Plant Resistance in Sorghums to the Sugarcane Aphid (Hemiptera: Aphididae)[1]

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Abstract Ten sorghum, *Sorghum bicolor* (L.) Moench, lines near or in commercial release were evaluated with the intent of identifying the phenotypic expression of host-plant resistance to the sugarcane aphid *Melanaphis sacchari* (Zehnter) (Hemiptera: Aphididae). Two of the 10 entries (OL2042 and SP7715) expressed a high degree of resistance to the sugarcane aphid, with damage ratings <3.0 (damage rating scale of 1.0 to 9.0, with 1.0 being no damage and 9.0 a dead plant) and were not significantly different than the known resistant Tx2783. Screening the four other entries (OL0029, SP74C40, SP78M30, and SP73B12) resulted in having very good expression of resistance scoring between <3.0 and >4.0 and were statistically lower than the susceptible check Tx7000. Chlorophyll loss and damage ratings exhibited a linear relationship ($R^2 = 0.87$), followed by a slight improvement ($R^2 = 0.89$) in the regression models when the difference in plant height was added as a second independent variable. The relationship helps explain the degree of tolerance when sorghum is challenged with high sugarcane aphid densities. These results provide sorghum producers with options for planting sorghums resistant to sugarcane aphid while allowing for more time to find and develop new sources of resistance.

Key Words sugarcane aphid, *Melanaphis sacchari,* plant resistance, grain sorghum, cross-resistance

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The sugarcane aphid *Melanaphis sacchari* (Zehnter) (Hemiptera: Aphididae) has developed into a serious pest of sorghum, *Sorghum bicolor* (L.) Moench, in the United States (Villanueva et al. 2014, Armstrong et al. 2015, Elliott et al. 2015). Since its discovery on sorghum near Beaumont, TX, in 2013, it has rapidly spread to 17 sorghum-producing states (Bowling et al. 2016) and into Mexico (Rodríguez-del-Bosque and Teran 2015). It is believed that the sugarcane aphid shifted its host range from sugarcane, *Saccharum officinarum* L., to sorghum. Sugarcane aphids in the United States have been reported on sugarcane in Florida (Mead 1978) and Louisiana (White et al. 2001). In sugarcane, it is a known vector of sugarcane yellow leaf virus, causing yellow leaf disease (Singh et al. 2004, Akbar et al. 2010, Nibouche et al. 2014). The host range of this aphid pest is not limited to sorghum and sugarcane. It is also found on Johnsongrass (*Sorghum halepense* [L.] Pers.),

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Sudan grass (*Sorghum bicolor* subsp. *drummondii* [Nees ex Steud.] de Wet & Harlan), and Columbus grass (*Sorghum almum* Parodi) (Armstrong et al. 2015, Medina et al. 2017). Although the sugarcane aphid has been considered a serious pest of sorghum since 2013, the economic relationship of aphid densities versus yield loss have not been completely established.

Sugarcane aphids feed by sucking sap from the phloem tissue of leaves and stems. Colonies increase quickly on the abaxial surface of the leaves (Villanueva et al. 2014). Heavy infestations cause leaves to turn from yellow to brown, followed by the appearance of dark leaf surfaces covered in sooty mold growth supported by honeydew produced by the aphids (Akbar et al. 2010, Elliott et al. 2015). Honeydew-covered sorghum is a problem during harvest, as equipment may become clogged because of the sugary substance (Knutson et al. 2016, Armstrong et al. 2017). On a comparative basis under ideal environmental conditions, the reproductive rate of the sugarcane aphid is reportedly double that of the greenbug *Schizaphis graminum* (Rondani) on susceptible sorghum (4.45 versus 2.30 nymphs/female/d) but not significantly different on resistant sorghum (3.09 versus 2.27) (Bayoumy et al. 2015). In a more recent study, the sugarcane aphid produced 3.6 nymphs/day on susceptible sorghum Tx7000 and 1.3 nymphs/d on the resistant Tx2783 (Limaje et al. 2017), demonstrating that the sugarcane aphid has one of the fastest reproductive rates of aphids that infest grain sorghum, which must be considered when developing economic thresholds. Identification and development of sorghum germplasm resistant to the sugarcane aphid is an ongoing process. Earlier work indicates that resistant sources thus far identified in grain and forage sorghum are likely carried over from greenbug-resistant sorghums (Armstrong et al. 2015, 2017, Bayoumy et al. 2015, Limaje et al. 2017). Herein, we report host-plant evaluations using conventional screening methods in order to accelerate the development of suitable commercial sorghum lines resistant to the sugarcane aphid.

Materials and Methods

Aphids. A clonal colony (parthenogenic female giving birth to females) of sugarcane aphids was initiated from a single female collected from infested grain sorghum in Matagorda Co., TX, in August of 2013. The colony is maintained on the susceptible Tx7000 seedlings grown in pots covered with sleeve cages in a greenhouse at 21 to 31°C under natural greenhouse light supplemented with two T-8 fluorescent lights. Aphids were transferred to new seedling plants every 2 weeks in the greenhouse to maintain viable colonies.

Resistance trials. Twelve sorghum entries, including a resistant sorghum Tx2783 and a known susceptible Tx7000, were evaluated in a free-choice flat-screen method. Entries 0L2042, 0L0029, and KS585 were owned and developed by Chromatin Inc. (Chicago, IL, USA), whereas entries SP74C40, SP78M30, SP73B12, SP70B17, SP68M57, SP7715, and SP6929 were owned and developed by Chromatin but sold under their marketing subsidiary Sorghum Partners® as commercial or near-commercial sorghums being evaluated for sugarcane aphid resistance. Each entry was randomized and replicated 20 times using Research Randomizer (2016).

The sorghum entries were planted in eight flats (plastic trays 60 × 90 cm with 128 individual cells; Growers Supply, Dyersville, IA, USA). Four of the eight flats were used for infesting, while duplicate sets of four flats were grown as not infested for comparing plant growth characteristics. When the plants entered the two-leaf stage (approx. 10 cm in height), they were infested as described by Starks and Burton (1977), where heavily infested sorghum seedlings from Tx7000 were laid down each row and across each alley of the flats. By this procedure, all entries were placed under tremendous pressure from the infesting aphids so that no ambiguity existed in the evaluation. The measured variables for infested and noninfested sorghums were plant height (cm), measured at the end of the trial, the number of formed leaves on the plant excluding the lower cotyledon leaf, and the difference in plant height where the infested entry heights were subtracted from the control entries. Difference in plant height is measured because sorghum growth curves are not the same across genotypes, and, thus, subtracting an infested sorghum versus the same entry that is not infested is more realistic in determining that the reduction in plant growth may have been due to aphid feeding. Total chlorophyll content (chlorophyll a + b) (Markwell et al. 1995), measured as μmol m^{-2}, was estimated using a SPAD-502 chlorophyll meter (Minolta, Ramsey, NJ, USA). Chlorophyll readings were taken from the sugarcane aphid infested entries as well as the noninfested entries so that the percent loss of total chlorophyll could be calculated using the formula (C–T)/C*100, where C = SPAD measurement from the noninfested or control, and T = SPAD measurement from corresponding infested plants. When the known susceptible Tx7000 was 90% to 100% dead based on the 20 replications of that entry, all plants in each flat were evaluated for damage by using a rating of 1–9, where 1 is a completely healthy plant with no necrotic tissue, 2 = 1%–5% chlorotic tissue, 3 = 5%–20%, 4 = 21%–35%; 5 = 36%–50%, 6 = 51%–65%; 7 = 66%–80%; 8 = 81%–95%, and 9 = 95%–100% or dead (Webster et al. 1991, Burd et al. 2006). The variables of damage rating, plant height, difference in plant height, number of leaves on a sorghum entry, and chlorophyll loss were subjected to PROC MIXED model analysis with sorghum entry means compared ($\alpha = 0.05$) using the least squared means pairwise comparisons procedure, and the degrees of freedom were calculated using Kenwood–Rodgers approximation method (SAS 9.3; SAS Institute 2010, Cary, NC, USA). The relationships of chlorophyll loss and damage ratings, differences in plant height, and number of leaves on a sorghum entry were further explored by using chlorophyll loss as a dependent variable and the others as independent variables using the PROC REG statement and the maximum R^2 best-fit model selection (SAS 9.3; SAS Institute 2010). This regression function selects the best related model and continues to add the other independent variables for a stepwise improvement in the correlation coefficient.

Results

From the 10 entries, excluding the known resistant Tx2783 and the susceptible Tx7000, 2 entries (OL2042 and SP7715) were considered highly resistant based on damage ratings being <3.0, whereas 4 entries (OL0029, SP74C40, SP78M30, SP73B12) exhibited resistance levels with damage scores between 3.0 and 4.0

Table 1. Free-choice evaluation for sorghum germplasm infested with sugarcane aphids and evaluated for plant resistance.

Variety/ Genotype	Damage Rating*	% Chlorophyll Loss**	Difference in Plant Height (cm)†	Number of Leaves/Plant‡
0L2042	2.8 ± 0.64 d	26.32 ± 7.8 d	6.9 ± 1.9 g	3.6 ± 0.18 a
0L0029	3.6 ± 0.66 d	31.30 ± 8.4 d	9.2 ± 1.5 fg	3.4 ± 0.15 a
SP74C40	3.4 ± 0.60 d	13.8 ± 2.2 d	9.5 ± 1.8 fg	3.5 ± 0.14 a
SP78M30	3.9 ± 0.69 d	20.5 ± 3.5 d	15.1 ± 1.4 cde	3.4 ± 0.15 a
SP73B12	3.4 ± 0.66 d	22.9 ± 7.9 d	13.4 ± 2.3 def	3.5 ± 0.66 a
SP70B17	6.2 ± 0.50 c	60.1 ± 7.7 c	18.7 ± 0.8 bc	2.9 ± 0.13 cd
SP7715	2.5 ± 0.44 d	15.2 ± 6.0 d	13.6 ± 2.2 c-f	3.3 ± 0.19 ab
SP68M57	6.1 ± 0.58 c	53.6 ± 8.1 c	21.4 ± 1.3 ab	2.9 ± 0.13 cd
SP6929	6.7 ± 0.61 b	65.6 ± 8.4 bc	18.5 ± 1.2 bcd	2.8 ± 0.09 d
KS585	8.0 ± 0.45 ab	80.3 ± 7.9 ab	23.7 ± 1.4 a	2.9 ± 0.15 cd
Tx 2783	2.5 ± 0.26 d	15.4 ± 3.2 d	10.8 ± 2.3 efg	3.2 ± 0.13 abc
Tx 7000	8.6 ± 0.37 a	96.8 ± 3.1 a	14.1 ± 0.8 c-f	2.5 ± 0.12 d

* Mean (±SD) damage ratings followed by the same lower case letter are not significantly different (least squares; $F = 15.04$; df = 11, 204; $P > F = <.0001$).

** Mean (±SD) % chlorophyll losses followed by the same lower case letter are not significantly different (least squares; $F = 10.14$; df = 11, 204; P > $F = <.0001$).

† Mean (±SD) difference in plant height followed by the same lower case letter are not significantly different (least squares; $F = 16.66$; df = 11, 189; $P > F = <.0001$).

‡ Mean (±SD) number of leaves followed by the same lower case letter are not significantly different (least squares; $F = 5.65$; df = 11, 204; $P > F = <.0001$).

(Table 1). The damage rating with SP7715 was identical to that of Tx2783, with a rating of 2.5, the lowest in the evaluation. However, Tx2783, SP7715, OL2042, OL0029, SP74C40, SP78M30, and SP73B12 with damage ratings between 2.5 and 3.9 did not differ statistically from those previously described. Traditionally, damage scores ≤3 have been considered highly resistant, whereas scores between 3.0 and 4.0 are considered resistant (Armstrong et al. 2015). The entries SP70B17, SP68M57, and SP6929 expressed what could be characterized as low levels of resistance, with ratings between 6.0 and 7.0, and were statistically lower than the susceptible Tx7000 and KS585 entries.

Percent chlorophyll loss, calculated from the comparison of the infested entries with noninfested entries, ranged from 96.8% for the susceptible Tx7000 to 13.8% for the SP74C40. The two lowest damage rated entries SP7715 and Tx2783 resulted in 15.2% and 15.4% losses, respectively (Table 1). Differences in plant height, where the height of the control (noninfested) was subtracted from that of the infested plants across the 20 replications, showed that the infested SP7715, OL2042, OL0029, SP74C40, SP78M30, and SP73B12 plants were, on average,

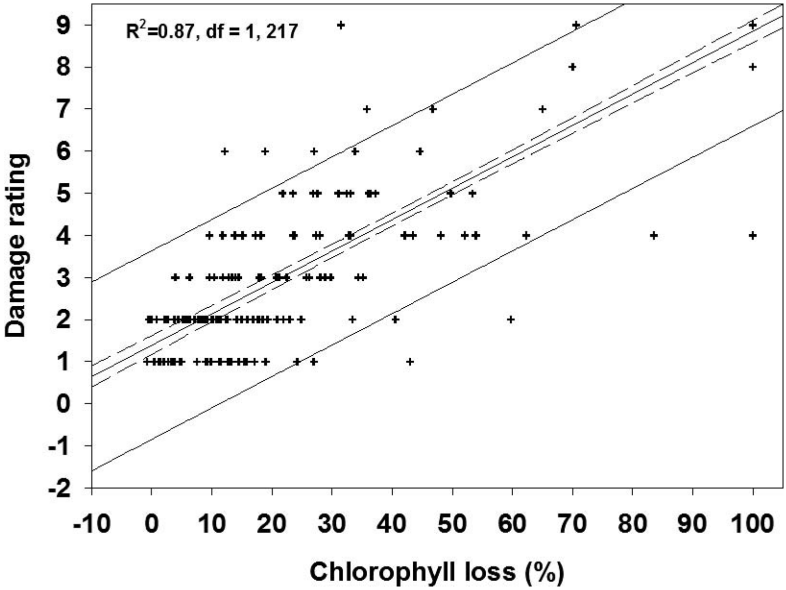

Fig. 1. Sugarcane aphid damage ratings (1.0 = no damage, 9.0 = dead plant, dependent variable) regressed against chlorophyll loss for 12 sorghum entries.

11.0 cm shorter than noninfested plants. Also, infested SP70B17, SP68M57, SP6929, and KS585 plants were, on average, 21.0 cm shorter in height than noninfested plants. The SP70B17, SP68M57, SP6929, and KS585 entries did not appear to exhibit the same degree of tolerance to sugarcane aphid feeding as the other entries previously listed.

The number of leaves on a sorghum entry can also be an indicator of sorghum susceptibility or tolerance in that sorghums exhibiting greater growth or more leaves may be less susceptible to sugarcane aphid feeding. Leaf numbers in this study ranged from 2.5 to 2.9 for the most susceptible Tx7000, SP6929, and SP70B17 entries and from 3.2 to 3.6 for the resistant Tx2783, SP7715, OL2042, OL0029, SP74C40, SP78M30, and SP73B12. Differences in plant height and the number of leaves on a sorghum entry appear to be related based on the means presented in Table 1.

There were few outliers that plotted outside the prediction intervals when chlorophyll loss was regressed against damage rating (Fig. 1), but an acceptable relationship explained the greatest portion of variation and resulted in a high correlation coefficient ($R^2 = 0.87$, $P = 0.0001$; Table 2). Some improvement in the model was detected when a stepwise addition for the difference in plant height was added ($P = 0.0012$, $R^2 = 0.89$). The next stepwise addition of the numbers of leaves on a sorghum entry did not improve the correlation coefficient ($P < 0.07$) as the third step in the model (Table 2).

Table 2. Stepwise regression for the additive effects of model improvement for damage ratings (dependent variable) regressed against independent variables chlorophyll loss, difference in plant height, and number of leaves per plant.

Dependent Variable Damage Rating Versus the Following Variables	Root Mean Squared Error	df	*F* Value	*P* > *F*	Adjusted R^2
Single model					
Chlorophyll loss	43.5	1, 217	152.4	.0001	0.87
Double model					
+ Plant height	30.9	1, 217	10.73	.0012	0.89
Triple model					
+ Number of leaves/plant	25.3	1, 217	3.2	.0760	0.89

Discussion

The methods of evaluations in this study for sugarcane aphid–resistant sorghums emphasize the detection of tolerance as a mechanism of resistance, as opposed to the other two forms of resistance, antibiosis or antixenosis. Tolerance has been an economically important form of resistance and has often been under appreciated for its value (Reese et al. 1994). The other forms of resistance should be determined, but what was clearly shown in this evaluation is that 6 of the 10 entries, excluding the known resistant Tx2783 and susceptible Tx700, contained very good levels of host plant resistance against the sugarcane aphid. At the time of this evaluation, the six entries SP7715, OL2042, OL0029, SP74C40, SP78M30, and SP73B12 were in development, but now are commercially available.

The strong correlation coefficient for the chlorophyll loss and damage ratings has been reported by Girma et al. (1998), where they challenged susceptible and tolerant sorghums to biotype E greenbugs. In this study, we also found a strong relationship for chlorophyll loss and damage ratings, with a slight improvement when difference in plant height was added and no improvement when numbers of leaves on a plant were included in stepwise regression.

We do not know the genetic background of the resistance expressed in the sorghums evaluated, but we can assume that at least some are related to the Tx2783 background that has been documented to have both tolerance and antibiosis expressed as resistance. Tx2783 was released in 1984 with resistance to greenbug biotypes C and E (Peterson et al. 1984), and it also was found to be cross resistant to the sugarcane aphid, with expression of both tolerance and antibiosis (Armstrong et al. 2015, 2017). Greenbug-resistant sorghums that are commercially available have been invaluable in suppressing sugarcane aphid epidemics. This, in turn, has allowed for the screening and discovery of new forms of resistance that are being developed into commercial varieties.

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