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Feeding Behavior of Aphis glycines (Hemiptera: Aphididae) on Soybeans Exhibiting Antibiosis, Antixenosis, and Tolerance Resistance

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Feeding behavior of *Aphis glycines* (Hemiptera: Aphididae) on soybeans exhibiting antibiosis, antixenosis, and tolerance resistance

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Abstract

Aphis glycines Matsumura (Hemiptera: Aphididae) is a serious pest of soybean in North America. Plant resistance is a valuable tool for the management of this pest, and a better understanding of the interactions between aphid and soybeans expressing varying levels and different categories of resistance can assist in the development of aphid resistant or tolerant genotypes. The electrical penetration graph (EPG) technique was used to evaluate the feeding behavior of *A. glycines* (biotype 1) on 4 soybean genotypes: (1) 'Dowling' (contains *Rag1* gene and exhibits antibiosis); (2) PI 200538 (contains *Rag2* gene and exhibits antixenosis); (3) KS4202 (exhibits tolerance); and (4) 'SD76RR' (susceptible to aphid). Aphids spent shorter periods in the sieve element phase on 'Dowling' and exhibited a greater number of pathway phases, non-probing events, and a longer time in non-probing events in PI 200538 and 'Dowling.' For 'SD76RR' and KS4202, aphids demonstrated more sustained phloem ingestion, spent shorter time in non-probing events, and exhibited fewer pathway phases and potential drops. These results indicate that resistance factors are present in the phloem of 'Dowling.' For PI 200538, it is suggested that antixenotic factors are involved in resistance to *A. glycines*. Because KS4202 is tolerant to biotype 1 of *A. glycines*, the suitability of this genotype was expected already. This study provides important data that contribute to the understanding of how soybean aphids (biotype 1) feed on soybean genotypes with various aphid resistant genes and categories. In addition to assisting in the distinction between resistance categories, these results are useful in soybean breeding programs focusing on developing genotypes with greater resistance to insects.

Key Words: host plant resistance; electrical penetration graph; soybean aphid

Resumo

Aphis glycines Matsumura (Hemiptera: Aphididae) é uma das principais pragas da cultura da soja na América do Norte. Dentre as técnicas de manejo da praga, a resistência de plantas a insetos se destaca como uma ferramenta valiosa. Assim, uma melhor compreensão sobre as interações entre o afídeo e plantas de soja que expressam variáveis níveis e diferentes categorias de resistência, pode auxiliar no desenvolvimento de genótipos resistentes ao inseto. A técnica de EPG foi utilizada para avaliar o comportamento alimentar de *A. glycines* (biótipo 1) em 4 genótipos de soja: (1) 'Dowling' (contém gene *Rag1* e expressa antibiose); (2) PI 200538 (contém gene *Rag2* e expressa antixenose); (3) KS4202 (expressa tolerância); e (4) 'SD76RR' (suscetível ao afídeo). Os afídeos demonstraram curtos períodos de alimentação na fase de seiva em 'Dowling' e exibiram uma grande quantidade de fases de caminhamento estiletar, número de períodos de não-prova e longos período de não-prova em PI 200538 e 'Dowling.' Para 'SD76RR' e KS4202, os afídeos mostraram um maior período de alimentação em vasos do floema, apresentaram curtos períodos de não-prova e exibiram poucas fases de caminhamento estiletar, além de menor número de quedas de potencial. Os resultados indicam que fatores de resistência estão presentes nos vasos floemáticos de 'Dowling.' Para PI200538, sugere-se que fatores antixenóticos estão envolvidos na resistência a *A. glycines*. Uma vez que KS4202 expressa tolerância ao biótipo 1 de *A. glycines*, a adequabilidade deste genótipo como fonte de alimento para o inseto já era esperada. Este estudo fornece importantes dados que contribuem para um melhor entendimento de como o pulgão-da-soja (biótipo 1) se alimentam de plantas de soja portadoras de diferentes genes e categorias de resistência. Além de auxiliar na distinção entre as categorias de resistência, esses resultados podem ser úteis em programas de melhoramento de soja, com intuito de selecionar genótipos mais resistentes a insetos.

Palavras Chave: resistência de plantas a insetos; gráfico de penetração elétrica (EPG); pulgão-da-soja

Aphis glycines Matsumura (Hemiptera: Aphididae), a major pest of soybean, *Glycine max* (L.) Merril (Fabaceae), is an invasive species in the United States. It causes direct and indirect injury by removing photo assimilates from plant vascular tissues, stunting plant growth, reducing photosynthetic rates (Beckendorf et al. 2008), and transmitting plant viruses (Clark & Perry 2002).

Plant resistance has been proposed as a valuable tool for managing *A. glycines* (Hill et al. 2012). Resistance mechanisms are classified into 3

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categories: antixenosis, antibiosis, and tolerance. Antixenosis is defined as a plant defense strategy in which the presence of morphological, physical, or chemical plant traits affect the behavior of arthropods. Antibiosis occurs when plant resistance mechanisms deleteriously affect the biology (physiology) of the arthropod without affecting its behavior. In tolerance, the plant does not cause any biological or behavioral change to the arthropod, but it has the ability to withstand or recover from the attack of the arthropod while maintaining its productive capacity (Smith 2005).

Soybean accessions from the USDA soybean collection have been screened to identify genes that confer resistance to *A. glycines* (Hill et al. 2006). At least 8 resistant soybean lines involving *Rag* (Resistance to *Aphis glycines*) genes linked to 4 chromosomes, numbered from *Rag1* to *Rag6*, have been documented (Hill et al. 2010; Zhang et al. 2017). In 'Dowling,' antibiosis is expressed and is controlled by the dominant gene *Rag1* (Hill et al. 2006). The gene *Rag2* was identified as responsible for antibiosis resistance to *A. glycines* in PI 243540 (Kang et al. 2008), and in PI 200538 (Hill et al. 2009; Kim et al. 2010). Studies evaluating genotypes with both *Rag1* and *Rag2* genes have demonstrated that *A. glycines* development was reduced relative to lines with either *Rag1* or *Rag2* alone (Hill et al. 2006, 2009). From the known *A. glycines* biotypes (defined by the capacity to develop on aphid-resistant soybeans genotypes), biotype 1 is unable to colonize soybean plants containing *Rag1* or *Rag2* (Hill et al. 2010; Chandran et al. 2013), biotype 2 can colonize *Rag1*, but does not colonize *Rag2* soybeans (Kim et al. 2008; Chandran et al. 2013), and biotype 3 is able to colonize *Rag2* plants as well as some others expressing different *Rag* genes (Hill et al. 2010, 2012). In other studies, virulent populations of *A. glycines* (biotype 4) were capable of overcoming either the *Rag1*, *Rag2*, or both genes (Kim et al. 2008; Hill et al. 2010; Alt & Ryan-Mahmutagic 2013).

The electrical penetration graph (EPG), using alternate current (AC), has been used to describe the feeding behavior of insects, and to analyze resistance expression in plant genotypes (Reese et al. 2000; Diaz-Montano et al. 2007; Crompton & Ode 2010; Zhu et al. 2011; Todd et al. 2016). The electrical penetration graph technique is based on an electrical circuit in which an insect and plant are integrated. Once the insect inserts its mouthparts into the plant, the circuit is complete and changes in electrical resistance, and bio-voltages (waveforms) are recorded over time, providing continuous information on feeding and probing phases that can be correlated to insect stylet penetration behavior (Sauvion & Rahbe 1999; Backus & Bennett 2009). The resulting waveforms can provide important information about physical location of plant resistance factors, and the time course of insect responses to these factors (Tjallingii 1985; Jiang et al. 2001).

During probing, the location of *A. glycines* stylets can be established, providing the basis for correlating feeding behavior with interactions within plant tissues, which is useful to determine the location of resistance factors in plant tissues (Jiang et al. 2001). The feeding process of the insects present specific waveforms (Reese et al. 2000). The waveforms described are the sieve element phase (represented by waveform E, and divided in the process of active salivation (E1) and phloem ingestion (E2), xylem feeding (G), pathway phase (P), potential drop (pd), and non-probing (Z) (Todd et al. 2016). The duration of the sieve element phase is longer in susceptible genotypes and shorter in resistant genotypes (Diaz-Montano et al. 2007). Researchers concluded that antibiotic metabolites reside in the phloem tissue (Crompton & Ode 2010). In PI 243540 (*Rag2*) and the susceptible genotype 'Wyandot,' it was observed that aphids had fewer prolonged phases of active salivation (E1), pathway events, and non-probing intervals, and that fewer insects reached the phloem on PI 243540 (Todd et al. 2016).

Although there are relevant studies using the electrical penetration graph technique to evaluate the feeding behavior of *A. glycines*, there is no study simultaneously evaluating soybean genotypes expressing *Rag1*, *Rag2*, and the tolerant KS4202. The contribution of a simultaneous study is the opportunity in evaluating the resistant expression of the soybean genotypes in the feeding behavior of *A. glycines* under a standardized condition (insect colony and generation; plant condition; and electrical penetration graph system). In addition, for the first time, KS4202 was used and compared to other genotypes as a tolerant, not a susceptible genotype, to *A. glycines*. Therefore, there is a need for electrical penetration graph studies in order to understand more thoroughly the feeding behavior of the aphids on resistant soybean lines. The objective of this work was to use the electrical penetration graph technique to compare the feeding behavior of *A. glycines* in soybean genotypes expressing antibiosis, antixenosis, and tolerance resistance.

Materials and Methods

SOYBEAN GENOTYPES

The soybean genotypes used in this study are listed in Table 1. Genotypes 'Dowling' and PI 200538 were selected because they express resistance to *A. glycines*, and because they carry the *Rag1* and *Rag2* gene, respectively (Hill et al. 2009; Crompton & Ode 2010). KS4202 was selected because it has been determined to express tolerance to

Table 1. Mean number and duration of electrical penetration graph feeding parameters (± SE) for *A. glycines* feeding on resistant and susceptible soybean entries for 15 h.

Feeding parameter	Genotypes[a]				df	P
	'SD76RR'	KS4202	'Dowling'	PI 200538		
Time to first probe[b]	16.14 ± 4.52 a	2.14 ± 4.52 b	2.30 ± 4.52 b	1.08 ± 4.52 b	3, 52	0.0005
Time to first sieve element phase	182.17 ± 33.36 a	188.32 ± 50.17 a	232.41 ± 56.46 a	173.25 ± 36.75 a	3, 52	0.9814
No. pathway phases	40.78 ± 7.84 b	40.57 ± 7.83 b	74.71 ± 7.84 a	66.29 ± 7.84 a	3, 52	0.0039
No. xylem phases	1.28 ± 0.28 a	1.14 ± 0.23 a	2.07 ± 0.38 a	1.28 ± 0.35 a	3, 52	0.2268
No. sieve element phases	4.14 ± 0.73 a	3.21 ± 0.68 a	2.86 ± 0.65 a	5.14 ± 0.76 a	3, 52	0.1144
No. non-probing events	36.07 ± 7.52 b	36.71 ± 7.52 b	70.36 ± 7.52 a	60.29 ± 7.52 a	3, 52	0.0031
Duration of pathway phases[b]	355.29 ± 60.01 a	180.35 ± 24.46 b	314.02 ± 40.07 a	322.31 ± 25.79 a	3, 52	0.0126
Duration of xylem phases	79.27 ± 15.47 a	57.38 ± 16.30 a	62.39 ± 13.77 a	65.81 ± 12.90 a	3, 52	0.5118
Duration of non-probing events	183.26 ± 45.75 c	236.52 ± 60.24 bc	500.15 ± 51.81 a	306.98 ± 50.12 b	3, 52	0.0003
No. of aphids that reached the sieve element phase[c]	12	13	10	13		

[a]Means within the same row followed by the same letter indicate no significant differences (*P* ≤ 0.05), LSD test.
[b]Time and duration calculated in minutes.
[c]All replicates are included in final analyses (*n* = 14).

A. glycines (Prochaska et al. 2013). 'SD76RR' was used as a susceptible genotype (Marchi-Werle et al. 2014).

Plants were grown in 5 L plastic pots (1 soybean plant per pot) (item 247P-FP, 247 Garden Hydroponics, Monterey Park, California, USA), filled with steam-sterilized potting mix (Premier Promix, Rivière-du-Loup, Québec, Canada), and maintained in greenhouse. The study was initiated when plants reached stage V1 (about 15 d after sowing) (Fehr et al. 1971).

APHIS GLYCINES STOCK COLONY

A colony was established with apterous adult females (biotype 1) collected in 2007 from commercial soybeans near the University of Nebraska, Haskell Agricultural Laboratory (Dixon County, Concord, Nebraska, USA) (42.3841°N, 96.9891°W). The colony was maintained in a growth chamber (25 ± 2 °C, 75 ± 5% RH, and photoperiod of 16:8 h [L:D]). Soybean plants (V4 to V6) were used as the food source, and were replaced once a wk.

BIOASSAYS WITH ELECTRICAL PENETRATION GRAPH

Aphid feeding behavior was characterized with the EPG-DC system (Giga-8 EPG model, EPG Systems, Wageningen, The Netherlands) with a 10^9 Ω resistance amplifier and adjustable plant voltage (Tjallingii 1978). To configure the setup, a copper plant electrode was inserted in the moist soil at the base of a potted plant. A sample rate of 100 Hz (100 samples per s) per channel was used to digitize the output from the electrical penetration graph using a built-in data logger (DI-710, Dataq Instruments Inc., Akron, Ohio, USA). Data was recorded on a computer containing the electrical penetration graph software (Stylet+, EPG Systems). Fluctuations of the substrate voltage were monitored on the computer and adjusted at ± 5 V, and the gain was adjusted from 50 to 100×.

The electrode was a gold wire (10 μm diam and 2 to 3 cm length; Sigmund Cohn Corp., Mount Vernon, New York, USA) linked to the dorsum of the insect with a silver conductive glue. The silver glue was made up with 4 mL water with 1 drop of Triton X-100 (Sigma-Aldrich, St. Louis, Missouri, USA), 4 g water soluble glue (clear paper glue, nontoxic; 3M, St. Paul, Minnesota, USA), and 4 g silver flake (99.95% purity; size 8–10 μm; Inframat Advanced Materials, Manchester, Connecticut, USA). The gold wire was attached with adequate length to allow free movement of the aphid within the leaf area (2 × 2 cm²). The end of the gold wire was connected to a copper wire (0.51 mm diam and 2 cm length) soldered to a copper nail (1.6 × 19.0 mm), which was then inserted into the electrical penetration graph probe. The electrical penetration graph probe system consisted of an amplifier with a 1 giga-ohm input resistance and 50× gain (Tjallingii 1985).

At the time of the bioassays, 14 apterous adult female aphids (fasted for 1 hr) per genotype were transferred from the insect stock colony and allowed to feed. The insects were placed on the abaxial leaf surface of the newest emerged trifoliate leaf. The feeding behavior was recorded for a 15 h period under continuous light (25 ± 2 °C, 75 ± 5% RH). Each plant was used once, then discarded. Faraday cages, constructed with aluminum mesh forming an aluminum frame and base (61 × 61 × 76 cm), were used to accommodate plants, aphids, and the electrical penetration graph equipment (Crompton & Ode 2010). A bioassay round consisted of 8 plants (2 per genotype), each with 1 aphid. A bioassay round was replicated 7 times. The corresponding channels were randomized in each replicate.

The following parameters were assessed: (1) time from beginning of recording to first probe, (2) time from the beginning of recording to first sieve element phase, (3) number of pathway phases, (4) number of xylem phases, (5) number of sieve element phases, (6) number of non-probing events (resting phase), (7) number of potential drops (intracellular stylet puncture), (8) duration of pathway phases, (9) duration of xylem phases, (10) duration of non-probing events, (11) duration of sieve element phases, and (12) number of *A. glycines* that reached the sieve element phase. The feeding behavior parameters were analyzed and calculated individually for the 4 soybean genotypes.

STATISTICAL ANALYSIS

Recordings of the electrical penetration graph files were transferred into a Microsoft Excel Workbook spreadsheet (Microsoft Corporation, Redmond, Washington, USA) and waveform durations were calculated. Data from all recordings were combined (replicate number [randomly selected], and waveform duration and number) before converting to comma-separated values. The comma-separated values files were checked for errors by using a beta-program designed for SAS software (Support SAS, SAS Institute 2017, Cary, North Carolina, USA) (SAS Institute 2006). Treatments were tested for significant differences by using analysis of variance (ANOVA) implemented in SAS PROC GLIMMIX. Means were separated using the Fisher least significant difference (LSD) test (P = 0.05). Residuals from the recordings were assessed for normality by using graphical analysis of the residuals and a Shapiro-Wilk test. In case of electrical penetration graph data that did not exhibit a normal distribution, data were tested for distribution fit and analyzed in a generalized linear model with a gamma or lognormal distribution, as appropriate.

Results

The electrical penetration graph feeding parameters of the 4 soybean genotypes are reported in Table 1. The time that *A. glycines* spent in the first sieve element phase, the number of xylem phases and sieve element phases, and duration of xylem phases did not differ among the genotypes. Aphids feeding on 'SD76RR' differed from the other genotypes by having a prolonged period for time to the first pathway (first probe).

The number of potential drops was higher in 'Dowling' and PI 200538, differing from KS4202 (Fig. 1). Genotypes 'SD76RR' and KS4202 had significantly fewer number of pathway phases than the other genotypes (Table 1). Additionally, aphids had a significantly greater number of non-probing events on 'Dowling' and PI 200538, relative to the other genotypes (Table 1).
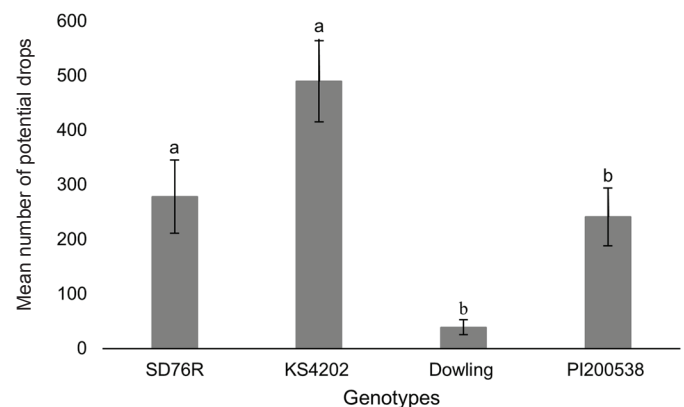

Fig. 1. Mean number of potential drops by *Aphis glycines* on soybean genotypes for a 15 h (900 min) period.

In relation to duration of the evaluated parameters (Table 1), the pathway phases were shorter for aphids feeding on KS4202 compared to the other genotypes. For duration of non-probing events, aphids feeding on 'Dowling' had the longest value while those on 'SD76RR' had the shortest.

Aphids fed on 'Dowling' had the shortest duration of sieve element phases (Fig. 2). The number of aphids that reached the sieve element phase were similar among the genotypes, although the number was lowest for 'Dowling.'

Discussion

The results of this study demonstrated that the different resistance categories present in the evaluated soybean genotypes significantly affect the feeding behavior of the aphid. There were no differences in the time that aphids took to reach the sieve element phase among the soybean genotypes. Similarly, 'Dowling,' PI 200538, and KS4202 did not differ from each other for the time to first probe. Because the genotypes had a short or similar period of time for these parameters, resistance factors do not appear to be present in the epidermis or mesophyll tissue layers (Crompton & Ode 2010).

Aphids spent similar periods in the xylem phase on all genotypes, as has been reported in another study involving other genotypes, as well as KS4202 and 'Dowling' (Diaz-Montano et al. 2007). Xylem ingestion is important for the insects to preserve a water balance (Spiller et al. 1990); however, xylem sap may not provide aphids with an adequate balance of nutrients as phloem sap (Powell & Hardie 2002).

The shortest duration of sieve element phase and the longest duration of non-probing occurred for aphids feeding on 'Dowling,' demonstrating the expression of resistance of 'Dowling' to *A. glycines*. This feeding behavior indicates the expression of antixenosis because the phloem provokes a feeding deterrence to the aphids (Roux et al. 2008).

In another study involving the *A. glycines* resistance genotypes 'Jackson,' 'Dowling,' Pioneer 95B97, and K1639, the aphids also spent less time in sieve element phases relative to KS4202 (Diaz-Montano et al. 2007). In a study examining *A. glycines* feeding behavior on plants treated with thiamethoxam, the insect spent less time feeding in the sieve element phase and had a long duration of non-probing events, which also demonstrates aphid feeding behavior under negative conditions (Stamm et al. 2013).

Although there were no significant differences, out of 14 replications for each genotype, fewer aphids reached the sieve element phase in 'Dowling' compared to the other genotypes. In another study,

'Dowling' also had the lowest number of aphids that reached the sieve element phase in 8 hr (Zhu et al. 2011), so we expected that fewer aphids would reach the sieve element phase on 'Dowling' in our study, and consequently, a higher difference in this parameter among the genotypes would be observed. However, it is important to point out that aphid feeding behavior in our study was evaluated for a period of 15 hr (Todd et al. 2016), while many other studies evaluated the aphids for approximately 8 or 9 hr (Diaz-Montano et al. 2007; Crompton & Ode 2010). This longer period of study would inform possible variation in feeding behavior and the insects would have more time to reach the phloem phase of the host plants.

The results of this study suggest that 'Dowling' expresses antixenosis or antibiosis to *A. glycines* through phloem resistance factors, the phloem characteristics being the principal component of induced resistance (Diaz-Montano et al. 2007; Crompton & Ode 2010). Several phloem characteristics have been correlated with antibiosis to *A. glycines*, including phloem protein coagulation and callose accumulation (Tjallingii 2006), deposition of lectins (Bostwick et al. 1992; Down et al. 1996), protease inhibitors (Kehr 2006), secondary metabolites (Dinant et al. 2010), or lack of sufficient nutrients in phloem sap (Smith 2005). These compounds or resistance factors may affect the aphids by affecting prolonged feeding, which would suggest antixenosis (Will et al. 2007), or by negatively affecting their development (e.g., weight and fecundity), suggesting antibiosis (Halkier & Gershenzon 2006; Kim et al. 2008).

In addition, 'Dowling' also showed long pathway phases, which are often described to reflect antixenosis (Diaz-Montano et al. 2007; Roux et al. 2008). Waves of pathway phases represent non-feeding behavior in the phloem or xylem. They comprise a sequence of feeding events where aphids puncture individual cells with their stylet tips to sample cell sap and sieve elements. This feeding behavior is fundamental in the process of accepting or rejecting a host plant (Jiang & Walker 2001).

Aphids feeding on PI 200538 had a greater number and duration of pathway phases, similar to 'Dowling'; however, those aphids had more sustained sieve element phases and spent less time in non-probing behavior. Although aphids fed on PI 200538 demonstrated inconsistent values under the conditions of this study, previous studies and the presence of the *Rag2* gene verify the resistance to *A. glycines* (including biotype 2). The PI 200538 is used as the male parent in crosses with 'Dowling' to obtain resistant genotypes in soybean breeding programs (Hill et al. 2009).

Due to the previous studies that verify tolerance of KS4202 to *A. glycines*, it was expected that this genotype would express different electrical penetration graph values compared to the resistant 'Dowling' and PI 200538. In this electrical penetration graph study, aphids feeding on KS4202 had long durations of sieve element phases and short durations of non-probing and pathway phase, supporting the expression of tolerance and not antibiosis or antixenosis in KS4202. In another electrical penetration graph study comparing KS4202 with other genotypes, including 'Dowling,' the results indicated that KS4202 had the longest duration of sieve element phase (Diaz-Montano et al. 2007). Similar results were documented when KS4202 was compared to KS16121, KS1613, and KS1642 (Zhu et al. 2011).

Genotype KS4202 had the lowest potential drop value compared to the other genotypes. The potential drops are considered brief intracellular punctures by the stylets along their pathway behavior (Tjallingii & Esch 1993). Aphids inject watery saliva into the punctured cell, using their stylets to collect sap samples (Martin et al. 1997). Different chemical cues can be present in these samples and thus be correlated to host plant acceptance by the insects (Tjallingii 2006). Because of this characteristic, a high number of potential drops is associated with
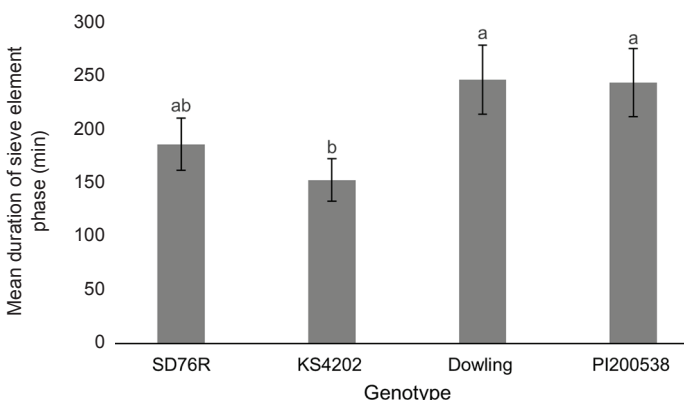

Fig. 2. Mean duration of sieve element phase by *Aphis glycines* on soybean genotypes for a 15 h (900 min) period.

greater difficulty of reaching phloem phases, and consequently, resistance of soybean genotypes (Chandran et al. 2013). On other hand, some research hypothesizes that a greater number of potential drops is an indication of host plant acceptance by the insect, enabling it to reach the sieve element phase quickly (Diaz-Montano et al. 2007).

The feeding behavior of soybeans aphids in KS4202 suggests that the insects do not suffer negative effects when feeding on this host plant. In addition, these results support that this genotype expresses tolerance to *A. glycines* (Prochaska et al. 2015). The expression of tolerance in this genotype may be associated with the upregulation of detoxification mechanisms, such as peroxidases and faster regulation of RuBP (ribulose-1,5-biphosphate) (Pierson et al. 2011). Recently, several genes were identified with roles that are connected to plant defenses and the scavenging of reactive oxygen species, which supports the hypothesis that these genes may be correlated to tolerance to *A. glycines* (Prochaska et al. 2015). The present study provides an important dataset describing *A. glycines* feeding behavior on *A. glycines* resistant soybean genotypes, and contributes to a better understanding of resistance mechanisms. New research should be conducted to investigate the tolerance response of KS4202 to *A. glycines*, which would contribute to soybean breeding programs aimed at developing *A. glycines* resistant soybean.

Acknowledgments

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