EXHIBIT C
Cover Sheet

Final Report
for
Texas Grain Sorghum Board (TGSB)

Project Title:	Assessing the impact of planting date, insecticide seed treatments, and resistant varieties of sorghum on timing and severity of SCA infestations in Texas High Plains
Institution or Organization:	Texas A&M AgriLife Research
Principal Investigator(s):	Ada Szczepaniec, Assistant Professor of Entomology, Texas A&M AgriLife Research, Amarillo, TX
Other Investigator(s):	Jourdan Bell, Extension Agronomist, Texas A&M AgriLife Extension, Amarillo; Pat Porter, Extension Entomologist, Texas A&M AgriLife Extension, Lubbock; Blayne Reed, IPM Agent, Texas A&M AgriLife Extension, Plainview
Cooperator(s):	Ed Bynum, Extension Entomologist, Texas A&M AgriLife Extension, Amarillo; Emi Kimura, Extension Agronomist, Texas A&M AgriLife Extension, Vernon; Kay Ledbetter, Ag Communications Specialist, Amarillo

<u>List of All Project Expenditures:</u>

Amarillo (Szczepaniec): Budget - $10,072.5
Expenditures: Salaries, wages, and benefits – $13,443.56 ($8,258.67 covered by this grant; $5,184 covered by Szczepaniec startup funds);
Travel: $748.44
Supplies and Materials $1,554.51:

Amarillo (Bell): Budget - $4,756.5
Expenditures: Wages and benefits - $4,600.6

Lubbock (Porter): Budget - $5,872
Expenditures: $0

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Executive Summary

This project was designed to evaluate the effect of plant date (early, common), sorghum varieties (susceptible, tolerant), and neonicotinoid seed treatments (present, absent) on the rate of increase and abundance of sugarcane aphids (SCA) and sorghum yield in the Texas High Plains. The protocol included detailed counts of the SCA once plots were colonized, and foliar insecticide applications when aphid numbers reached the 50-125 SCA/leaf threshold developed and used successfully in South Texas. We proposed to execute these experiments in Bushland (Amarillo PIs) and in Lubbock (Lubbock Co-PIs). Owing to the Co-PI reported issues with SCA colonization of sorghum at the Lubbock location, however, this report contains only data collected from the Bushland location.

We report several key outcomes from the first year of this experiment that will be relevant to sorghum producers in the Texas High Plains. We observed that once SCA colonized the plots, they increased in numbers very rapidly, and foliar applications of insecticides were required only two weeks following colonization. Notably, we used the threshold of 50-125 SCA/leaf to trigger insecticide applications (Sivanto® at 4 oz/A) and we achieved an effective and long-lasting suppression by adhering to this threshold. Moreover, as has been demonstrated elsewhere, tolerant sorghum variety (DKS37-07) had significantly fewer aphids than the susceptible variety. Remarkably, even when the early-planted DKS37-07 plots were never treated with foliar insecticides the yield was comparable to the insecticide-treated plots. Insecticide seed treatments, on the other hand, offered some protection against aphids, but only in sorghum planted at common planting date, and not in early-planted sorghum.

It is important to note that this is only one year of field data, and additional work is necessary to validate these outcomes. However, the early recommendations based on this research would be to 1) scout often, especially at the end of July based on the 2015 and 2016 SCA reports, and apply insecticides when thresholds are reached; 2) use tolerant varieties of sorghum if they are agronomically sound for the area; 3) omit insecticide seed treatments if planting sorghum early.

Technical Objectives

The goal of this research was to examine the effectiveness of several cultural practices and management tactics as viable strategies to reduce the impact of SCA on sorghum production in Texas Panhandle. Our objectives were to quantify the effects of 1) planting date, 2) insecticide seed treatments, and 3) tolerant varieties of sorghum on timing and severity of SCA infestations in replicated experiments at two locations. Data from one of the locations (Lubbock) were not collected owing to lack of SCA in plots and failed attempts to artificially infest the plots.

Background

This project was a part of an integrative effort to address the unique challenges of managing SCA in Texas Panhandle. In order to address how a suite of cultural practices affects SCA in the Panhandle of Texas, replicated experiments were carried out at the Bush Research Farm associated with the Texas A&M AgriLife Research. We evaluate the effect of the following treatments on timing and severity of SCA infestations:

1) Early (May 11) and common (June 23) planting date;
2) SCA-resistant (DKS37-07) and susceptible (DKS44-20) varieties of sorghum;
3) Effect of an insecticide seed treatment (Poncho®) and untreated seed on SCA; and
4) SCA numbers and sorghum yield in presence and absence of foliar insecticide applications (triggered at 50-125 SCA/leaf)

Each treatment combination (a total of eight treatment combinations) was replicated four times for each plant date (total number of plots at Bushland – 64). Each plot measured 8 rows by 50 feet. Plots were scouted for SCA twice a week from emergence and until SCA colonized the plants. Following colonization, numbers of SCA per leaf on two leaves from five randomly selected plants in each plot were established every 3-4 days until end of August. Once plots reached the average of 50-125 SCA/leaf, Sivanto® foliar insecticide was applied using a backpack sprayer at 4 oz/A. Aphid counts continued weekly from insecticide applications until harvest. SCA in untreated plots were counted weekly. Yield data were collected by manually removing heads from 1 m^2 area of each plot. Prior to harvest, heads were protected from bird herbivory using fine mesh cages that allowed insect movement but prevented bird access. In all plots and throughout the scouting and sampling period, data on numbers and diversity of major aphid predators were collected as well.

In order to disseminate information about the project and early outcomes, two radio interviews and one media report was prepared by PI Szczepaniec (http://today.agrilife.org/2016/08/24/sugarcane-aphid-research-aimed-planting-timing-variety-selection/).

Results

Substantial amount of data were collected at the Bushland location, and for clarity the major results are summarized in a list form along with figures that correspond to each outcome.

RESULTS I: We observed a rapid increase in aphid numbers across all plots shortly after colonization. Specifically, SCA reached threshold (50-125 aphids/leaf) only two weeks after they were first observed in plots. It is important to point out how quickly the aphids increased in numbers on DKS44-20 (susceptible hybrid). SCA abundance rose considerable slower in the tolerant hybrid plots. Insecticide seed treatments decreased numbers of aphids in sorghum planted at common planting date, but not in early-planted sorghum. Once applications of Sivanto® (4 oz/A) triggered by this threshold were made, however, we noted an effective and long-lasting reduction in aphid numbers. This was evident across planting dates (Figure 1A,B). Interestingly, season-long average numbers of SCA were greater in early-planted sorghum across varieties (Figure 1C).

Figure 1A. Impact of variety and seed treatments on SCA densities and population increase in early planted sorghum. Aphid colonization coincided roughly with flowering stage of the crop and foliar insecticide treatments were applied at soft dough stage. Numbers of aphids in DKS44-20 plots with and without insecticide seed treatments were quite high, and it is evident that SCA numbers decreased slower in these plots after foliar insecticide was applied. Nonetheless, six days after treatment effective control was achieved. Individual points on each graph are averages and vertical lines designate measure of variation among individual data points within each average – standard error of the mean. Simplified way to interpret the variation: if standard error bars overlap the means are not statistically different from each other.

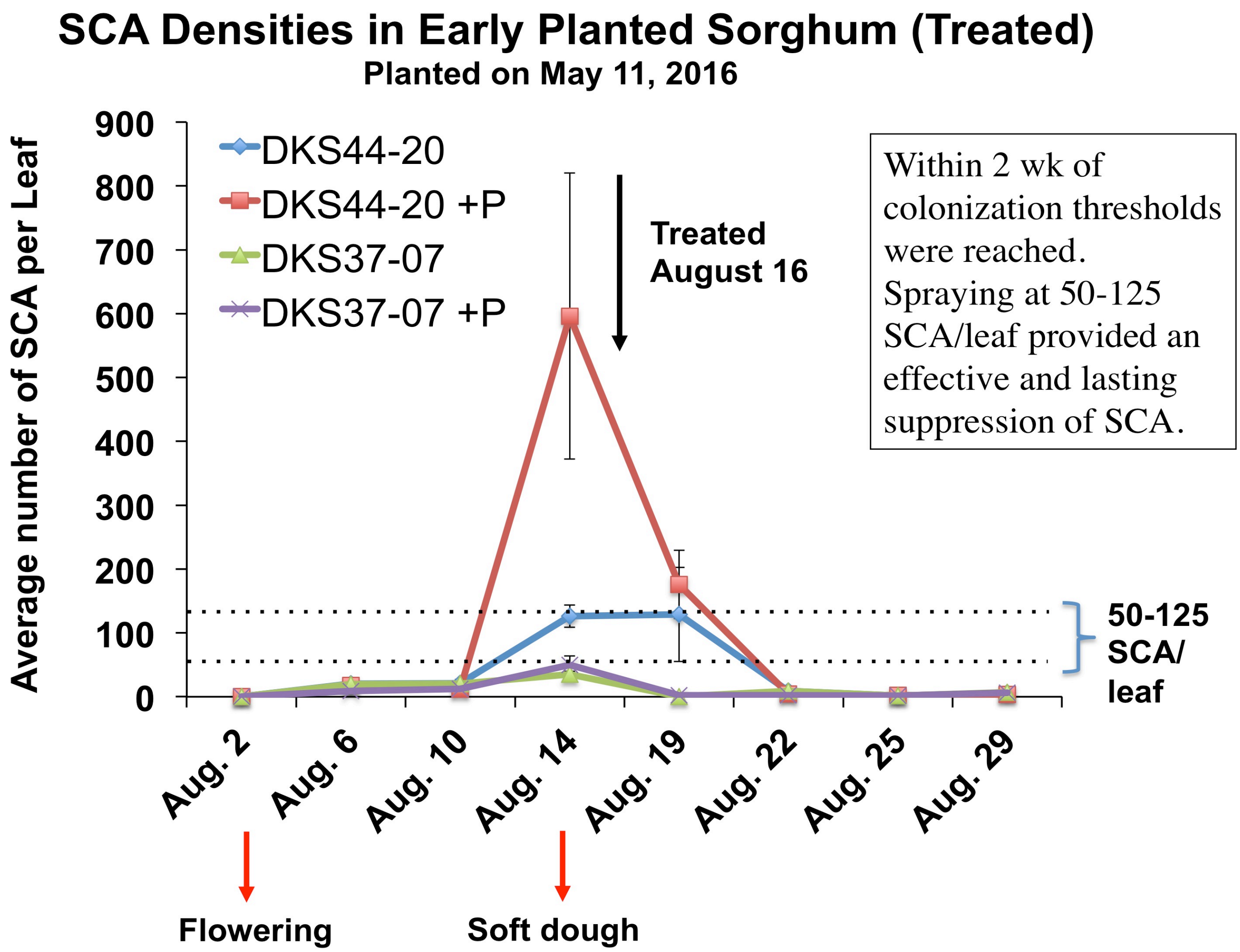


Figure 1B. Impact of variety and seed treatments on SCA densities and population increase in sorghum planted at common planting date. Aphid colonization coincided roughly with boot stage of the crop and foliar insecticide treatments were applied at flowering. Individual points on each graph are averages and vertical lines designate measure of variation among individual data points within each average – standard error of the mean. Simplified way to interpret the variation: if standard error bars overlap the means are not statistically different from each other.

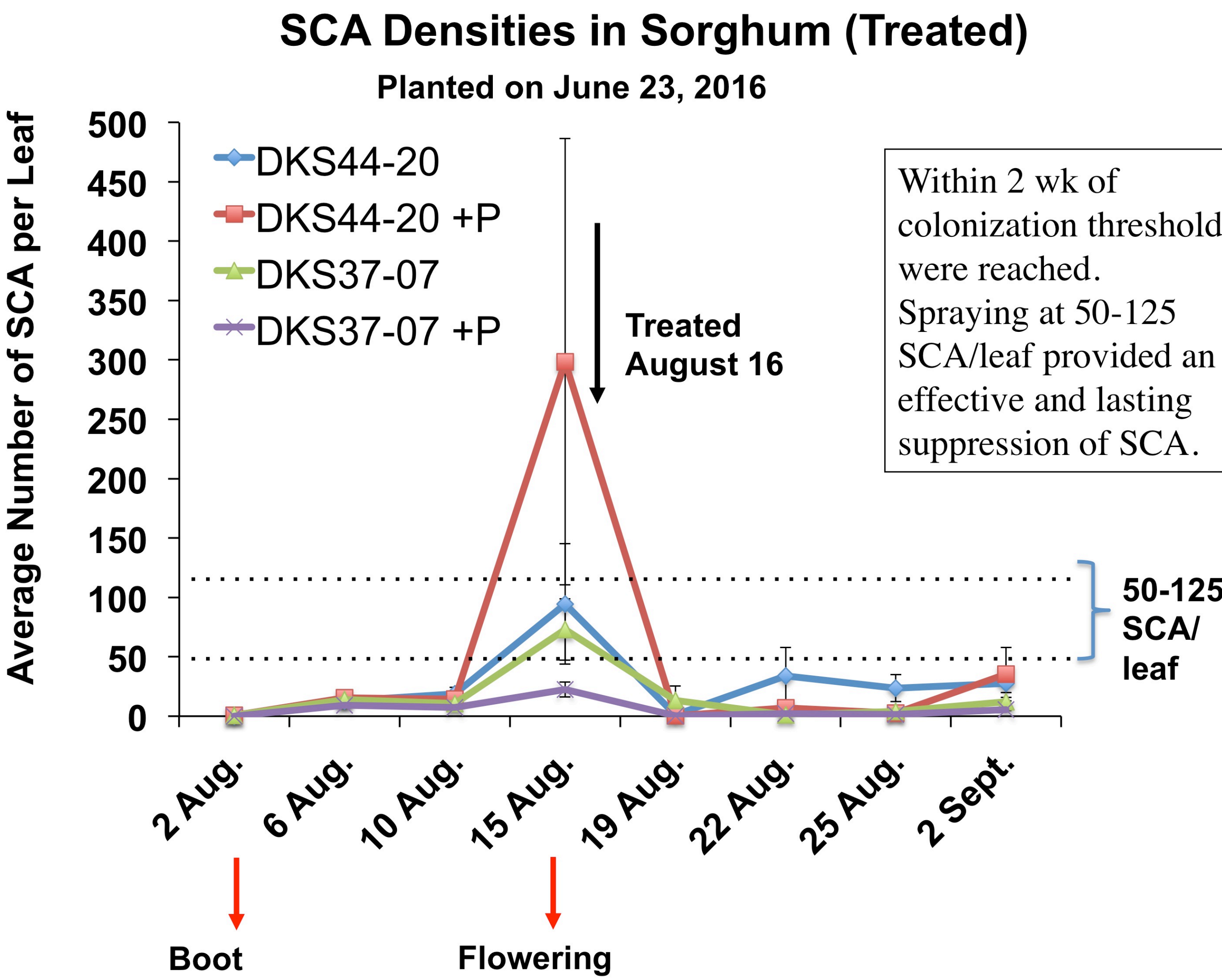
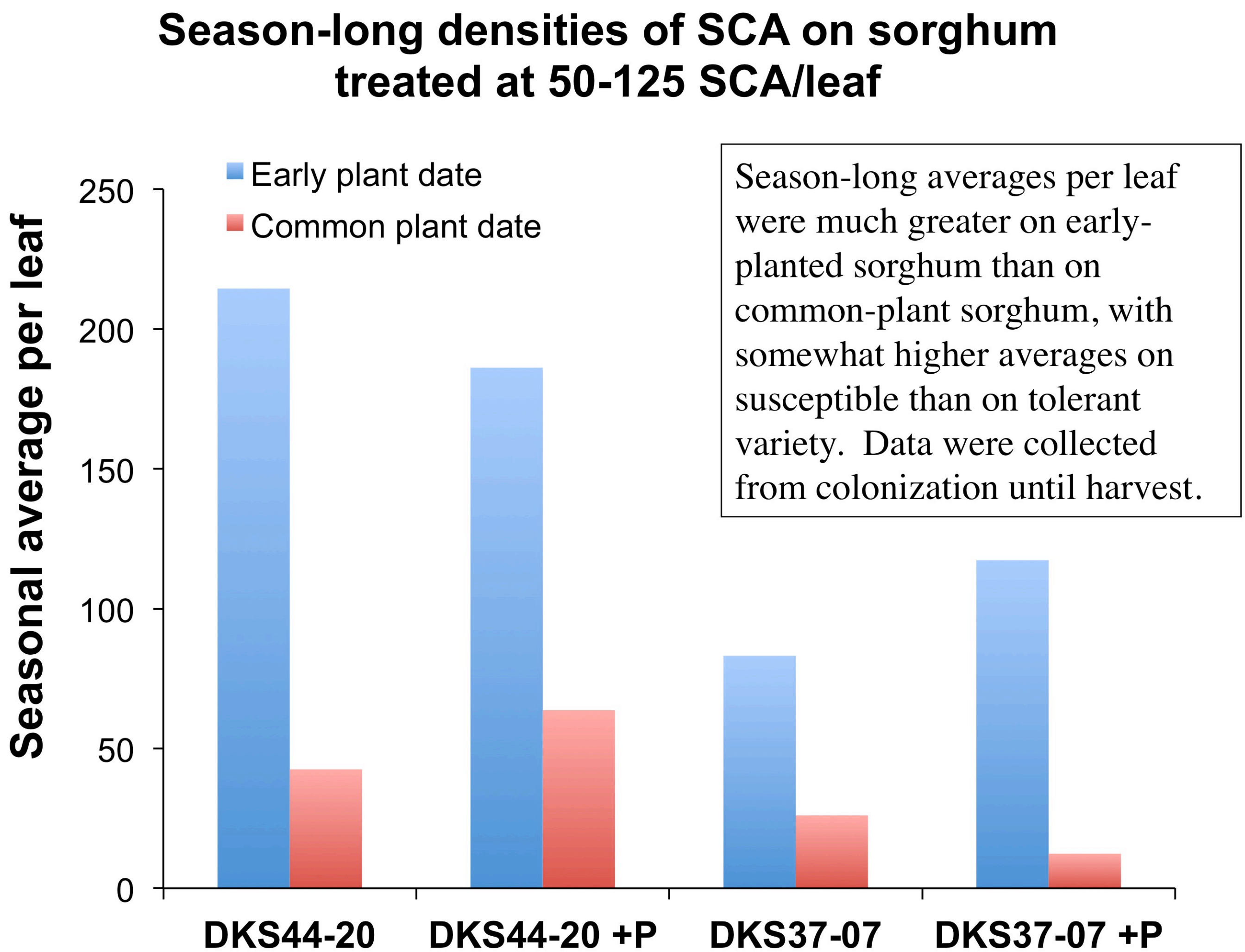


Figure 1C. Impact of sorghum varieties and seed treatments on season-long average densities of sugarcane aphids in sorghum treated with foliar insecticide at 50-125 SCA/leaf. Despite predictions, the season-long averages were much higher on early-planted sorghum. It is noteworthy that despite trends depicted in this figure the only factor that affected SCA across the entire growing season was planting date. Variability was very high for all other factors, i.e., tolerant/susceptible hybrid and insecticide seed treatments.



RESULTS II. We modified the protocol somewhat and included an unsprayed control. The goal of this modification was to evaluate differences between the hybrids with and without insecticide seed treatments across planting dates in terms of SCA capacity to reach density of 500 per leaf that has been associated with severe yield losses. We also wanted to evaluate when SCA reach exponential population growth, a turning point in SCA management after which it is extremely difficult to successfully control them. We note a significant variability in the data, but a few trends emerged. Tolerant variety treated with insecticide seed application supported fewer aphids than the other treatments regardless of planting date and did not reach the 500 SCA/leaf or exponential increase phase until four weeks after colonization (Figures 2A,B). The same held true for the tolerant hybrid free of insecticide treatment but only when planted at the common plant date, not when planted early. Susceptible hybrid with and without seed treatment had more than 500

SCA/leaf 2.5 to 3 weeks following aphid colonization ((Figures 2A,B). Season-long average densities of SCA tended to be higher in sorghum planted at the common plant date, but variance was high and differences were not statistically significant (Figure 2C).

Figure 2A. Impact of variety and seed treatments on SCA densities and population increase in early planted sorghum that was not treated with foliar insecticide. Individual points on each graph are averages and vertical lines designate measure of variation among individual data points within each average – standard error of the mean. Simplified way to interpret the variation: if standard error bars overlap the means are not statistically different from each other.

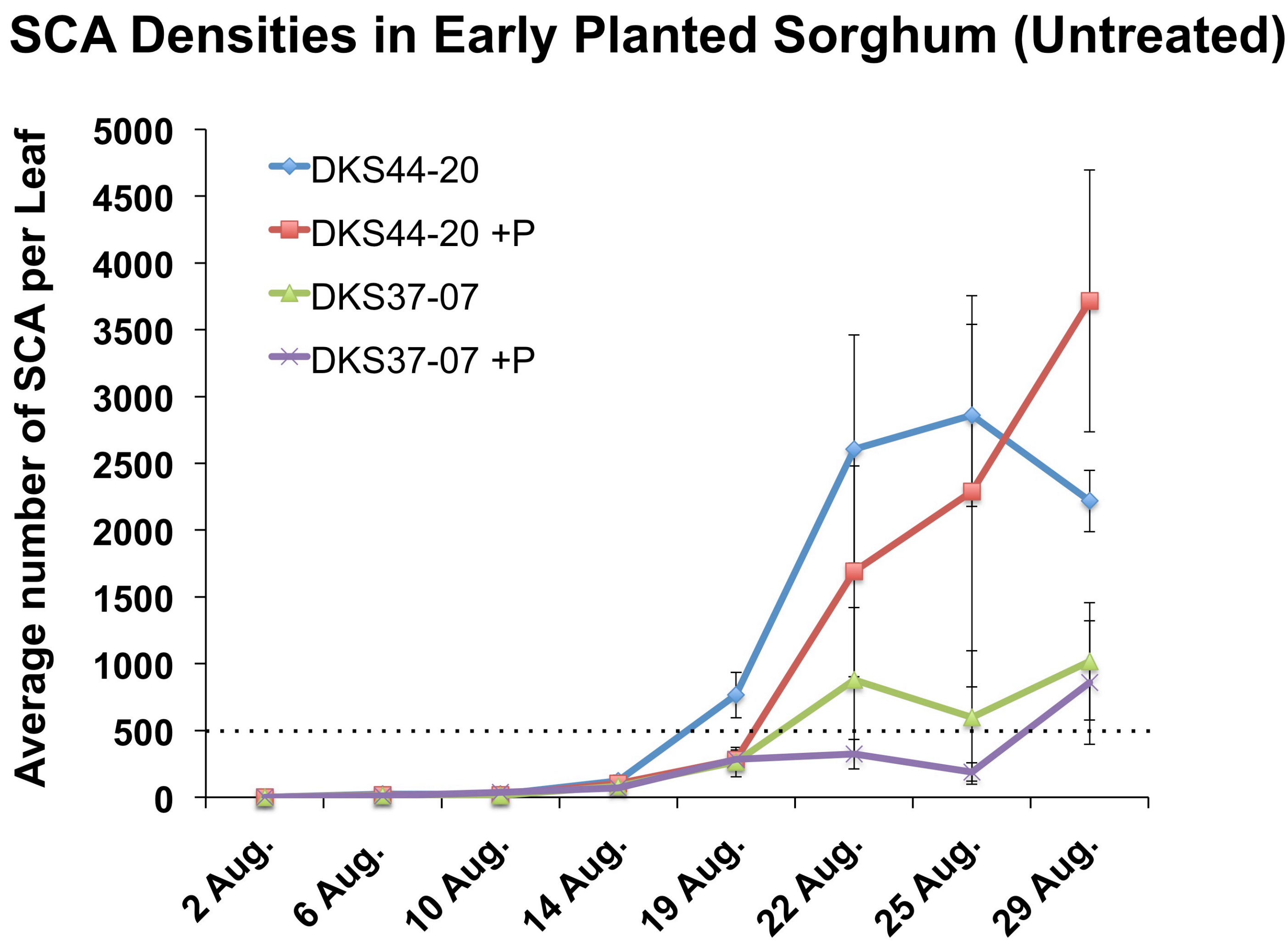


Figure 2B. Impact of variety and seed treatments on SCA densities and population increase in sorghum planted at common planting date that was not treated with foliar insecticide. Individual points on each graph are averages and vertical lines designate measure of variation among individual data points within each average – standard error of the mean. Simplified way to interpret the variation: if standard error bars overlap the means are not statistically different from each other.

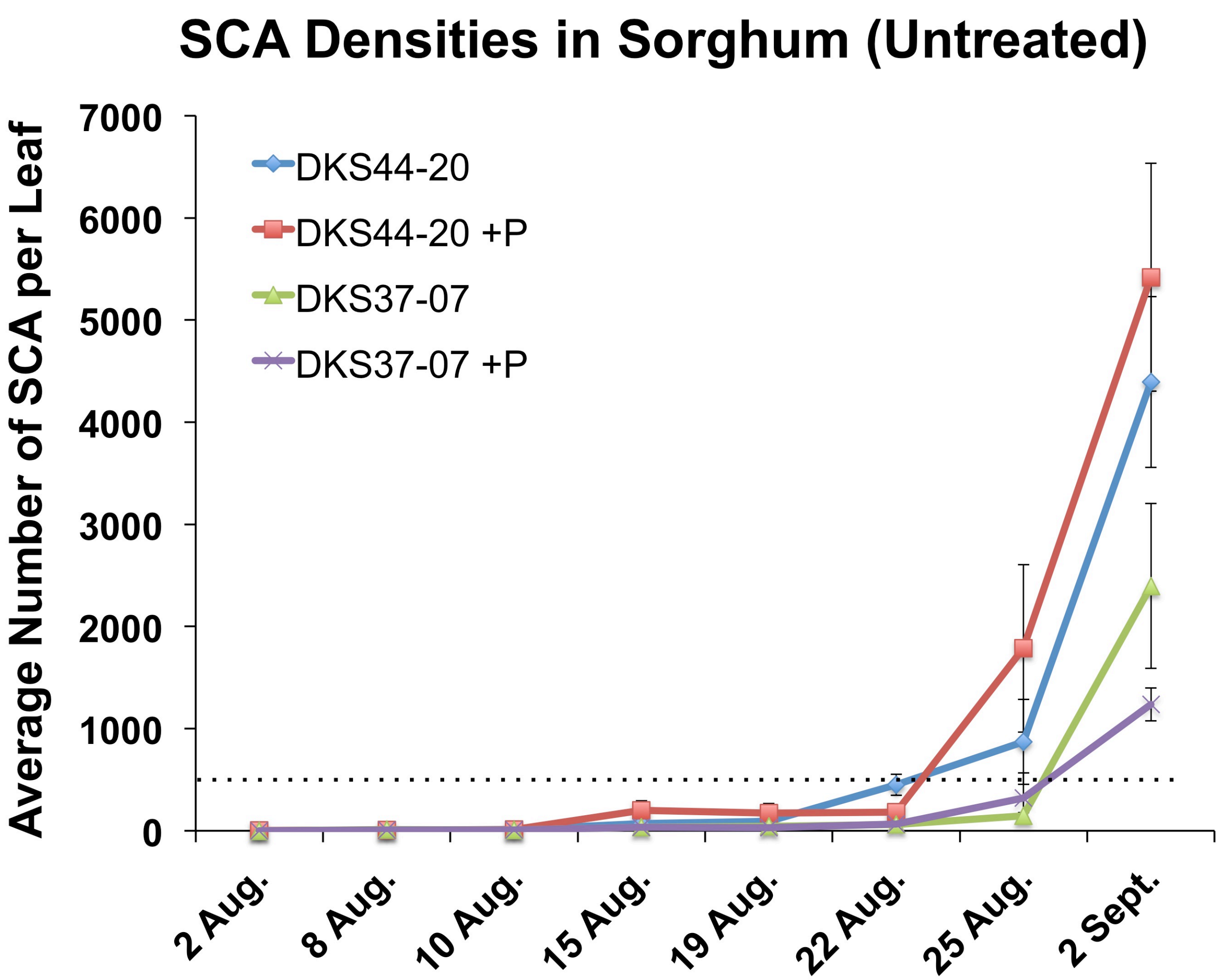
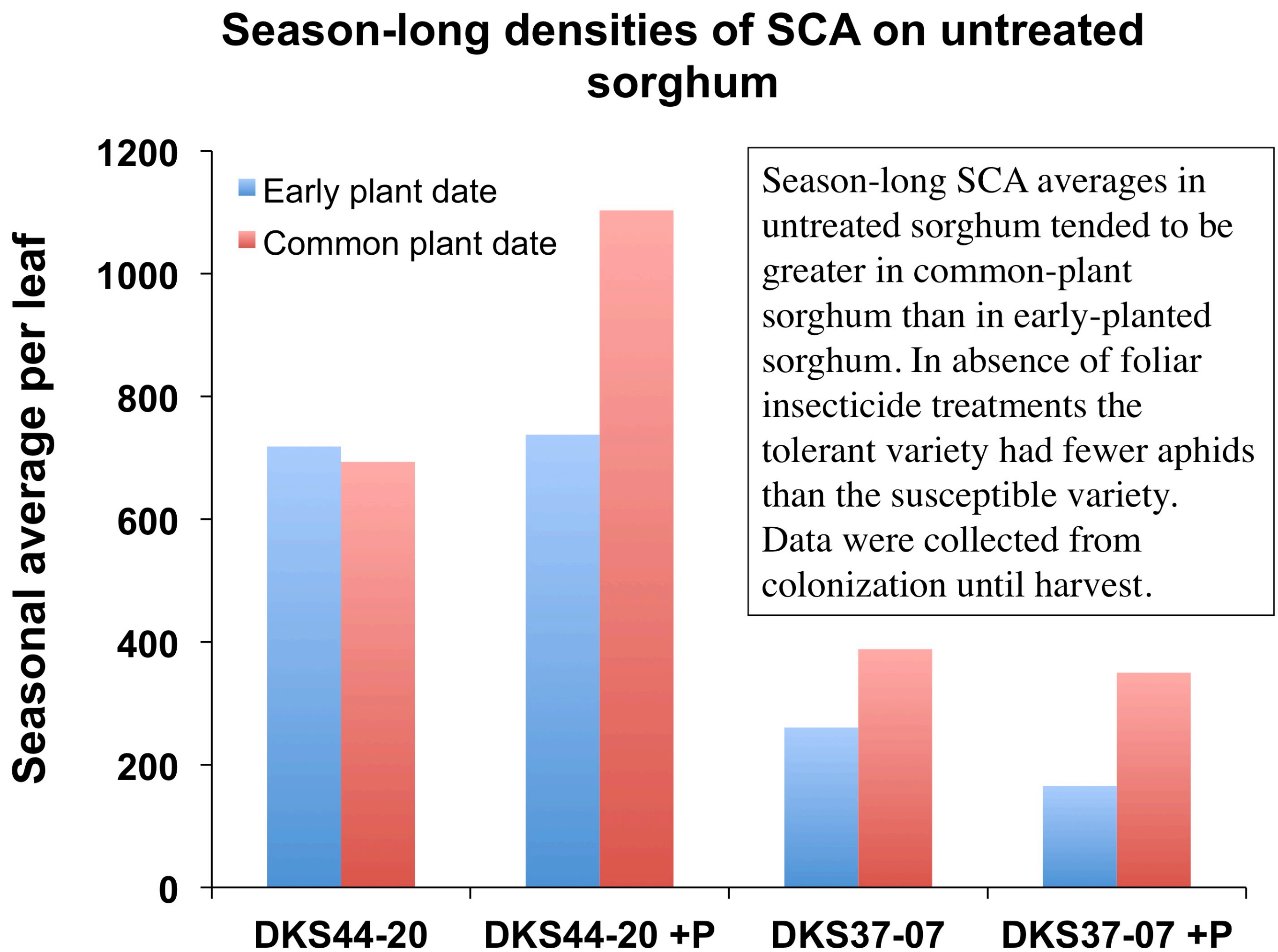


Figure 2C. Impact of sorghum varieties and seed treatments on season-long average densities of sugarcane aphids in untreated sorghum. When left untreated, SCA abundance was 2.5-3 times greater on susceptible hybrids than on tolerant hybrid. There was substantial variance in the data, however.



RESULTS III. When foliar applications were made at 50-125 SCA/leaf there were no statistical differences between yield of tolerant and susceptible hybrids with and without seed treatments whether they were planted early or at the common planting date (Figure 3A,B). We note that while not significant, DKS44-20 yields tended to be lower when planted later, and this may well be caused by hybrid differences and their performance in this region.

Figure 3A. Yield of early-planted sorghum treated with foliar insecticide at 50-125 aphids/leaf. Bars are averages and vertical lines designate measure of variation among individual data points within each average – standard error of the mean. Simplified way to interpret the variation: if standard error bars overlap the means are not statistically different from each other.

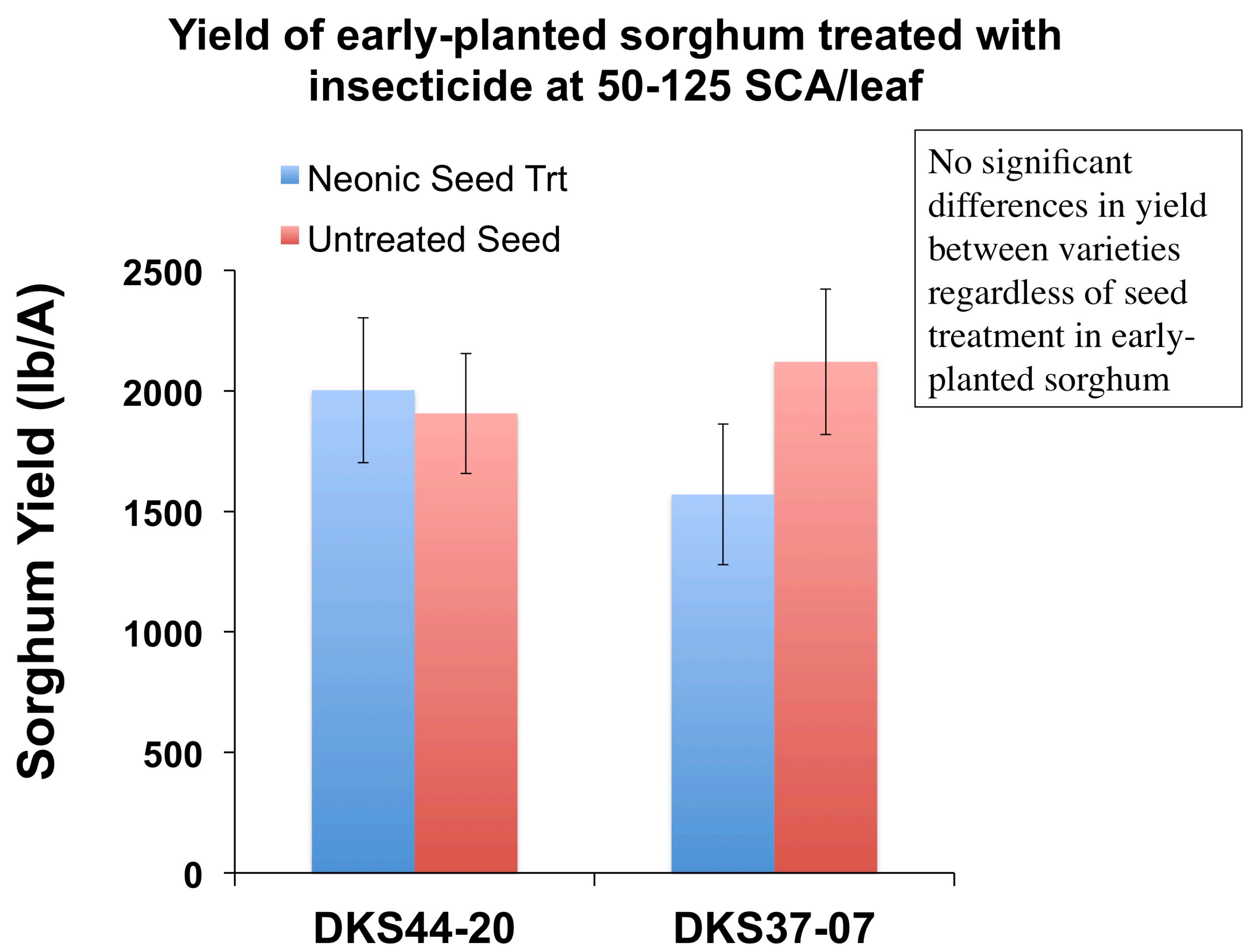
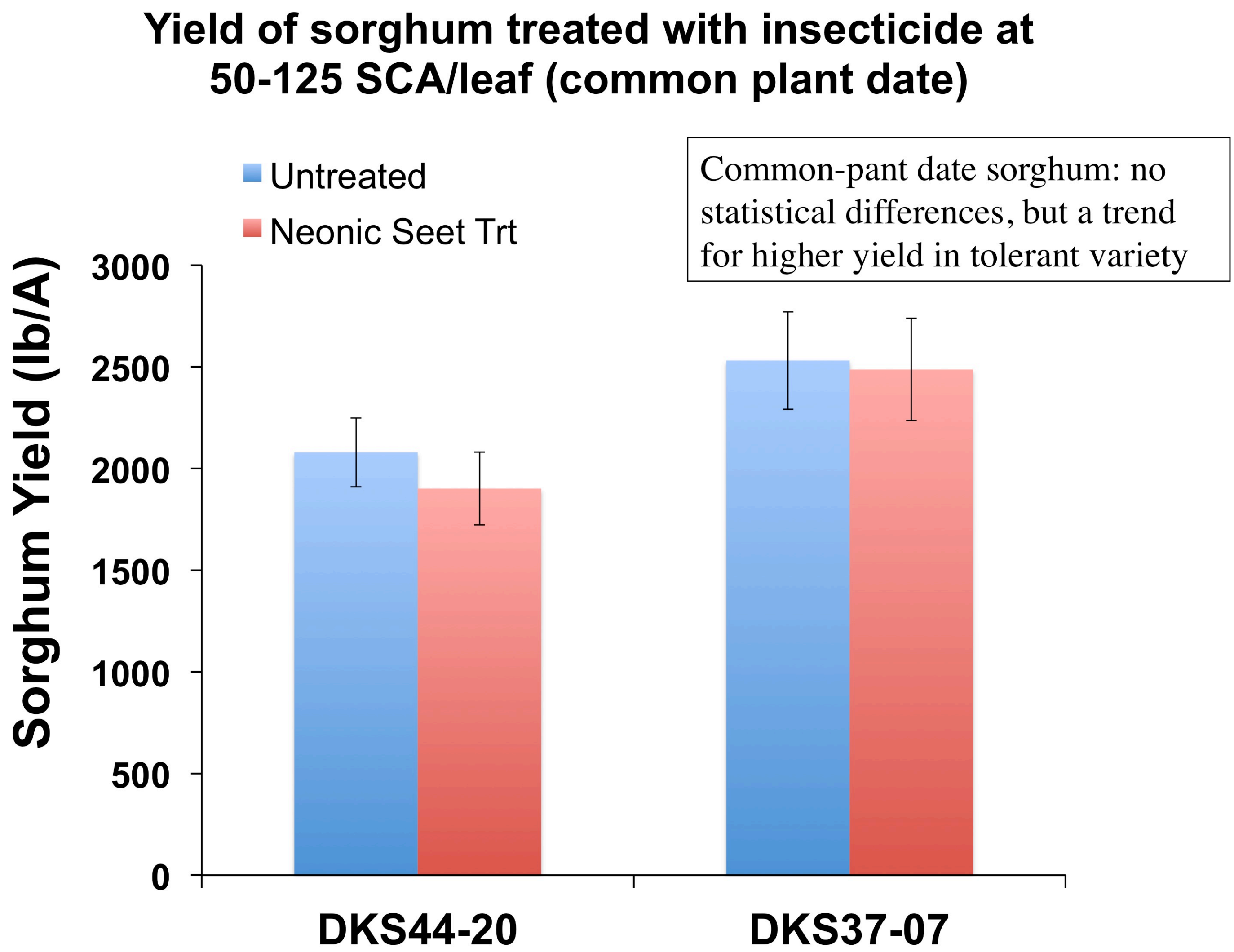


Figure 3B. Yield of early-planted sorghum treated with foliar insecticide at 50-125 aphids/leaf. Bars are averages and vertical lines designate measure of variation among individual data points within each average – standard error of the mean. Simplified way to interpret the variation: if standard error bars overlap the means are not statistically different from each other.



RESULTS IV. When foliar insecticides were not applied, the yield of tolerant sorghum hybrid with and without seed treatment surpassed that of susceptible sorghum variety (Figure 4A,B). In fact, untreated susceptible hybrid planted at the common planting date did not reach maturity and no yield data were collected from any of the plots (Figure 4B). It is noteworthy that even when not sprayed for aphids, yields of the early-planted tolerant hybrid were comparable to yields of sorghum that was sprayed for aphids (compare Figures 3A and 4A).

Figure 4A. Yield of early-planted sorghum not treated with foliar insecticide. Tolerant hybrid had higher yields than susceptible hybrid regardless of seed treatment. Yields of early-planted tolerant hybrid never sprayed for aphids were comparable to yields of sorghum that was sprayed with foliar insecticide when aphids reached densities of 50-125 SCA/leaf. Bars are averages and vertical lines designate measure of variation among individual data points within each average – standard error of the mean. Simplified way to interpret the variation: if standard error bars overlap the means are not statistically different from each other. Asterisk marks means that were statistically different.

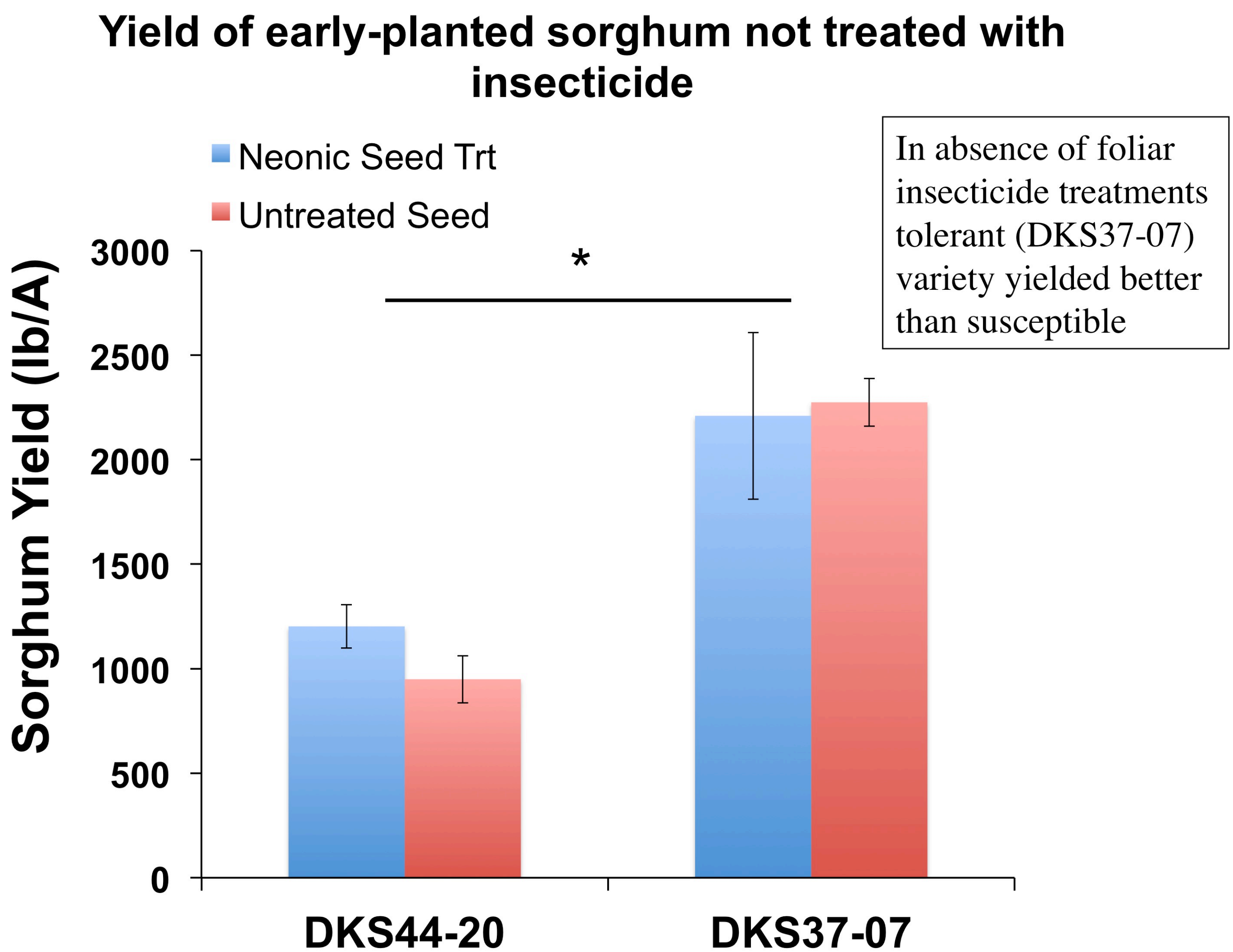
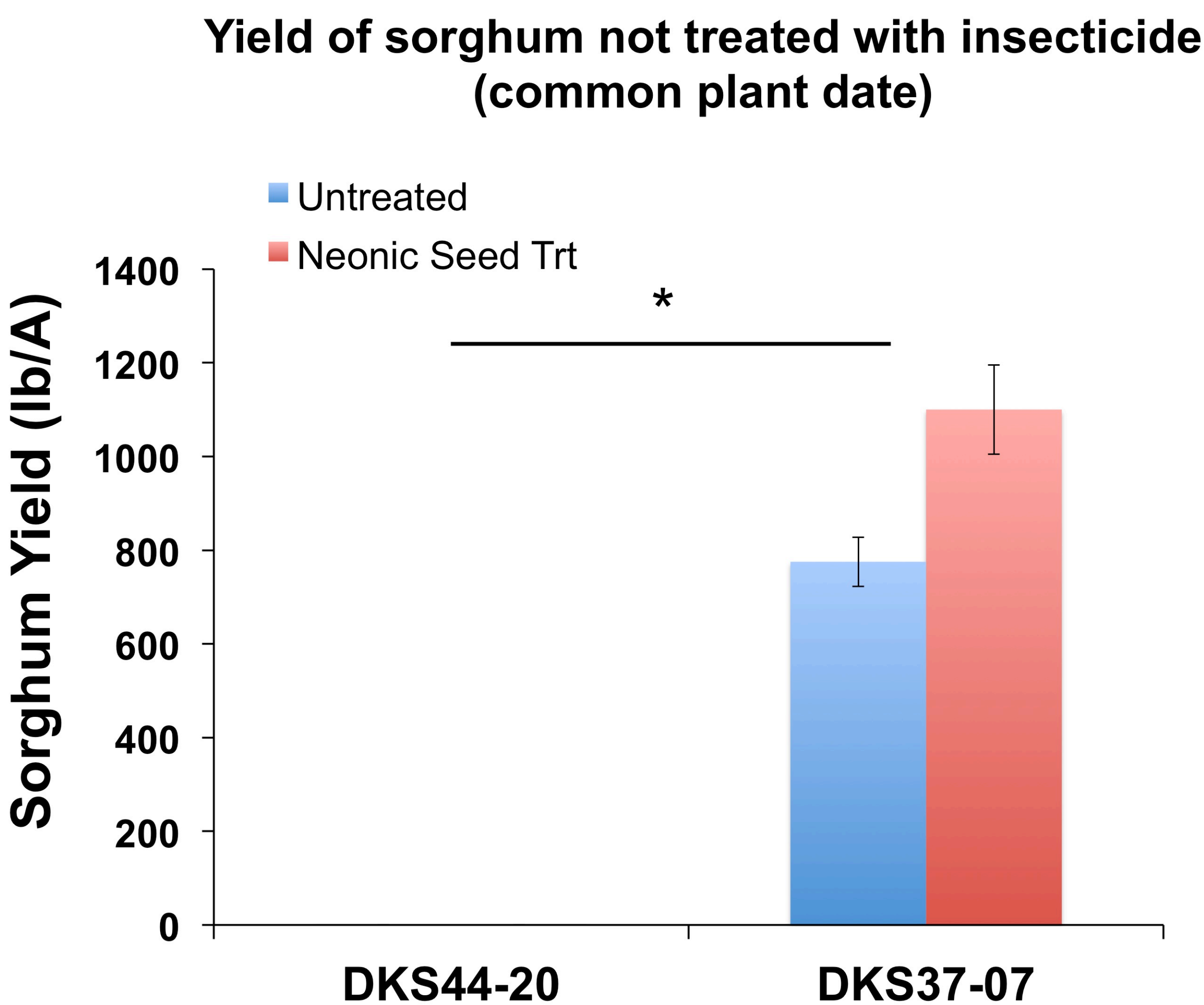


Figure 4A. Yield of sorghum planted at common planting date and not treated with foliar insecticide. Tolerant hybrid had higher yields than susceptible hybrid regardless of seed treatment, but yield overall was very low. Susceptible hybrid did not reach maturity. Bars are averages and vertical lines designate measure of variation among individual data points within each average – standard error of the mean. Simplified way to interpret the variation: if standard error bars overlap the means are not statistically different from each other. Asterisk marks means that were statistically different



RESULTS V. In addition to the sugarcane aphids, densities of the major aphid predators were recorded over the season. Lady beetles were the most numerous predators and owing to their high mobility their numbers were estimated for entire plant rather than leaf. All other predator densities were established per leaf. Overall the predator numbers were relatively high, which may have been driven by close proximity of unsprayed plots abundant in aphids. However, we noted lady beetles in plots throughout the season and well before SCA colonization. The average numbers of predators were roughly comparable among hybrids, seed treatments, and planting dates, but lady beetles tended to be the most numerous predators in early-planted sorghum while lacewings were very abundant in sorghum planted at common planting date (Figures 5A, B). Overall, we noted a robust and fairly diverse community of predators that were abundant despite foliar application of Sivanto®.

Figure 5A. Abundance of major aphid predators in early-planted sorghum treated with foliar insecticide

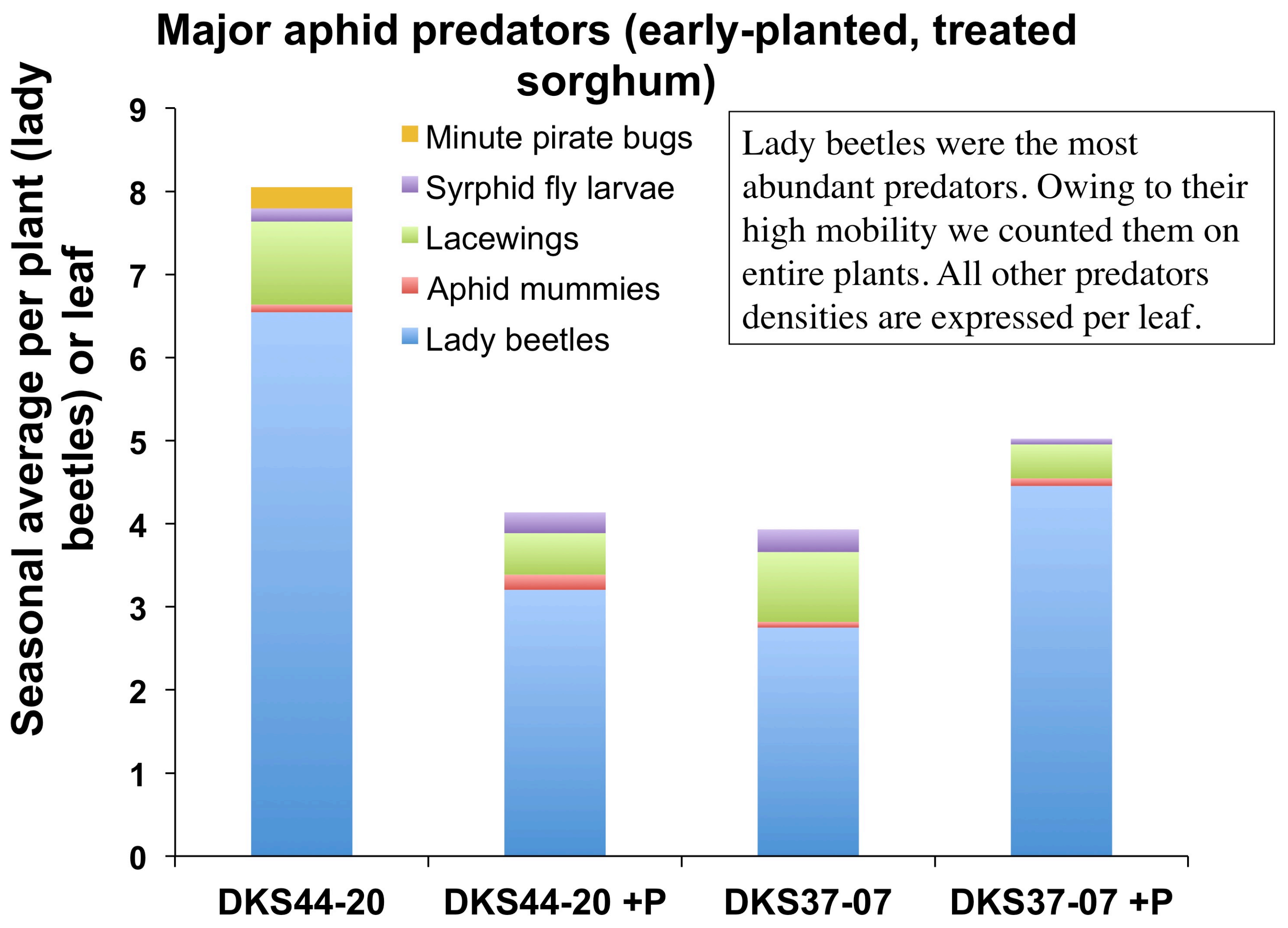
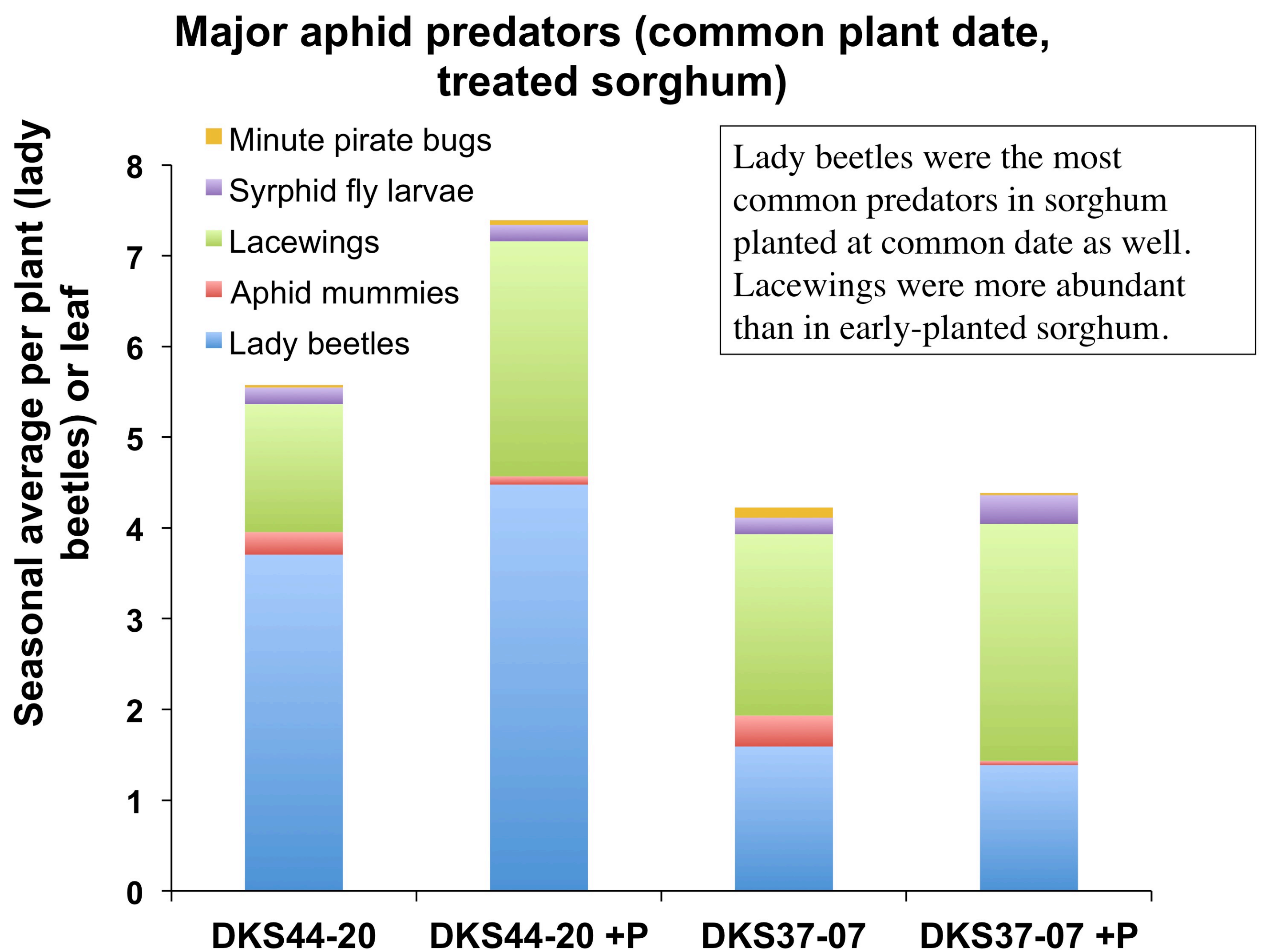


Figure 5B. Abundance of major aphid predators in sorghum planted at the common planting date and treated with foliar insecticide



Conclusions

Based on the first year of data we conclude that the population increase of SCA in the Texas High Plains is very rapid – much more so than in South Texas. Mere two weeks after colonization the susceptible hybrid reached or exceeded the threshold of 50-125 SCA/leaf and required insecticide applications. Tolerant hybrids offered about an additional week of time before thresholds were exceeded. The advantage of tolerant hybrid was also evident with respect to yield – the unsprayed tolerant hybrid plots yielded as well as the sprayed plots. It is important to note that we hand-harvested all of the plots – had we used mechanical equipment to harvest the unsprayed plots we likely would have experienced harvesting issues and suffered losses in yield. Nonetheless, we conclude that the aphids increased in numbers much slower on the tolerant hybrid DKS37-07 and it offered additional time before thresholds were exceeded. SCA on the tolerant hybrid with insecticide seed treatments, particularly when it was planted at common planting date, also did not reach the exponential population growth until approximately four weeks after colonization compared to three weeks on the susceptible hybrid. This is relevant because once the aphids reach this phase it is extremely hard if not impossible to control them.

Owing to lack of any guidance specific to the Texas High Plains with respect to SCA threshold we used the already established guidelines from South Texas. Data presented in this report indicate that using this threshold simply worked – we suppressed the aphids and their numbers were very low for three weeks after treatments. Additional research is necessary before the validity of the threshold with respect to actual impact of SCA on yield of sorghum at flowering and soft dough can be established. We also noted that SCA infestations were very uniform across the plots – once the aphids arrived close to 100% of the plants had one or more aphids. We also did not note honeydew on plants before aphid numbers exceeded >150 SCA/leaf. This means that looking at undersides of individual leaves to assess SCA numbers is likely to be key to spotting their densities before their populations explode. Lastly, numbers and diversity of aphid predators were relatively high even in plots that received foliar insecticide applications. It is not possible to estimate if these predators play large enough role in suppressing aphids until additional data are collected, but their presence is highly desirable especially after foliar insecticides are applied.

These conclusions will be much more robust after at least one additional year of data. Wealth of information important to sorghum production in the Texas High Plains was collected in 2016, but variation was very high. Additional year of data would provide more resolution and strengthen the validity of the outcomes.

Impact

Economic Impact:

- **Industry:** The economic impact of these outcomes to industry include data on the effectiveness of the tolerant hybrid in suppressing SCA populations in the Texas High Plains, and high efficacy and long residual suppression of the aphids using Sivanto® when applied at 50-125 SCA/leaf. We demonstrated that use of a hybrid with tolerance has significant economic benefits through slowing down the growth of SCA population and thus decreased costs of managing these pests if only one well-timed application of insecticides is needed. Well-timed insecticide applications along with tolerant hybrids are likely to be key tactics in managing the aphids, and industry efforts to develop agronomically well-suited hybrids with aphid tolerance are extremely important, especially in the High Plains.
- **Producer:** We demonstrate that the producers can decrease input costs by eliminating expenses on insecticide seed treatments to suppress SCA if they plant sorghum early. We also demonstrate that sorghum producers in the region can benefit economically by incorporating tolerant sorghum varieties that slow down SCA population growth and thus improve the likelihood of proper timing of insecticide applications. Above all and regardless of hybrid selected or insecticide seed treatment presence, we report that scouting early and often once reports of SCA presence in the region emerge are pivotal to reducing economic losses caused by this invasive pest.

Economic Feasibility: Selecting a hybrid with tolerance to SCA that yields well in the region may be very challenging and more research is needed before economic feasibility of this control tactic is evaluated. However, a combination of planting early and omitting insecticide seed treatments if SCA are the major pest of concern is likely to be economically sound. Scouting costs are essential but data we collected suggest that a more focused effort to scout is only needed for a short period of time, and if well-timed foliar insecticide application provides significant economic benefits.

Return on Investment: We requested $20,700 to support this project, $5,800 of which was assigned to the Lubbock Co-PI and was not expended, bringing the support to $14,900. These funds were spent and additional funds from other sources were used to complete this project. We delivered comprehensive information on the impact of four different factors (plant date, hybrids, seed treatments, foliar insecticides) on timing of initial colonization and SCA population dynamics, yield, and predatory insects from one location. These outcomes can be immediately used by sorghum producers and have a significant potential to aid in mitigating the costs of SCA to sorghum production in the region.

Next Steps

Experiments that were conducted in 2016 have to be repeated – there is too much variability in field research to rely solely on a single year of data. There is also a significant need to measure in more precise manner the economic threshold of SCA in sorghum colonized at flowering and beyond. If sorghum can tolerate higher SCA numbers at later growth stages without impact on yield it would improve our ability to properly time insecticides to control SCA populations and 'buy' more time from initial SCA colonization to insecticide application. It would also be of benefit to sorghum production in the region if more tolerant hybrids well-suited to the growing conditions were evaluated.

List of Abbreviations

SCA – sugarcane aphid