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Host suitability for the sugarcane aphid *Melanaphis sacchari* (Hemiptera: Aphididae) on sorghum, sorghum-sudangrass, millets and other forage grass species

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ABSTRACT

The sugarcane aphid (SCA), *Melanaphis sacchari* (Zehntner) became a perennial pest of grain sorghum in the United States beginning in the summer of 2013. Susceptible grain sorghum has been considered one of the most optimum hosts utilized by SCA although it is widely known that other grass species used for grazing, haying and silage also serve as hosts. We evaluated the reproductive capacity, preference/non-preference, and feeding damage caused by SCA on eleven forage and grass species, including pearl millet, proso millet, forage sorghums, sorghum x Sudan grass hybrids and a related grass, S*accharum ravennae*. The study was conducted on younger plants, infested one week after emergence, and an identical evaluation was conducted by infesting at two weeks post emergence, with both experiments carried out for five consecutive weeks post-infestation. Sorghums, sorghum-sudangrass forages such as TX 7000, SPX 46214, SPX 49313, Trudan and Sordan supported a high reproductive potential and were highly preferred over millets sp., wheat and barley. The sorghums, sorghum-sudangrass forages sustained the highest damage ratings when the plants were infested at one week and two weeks of age. Sugarcane aphids had a mid-level reproductive potential on millets Leafy 22 and Millet 32 when plants were infested at one week of age but were lower in aphid numbers at two weeks of age. Millets were not as suitable in terms of hosting sugarcane aphids when compared to the sorghums. Barley Aberdeen 812, soft red winter LA 841, and millet Parakeet did not support any sugarcane aphids when infested at two weeks, and thus are not considered sustainable hosts. These findings identify the forage grass species that could be used in the sorghum production regions that would help reduce threatening populations of the sugarcane aphids and demonstrates the need for developing resistant SCA sources for Sudan and sorghum-sudangrass forages.

KEYWORDS: *Melanaphis sacchari,* resistance, millet, forage sorghum, host plants.

INTRODUCTION

Following the initial infestations of sorghum by the sugarcane aphid (SCA) *Melanaphis sacchari*, (Zehntner) (Homoptera: Aphididae) in the US in the summer of 2013, the identification, breeding and release of SCA-resistant sorghum varieties has had a significant impact on reducing the impact of SCA populations [1, 2]. Grain sorghum

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producers currently have > 40 commercial varieties that are resistant to the SCA (JSA, unpublished data). The contributions to SCA-resistant grain sorghums originated from different sources. The parental line RTx2783 known to be resistant to greenbug biotypes C and E [3] was found to be cross-resistant to the SCA [4]. This cross-resistance for greenbug and sugarcane aphid is also reported in P.I. 550610 [5, 6], which has a completely different resistance genes. In 2016, Tx3408 and Tx3409 were developed and released by the Texas A&M sorghum breeding program and released as seed parental lines with sterile versions developed using the A1 cytoplasmic male sterility system (A1 CMS) [7]. In 2018, eighteen pollinator parents resistant to the SCA and designated as RTx3410 through RTx3428 were released [8]. Later in that same year, two additional lines, R.LBK1 and R.LBK2 from the USDA-ARS breeding program in Lubbock, TX were registered and released [9]. For forage sorghum breeding, the industry commonly uses a limited choice of public seed parents (A/BTx623, A/BTx631, A/BTx378) to produce forage sorghum and sorghum x sudangrass hybrids [10, 11]. These plants are widely adapted and high yielding but are not SCA-resistant. SCA resistance is a dominant genetic trait [9], but if the forage pollinator parent is SCA-susceptible, the hybrid generated between the inbreeds will also be SCA-susceptible.

Given that there are numerous varieties of SCA-resistant grain sorghum available for producers to choose from, what needs further investigation is the host suitability of forage grasses that provide hay, silage, green chop, or grazing forage during the summer or winter. Sorghum x sudangrass hybrids have the potential to produce up to 4 tons of haylage per acre if planted in July and harvested for hay in September, while pearl millet (*Pennisetum glaucum*) used for forage has the potential to produce 2 to 3 tons by September [12]. While both sorghum-sudangrass and pearl millet can be used for silage, forage sorghum may have an advantage with a higher yield potential [13, 14]. An early effort to determine the host range of the sugarcane aphid within the United States found that grasses from the genus *Sorghi* and *sacchari* are favored hosts, which includes the weed species Johnsongrass, *Sorghum halepense* [15]. Our studies were carried out to determine SCA host suitability of common planted grass species used for grazing, hay and silage, and to determine which of the species would limit the generation of high numbers of SCA that could also infest grain sorghum when grown near each other. Identification of grass species that support low SCA reproductive capacity could be examined for resistant traits in grass/forage breeding efforts and could be utilized in areas where grain sorghum is planted to help minimize threatening populations.

MATERIALS AND METHODS

Grass species

Eleven different grass species including proso millet (*Panicum miliaceum* L.), pearl millet (*Pennisetum glaucum*), grain sorghum [*Sorghum bicolor* (L.) Moench], sorghum × sudangrass hybrids (*Sorghum* × *drummondii*), forage sorghum [*Sorghum bicolor* (L.) Moench], hard red winter wheat (*Triticum aestivum* L.), Winter barley (*Hordeum vulgare*), and a Ravenna grass [*Saccharum ravennae* L.] were selected to determine the reproductive potential, preference and damage potential when threatened by SCA populations (see Table 1).

Aphid collection and culture

A colony of SCA was originally collected in August 2013 on grain sorghum near Bay City, Matagorda County, TX. From this collection, a single parthenogenic female was continuously maintained on RTx7000, a susceptible sorghum used in greenbug biotype determinations [16]. The nymphs produced from this female were reared on RTx7000 seedlings grown in 4.4 L pots fitted with 45 cm tall cylinder Lexan® (SABIC Polymershapes, Tulsa OK) sleeve cages ventilated with organdy cloth covering the top. This clonal colony was maintained by changing to new seedling plants every 2 weeks in the greenhouse. The pots and seedling sorghum were maintained on greenhouse benches with two T-8 flourescent lights that provided supplemental light and a 24 h temperature range of 31 °C to 21 °C.

Table 1. Grass genotypes evaluated for sugarcane aphid host suitability.

Entry	Type	Utility
Leafy 22	Hybrid Pearl Millet	Livestock Forage and Hay
Millet 22	Hybrid Pearl Millet	Livestock grazing & pasture
Aberdeen 812	Winter Barley	forage/cover crop, feed grain, malting barley
LA 841	Soft red winter wheat	Grain, pastries & crackers
Parakeet	White Millet	Bird feed
TX 7000	Parental breeding line	Grain sorghum
SPX 46214	Sudan forage sorghum	Grazing, haying
SPX 49313	Hybrid Sudangrass	Livestock grazing & pasture
Trudan headless	Sorghum x Sudangrass	Hay, Haylage & Grazing Livestock
Sordan headless	Sorghum x Sudangrass	Hay, Haylage & Grazing Livestock
Saccharum ravennae	Ornamental grass considered invasive weed in some states	Aesthetics, ornamental, prevent soil erosion

Reproductive potential

A no-choice evaluation was conducted to determine the reproductive potential of the SCA on the 11 grasses presented in Table 1. Two identical evaluations were conducted with the only difference being in the first experiment, seedlings were infested at one week of age with 5 nymphal SCA, whereas in the second evaluation, plants were infested at two weeks of age with 5 nymphal SCA. In both experiments, plants were evaluated for aphid number for five consecutive weeks following the initial infestation. Each of the eleven grass entries were planted in a 4.4 L pots filled with potting soil (Sun-gro Propagation Mix, Pine Bluff, AR 71601) and replicated seven times for a total of 77 no-choice experimental units. A single replicate from each entry was blocked together and randomized on greenhouse bench tops using research randomizer [17]. After infestation for both experiments, each pot was fitted with 85 cm tall cylinder Lexan® (SABIC Polymershapes, Tulsa, OK) sleeve cage ventilated with organdy cloth. All 7 replications for each grass species were examined once a week starting from the day of infestation and all aphids (nymphs and adults) on each plant were counted using 3x magnifying visors (Carson Optical Pro Head magnifier, Desoto TX, 75115) and Sparco® hand tally counters (Sparco Industries, Tawain). The grass pots were maintained on greenhouse benches with two T-8 fluorescent lights that provided supplemental light and a 24 h temperature range of 21 °C to 28 °C. Cumulative Aphid days (CAD's) were calculated for each entry once a week for 5 consecutive weeks. The number of aphids per plant was averaged per entry and then converted to CADs over sample dates using the formula:

$$\mathrm{CAD} = \sum_{i=1}^{n} \left(\frac{\mathrm{N}_i+\mathrm{N}_{i+1}}{2}\right) \times T,$$

where N_i is the total number of SCA on a given sample date and N_{i+1} is the total number of aphids on the same plant for the following sample date, and T is the number of days between the two evaluations [18]. The younger group (one week old) plants were infested on June 10, 2020 and examined for SCA on June 16, 23, 30, July 7, and 14. The older set of plants was infested on June 25, and examined for SCA on July 3, 8, 14, 21, and 29.

At the conclusion of the trial, the number of aphids by entry for each weekly evaluation was subject to two-way Analysis of Variance (ANOVA), with grass entry means compared ($\alpha = 0.05$) using least squared means pair-wise comparisons procedure [19]. However, a test for normality showed the data were not normally distributed so prior to SAS analysis, the data were square root transformed but the actual means and variance are presented. Chlorotic damage ratings for each entry and for the one-week old, and two weeks of age when infested were evaluated using a rating of 1-9 where; 1 is completely healthy; 2, 1-5% chlorotic; 3, 5-20%; 4, 21-35%; 5, 36-50%; 6, 51-65%; 7, 66-80%; 8, 81-95%; and 9, 95-100% or dead [20, 21]. The data for leaf damage ratings was analyzed by the non-parametric Kruskal-Wallis One Way Analysis of Variance, followed by a Tukey-test [22] to compare rating estimates by grass type.

Antixenosis

The 11 grass genotypes listed in Table 1 were evaluated for antixenosis (non-preference) using the methods described in [23] where several Ethiopian sorghums were evaluated for yellow sugarcane aphid S*ipha flava* (Forbes) antixenosis. In the first test for antixenosis, the eleven entries were planted in an evenly spaced circular pattern around the edge of a 20.3 cm (d) pot. When the plants were one week of age, they were infested with approximately 3,000 SCA by releasing them in the center area of the pot for a 48 h of period, followed by counting all aphids that had settled on each of the plants to determine antixenosis. An identical experiment was conducted with the exception that the plants were 2 weeks of age at the time of infesting, followed by counting all nymphs and adults after 48 h. There were 7 replications (pots) of the 11 entries randomly planted in equal distances around the edge of each pot. SCA's were counted from each plant in a pot, and the numbers of nymphs were arcsine square root transformed and analyzed with two-way (grass species * week of evaluation) ANOVA. Aphid numbers were compared ($\alpha = 0.05$) using least squared means pair-wise comparison procedure [19]. The LS means statement was adjusted using Tukey's HSD test.

RESULTS

Reproductive potential

The SCA reproductive potential was measured by the use of cumulative aphid days (CAD) and varied by grass species and across the five weeks of the study (Tables 2 & 3). As a reference, a susceptible grain sorghum, TX 7000, has been used in numerous evaluations in the effort to identify resistance to sugarcane aphids [15, 11]. During the first two weeks of evaluation for the grasses infested at one week of age, numbers of SCA on TX 7000 were lower than SPX 49313, Trudan and Sordan, but by the third week TX 7000 accumulated 10,671 CAD which was similar to Sordan with 8,251 CAD, both were significantly higher than all others (Table 2). SCA reproduction on millets Leafy 22, Millet 32, Parakeet, barley Aberdeen 812 and hard red winter wheat LA 841 started slowly with all having fewer than the 5 aphids they were originally infested with; however by the third, fourth and fifth week the Leafy 22, Millet 32 had a mid-range of reproduction, while Aberdeen 812, LA 841 and Parakeet had the lowest numbers of SCAs produced. Trudan had the fewest aphids of any grass species by the end of the fifth week but this was a result of the plant being dead and thus, unable to support aphids.

In the second experiment when grass entries were infested at two weeks of age, by the third, fourth and fifth week the highest sustained numbers of SCA were produced by TX 7000, Sordan and SPX 49313 followed by SPX 46214 and Trudan. Interestingly, SCAs did not establish on Aberdeen 812, Parakeet or LA 841when grass entries were infested at two weeks of age, and for the Millet 32 and Leafy 22 the CAD accumulation was greatly reduced across the 5 weeks of study when compared to plants infested at one week of age (Tables 1 & 2).

A summary of the results of reproduction for one week old and two-week-old infested plants (Figure 1A & B) shows that the known susceptible TX 7000 had the highest accumulated CAD in both experiments followed by Millet 32 at one week infestation, Sordan, Leafy 22, SPX 49313, SPX 46214, Trudan, Aberdeen 812, Ravenna grass, Parakeet and LA 841. The significance of plants being older at the time of infesting is highlighted in Figure 1B where SCAs failed to establish on Aberdeen 812, Parakeet and LA 841, and had greatly reduced numbers on Millet 32 and Leafy 22. The sorghum and sorghum-sudangrass entries SPX 49313, SPX 46214 and Trudan

Table 2. Cumulative aphid days (CAD) for sugarcane aphid progeny produced from different millet and grass species when plants were infested at one week of age and evaluated weekly for the next five consecutive weeks.

Genotype	Entry	Week 1	Week 2	Week 3	Week 4	Week 5
Leafy 22	1	3.8 ± 2.4 d	281.0 ± 11.5 e	5,698.0 ± 216.5 bc	10,705.0 ± 423.7 bc	4,175.5 ± 207.3 b-d
Millet 32	2	1.8 ± 1.9 d	469.0 ± 18.2 de	4,900.0 ± 114.3 bcd	15,136.0 ± 227.8 ab	12,295.5 ± 163.7 ad
Aberdeen 812	3	1.5 ± 0.3 d	95.8 ± 39.8 e	1,431.5 ± 22.3 def	3,018.3 ± 65.6 bc	1,533.0 ± 73.5 cde
LA 841	4	1.8 ± 0.3 d	55.3 ± 16.0 e	278.2 ± 27.8 f	417.8 ± 8.6 d	217.5 ± 5.8 e
Parakeet	5	1.5 ± 0.3 d	112.3 ± 10.7 e	689.5 ± 32.9 ef	984.5 ± 60.9 d	462.3 ± 27.8 e
TX 7000	6	32.5 ± 16.7 bc	830.8 ± 14.7 cd	10,671.8 ± 153.2 a	18,099.9 ± 178.8 a	6,335.4 ± 29.6 b
SPX 46214	7	34.0 ± 9.4 bc	964.5 ± 9.4 c	4,077.5 ± 95.2 cde	5,001.0 ± 29.4 cd	1,970.7 ± 115.2 cde
SPX 49313	8	40.5 ± 17.3 bc	1,481.4 ± 25.0 ab	4,670.8 ± 113.7 bcd	6,145.7 ± 159.4 ab	4,385.0 ± 43.4 bc
Trudan headless	9	55.0 ± 15.8 ab	1,214.8 ± 140.9 bc	4,257.5 ± 134.9 cde	3,352.5 ± 166.9 cd	42.0 ± 19.8 e
Sordan headless	10	100.5 ± 28.6 a	1,775.0 ± 290.3 a	8,251.3 ± 160.7 ab	9,502.2 ± 183.7 bc	1,858.5 ± 161.9 cde
Saccharum ravennae	11	11.5 ± 4.3 c	434.3 ± 9.2 de	1,039.5 ± 10.8 ef	562.0 ± 68.3 d	556.5 ± 23.1 de

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD.
Overall model df = 54, 219, $F = 33.09$; $P > F = < 0.0001$.
Entry df = 10, $F = 31.68$; $P > F = 0.0001$.
Week df = 4, $F = 315.68$; $P > F = 0.0001$.
Entry*Week df = 40, $F = 5.25$; $P > F = 0.0001$.

Table 3. Cumulative aphid days (CAD) for sugarcane aphid progeny produced from different millet and grass species when plants were infested at two weeks of age and evaluated weekly for five consecutive weeks.

Genotype	Entry	Week 1	Week 2	Week 3	Week 4	Week 5
Leafy 22	1	19.8 ± 4.8 d	402.0 ± 14.8 d	831.0 ± 16.5 d	418.0 ± 42.7 c	448.5 ± 20.3 b
Millet 32	2	5.0 ± 2.3 d	99.3 ± 18.2 d	329.0 ± 14.3 d	249.8 ± 22.8 d	91.5 ± 163.7 c
TX 7000	3	34.8 ± 5.2 bc	2,767.8 ± 31.4 c	26,047.8 ± 153.2 b	33,099.9 ± 178.8 a	3,297.4 ± 39.5 cb
SPX 46214	4	113.8 ± 13.3 a	2,948.5 ± 99.4 c	11,697.5 ± 54.7 c	10,440.0 ± 29.4 ab	802.7 ± 115.2 ab
SPX49313	5	80.5 ± 19.6 ab	6,387.0 ± 36.8 a	33,399.8 ± 459.7 a	33,041.7 ± 159.4 ab	1,852.0 ± 43.4 ab
Trudan headless	6	38.0 ± 15.8 ab	3,245.8 ± 175.9 c	10,183.5 ± 96.9 c	10,850.5 ± 142.9 ab	6,549.0 ± 19.8 a
Sordan headless	7	64.5 ± 16.5 abc	5,301.0 ± 290.3 b	21,819.3 ± 160.7 b	18,173.2 ± 193.7 b	900.5 ± 161.9 b
Saccharum ravennae	8	53.8 ± 4.3 bcd	946.3 ± 15.8 d	2,875.5 ± 10.8 d	4,531.0 ± 98.3 b	3,633.5 ± 23.1 b

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD.

Overall model df = 15, 159, $F = 53.09$; $P > F = < 0.0001$.

Entry df = 7, $F = 12.38$; $P > F = 0.0001$.

Week df = 4, $F = 62.28$; $P > F = 0.0001$.

Entry*Week df = 24, $F = 10.25$; $P > F = 0.0001$.

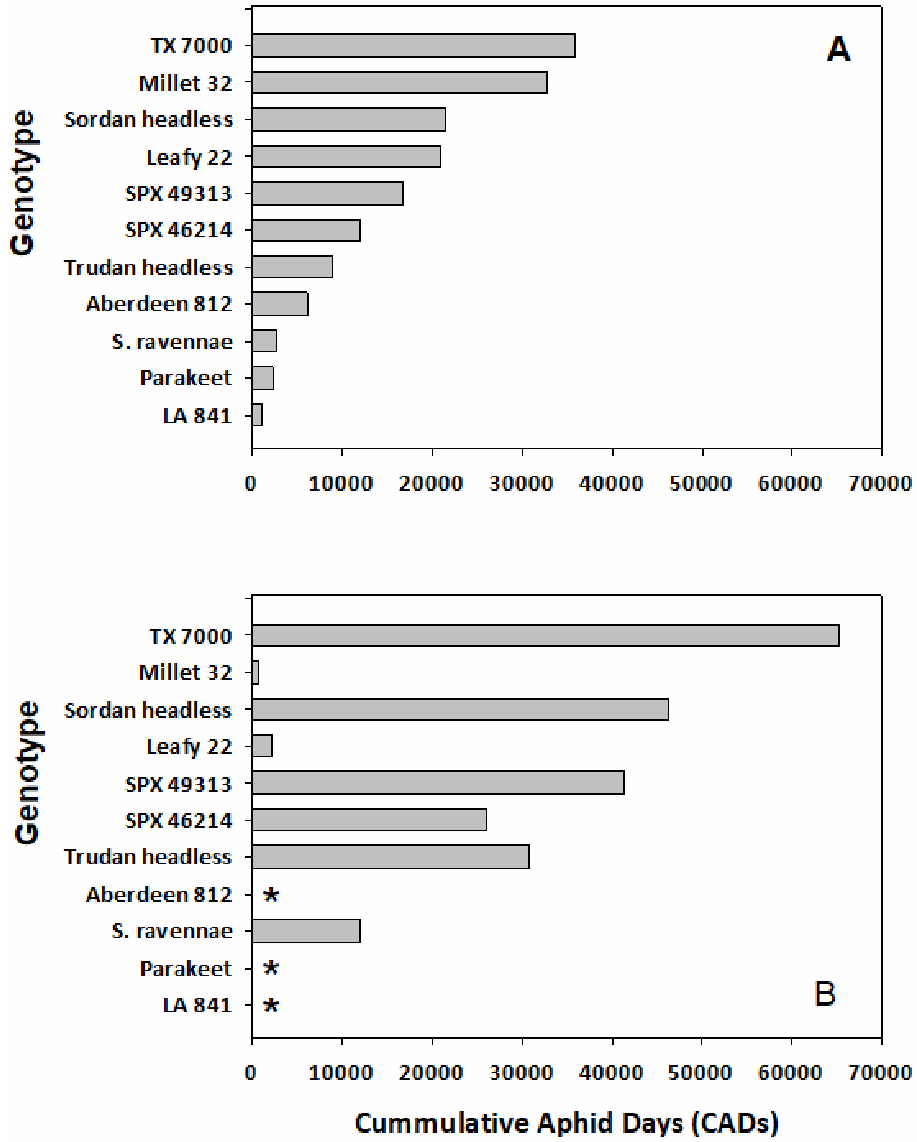

Figure 1. Total cumulative aphid days (CAD) for the sugarcane aphid when infested at one week of plant age (A) and two weeks of plant age (B) and counted once per week for five consecutive weeks. Sugarcane aphids did not establish (survive) when offered Aberdeen 812, Parakeet millet or LA 841 soft red winter wheat that was two weeks old at the time of infesting as indicated by the asterisks.

supported between 30,000 and 50,000 accumulated CAD across the five weeks of study (Figure 1B).

Antixenosis

A clear pattern was exhibited when the eleven grass entries were challenged at one week of age vs two weeks of age at the time of infesting (Figure 2 and Figure 3). SCA's established on all entries when plants were one week old; however the Aberdeen 812, LA 841, and Parakeet were the least preferred, while the Leafy 22, Millet 32, SPX 46214, Trudan, Sordan and Ravenna grass were preferred hosts. The most preferred entry for plants that were one week of age at the time of infesting plants was the hybrid sudangrass SPX 49313 hosting slightly over 100 aphids, followed by our known susceptible sorghum TX 7000 that had a mean of 85 SCA's at the time of evaluation (Figure 1A) in this study. When plants were two weeks old, a similar pattern of preference was

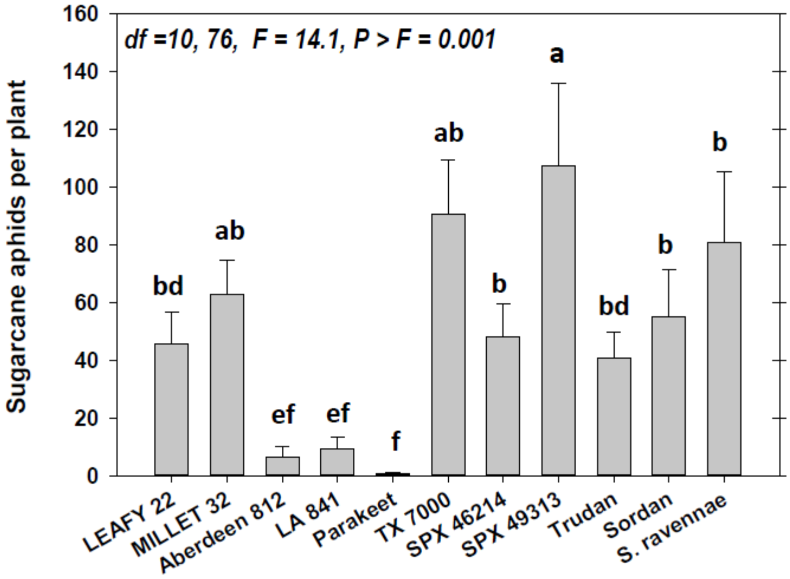

Figure 2. Antixenosis test for sugarcane aphid counts (n = 7 replications) for 11 grass entries. All aphids (nymphs and adults) were counted 48 h after releasing in the center of a circular arena.

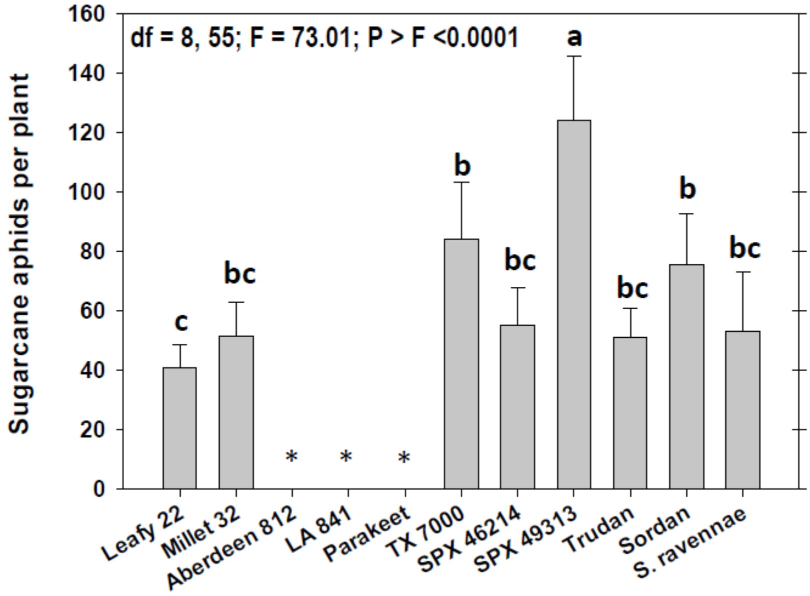

Figure 3. Antixenosis test for sugarcane aphid counts (n = 7 replications) for 11 grass entries. All aphids (nymphs and adults) were counted 48 h after releasing in the center of a circular arena when grasses were two weeks of age. Sugarcane aphids did not prefer Aberdeen 812, Parakeet millet or LA 841 soft red winter wheat that was two weeks of age at the time of infesting as indicated by the asterisks.

observed except that SCAs did not establish on barley Aberdeen 812, soft red winter wheat LA 841, or the millet Parakeet. It appears that plants that are two weeks of age express more antixenosis when compared to plants one week of age. Damage ratings taken at the end of five weeks for grasses infested at one week, or at two weeks also showed antixenosis (Figures 4 & 5).

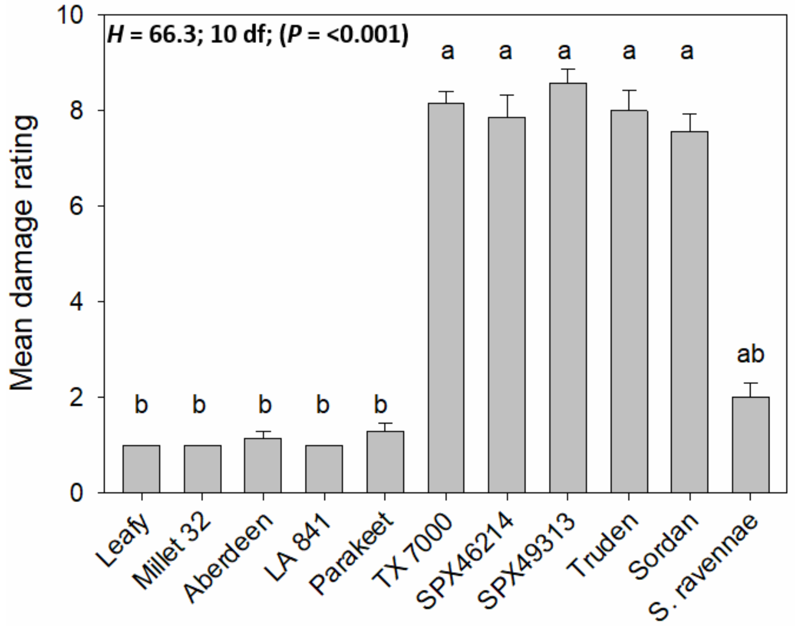

Figure 4. Mean ± 1 S.E. damage ratings for 11 different grass species when infested with sugarcane aphids following a 5-week period from the time of infesting. Bars with different letters are significantly different from Kruskal-Wallis One Way Analysis of Variance followed by a Tukey's Test.

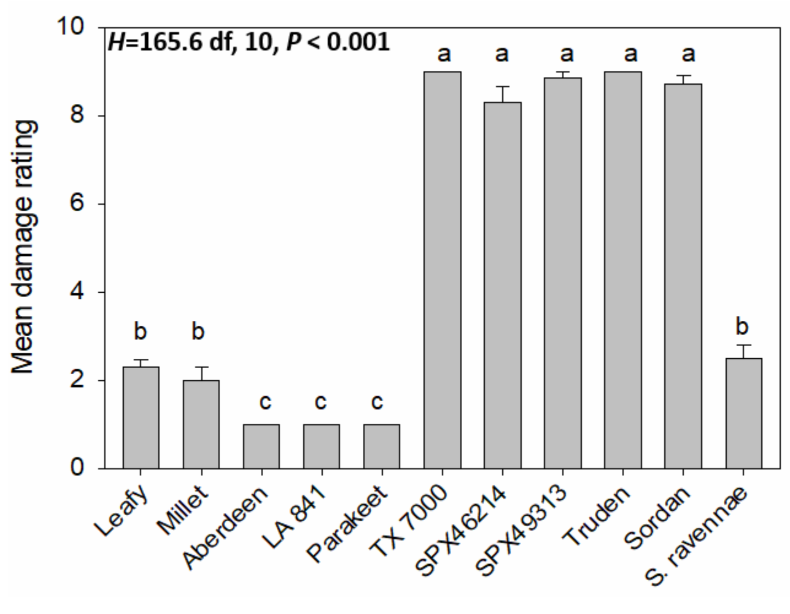

Figure 5. Mean ± 1 S.E. damage ratings for 11 different grass species when infested with sugarcane aphids following a five-week period from the time of infesting. Bars with different letters are significantly different from Kruskal-Wallis One Way Analysis of Variance followed by a Tukey's Test.

The millets Leafy 22 and Millet 32 sustained damage ratings slightly over 2, while the soft red winter wheat LA 841, the barley Aberdeen 812, and the millet Parakeet all had the lowest possible damage rating of 1, which is indicative of no damage from the 1-9 scale rating scale.

DISCUSSION

Host plant suitability for the eleven entries against SCA varied by host and the age of the plant at the time of infestation (1 week old vs 2 weeks of age). The known susceptible sorghum TX 7000 clearly demonstrates the serious threat that SCA is to susceptible grain sorghums. Following the TX 7000 in host suitability were Sordan, a sorghum-sudangrass hybrid, SPX 49313 a hybrid sudangrass, SPX 46214, a hybrid sudangrass and Trudan, a sorghum-sudangrass hybrid. All of the grasses generated high numbers of aphids regardless of the age of the plant at initial infestation and all sustained heavy injury by the end of a 5-week infestation. The hybrid millets Millet 32 and Leafy 22 supported large numbers of SCA, especially when infested at one week of age; however when infested at 2 weeks of age they supported very few SCA. The remaining millet Parakeet only supported a few SCA with infestation at one week and did not support any at two weeks of age.

It was quite apparent throughout this study that the millets are covered in plant hairs or trichomes [24, 25]. In fact, we video recorded a sugarcane aphid nymph trying to negotiate through the plant hairs of the "Parakeet" millet when it was 4 weeks of age. The nymph was unable to access the surface of the millet leaf or main stems because the trichomes were too dense. The hairiness of the millets accounts for at least a portion of the antibiosis expressed for the millets. The three grasses, Aberdeen 812 barley, Parakeet (white) millet, and the LA 841 soft red winter wheat expressed the highest degree of antixenosis where the aphids did not establish on the plants at 2 weeks of age. The Ravenna grass served as an interesting host for the SCA when compared to all other hosts because it supported higher numbers when infested at 2 weeks as opposed to being infested at one week. Even though the Ravenna grass supported SCA throughout the study, it sustained a low damage rating of only slightly higher than 2, which means it will support SCA for weeks at a time but not be significantly damaged.

CONCLUSION

As was stated earlier, the many forage grasses are planted and utilized in the same production areas of grain sorghum on the Great Plains. Many of these grasses have the capacity to support a significant number of SCA. We have shown that most sorghums, Sudan sorghums, Sudan grasses can be planted near the susceptible sorghums and should not be used where resistant grain sorghums are planted. The hybrid Pearl millets Millet 32 Leafy 22 will support SCAs in the very early growth stages but with age, will decrease in host importance, while wheat, barley and white millet represent forage grasses that are not palatable and will not support SCA populations. Ravenna grass is widely cultivated as an ornamental grass and has escaped cultivation becoming noxious weed in riparian areas. This grass can sustain large populations of SCA with little or no damage to itself and may serve as an alternate host for SCA.

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CONFLICT OF INTEREST STATEMENT

The authors of this manuscript have no conflict of interest of any kind with the research reported and presented in this manuscript. The conceptualization, design and representation of this research is an original product produced solely by the authors.

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