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Lia Marchi-Werle
University of Nebraska–Lincoln, lia.werle@unl.edu

Edson L.L. Baldin
São Paulo State University, elbaldin@fca.unesp.br

Hillary D. Fischer
University of Arkansas, Fayetteville, hdfische@uark.edu

Tiffany Heng-Moss
University of Nebraska - Lincoln, thengmoss2@unl.edu

Thomas Hunt
University of Nebraska-Lincoln, thunt2@unl.edu

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Lia Marchi-Werle,[1] Edson L. L. Baldin,[2] Hillary D. Fischer,[3] Tiffany M. Heng-Moss,[4] and Thomas E. Hunt[5]

1 Department of Entomology, University of Nebraska–Lincoln, 103 Entomology Hall, Lincoln, NE 68583
2 Department of Crop Protection, Sao Paulo State University (UNESP), Botucatu, SP 18610, Brazil
3 Department of Entomology, University of Arkansas, 319 Agriculture Building, Fayetteville, AR 72701
4 College of Agricultural Sciences & Natural Resources, University of Nebraska–Lincoln, 103 Agricultural Hall, Lincoln, NE 68583
5 Department of Entomology, NEREC Haskell Agricultural Laboratory, University of Nebraska—Lincoln, 57905 866 Road, Concord, NE 68728

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

Abstract

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an invasive species from Asia that has been the major economic insect pest of soybeans, *Glycine max* (L.) Merrill, since 2000. While use of soybeans expressing antibiosis and antixenosis is a well-studied strategy to manage this pest, aphid-tolerant soybeans remain underexplored. This study examined the relationship between cumulative aphid-days (CAD) and yield loss in the tolerant soybean KS4202 during two growing seasons to determine the economic injury levels (EILs) for soybean aphids on KS4202. Soybean aphid infestations were initiated during the soybean reproductive stages. A range of CAD treatments (3,000–45,000 CADs) were applied during the growing seasons. Aphid populations reached 45,000 CAD in 2011 and 38,000 CAD in 2013 in plots that were not treated with insecticides. It was estimated that the population doubling time was 9.4 d. In infested plots, soybean yield was reduced by 1.4–13.3%, equivalent to a 3.1% yield loss for every 10,000 CAD. Overall, most CAD treatments did not affect yield parameters, although CAD > 39,000 caused a significant reduction in most yield parameters. The EILs calculated for KS4202 ranged from 526 to 2,050 aphids/plant, which were approximately 2.5-fold higher when compared to EILs previously calculated for susceptible soybean. The adoption of soybean aphid tolerant soybean with higher EILs may help mitigate treatment delay problems by lengthening the treatment lead-time and possibly reduce the number of insecticide applications.

Keywords: IPM, plant resistance, tolerance, Glycine max, soybean aphid

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is an invasive pest of soybean, *Glycine max* (L.) Merr., first reported in North America in 2000 (Alleman et al. 2002). The insect has been reported in the majority of the soybean growing regions in the United States (Ragsdale et al. 2011), and remains the most important economic pest of this crop (Hurley and Mitchell 2017). Soybean aphids have a complex life cycle, known as heteroecious holocyclic, where the insect alternates sexual reproduction on its primary and secondary hosts. In North America, common buckthorn (*Rhamnus cathartica*) is considered the primary overwintering host (Voegtlin et al. 2005). Soybean is the alternative host, where females feed and reproduce in the absence of males (i.e., parthenogenic viviparae) during most of the growing season (Ragsdale et al. 2004).

The feeding damage caused by soybean aphids has a significant economic impact on soybean yield. Reductions in plant height, pod development, number of seeds and oil content are commonly reported (DiFonzo and Hines 2002, Ragsdale et al. 2007, Beckendorf et al. 2008). Initially, soybean aphid management relied heavily on foliar-applied pyrethroid insecticides, resulting in a sharp increase in pesticide applications for soybeans and increased production costs (Ragsdale et al. 2007, 2011; NASS [National Agricultural Statistics Service] 2016).

Integrated pest management (IPM) is a well-established strategy for managing agronomically important insect pests (Pedigo et al. 1986), and has been identified as the most cost-efficient tool to reduce soybean aphid outbreaks (Johnson et al. 2009, Ragsdale et al. 2011). The economic injury level (EIL) and economic threshold (ET) are key IPM concepts. Soybean aphid EILs and ETs were determined in a 3-yr multistate project (Ragsdale et al. 2007) considering soybean aphid population growth rate, control costs, market values and expected yield. The average estimated ET was 273 aphids per soybean plant. Once aphid densities reach the ET, and there is evidence that the population is increasing, treatment is recommended to avoid reaching the EIL (average of 674 aphids/plant). Under favorable conditions, soybean aphid asexual reproduction allows populations to grow dramatically. The treatment window based on the soybean aphid growth rate is approximately 7 d (Ragsdale et al. 2007), requiring that growers remain alert and mobilize the necessary resources to avoid economic losses.

Host-plant resistance (HPR) is an important component of soybean aphid IPM. Currently, five soybean genes have been reported to provide some level of resistance to soybean aphids (Hill et al. 2006a,b; Zhang et al. 2010; Jun et al. 2013). Named *Rag* genes (Resistance to *A. glycines*), these genes negatively affect soybean aphid biology (antibiosis) and under certain circumstances (e.g., genotype and gene type), may affect the insect's host preference (antixenosis). In the United States, varieties that contain *Rag1* and *Rag2* either containing a single gene or pyramided (both genes) have been commercialized; however, their availability to growers is limited. In addition, *Rag* soybeans have been threatened by the presence of three virulent soybean aphid populations. Referred as biotype 2, this population is capable of overcoming resistance imposed by the *Rag1* gene, with biotype 1 considered susceptible to this gene (Kim et al. 2008). Not long after biotype 2, Hill et al. (2010) characterized soybean aphid biotype 3 as populations that readily colonized *Rag2* soybeans but still susceptible to *Rag1*. The last biotype reported to date is biotype 4, which is virulent towards *Rag1*, *Rag2* and *Rag1/Rag2* pyramid (Alt and Ryan-Mahmutagic 2013). Although soybean aphid virulence has limited the durability of Rag genes, studies have found that implementing refuge areas as well as breeding three-gene pyramid soybean may extend the durability of these genes (Varenhorst et al. 2015, Ajayi-Oyetunde et al. 2016).

Tolerance is another category of HPR conferred by polygenic traits, which enables plants to withstand insect feeding without incurring excessive yield losses (Smith 2005). The deployment of tolerant plants may benefit IPM programs in several ways. A higher EIL may allow the adoption of a higher ET, resulting in fewer insecticide applications and greater cost effectiveness. Additionally, tolerant plants do not impose the same levels of selection pressure as antibiotic or antixenotic plants, minimizing the selection of biotypes (Smith 2005). Tolerance can be more compatible with biological control agents, reducing soybean aphid outbreaks (Costamagna and Landis 2006, Schmidt et al. 2008) and maintaining populations below the ET.

Studies have reported that the soybean genotype KS4202 has moderate levels of tolerance to soybean aphids in both vegetative and reproductive stages (Pierson et al. 2010, Marchi-Werle et al. 2017). Furthermore, field evaluations that included KS4202 have reported yield losses of 13% at a range of 35,000–50,000 cumulative aphid-days (CAD) (Prochaska et al. 2013), when at that same CAD, Ragsdale et al. (2007) estimated approximate yield reductions of 24–36%. Considering these findings, deployment of tolerant soybeans to manage soybeans aphids requires refinement of current soybean aphid IPM. Thus, the objective of this research was to quantify the relationship between CAD and yield loss in the tolerant KS4202 and discuss the use of tolerance in soybean aphid IPM.

Materials and Methods

Agronomic Practices and Plant Material

The field studies were conducted in 2011, 2012, and 2013 at the University of Nebraska Haskell Agricultural Laboratory, Concord, NE. Aphid numbers in 2012 were low and required infestation levels where not met; therefore, only 2011 and 2013 studies are reported. The studies were performed with the soybean KS4202, an F4 plant selection from KS4694 × C1842. KS4202 is an early maturity group IV soybean with indeterminate growth habit. In 2011 and 2013, soybeans were planted in a corn-soybean rotation in an Alcester-silt loam soil. Soil was disked prior to planting, following agronomic practices for northeastern Nebraska. Soybean seeds were planted at a density of 425,000 seeds/ha. Due to the wet conditions in May, and because soybean aphids are attracted to late planted soybeans, planting occurred on 3 June 2011 and 11 June 2013. In the first season, plots were not irrigated, as the irrigation system was inoperative, whereas plots in 2013 were irrigated via lateral irrigation system. Weeds were managed with glyphosate (Durango, Dow AgroSciences LCC, Indianapolis, IN), flumioxazin (Valor, Valent U.S.A Corporation, Walnut Creek, CA) and 2,4-D ester (Weedone, Nufarm Inc., Alsip, IL) herbicides on 3 May 2011, following manufacturer's recommendations. In 2013, flumioxazin + glyphosate (Flexstar GT, Syngenta Crop Protection, Greensboro, NC), 2,4-D ester and glyphosate were applied on 29 April, and fluthiacet-methyl (Cadet, FMC Corporation, Philadelphia, PA), fomesafen (Reflex, Syngenta Crop Protection), and clethodim (Select Max, Valent U.S.A Corporation) were applied on 18 June, according to label recommendations.

Field Plot Design

The experimental design for each year was a randomized complete block with four replications. Each plot consisted of four rows, measuring 15.2 m long and 3 m wide with 76.2 cm row spacing. There were five CAD treatments designed for each season. Data was collected from the two center rows of each plot. CAD provides a good estimation of aphid pressure over time, and is more informative than aphid number. CAD was calculated using the formula:

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

where n is the number of sample dates, x_i is the mean number of aphids per plant (i.e., average per plot) on sample date i, and $(t_i - t_{i-1})$ is the number of days between two consecutive sample dates (Hanafi et al. 1989). In 2011, the CAD treatments were 0 CAD (control = aphid free), 3,000, 8,000, and 13,000 and untreated (= not treated with insecticide), whereas in 2013 they were 0 (control) 5,000, 13,000, and 22,000 and untreated. The difference in the CAD treatments between growing seasons reflects the soybean aphid's growth rate, which was slightly higher in 2013 (**Table 1**). The treatments designated as 'untreated' were conducted to simulate the natural soybean aphid population cycle for that given year. Once the desired CAD treatment level was achieved (average across the blocks), a foliar insecticide lambda-cyhalothrin at 28.0 g ai/ha (Warrior with Zeon Technology, Syngenta Crop Protection) was applied using standard ground equipment. Although the complete eradication of aphids is not feasible, regular monitoring of the control plots (0 CAD) and CAD targeted plots was

Table 1. Growth rate, discrete daily increase and population doubling time of soybean aphids on KS4202 during 2011 and 2013 growing seasons

Season	*Y intercept ± SEM*	R^2	*Growth rate (r) ± SEM*[a]	*P*	*Discrete daily increase rate (λ)*[b]	*DT (d)*[c]
2011	1.742 ± 0.104	0.96	0.0719 ± 0.006	0.0004	1.0745	9.64
2013	1.188 ± 0.230	0.91	0.0759 ± 0.001	0.0003	1.0788	9.13

a. Aphid population growth rate in untreated plots using the equation $N_t = N_0e^{rt}$, where N_0 = initial population density, r = population growth rate (linear regression slope), and t (in days) is based on the interval when 80% of the plants were infested until aphid densities reached a peak.

b. Discrete daily increase rate = e^r.

c. DT = Population doubling time (days); DT = ln(2)/r.

conducted to ensure that populations remained close to zero aphids per plant. Insecticide interventions were performed as needed.

Aphid Infestation, Evaluations and Harvest

Soybean aphids naturally occurred and colonized soybeans in 2011. In 2013, populations of soybean aphids were low and intermittent in northeast Nebraska, so plots were artificially infested. As a precautionary measure due to low aphid population in 2012, two mesh cages measuring 2 × 2 × 2 m were installed in an adjacent soybean field in June. Aphid infested plants from a laboratory colony were introduced in the cages for population expansion and acclimatization prior to artificial infestation. The initial aphids used for the artificial infestation were from a colony maintained in a growth chamber (23 ± 2°C and 16:8 [L: D] h), and were progeny of a Nebraska isolate (biotype 1), collected in a nearby commercial field in 2011. The artificial infestation occurred on 30 July 2013 to mimic a typical infestation of soybean aphids in northeast Nebraska. Leaf sections containing 10–50 nymph and adult aphids were placed approximately 60 cm apart on the top trifoliate of one soybean plant in the two center rows.

Evaluations were performed every 5 to 7 d after the initial detection or artificial introduction of soybean aphids and were terminated once the number of insects per plant was close to zero. In each plot, five plants were destructively sampled for estimating aphid densities. Once the targeted CAD treatment levels were reached, plots were sprayed within 48 h.

At maturity, 10 plants from the treatment rows (two center rows) from each plot were manually cut at the base of the stem and stored in a cold walk-in chamber for further processing. The sampled material was oven-dried and the following yield parameters were determined: number of pods per plant, number of seeds per pod, average seed weight, average pod weight, and total biomass (Hill et al. 2004). Upon maturity, all treatment rows were harvested on 4 October 2011 and 29 October 2013 with a small plot combine, and yield was adjusted to seed moisture of 13%.

Aphid Population Growth and EIL Calculation

Soybean aphid population growth rates (*r*) across 2011 and 2013 were calculated within the time interval of when 80% of the plants were infested and populations reached peak densities (Ragsdale et al. 2007). Aphid densities were natural log transformed and graphed against time to determine the growth rate (i.e., slope of the linear regression). In addition, discrete daily growth rate (λ) was calculated based on the average of both growing seasons, using the expression:

$$\lambda = e^r \tag{2}$$

Prior to calculating the EILs, a gain threshold (GT) in percentage yield loss was determined using the equation by Pedigo et al. (1986): GT (% yield loss):

$$\frac{C}{V \times Y} \times 100 \tag{3}$$

where *C* is treatment cost ($/ha) of soybean aphid infested fields, *V* is the crop value ($/ton) and *Y* is the maximum yield (i.e., aphid-free plots) from both seasons. To provide direct comparison, the control costs used in this study were based on the survey conducted by Ragsdale et al. (2007). The crop value was determined based on the current U.S. soybean prices by the NASS (USDA NASS 2016); soybean prices reported in Ragsdale et al. (2007) were also included in this calculation. The EIL, expressed as CAD was calculated as outlined by Ragsdale et al. (2007):

$$\frac{\beta_0 - (\%\ \text{yield potential})}{\beta_1} \qquad (4)$$

using the slope (β_1) and intercept (β_0) from the regression curve built of CAD versus percentage of yield loss, and the percentage of yield potential. The percentage of yield potential was obtained by deducting the GT (equation 3) from the maximum yield potential (Stone and Pedigo, 1972). In practical terms, GT indicates the soybean yield that will pay for control costs. The conversion of EIL in CAD to aphids per plant (*l*) also proceeded as outlined by Ragsdale et al. (2007) with the expression

$$l = \frac{s(\lambda - 1) + 1}{\lambda} \qquad (5)$$

where *s* is the EIL in CAD (per plant) and λ is the discrete daily population growth rate. The time (in days) that a given population feeding on KS4202 would require to reach the EIL once the ET (average of 273 aphids per plant from Ragsdale et al. 2007) was calculated with the population growth model: $Nt = N_0e^{rt}$ or $\ln(Nt) = \ln(N_0) + rt$ (equation 6), where N_0 is the initial aphid density, *r* is the population growth rate, and *t* is time expressed in days.

Statistical Analysis

An analysis of variance (ANOVA) was used to analyze yield parameters and plot yield in PROC GLIMMIX in SAS 9.3 (SAS Institute, Cary, NC). Experimental treatments for both seasons were treated as fixed factors; whereas, replication blocks nested within experimental runs were treated as random factors. Means were separated when the interaction or main effect was significant ($P < 0.05$). The results presented for each growing season were originated from the same mixed model analysis.

To evaluate the treatment effect (CAD) and percentage of maximum yield for KS4202, an '*F*-test' was performed in R version 2.15.1 (R Foundation for Statistical Computing, Wien, Austria), according to Ritz and Streibig (2008). This statistical analysis computes the difference between residual sum of squares (RSS) for two considered models. The models need to be fitted to the data: a full model (full) and a sub-model (sub) of the full model. In the full model, the CAD treatments for each year were estimated separately; whereas, the sub-model estimated the parameters for a single model fit to the data of all treatments combined. Models were fitted to the data and parameters estimated using the nls function of R (version 2.15.1, R Foundation). The following equation represents the *F*-test performed:

$$\frac{(\text{RSSsub} - \text{RSSfull})/(\text{dfsub} - \text{dffull})}{\text{RSSfull}/\text{dffull}}$$

where RSSsub and RSSfull indicate the minimized RSS for the CAD and yield estimates of the sub-model and full model, respectively; dfsub and dffull represent the degrees of freedom for the sub-model and full model, respectively. A large *F*-value indicates that two nested models are different, whereas a small *F*-value indicates that both models provide similar fit to the data. Next, the *F*-value was converted to a *P*-value from the *F*-distribution (dfsub − dffull, dffull). A significant analysis ($P < 0.05$) indicates that models are statistically different. If statistically significant, the full model can be used along with the parameters for each treatment level; whereas, a nonsignificant test ($P > 0.05$) indicates that nested models are not different and that a sub-model may be used.

Results and Discussion

Soybean Aphid Population Density and CAD

The ET established for soybean aphids of 273 insects per plant (Ragsdale et al. 2007) was surpassed in all treatments in 2011 and 2013, with the exception of 0 CAD (control = aphid-free) treatment (**Figs. 1 and 2**). Infestation began in late July when plants were in the early reproductive stage (R2) (Fehr and Caviness 1977**).** In the untreated plots, where soybean aphids were allowed to colonize soybeans throughout the season, the mean peak aphid number of both seasons was 2,513 ± 59, which corresponds to 3,108 and 1,918 aphids per plant in 2011 and 2013, respectively. Aphid peak density occurred on 18 August 2011 and 6 September 2013 when KS4202 plants were within R4 (full-size pods) and R5 stage (beginning seed).

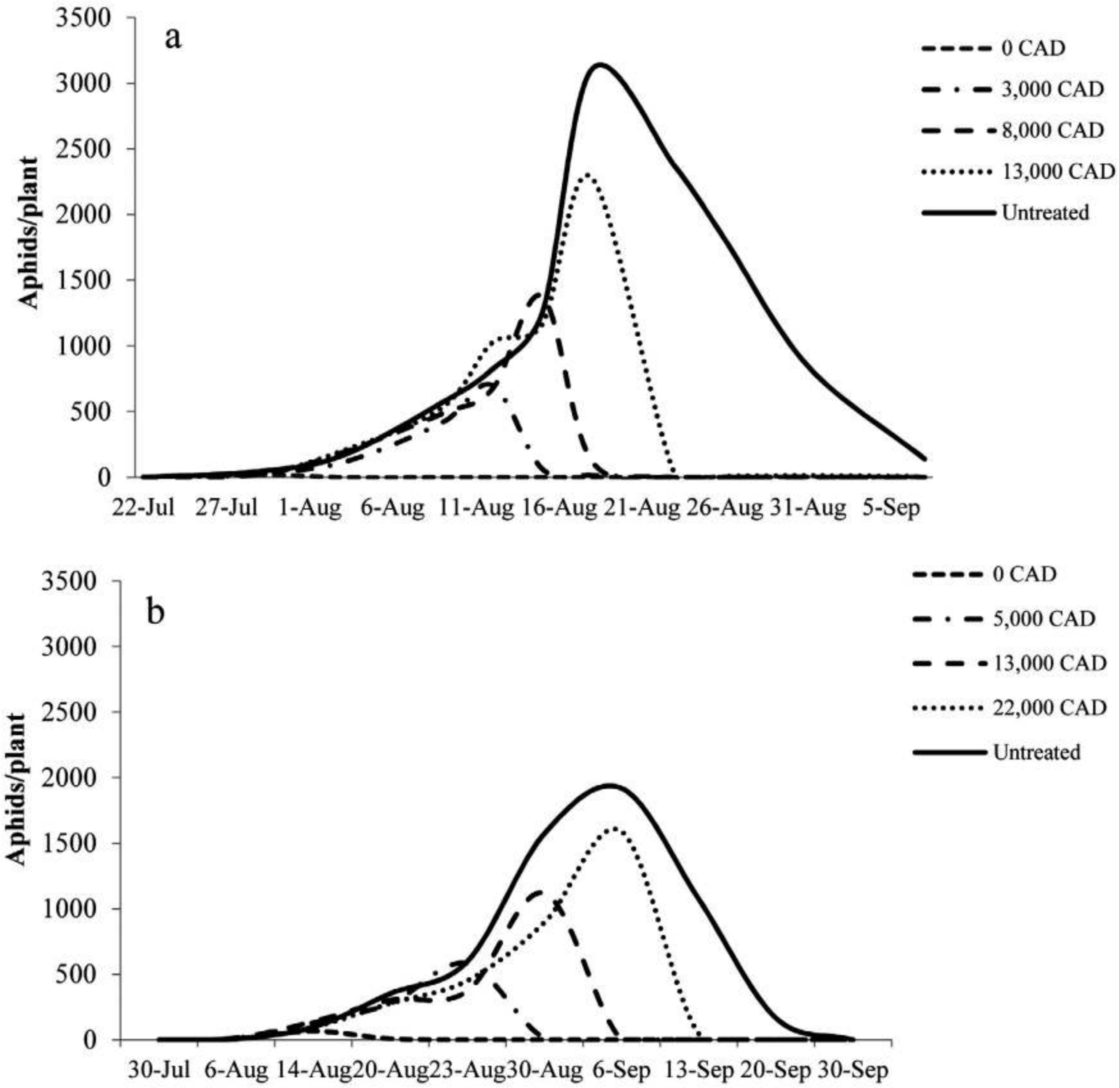

Fig. 1. Mean aphid number for KS4202 during the weekly evaluations in the growing season of 2011 (a) and 2013 (b) in each respective target CAD treatment.

Soybean aphid growth rate and discrete daily increase rate were consistent for the two seasons (Table 1), resulting in population doubling time of 9.64 d and 9.13 d, in 2011 and 2013, respectively. Peak aphid numbers in 2013 (Fig. 1b) were generally lower than 2011 (Fig. 1a), however, aphid infestation was prolonged in 2013 (Fig. 2a and b). In 2011, the targeted CAD treatments of 0, 3,000, 8,000, and 13,000

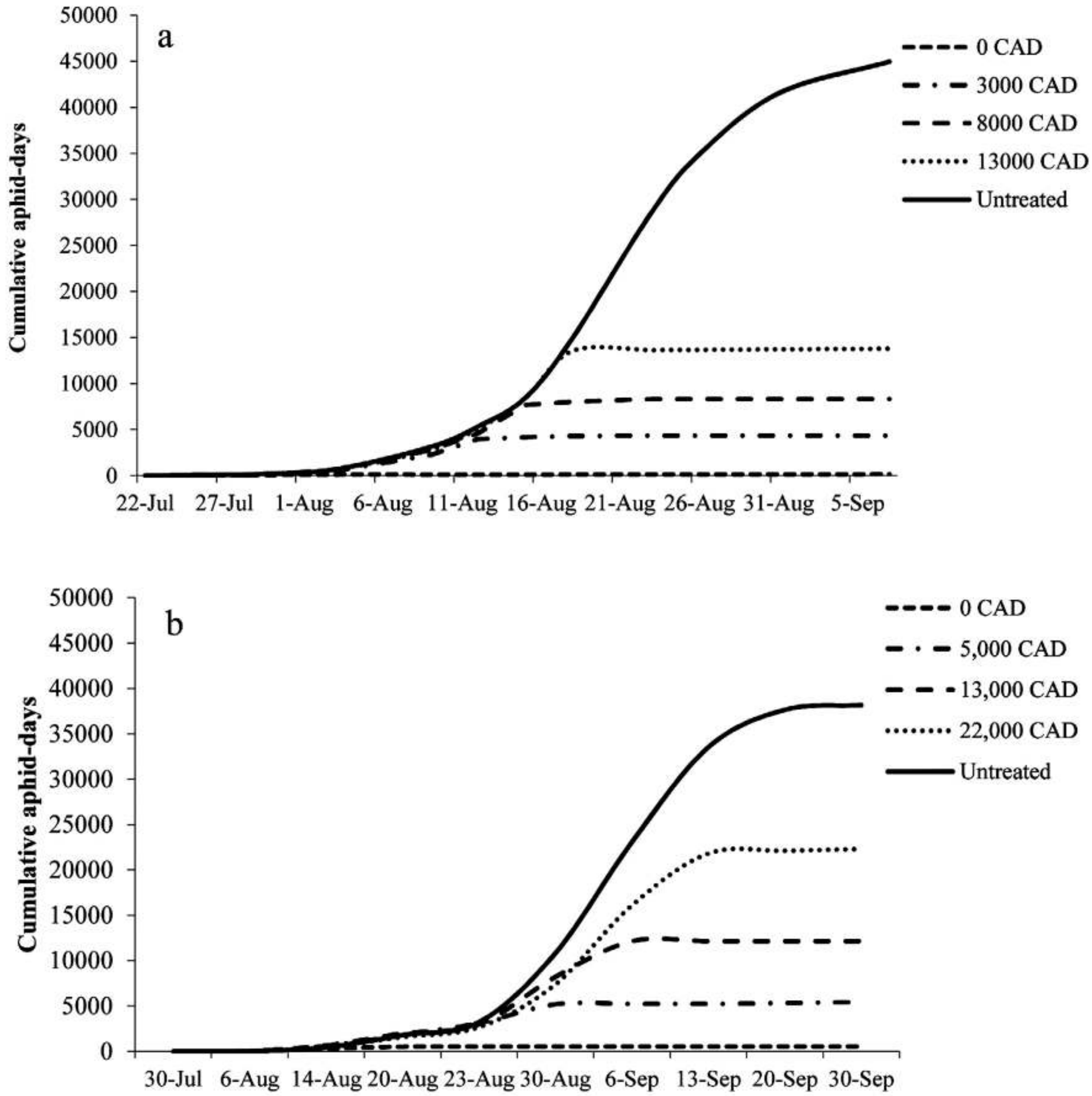

Fig. 2. CAD in the target treatments in 2011(a) and (b) 2013.

had an actual CAD mean of 163 ± 13; 4,354 ± 405; 8,313 ± 506; and 13,776 ± 1,044, respectively. The actual CAD means in 2013 for the treatments of 0, 5,000, 13,000, and 22,000 CAD were 542 ± 62; 5,458 ± 330; 12,138 ± 234; and 22,303 ± 2,779. In untreated plots, CAD reached 44,959 ± 4,148 in 2011 and 38,174 ± 4,790 in 2013.

KS4202 Yield Response to Soybean Aphids

There were no differences in total yield among 0 (control), 3,000, 8,000, and 13,000 CAD treatments in 2011. However, untreated plots had a yield reduction of 13.33%, which was statistically different from the remaining treatments (Table 2). A similar pattern occurred in 2013, although there was not a significant difference in total yield among the treatments, even when soybean aphids were allowed to colonize the field throughout the season (**Table 2**). In the untreated plots, yield was reduced by 12.60% when compared to 0 CAD treatments (*P* = 0.06), which is also consistent with the data from the previous season.

Yield parameters were also evaluated. In 2011, total pod weight, total seed weight, and total plant biomass for CAD treatments of 3,000, 8,000, and 13,000 treatments were not statistically different from the 0 CAD (control) treatment (**Table 3**). However, there was a significant reduction in those parameters when compared to untreated plots, where CAD levels were near 45,000. Although plants from 3,000

Table 2. Estimated yield (ton/ha) for KS4202 under different CAD treatments in 2011 and 2013

Treatment	*CAD ± SEM*	*Yield ± SE*	*Yield reduction (%)*[a]
2011			
0 CAD	163 ± 13	2.85 ± 0.10 a	—
3,000 CAD	4,354 ± 405.2	2.85 ± 0.00 a	0
8,000 CAD	8,313 ± 506.9	2.81 ± 0.04 a	1.40
13,000 CAD	13,776 ± 1,044	2.76 ± 0.06 a	3.15
Untreated	44,958 ± 4,148	2.47 ± 0.03 b	13.33
2013			
0 CAD	542 ± 62	3.49 ± 0.09 a	—
5,000 CAD	5,458 ± 330	3.43 ± 0.10 a	1.72
13,000 CAD	12,138 ± 234	3.29 ± 0.18 a	5.73
22,000 CAD	22,303 ± 2,779	3.21 ± 0.15 a	8.02
Untreated	38,174 ± 4,790	3.05 ± 0.20 a	12.60

Means within the same column followed by the same letter are not statistically different (*P* > 0.05), LSD test.

a. Yield reduction (%) relative to aphid-free (control) plots for each growing season.

CAD and untreated treatments had significantly fewer pods than the 0 CAD treatment, no differences were observed when comparing 0 CAD with 8,000 and 13,000 CAD treatments (Table 3). Untreated (P = 0.01) and 3,000 CAD (P = 0.08) treatments also had fewer seeds than 0 CAD treatment. Furthermore, the single seed weight for the 8,000 and 13,000 CAD treatments did not differ from 0 CAD treatment, but the untreated treatment produced smaller seeds than 0 CAD treatment (P = 0.01). Seeds from 3,000 CAD plots were approximately 8% heavier than seeds from control plot (Table 3), indicating that plants exposed to this treatment may be compensating for a reduction in seed number by producing heavier seeds and thus no differences were observed in total yield (Table 2).

Total biomass, number of pods, pod weight, number of seeds and total seed weight were not significantly different among any of the treatments in 2013 (**Table 4**), although single seed weight for untreated plots (CAD ~38,000) was significantly lower than 0, 5,000, 13,000, and 22,000 CAD treatments.

Table 3. Mean ± SEM of yield parameters of KS4202 under different CAD treatments harvested in 2011

Treatment	*Total biomass/plant (g)*	*No. of pods/plant*	*Total pod weight/plant (g)*
0 CAD	18.87 ± 1.52 a	38.10 ± 3.95 a	11.95 ± 0.97 a
3,000 CAD	17.40 ± 0.95 a	32.78 ± 1.55 bc	11.21 ± 0.70 a
8,000 CAD	18.84 ± 0.80 a	37.48 ± 2.01 a	11.92 ± 0.56 a
13,000 CAD	17.62 ± 0.52 a	35.70 ± 1.91 ab	11.37 ± 0.39 a
Untreated	14.76 ± 0.51 b	30.98 ± 0.94 c	9.11 ± 0.31 b
Treatment	*No. of seeds/plant*	*Total seed weight/plant (g)*	*Single seed weight (g)*
0 CAD	74.38 ± 6.59 a	8.34 ± 0.60 a	0.113 ± 0.002 b
3,000 CAD	65.93 ± 3.97 ab	7.95 ± 0.51 a	0.121 ± 0.001 a
8,000 CAD	74.28 ± 3.63 a	8.38 ± 0.35 a	0.113 ± 0.003 b
13,000 CAD	71.18 ± 3.58 a	7.98 ± 0.26 a	0.113 ± 0.003 b
Untreated	58.68 ± 0.84 b	6.29 ± 0.25 b	0.107 ± 0.003 c

Means within the same column followed by the same letter are not statistically different (P > 0.05), LSD test.

Table 4. Mean ± SEM of yield parameters of KS4202 under different CAD treatments harvested in 2013

Treatment	*Total biomass/plant (g)*	*No. of pods/plant*	*Total pod weight/plant (g)*
0 CAD	31.52 ± 3.41 a	53.50 ± 5.31 a	25.18 ± 2.88 a
5,000 CAD	33.46 ± 2.90 a	56.70 ± 5.15 a	27.15 ± 2.52 a
13,000 CAD	32.79 ± 3.46 a	56.25 ± 6.10 a	26.37 ± 3.04 a
22,000 CAD	34.78 ± 4.43 a	59.08 ± 7.34 a	28.07 ± 3.91 a
Untreated	32.87 ± 3.63 a	61.93 ± 6.53 a	26.61 ± 3.23 a
Treatment	*No. of seeds/plant*	*Total seed weight/plant (g)*	*Single seed weight (g)*
0 CAD	111.69 ± 11.75 a	19.54 ± 2.23 a	0.173 ± 0.005 a
5,000 CAD	121.03 ± 11.49 a	21.05 ± 1.93 a	0.175 ± 0.005 a
13,000 CAD	118.90 ± 13.30 a	20.46 ± 2.33 a	0.173 ± 0.004 a
22,000 CAD	126.13 ± 16.61 a	21.79 ± 2.98 a	0.173 ± 0.005 a
Untreated	127.95 ± 14.55 a	20.28 ± 2.47 a	0.158 ± 0.005 b

Means within the same column followed by the same letter are not statistically different ($P > 0.05$), LSD test.

KS4202 tolerance to soybean aphids was initially documented in greenhouse studies (Pierson et al. 2010, Marchi-Werle et al. 2017). Pierson et al. (2010) examined tolerance in the reproductive stages of KS4202, and found no impact on the average seed weight or number of seeds per pod in the presence of soybean aphids. Marchi-Werle et al. (2017) also reported KS4202 tolerance in the early vegetative and reproductive stages, where most of the yield parameters for plants infested during the V3 and R1 stages were unaffected at 1,000 or 2,000 aphids per plant (corresponding range of 4,000–8,500 CAD). In field trials, Prochaska et al. (2013) corroborated the presence of tolerance in KS4202. Their research included multiple field seasons, and found that KS4202 tolerated soybean aphid feeding without the expected severe impact on yield. Moreover, KS4202 tolerance to silverleaf whitefly (*Bemisia tabaci* Genn.) feeding has been reported in Brazil (Cruz et al. 2016).

To standardize the yield data from both years and permit a direct statistical comparison, the proportion of maximum yield (relatively to 0 CAD treatment) was calculated (**Fig. 3**). An *F*-test indicated there was no significant difference in the proportion of maximum yield by

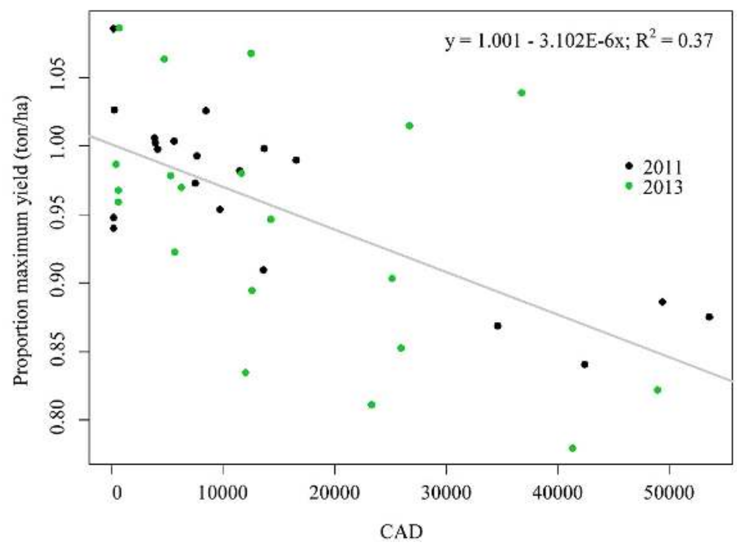

Fig. 3. Percentage of maximum yield comparing aphid-free (control) plots with the target CAD treatments in 2011 and 2013 seasons. $F = 23.91$; df = 1, 38; $P < 0.0001$.

CAD across seasons ($P = 0.39$), so 2011 and 2013 were included in one model. An inverse relationship between CAD and yield was detected (Fig. 3; $F = 23.91$; df = 1, 38; $R^2 = 0.37$; $P < 0.0001$). The intercept of the equation $y = -3.102E^{-6}x + 1.001$ passes through 100% of the proportion maximum yield (Fig. 3); this indicates that linear regression was adequate to explain the relationship between yield loss-CAD. No evidence of feeding by bean leaf beetle, *Cerotoma trifurcata* (Forster), or injury caused by other pests or diseases was observed, indicating that yield losses observed were caused by soybean aphid feeding.

The CAD treatment over two growing seasons in this study varied from 3,000 to 44,000. A visual comparison between CAD and proportion of maximum yield from Ragsdale et al. (2007) multi-state study and this research is provided on Fig. 4. Ragsdale et al. (2007) calculated that soybean yield is reduced by 6.88% for every 10,000 aphid-days accumulated for soybean aphid susceptible soybeans. In contrast, the slope of the regression obtained for KS4202 was -3.102×10^{-6}, indicating that yield was reduced by 3.10% (95% CI of 1.82–4.38%) for every 10,000 aphid-days accumulated (**Fig. 4**), so yield loss in KS4202 is approximately 45% of the yield loss of the susceptible soybean varieties used in the Ragsdale et al. (2007) multi-state study.

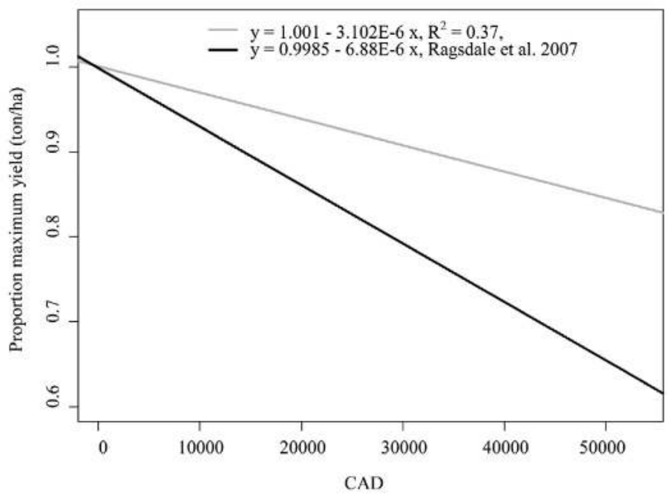

Fig. 4. Comparisons of simple regressions of proportion of maximum yield (ton/ha) and CAD of soybean KS4202 and multi-state study by Ragsdale et al. (2007).

Economic Injury Levels

The EILs calculated ranged from 526 to 2,050 aphids per plant (CAD = 8,580 to 16,898), averaging 1,177 aphids per plant (CAD = 9,699) (Table 5). Considering a generalized commodity price of $202.09/ton used by Ragsdale et al. (2007) and the control cost of $16.41/ha, the EIL for KS4202 is 1,041 per plant (**Table 5**), when under the same parameters is at most 684 aphids per plant in the aforementioned study.

The establishment of an ET prevents pest populations from reaching the EIL (Pedigo et al. 1986). The ETs presented in the Ragsdale et al. (2007) multi-state study are based on the mean rate of soybean aphid population growth (r = 0.127), and provide a lead-time of 3–7 d to arrange curative action (i.e., apply insecticide). The soybean aphid growth rate is this study (Table 1; r = 0.074 and DT = 9.38) was lower than the multi-state average (r = 0.127 and DT = 6.8), but within the range reported by Ragsdale et al. (2007). Lead-time is particularly important with respect to soybean aphid because of the soybean aphid rapid population growth potential. Soybean aphid populations cannot only reach the EIL in a relatively short time, but also increase well beyond the EIL to levels that frequently result in yield losses >20%. However, even with a recommended lead-time of 7 d (Ragsdale et al. 2007, Hodgson et al. 2012), this can pose significant problems for farmers, where weather and scheduling delays, or even late decision-making

Table 5. EILs for soybean aphids on tolerant KS4202 soybean

Soybean market price ($/ton)[b]	*Control cost ($/ha)*[c]	*EIL: CAD*	*EIL: aphids per plant*[a]	
			Growth rate = 0.074 (Nebraska)	*Growth rate = 0.127 (Multi-state)*
202.09	16.41	8,580	612	1,041
	24.51	12,656	902	1,536
	32.94	16,898	1,205	2,050
220.46	16.41	7,892	563	958
	24.51	11,628	829	1,411
	32.94	15,517	1,106	1,883
238.83	16.41	7,310	522	887
	24.51	10,759	767	1,306
	32.94	14,348	1,023	1,741
376.66	16.41	4,753	340	577
	24.51	6,940	495	843
	32.94	9,216	657	1,119
416.66	16.41	4,328	309	526
	24.51	6,305	450	765
	32.94	8,362	597	1,015
Mean		9,699	691	1,177

a. For comparison purposes, the EILs in aphids per plant were calculated based upon the growth rates observed during 2011 and 2013 in Concord, NE (r = 0.074) and the multi-state growth rate (r = 0.127) from Ragsdale et al. 2007.

b. Market price in $/bu equivalents are $5.50, 6.00, 6.50, 10.25, and 11.34, respectively.

c. Control cost in $/ac equivalents are $6.64, 9.92, and 13.33, respectively.

(i.e., initiating scouting after populations reach the ET) can result in treatment well after populations reach and exceed the EIL.

The higher EILs of soybean aphid tolerant varieties, such as KS4202, can help mitigate treatment delay problems by lengthening the treatment lead-time. For example, the mean ET for soybean aphid from Ragsdale et al. (2007) is 273 aphids per plant with a corresponding mean EIL of 674 aphids per plant. The lead-time for aphid populations to increase from 273 aphids per plant to 674 aphids per plant is 7 d. For the soybean aphid tolerant KS4202, a corresponding lead-time would be on average 11 d. The time interval between scouting and employment of control strategies is of importance especially when dealing with pests of rapid growth rates and high economic impact. While most management tactics are employed within 7 d of determining the need, difficulties such as inclement weather, equipment malfunction, or scheduling difficulties can delay insecticide application and result in economic loss. In this case, the advantage of using tolerant plants is the flexibility to schedule chemical control.

Different from other insect-pests, the soybean aphid ET is a comprehensive value based on the population doubling time (Ragsdale et al. 2007). This means that even when commodity price is high, ET is constant. Considering that the damage boundary (i.e., lowest pest population that causes measurable yield loss) for soybean aphids is estimated at about 4,000–5,000 CAD or 485 to 600 aphids per plant, control actions beforehand would adversely affect natural enemies (Tilmon 2014). In the case of soybean aphids, the ET established has been set high enough to permit maximum response by natural enemies and avoid unnecessary insecticide interventions (Ragsdale et al. 2007), but at the same time is set to be lower than the damage boundary.

Although a case can be made for keeping the practical and widely adopted soybean aphid ET (273 aphids per plant) and benefiting from the more flexible insecticide application lead-time associated with a soybean aphid tolerant soybean, increasing the ET could be argued. For example, assuming that soybean aphid population growth of $r = 0.0127$ and a lead-time of 7 d, the average ET for KS4202 is 476 aphids per plant (based on the range of commodity prices and control costs), when the same calculations resulted in an average of 198 aphids per plant in Ragsdale et al. (2007). As a basic component of decision making in pest management, the ET is set to guide growers on when to take control action. Redefining (i.e., increasing) the ET for tolerant soybeans would result in delayed control applications and possibly fewer applications and associated costs. Although insecticide resistance has not been confirmed in the United States, it's crucial to consider the impacts of repeated exposure of these chemicals as aphids have a high capacity of reproduction and dispersion (McCornack et al. 2004, Zhang et al. 2008). In that sense, the use of tolerance in general may result in reduced insecticide application. This has long-term benefits, as minimizing chemical control enhances the conservation of natural enemies. The establishment of a strong predator and parasite community enhances soybean aphid IPM, extending soybean aphid biological control even after winged forms return to the overwintering host (Yoo et al. 2005).

Future research should focus on the implementation of KS4202 as a platform to backcross antibiotic/antixenotic (single or pyramided) genes. The combination of tolerance with traits that are biologically detrimental or affect soybean aphid's host preference may provide a more stable management approach by keeping its population below economic damaging levels. Although, in theory, it is possible that tolerance could affect herbivore performance (Stinchcombe 2002), researchers generally believe that arthropods on tolerant plants experience lower selection pressure than those on antibiotic or antixenotic plants, which minimizes the likelihood of the emergence of a virulent population (biotype) (Stinchcombe 2002, Smith 2005). Tolerant plants would not need the same level of antibiosis or antixenosis as non-tolerant plants when considering the total effect of the resistant plant on the insect, and may be more durable because it is conferred by a collection of plant characteristics (Smith 2005). Even if virulent aphid populations emerge in response to the higher pressure imposed by antibiotic and antixenotic traits, the aphid tolerant background in these plants is likely to prevent substantial yield losses.

The integration of tolerant plants into IPM programs is a valuable tactic that remains underexplored. Difficulties in identifying tolerance mechanisms for incorporation in breeding programs or perhaps the ability of harboring large insect populations may have caused tolerance to receive little attention. This work represents the first attempt to develop EILs for aphid-tolerant soybeans and provides support for the proper deployment use of tolerance in the field.

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