from endosymbiotic bacteria can also be transmitted to the host plant and affect the survival of the aphid host (Chaudhary et al. 2014). However, to date this phenomenon has been limited to an endosymbiont-produced protein (GroEL) that induces resistance in the host plant to aphids. We are unaware of endosymbionts that produce proteins that alter plant physiology such that it is a better host for the aphid. Regardless of the source, the *A. glycines*-soybean system suggests that the impact of these proteins is systemic and alters the plant for at least 120 h.

The short duration and apparent degradation of the effect between 24 h PII and 120 h PII for obviation of resistance further support the role of effector proteins in this aphid–plant system. The decline of obviation of resistance that was observed over time in our experiment may be attributed to an aphid-produced enzyme or protein present in the host plant and its subsequent degradation by the plant (Boyes et al. 1998; Martin et al. 2003). Therefore, we hypothesize that the effect of obviation of resistance is strongest when the inducer and response populations are present on the plant simultaneously, but the effect persists until the putative effector proteins are degraded. This is likely a function of the density of the aphids that are injecting effector proteins and the capacity of the plant to recognize and/or degrade them.

Previous studies in which the transcriptional response of either *A. glycines* or soybean to each other have focused on either the immediate response within a 24-h period or delayed responses of several days. Studham and Macintosh (2013) measured the response of susceptible and resistant soybean to *A. glycines* feeding after plants were infested for 1 or 7 days. Bansal et al. (2014) measured the response of biotype-1 *A. glycines* feeding on *Rag1* soybean after 12 h. Given our results, these studies with avirulent *A. glycines* most likely observed the effect of both feeding facilitation (e.g., avirulent aphids feeding on resistant soybean) and obviation of resistance (e.g., virulent aphids feeding on susceptible soybean). If future studies are focused on the mechanism of how virulent aphids overcome *Rag* resistance, then the amount of time in which the plant is allowed to respond to *A. glycines* feeding should be adjusted to account for only obviation of resistance. Our data suggest that this impact may be most noticeable at 120 h post-infestation.

The results from this paper provide a framework for future research on the mechanism of *A. glycines* virulence. Future work should investigate effector protein candidates and determine the mechanism of these effector proteins as potential targets for novel pest control technologies. We predict that if effector proteins are the cause of the biotypic variation in virulence toward *Rag* genes, then variation within the effector proteins among these biotypes should also be present. This variation may not only be responsible for the virulence of a biotype toward a resistance gene, but may also affect the duration of the obviation of resistance effect (i.e., the legacy of effector proteins may differ by biotype). Finally, the study of induced susceptibility within the *A. glycines*-soybean system has been limited to microcosms within a laboratory setting. The study of this phenomenon in a field setting will have to account for the impact of natural enemies on *A. glycines*, which suffers significant mortality from predators commonly found in North America (Ragsdale et al. 2011).

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