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Lia Marchi-Werle

*University of Nebraska-Lincoln, lia.werle@unl.edu*

Renata Ramos Pereira

*Universidade Federal de Vicosa*

John C. Reese

*Kansas State University, jreese@ksu.edu*

Thomas Hunt

*University of Nebraska-Lincoln, thunt2@unl.edu*

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# Yield Response of Tolerant and Susceptible Soybean to the Soybean Aphid

Lia Marchi-Werle,<sup>1</sup> Renata Ramos Pereira,<sup>2</sup>  
John C. Reese,<sup>3</sup> Tiffany M. Heng-Moss,<sup>1</sup>  
and Thomas E. Hunt<sup>4</sup>

<sup>1</sup> Department of Entomology, University of Nebraska-Lincoln, Lincoln, NE 68583

<sup>2</sup> Department of Entomology, Universidade Federal de Vicosa, Vicosa, MG, Brazil

<sup>3</sup> Department of Entomology, Kansas State University, Manhattan, KS 66506

<sup>4</sup> NEREC Haskell Agricultural Laboratory, Department of Entomology,  
University of Nebraska-Lincoln, Concord, NE 68728

*Corresponding author* — L. Marchi-Werle, *email* lia.werle@unl.edu

## Abstract

Soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), is the most economically important soybean [*Glycine max* (L.) Merr.] pest of North America. Multiple studies have identified soybean expressing antibiosis and/or antixenosis; however, soybean tolerance remains underexplored. Tolerance to soybean aphid injury was previously identified in soybean KS4202. This research examined the yield response of KS4202 infested with soybean aphid at specific plant stages and identified at what plant stage tolerance initiates. A preliminary study evaluated the yield parameters of the tolerant genotype at low (4000–5500 cumulative aphid-days [CAD]) and high aphid pressure (7500–8500 CAD) at different growth stages (V1, V3, and R1). A second study compared the yield response of the tolerant and a susceptible genotype (K03-4686) at both V1 and V3 stages. In addition, low and high aphid pressure increased to 9,000 to 12,000 and 18,000 to 25,000 for V1 and V3 stages, respectively. Preliminary evaluations indicated that the yield parameters of the tolerant genotype infested at V3 and R1 were not significantly different from the respective controls. Conversely, plants were unable to compensate for high aphid pressure at the V1 stage. In study 2, high aphid pressure negatively influenced yield of both tolerant and susceptible V1-plants infested, although the tolerant genotype compensated for low aphid pressure. Aphid pressures applied at the V3 stage did not

influence the yield parameters of tolerant genotype; however, both aphid pressures were detrimental to the susceptible genotype. Tolerance in KS4202 begins as early as V3, and maintains as plants mature.

**Abbreviations:** **CAD**, cumulative aphid-days; **EIL**, economic injury level; **ET**, economic threshold; **V1**, fully developed leaves at unifoliate node, first trifoliate leaf unrolled; **V3**, fully developed leaf at second trifoliate node, third trifoliate leaf unrolled; **R1**, reproductive stages from first flower.

### Core Ideas

- Soybean aphid-tolerance in KS4202 soybean is plant age dependent.
- Soybean aphid infestation occurring at the V1 stage impacts both susceptible and tolerant soybean.
- KS4202 during late vegetative and early reproductive stage tolerated high aphid pressure.

Soybean is an important commodity in world trade and represents the majority of the oilseed produced in the United States (Bilyeu et al., 2010). The first report of soybean aphid in North America was in 2000. The most current report shows that soybean aphids are present in 30 states in the United States as well as southeastern Canada (Ragsdale et al., 2011). Soybean aphids feed by withdrawing the phloem contents through piercing-sucking mouthparts. Feeding injury can result in plant stunting, leaf distortion, along with significant reductions in the photosynthetic rates (Macedo et al., 2003; Pierson et al., 2011). High aphid infestation favor the growth of sooty mold on the sugary excretions or "honeydew" that aphids excrete during feeding (Tilmon et al., 2011). Ultimately, the injury caused by soybean aphids manifests as yield reduction by reducing the number of pods and seed, and also seed weight (Rhainds et al., 2007; Beckendorf et al., 2008; Pierson et al., 2010). Although the severity of soybean yield losses depend on the aphid pressure and the physiological conditions of the plants, yield losses from 20 to 50% have been reported (Wang et al., 1996; Ragsdale et al., 2004; Myers et al., 2005). A comprehensive, multi-state research project estimated a yield loss of 6.88% for every 10,000 CAD during the reproductive stages from first flower (R1) to full pod (R5) (Fehr and Caviness 1977), and an average economic injury level (EIL) of  $674 \pm 95$  aphids per plant (or  $\approx 5563$  CAD) (Ragsdale et al., 2007).

In North America, soybean aphid management has relied heavily on foliar-applied insecticides. Before the arrival of soybean aphids in North America, less than 1% of the soybean fields were treated with insecticides (NASS, 1999). This scenario drastically changed by 2006, when a sharp increase (estimated 130-fold) in insecticide applications was associated with the introduction of the soybean aphid to North America (Ragsdale et al., 2011). As a result, production costs increased (Ragsdale et al., 2007), which

has stimulated the development of alternative pest management methods for this pest.

Host plant resistance provides an effective, economical, and environmentally safe pest management approach and is considered an important component of integrated pest management (Smith, 2005). Since soybean aphids were detected in North America, accessions from the USDA Soybean Germplasm collection have been screened with significant progress made in identifying sources of resistance to the soybean aphid. Resistance was first identified in Dowling, Jackson, and PI 71506 (Hill et al., 2004). Antibiosis was the major contributor for resistance in Dowling and Jackson (Hill et al., 2004); whereas, resistance in PI 71506 was attributed to antixenosis (Hill et al., 2004, Mian et al., 2008). Other studies have identified antibiosis in PI 567541B, PI 567598B, PI 243540 and PI 597732 (Mensah et al., 2005; Zhang et al., 2009; Kim et al., 2014). Antixenosis was also reported in Dowling, PI 230977 (Hessler et al., 2007), PI 567453C and PI 567597C (Mensah et al., 2005; Zhang et al., 2010). Resistance in those genotypes was attributed to a series of single dominant genes, named *Rag* (Resistance to *Aphis glycines*). Three additional resistance genes (*rag1b*, *rag1c*, and *rag4*) have been characterized as recessive (Bales et al., 2013; Zhang et al., 2009, 2010).

Not long after the identification of aphid resistance genes, virulent populations of soybean aphids were observed colonizing *Rag*-containing soybean. Kim et al. (2008) reported soybean aphid populations overcoming the resistance imposed by *Rag1* gene, later designated as biotype 2, with biotype 1 referred as aphid populations that colonize plants lacking *Rag* genes (Hill et al., 2009). Soybean aphid biotype 3 has been characterized by populations that readily overcome resistance of *Rag2* while remaining susceptible to *Rag1* from Dowling (Hill et al., 2010). To date, the latest biotype reported is biotype 4, which can colonize both *Rag1* and *Rag2* genes and *Rag1/Rag2* pyramid (Alt and Ryan-Mahmutagic, 2013). Because of the threat posed by soybean aphid biotypes, researchers have been focusing on strategies to increase the effectiveness and durability of the resistance genes. Varenhorst et al. (2015) found that employing refuge areas with susceptible soybean could decline the risk of virulence by imposing a fitness cost. Moreover, breeding three-gene pyramid soybean could enhance the effectiveness of *Rag* genes; however, field studies to confirm this finding and evaluate the impact of this trait on soybean's yield and agronomic aspects are still pending (Ajayi-Oyetunde et al., 2016).

Tolerance is a polygenic form of resistance defined as the ability of a host plant to withstand arthropod feeding without suffering excessive injury (Smith, 2005). This type of resistance has several advantages from an ecological and agronomical viewpoint. Unlike antibiosis and antixenosis, tolerance does not interfere with the insect's biology or behavior, which presumably limits the emergence of virulent biotypes (Smith, 2005). Because of its ability

to withstand greater arthropod injury, tolerant plants have higher EILs than a referenced susceptible plant, and can alleviate yield losses resultant from arthropod herbivory when associated with integrated pest management practices. Limited studies have focused on identifying soybean aphid-tolerant soybean. In a controlled environment, studies by Pierson et al. (2010) first reported later vegetative and reproductive KS4202 soybean to be tolerant to soybean aphid injury. Moreover, field trials conducted over three growing seasons supported that KS4202 is tolerant to soybean aphid during the reproductive stages (Prochaska et al., 2013). In that study, aphid infestations on KS4202 reached approximately 53,000 CAD, which resulted in yield losses of 13% when 24 to 36% would have been expected (Prochaska et al., 2013). Although soybean aphids generally infest Nebraska soybean fields when plants are in the reproductive stages (Prochaska et al., 2013), soybean aphids may infest soybean in other regions earlier in the season (Brosius et al., 2007).

A preliminary study based on visual evaluations (i.e., scale 1–5; Pierson et al., 2010) of leaf chlorosis documented that KS4202 is highly susceptible to the soybean aphid during emergence (VE); unifoliate leaves unrolled (VC); whereas, V1 is moderately susceptible, and V3, V4, and V5 are moderately resistant to this aphid (Marchi, 2012). Because leaf chlorosis may not directly correspond with the actual yield losses, further studies to characterize the yield response of KS4202 when infested during early vegetative stages are necessary. Therefore, the objectives of this research were to investigate the yield response of the tolerant soybean KS4202 at specific vegetative plant stages and aphid infestation levels, and to identify at what stage tolerance begins to be expressed.

## **Materials and Methods**

Two greenhouse studies were performed to evaluate the impact of soybean aphids and plant stage on the yield response of KS4202. In the first study, soybean aphids were introduced at three vegetative stages: fully developed leaf at unifoliate node (V1), fully developed leaf at third node (V3) and reproductive stage from first flower (R1). Three levels of aphid infestation were implemented, control (aphid-free), low (4000–5500 CAD), and high (7500–8500 CAD) aphid pressure. The treatment design was a 3 × 3 factorial arranged in a completely randomized design with 10 replications. The low level, equivalent to 1000–1500 insects per plant, represented the high EILs for conventional soybean (i.e., non-tolerant) calculated by Ragsdale et al. (2007). Although these thresholds were determined for R1 to R5 soybean, yield losses may occur for tolerant KS4202 soybean, although possibly not economic. The higher level represented a range where significant yield loss

would be expected for KS4202. The results of this study were used to optimize the methods for second study, which included the soybean aphid susceptible genotype K03-4686 (Prochaska et al., 2015).

The second study design was a factorial with two genotypes (KS4202 and K03-4686) × two soybean stages (V1 and V3) × three infestation levels (control– aphid-free, low and high aphid pressure). Based on the results from the first study, low and high CAD treatments increased to 9000 to 12,000 and 18,000 to 25,000 CAD, respectively. The experimental design was a completely randomized design with at least eight replications.

### ***Plant and Insect Source***

The seeds of each genotype (KS4202 and K03-4686) were planted in 15-cm diam. round plastic pots at a depth of approximately 3 cm. The potting media was a mixture of 34% peat, 31% perlite, 31% vermiculite, and 4% soil. Planting dates were staggered to ensure that plants in each study would reach the designated plant stage at the same time. Upon germination, plants were thinned to one plant per pot and placed in a plastic tray (35 by 50 cm). Watering schedule was performed according to the physiological need of the plants; whereas, fertilization with a soluble (20:10:20 N/P/K) fertilizer occurred every 2 wk. The greenhouse temperature was maintained at 23±3°C, with lighting supplemented by 400-W high intensity lamps to produce a photoperiod of 16:8 (light:dark) h.

Once the plants were at the desired stage, 10 (low aphid pressure) or 20 (high aphid pressure) soybean aphids (fourth instars and adults) were placed on the youngest fully expanded leaf of the designated aphid-infested treatments using a soft paintbrush. Aphids used in this study were progeny of a Nebraska isolate (biotype 1) initially collected during the 2011 growing season from commercial soybean near the University of Nebraska Haskell Agricultural Laboratory, Concord, NE (42°23'3" N, 96°59'21" W). The soybean aphid colony was maintained on KS4202 plants in a growth chamber at 21±2°C and a photoperiod of 16:8 (L:D) h. Upon aphid introduction, tubular polycarbonate plastic cages (15 cm of diameter and 61 cm of height, Lexan, E-plastics, San Diego, CA) were placed on both infested and control (aphid-free) plants.

### ***Evaluations and Yield Parameters***

Plants were evaluated twice a week by recording aphid number and plant stage. After each evaluation, aphid number was used to calculate CAD, which provides an estimate of accumulated aphid pressure. The CAD was calculated using the formula:

$$\sum_{i=1}^n = [(x_i + x_{i-1})/2 + (t_i - t_{i-1})]$$

where  $n$  is the number of sample dates,  $x_i$  is the mean number of aphids per plant on sample date  $i$ , and  $(t_i - t_{i-1})$  is the number of days between two consecutive sample dates (Hanafiet al., 1989; Ragsdale et al., 2007).

For both studies, aphids remained on the plants until the targeted infestation levels were reached, after which plants were sprayed with the synthetic pyrethroid insecticide lambda-cyhalothrin (Warrior with Zeon technology, Syngenta Crop Protection, Greensboro, NC). Plants were monitored closely within the next 24 to 48 h after insecticide application, and cages were removed once aphid populations were completely eradicated. Plants were then tied to a bamboo stick (approximate length of 1 m) to ensure the main stem was properly supported.

Upon maturation (i.e., pods were completely yellow or brown), soybean pods were harvested and placed in a paper bag and oven dried. The yield parameters of each plant (number of pods, number of seeds/pod, average seed weight and average pod dry weight) were recorded (Beckendorf et al., 2008; Pierson et al., 2010). Analysis of variance was conducted for all plant stages and infestation levels to assess differences in yield parameters using PROC GLIMMIX in SAS 9.3 (SAS Institute, Cary, NC). When pertinent ( $\alpha = 0.05$ ), means were separated using Fisher's least significant difference (LSD) test.

## Results and Discussion

### Study 1

Tolerant plants (KS4202) exposed to low aphid pressure treatment during V1, V3, and R1 exceeded the average EIL of 674 aphids per plant reported by Ragsdale et al. (2007). Low aphid pressure treatment ( $3710 \pm 304.7$  CAD) did not influence the yield parameters of V1 infested plants, although high aphid pressure ( $7790 \pm 769.1$  CAD) resulted in a significant reduction in total seed weight, total pod weight, seed number, and pod number (33.8, 21.74, 32.3, and 27.2% reduction, respectively) (Tables 1 and 2).

Despite aphid numbers exceeded the typical CAD EILs presented by Ragsdale et al. (2007), V3 and R1 plants exposed to low (CAD =  $4530 \pm 245.8$  and  $5300 \pm 525.5$ , respectively) and high aphid pressure (CAD =  $7490 \pm 803.4$  and  $8385 \pm 498.8$ , respectively) were not different from their respective control plants for most of the yield parameters evaluated (Tables 1 and 2). It appears that KS4202 soybean overcompensated for soybean aphid by increasing some yield parameters. There was a 58% increase in single seed weight for plants in the V3 stage treated with high aphid pressure (Table 1;  $P = 0.06$ ). Similarly, R1 plants exposed to low aphid pressure had an increase in the single pod weight (Table 2;  $P = 0.002$ ), and a slight increase in total seed weight and seed number (Tables 1 and 2).

**Table 1.** Means ± SEM of seed-related parameters in soybean aphid-infested (low/high aphid pressure) and control KS4202 soybean (study 1).

| Stage of infestation         | Control        | Low aphid pressure | High aphid pressure | Control × low aphid pressure | P value†                      |                           |
|------------------------------|----------------|--------------------|---------------------|------------------------------|-------------------------------|---------------------------|
|                              |                |                    |                     |                              | Control × high aphid pressure | Low × high aphid pressure |
| <b>Single seed weight, g</b> |                |                    |                     |                              |                               |                           |
| V1                           | 0.1491 ± 0.017 | 0.1509 ± 0.005     | 0.1465 ± 0.007      | 0.97                         | 0.97                          | 0.93                      |
| V3                           | 0.1703 ± 0.006 | 0.1593 ± 0.008     | 0.2694 ± 0.108      | 0.81                         | 0.08                          | 0.06                      |
| R1‡                          | 0.1439 ± 0.004 | 0.1619 ± 0.006     | 0.1448 ± 0.008      | 0.78                         | 0.99                          | 0.79                      |
| <b>Total seed weight, g</b>  |                |                    |                     |                              |                               |                           |
| V1                           | 9.92 ± 3.16    | 9.24 ± 3.59        | 6.57 ± 3.36         | 0.68                         | 0.04                          | 0.11                      |
| V3                           | 14.70 ± 1.30   | 11.92 ± 1.19       | 12.94 ± 1.23        | 0.10                         | 0.27                          | 0.55                      |
| R1‡                          | 7.11 ± 0.60    | 10.27 ± 1.24       | 7.44 ± 1.15         | 0.10                         | 0.86                          | 0.13                      |
| <b>Seed number</b>           |                |                    |                     |                              |                               |                           |
| V1                           | 66.60 ± 6.91   | 60.55 ± 6.50       | 45.10 ± 8.29        | 0.56                         | 0.04                          | 0.14                      |
| V3                           | 86.60 ± 6.99   | 74.50 ± 6.35       | 74.62 ± 6.35        | 0.26                         | 0.23                          | 0.99                      |
| R1‡                          | 49.43 ± 3.85   | 63.57 ± 7.88       | 49.25 ± 4.84        | 0.25                         | 0.98                          | 0.23                      |

† Treatment means significantly different at  $P < 0.05$  by LSD test.

‡ R1: Tolerant control (Pierson et al. 2010).

**Table 2.** Means ± SEM of pod-related parameters in soybean aphid-infested (low/high aphid pressure) and control KS4202 soybean (study 1).

| Stage of infestation        | Control        | Low aphid pressure | High aphid pressure | Control × low aphid pressure | P value†                      |                           |
|-----------------------------|----------------|--------------------|---------------------|------------------------------|-------------------------------|---------------------------|
|                             |                |                    |                     |                              | Control × high aphid pressure | Low × high aphid pressure |
| <b>Single pod weight, g</b> |                |                    |                     |                              |                               |                           |
| V1                          | 0.4477 ± 0.018 | 0.4387 ± 0.012     | 0.4400 ± 0.023      | 0.74                         | 0.77                          | 0.96                      |
| V3                          | 0.4646 ± 0.016 | 0.4577 ± 0.017     | 0.4644 ± 0.013      | 0.81                         | 0.99                          | 0.82                      |
| R1‡                         | 0.3751 ± 0.016 | 0.4758 ± 0.021     | 0.4167 ± 0.028      | 0.002                        | 0.19                          | 0.06                      |
| <b>Total pod weight, g</b>  |                |                    |                     |                              |                               |                           |
| V1                          | 14.67 ± 1.33   | 21.51 ± 1.53       | 11.48 ± 1.59        | 0.73                         | 0.04                          | 0.10                      |
| V3                          | 13.92 ± 1.70   | 17.75 ± 1.59       | 12.11 ± 1.53        | 0.10                         | 0.21                          | 0.61                      |
| R1‡                         | 10.29 ± 0.86   | 18.88 ± 1.63       | 15.23 ± 1.44        | 0.14                         | 0.79                          | 0.21                      |
| <b>Pod number</b>           |                |                    |                     |                              |                               |                           |
| V1                          | 32.80 ± 2.94   | 32.11 ± 3.75       | 23.70 ± 4.27        | 0.88                         | 0.06                          | 0.09                      |
| V3                          | 46.60 ± 3.77   | 38.87 ± 3.55       | 40.50 ± 2.89        | 0.13                         | 0.20                          | 0.74                      |
| R1‡                         | 31.00 ± 2.29   | 32.14 ± 3.31       | 28.87 ± 2.57        | 0.84                         | 0.70                          | 0.55                      |

† Treatment means significantly different at  $P < 0.05$  by LSD test.

‡ R1: Tolerant control (Pierson et al. 2010).

There were no statistical differences in yield parameters for V1 aphid infested plants between the aphid pressure treatments, although there was a trend for plants exposed to high aphid pressure to have lower total seed weight, number of pods, total pod weight, and number of seeds (Tables 1 and 2).

At the V3 stage, there were no significant differences in the yield parameters between the aphid treatments (Tables 1 and 2). Although not at the 5% significance level, single seed weight under high aphid pressure was higher than low aphid pressure (Table 1;  $P = 0.06$ ). Yield parameters for R1 infested soybean were not different between the aphid pressure treatments; however, means for the yield parameters tended to be lower in the high aphid pressure treatment (Tables 1 and 2).

Plants infested at different vegetative stages with low aphid pressure had similar yield parameters, although there was a trend for total seed weight (22.5%), number of pods (17.4%) and total pod weight (17.5%) to be lower in V1-infested plants (Table 3). Conversely, the yield parameters of V1 plants exposed to high aphid pressure were 30% lower than V3 plants under the same treatment (Table 3). A similar trend occurred when comparing R1 × V3 infested plants with significant reductions for most of the R1 yield parameters under high aphid pressure (Table 3). Lastly, there were no differences in the yield parameters for the comparison R1 × V1 for either aphid pressure.

**Table 3.** Effect of infestation level (low/high aphid pressure) and different plant stages (V1, V3, and R1) on yield parameters of the tolerant genotype (KS4202) (study 1).

| <b>Single seed weight, g</b> |         |                  |          | <b>Total seed weight, g</b> |         |                |          |
|------------------------------|---------|------------------|----------|-----------------------------|---------|----------------|----------|
|                              | V1 × V3 | R1† × V1         | R1† × V3 |                             | V1 × V3 | R1† × V1       | R1† × V3 |
| <i>Infestation level</i>     |         | <i>P value</i> ‡ |          | <i>Infestation level</i>    |         | <i>P value</i> |          |
| Low aphid pressure           | 0.889   | 0.860            | 0.96     | Low aphid pressure          | 0.126   | 0.569          | 0.37     |
| High aphid pressure          | 0.029   | 0.976            | 0.03     | High aphid pressure         | <0.001  | 0.609          | 0.002    |
| <b>Single pod weight, g</b>  |         |                  |          | <b>Total pod weight, g</b>  |         |                |          |
|                              | V1 × V3 | R1† × V1         | R1† × V3 |                             | V1 × V3 | R1† × V1       | R1† × V3 |
| <i>Infestation level</i>     |         | <i>P value</i>   |          | <i>Infestation level</i>    |         | <i>P value</i> |          |
| Low aphid pressure           | 0.519   | 0.227            | 0.56     | Low aphid pressure          | 0.100   | 0.586          | 0.31     |
| High aphid pressure          | 0.368   | 0.419            | 0.10     | High aphid pressure         | <0.01   | 0.412          | 0.003    |
| <b>Pod number</b>            |         |                  |          | <b>Seed number</b>          |         |                |          |
|                              | V1 × V3 | R1† × V1         | R1† × V3 |                             | V1 × V3 | R1† × V1       | R1† × V3 |
| <i>Infestation level</i>     |         | <i>P value</i>   |          | <i>Infestation level</i>    |         | <i>P value</i> |          |
| Low aphid pressure           | 0.194   | 0.995            | 0.22     | Low aphid pressure          | 0.211   | 0.793          | 0.35     |
| High aphid pressure          | <0.001  | 0.308            | 0.02     | High aphid pressure         | 0.005   | 0.702          | 0.02     |

† R1: Tolerant control (Pierson et al. 2010).

‡ Treatment means significantly different at  $P < 0.05$  by LSD test.

**Study 2**

The three-way interaction (genotype × soybean stage × infestation level) significantly impacted the yield parameters in this study: single seed weight ( $F = 6.25$ ;  $df = 2, 110$ ;  $P = 0.02$ ), seed number ( $F = 3.31$ ;  $df = 2, 110$ ;  $P = 0.04$ ), total seed weight ( $F = 6.02$ ;  $df = 2, 110$ ;  $P = 0.003$ ), single pod weight ( $F = 4.09$ ,  $df = 2, 110$ ,  $P = 0.02$ ), pod number ( $F = 4.13$ ;  $df = 2, 110$ ;  $P = 0.02$ ) and total pod weight ( $F = 6.48$ ,  $df = 2, 110$ ;  $P = 0.002$ ). Therefore, comparisons relative to the simple effects are presented. At V1, tolerant (KS4202) and susceptible (K03-4686) plants under low aphid pressure accumulated  $11,623 \pm 464.9$  and  $10,392 \pm 461.1$  aphid-days, respectively. Under high aphid pressure, tolerant and susceptible plants accumulated  $25,031 \pm 1845.4$  and  $25,988 \pm 1402.7$  aphid-days, respectively. The low aphid pressure treatment had a negative impact on the yield parameters (Tables 4 and 5) of both genotypes, with stronger impacts in the pod number, total and single pod weight (percentage reduction relative to the control treatment of 29.7, 39.9, and 28.8%, respectively) of the susceptible genotype. Soybean plants exposed to high aphid pressure experienced greater deleterious effects in the yield parameters than low aphid pressure, regardless of the genotype

**Table 4.** Means ± SEM of the seed-related parameters of the susceptible (K03-4686) and tolerant (KS4202) genotypes infested at V1 and V3 stages (study 2).

| Stage of infestation         | Genotype | Control       | Low aphid pressure | High aphid pressure | P value†                     |                               |                           |
|------------------------------|----------|---------------|--------------------|---------------------|------------------------------|-------------------------------|---------------------------|
|                              |          |               |                    |                     | Control × Low aphid pressure | Control × High aphid pressure | Low × High aphid pressure |
| <b>Single seed weight, g</b> |          |               |                    |                     |                              |                               |                           |
| V1                           | K03-4686 | 0.104 ± 0.006 | 0.098 ± 0.008      | 0.079 ± 0.011       | 0.57                         | 0.03                          | 0.12                      |
|                              | KS4202   | 0.136 ± 0.006 | 0.143 ± 0.005      | 0.130 ± 0.009       | 0.43                         | 0.66                          | 0.25                      |
| V3                           | K03-4686 | 0.105 ± 0.006 | 0.112 ± 0.006      | 0.092 ± 0.005       | 0.47                         | 0.21                          | <0.0001                   |
|                              | KS4202   | 0.147 ± 0.005 | 0.130 ± 0.008      | 0.130 ± 0.006       | 0.07                         | 0.06                          | 0.97                      |
| <b>Total seed weight, g</b>  |          |               |                    |                     |                              |                               |                           |
| V1                           | K03-4686 | 9.33 ± 1.14   | 6.03 ± 0.67        | 5.63 ± 2.11         | 0.12                         | 0.08                          | 0.85                      |
|                              | KS4202   | 17.18 ± 1.40  | 14.96 ± 1.46       | 12.15 ± 1.83        | 0.24                         | 0.01                          | 0.17                      |
| V3                           | K03-4686 | 12.38 ± 1.05  | 13.91 ± 1.08       | 7.37 ± 1.02         | 0.46                         | 0.01                          | 0.002                     |
|                              | KS4202   | 18.16 ± 1.23  | 16.83 ± 1.97       | 17.80 ± 1.09        | 0.45                         | .83                           | 0.58                      |
| <b>Seed number</b>           |          |               |                    |                     |                              |                               |                           |
| V1                           | K03-4686 | 89.50 ± 8.38  | 62.71 ± 5.98       | 57.85 ± 6.16        | 0.07                         | 0.02                          | 0.78                      |
|                              | KS4202   | 128.41 ± 8.04 | 105.55 ± 11.55     | 94.28 ± 15.05       | 0.14                         | 0.04                          | 0.50                      |
| V3                           | K03-4686 | 118.35 ± 7.04 | 124.28 ± 7.17      | 84.33 ± 12.18       | 0.65                         | 0.008                         | 0.007                     |
|                              | KS4202   | 124.00 ± 8.13 | 125.10 ± 8.67      | 136.00 ± 5.66       | 0.92                         | 0.29                          | 0.34                      |

† Treatment means significantly different at  $P < 0.05$  by LSD test.

**Table 5.** Means  $\pm$  SEM of pod-related parameters of the susceptible (K03-4686) and tolerant (KS4202) genotypes infested and control at V1 and V3 stages (study 2).

| Stage of infestation        | Genotype | Control          | Low aphid pressure | High aphid pressure | <i>P</i> value†                     |                                      |                                  |
|-----------------------------|----------|------------------|--------------------|---------------------|-------------------------------------|--------------------------------------|----------------------------------|
|                             |          |                  |                    |                     | Control $\times$ Low aphid pressure | Control $\times$ High aphid pressure | Low $\times$ High aphid pressure |
| <b>Single pod weight, g</b> |          |                  |                    |                     |                                     |                                      |                                  |
| V1                          | K03-4686 | 0.285 $\pm$ 0.02 | 0.203 $\pm$ 0.03   | 0.142 $\pm$ 0.02    | 0.02                                | 0.0001                               | 0.08                             |
|                             | KS4202   | 0.429 $\pm$ 0.02 | 0.425 $\pm$ 0.01   | 0.375 $\pm$ 0.02    | 0.83                                | 0.17                                 | 0.13                             |
| V3                          | K03-4686 | 0.295 $\pm$ 0.01 | 0.321 $\pm$ 0.02   | 0.231 $\pm$ 0.01    | 0.47                                | 0.06                                 | 0.01                             |
|                             | KS4202   | 0.462 $\pm$ 0.02 | 0.407 $\pm$ 0.02   | 0.407 $\pm$ 0.02    | 0.08                                | 0.08                                 | 0.99                             |
| <b>Total pod weight, g</b>  |          |                  |                    |                     |                                     |                                      |                                  |
| V1                          | K03-4686 | 15.18 $\pm$ 1.67 | 9.12 $\pm$ 3.06    | 10.73 $\pm$ 0.86    | 0.04                                | 0.05                                 | 0.61                             |
|                             | KS4202   | 25.70 $\pm$ 1.81 | 22.15 $\pm$ 2.57   | 17.97 $\pm$ 2.57    | 0.20                                | 0.01                                 | 0.17                             |
| V3                          | K03-4686 | 19.89 $\pm$ 1.51 | 21.92 $\pm$ 1.51   | 12.65 $\pm$ 1.65    | 0.51                                | 0.01                                 | 0.003                            |
|                             | KS4202   | 27.13 $\pm$ 1.94 | 25.28 $\pm$ 2.68   | 27.04 $\pm$ 1.72    | 0.48                                | 0.97                                 | 0.50                             |
| <b>Total pod number</b>     |          |                  |                    |                     |                                     |                                      |                                  |
| V1                          | K03-4686 | 53.25 $\pm$ 4.39 | 37.42 $\pm$ 5.20   | 40.71 $\pm$ 5.69    | 0.03                                | 0.04                                 | 0.29                             |
|                             | KS4202   | 62.10 $\pm$ 3.27 | 52.22 $\pm$ 5.39   | 47.14 $\pm$ 6.18    | 0.19                                | 0.07                                 | 0.54                             |
| V3                          | K03-4686 | 67.62 $\pm$ 4.24 | 69.14 $\pm$ 4.13   | 54.11 $\pm$ 6.21    | 0.81                                | 0.02                                 | 0.01                             |
|                             | KS4202   | 58.70 $\pm$ 3.10 | 61.40 $\pm$ 3.76   | 66.50 $\pm$ 2.24    | 0.62                                | 0.15                                 | 0.35                             |

† Treatment means significantly different at  $P < 0.05$  by LSD test.

(Tables 4 and 5). For example, high aphid pressure reduced seed number of both genotypes by 33% (Table 4), and reduced single seed weight by 50% in the susceptible genotype (Table 4;  $P = 0.03$ ).

It is noteworthy that the high aphid pressure treatment applied in study 2 ranged from 18,000 to 25,000 CAD, which is two to threefold higher than the same treatment applied in study 1; however, the proportion of yield reduction in KS4202 (relative to the control treatment) remained similar between these studies. Moreover, when comparing low  $\times$  high aphid pressure treatments at V1 within each genotype, no differences in yield parameters were observed (Tables 4 and 5). This demonstrates that soybean was susceptible to aphid infestation occurring at the V1 when CAD surpassed 10,000, independent of the genotype.

The mean CAD for V3-soybean infested under low aphid pressure was 9609  $\pm$  882.1 for the tolerant genotype and 11,537  $\pm$  576.2 for the susceptible genotype. Under high aphid pressure, the mean CAD for tolerant plants was 24,079  $\pm$  1332.3; whereas, the susceptible was 17,376  $\pm$  899.3. Yield parameters of both genotypes at low aphid pressure remained similar to the respective control; however, high aphid pressure had a detrimental impact

(i.e., yield parameters reduced by 27%) on the susceptible genotype (Tables 4 and 5). Conversely, high aphid pressure (~24,000 CAD) did not influence the yield parameters of tolerant plants (Tables 4 and 5).

The comparison between infestation levels (i.e., low × high aphid pressure) for V3-infested plants indicated that higher aphid pressure did not affect the yield parameters on the tolerant, but had a significant effect on the susceptible genotype (Tables 4 and 5). The data for the tolerant genotype were consistent with study 1, indicating that KS4202 tolerated various levels of aphid pressure during V3 while the susceptible genotype experienced yield losses when aphid pressure exceeded 10,000 CAD.

Despite the yield losses observed at the highest CAD treatment (25,000 CAD), when plants were infested at V1, lower CAD pressure (4000–9000 CAD) caused reductions in the yield parameters of tolerant genotype that varied from 0.9 to 17.7%. The same comparisons in the susceptible genotype resulted in reductions that ranged from 24 to 39%. These data demonstrate that the tolerant genotype withstood soybean aphid injury better than the susceptible at these lower CAD levels during V1.

As the soybean's vegetative phase progressed, plants infested at V3 were more resilient to aphid pressures above 10,000 CAD. Low aphid pressure (average of 10,000 CAD) had little or no impact on yield parameters of the susceptible genotype. This is consistent with previous studies, which found that minor injury or severe injury quickly managed in early stages of soybean development had no significant impact on soybean yield (He et al., 1991). Interestingly, some researchers found a positive relationship between low CAD and yield (Liere et al., 2015; Kucharik et al., 2016), suggesting some degree of overcompensation. We also observed a slight increase in some yield parameters of the susceptible soybean at the V3 stage; however, these differences were not significant. Conversely, increased aphid pressure (>10,000 CAD) restricted yield on the susceptible genotype, where yield parameters were reduced by 17 to 40% when compared with healthy, aphid-free plants. This finding compares favorably with other studies, where continuous infestation in the early vegetative stages caused a 20 to 30% yield reduction (Rhainds et al., 2007). Most importantly, the tolerant genotype withstood aphid pressure within the 17,000 to 25,000 CAD without a negative impact to the yield parameters evaluated.

This research also demonstrated that under higher aphid pressure, the tolerant genotype infested at the R1 was less tolerant than the same pressure at V3. This could be a result of the plant's physiological condition at the time of aphid introduction, and possibly the ability to compensate for early injury. Generally, soybean is less sensitive to stress in the vegetative stages than in the reproductive stages. Soybean can often compensate for bean leaf beetle, *Cerotoma trifurcata*, injury (e.g., defoliation) during the early vegetative stages (Hunt et al., 1994); however, stress during the reproductive period

can cause a significant impact on yield, particularly in the later stages due to the reallocation of photosynthates from vegetative to reproductive structures (Ostlie 1984; Singer 2001).

From an integrated pest management perspective, plant tolerance to insect injury has several advantages (Smith, 2005). Different from antibiosis and antixenosis, plant tolerance is conferred by a collection of plant characteristics and may not impose the same constraints on the arthropod's biology and/or behavior. Although it is possible that tolerance could affect herbivore performance (Stinchcombe, 2002), it is presumed that arthropods exposed to tolerant plants experience lower selection pressure than those on antibiotic or antixenotic plants. Hence, the likelihood of the emergence of virulent population (biotype) in response of a tolerant plant is minimized (Stinchcombe, 2002; Smith, 2005). The cultivation of tolerant plants favors the establishment of beneficial arthropods. Although the abundance and diversity of these organisms vary geographically, natural enemies play an important role in regulating the population growth and preventing soybean aphid outbreaks, particularly when they occur early in the season and at high densities (Rutledge et al., 2004; Costamagna and Landis 2006; Schmidt et al., 2008). Because tolerant plants have higher EILs and possibly higher economic thresholds (ETs), there is an increased opportunity for beneficial organisms to act, thereby decreasing the likelihood of an early pest management intervention. When curative treatments become necessary, the employment of a tolerant soybean translates into a greater lead-time (i.e., time to implement control strategies once populations reach the ET). This is relevant considering that growers often face delays with insecticide applications (e.g., weather conditions, equipment malfunction, or scheduling logistics), missing the lead-time of 7 d (Ragsdale et al., 2007). These plants may also serve as a platform for backcrossing resistance genes (e.g., *Rag* genes). In the emergence of a virulent biotype, a tolerant platform will minimize yield losses and allow time for the development and release of an additional antibiotic/antixenotic trait.

The results from this research support Pierson et al. (2010) and Prochaska et al. (2013), which found that KS4202 is tolerant to soybean aphids during the reproductive stages. In addition, this research documents tolerance also occurs in the early vegetative stages (i.e., V3) of this genotype. This research will contribute to the development of management alternatives to mitigate the impacts of soybean aphid injury and for establishing EILs for vegetative and reproductive stages of soybean aphid tolerant KS4202 soybean.

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