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Interactions between Sudangrass Lines Selected for Differing Nitrate Expression and Sorghum Aphid

Rafael Hayashida [1,*], Camille Carey [2], Tim Springer [3], Bailey Knighten [4], J. Scott Armstrong [4] and W. Wyatt Hoback [1,*]

[1] Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078, USA
[2] Department of Rangeland, Wildlife and Fisheries Management, Texas A&M University, College Station, TX 77840, USA; camille.carey@tamu.edu
[3] U.S. Department of Agriculture, Southern Plains Range Research Station, Woodward, OK 73801, USA; tim.springer@ars.usda.gov
[4] Wheat, Peanut and Other Field Crops Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Stillwater, OK 74075, USA; bailey.knighten@usda.gov (B.K.); scootamous@gmail.com (J.S.A.)
* Correspondence: rafael.hayashida@okstate.edu (R.H.); whoback@okstate.edu (W.W.H.)

Abstract: Sudangrass (*Sorghum sudanense* Stapf) is widely cultivated as a summer annual forage across the southern Great Plains because of its robust forage yield potential. However, the accumulation of nitrates and the potential harm to livestock have restricted the use of Sudangrass for feeding ruminants. Since 2013, the sorghum aphid (SA), *Melanaphis sorghi* (Theobald) (Hemiptera: Aphididae), has been damaging sorghum and Sudangrass production. However, the interaction between SA feeding and nitrate accumulation in Sudangrass has not been determined. In this study, we evaluated the effect of SA feeding on different Sudangrass lines, comparing them to a susceptible and a resistant sorghum variety and measuring the physiological responses and nitrate concentration after aphid feeding. Additionally, we evaluated the use of these grass lines on SA demographics. Initial infestations of 100 SA per plant that were allowed to feed and proliferate for 20 days increased nitrate concentrations in Sudangrass by more than 60% compared to controls. Concurrently, the Sudangrass lines selected for high nitrate levels drastically reduced SA demographic parameters, comparable to those of the resistant sorghum control. Although the adoption of resistant cultivars is recommended for aphid management, the Sudangrass lines selected may not be the best option for SA management because their accumulation of nitrates in response to herbivory can cause ruminant poisoning.

Keywords: plant resistance; secondary plant compounds; sorghum germplasm; ruminant poisoning

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1. Introduction

Sudangrass (*Sorghum sudanense* Stapf) is widely cultivated as a summer annual forage across the southern Great Plains because of its robust forage yield potential and ease of establishment. Additionally, this forage exhibits notable potential benefits, including erosion control, soil carbon sequestration, organic matter augmentation, soil compaction reduction, weed suppression, nutrient cycling enhancement, saline soil tolerance, attraction of beneficial insects and pollinators, and increase in biodiversity [1,2]. Selective breeding also produces Sudangrass lines that accumulate nitrogen, potentially improving soil nutrients [1].

However, a drawback of the adoption of Sudangrass as a forage crop for ruminants is its potential to cause nitrate poisoning in ruminants. Nitrate poisoning occurs when there is an overconsumption of nitrate (NO_3) or nitrite (NO_2) by ruminants, including cattle. Typically, nitrates transform into nitrites during digestion, which then change into ammonia and subsequently into protein. However, when an animal ingests high levels of nitrates, nitrites accumulate in the rumen. Because nitrites are up to ten times more harmful than nitrates, this can lead to poisoning [3]. At lower amounts, chronic nitrate

intoxication is often associated with reproductive problems in cattle, such as abortions and other complications [4,5]. Acute nitrate poisoning in cattle primarily manifests as methemoglobinemia, leading to anoxia and death [5].

The build-up of nitrates in plants results from an imbalance in the absorption and assimilation processes. Some abiotic factors contribute to nitrate accumulation, including drought stress, accumulation of organic matter in soil, and chemical fertilization [6]. Additionally, events like regrowth after cutting or hail can increase nitrate levels, although nitrates generally decrease as plants mature [1]. Feeding by herbivores can also increase plant nitrates either by affecting underlying metabolic pathways or as a secondary metabolite in response to herbivory [7,8].

Since the summer of 2013, Sudangrass fields in the southern Great Plains of the US have been impacted by the sorghum aphid (SA), *Melanaphis sorghi* (Theobald) (Hemiptera: Aphididae) [9]. By 2015, the aphid broadened its geographical distribution, posing a significant risk to more than 90% of sorghum cultivation in North America [10]. SA causes injury to sorghum by extracting sap from plants, leading to a direct reduction in yield. Additionally, the build-up of honeydew on leaves and in the panicle increases sooty mold and disrupts harvest operations, contributing to further declines in yield [10].

In addition to causing yield losses, the feeding habits of SA could potentially influence the nitrate concentration in Sudangrass. As the aphids extract sap from the plants, they modify the plant physiology [11,12], which can impact the plant's nitrate absorption and assimilation processes, potentially resulting in an increase in nitrate levels. Furthermore, the nitrate concentration in the grass can affect SA demographics. High nitrate levels might either reduce aphids' fitness, leading to a decrease in their population or increase their fitness, resulting in a population surge. However, we are unaware of previous work that examines the effect of aphid feeding on the nitrate concentration of Sudangrass, or on the role of nitrate concentration on aphid populations feeding on this species.

Thus, the objectives of this study were to evaluate the effect of SA feeding on different Sudangrass lines previously selected for nitrate concentration and compare them with a susceptible and resistant sorghum variety. Additionally, we characterized the effect of these lines on the demographics of SA.

2. Materials and Methods

2.1. Nitrate Selections in 'Piper' Sudangrass

Divergent recurrent selection at the USDA was used to develop lines of Piper Sudangrass [*Sorghum sudanense* (Piper) Stapf] for low and high nitrate accumulation over two successive generations. The base population (C0) was the original Piper release of Sudangrass [13]. Two cycles of recurrent selection were used. Cycle 1 (C1) selection consisted of seeds that originated from Piper C0. Plants were grown in 100 pots (8 L) in a greenhouse seedling mixture. Each pot contained one plant and was fertilized with ammonium nitrate (NH_4NO_3) at an equivalent rate of 112 kg ha^{-1}.

When plants were approximately 1 m in height, three leaves were removed from the plant and crushed to collect approximately 5 mL of sap. The nitrate concentration of the sap was obtained by using a handheld nitrate meter [14]. The plants were sorted from lowest nitrate concentration to highest concentration. Six plants with the lowest nitrate concentrations and eight plants with the highest nitrate concentrations were selected. The low-nitrate lines were transplanted into 19 L buckets, isolated in a greenhouse bay, and allowed to produce seeds. Similarly, the high-nitrate lines were transplanted into 19 L buckets and isolated in a different greenhouse bay and allowed to produce seeds.

The seeds of the first selection were designated Piper C1 lo and Piper C1 hi for the low and high ability to concentrate nitrate, respectively. Seeds from the second generation were designated Piper C2 lo and Piper C2 hi, which were similarly created using the methods above, except that Piper C1 lo seeds were used to develop Piper C2 lo, and Piper C1 hi was used to create Piper C2 hi. Nine plants were retained from the Piper C1 lo population, and seeds harvested from these plants were designated Piper C2 lo. Similarly, six plants

were retained from the Piper C1 hi population, and seeds harvested from these plants were designated Piper C2 hi.

2.2. Aphid Culture

Aphids were collected from a post-harvested grain sorghum field near Bay City, Matagorda County, Texas, in August 2013. In 2019, a preferred sorghum biotype of 'sugarcane aphid' named 'SoSCA' biotype MLL-F was phenotyped and genotyped [15,16]. Subsequently, the biotype MLL-F was recognized as a population of sorghum aphid, *Melanaphis sorghi* [Theobald, 1904] [17] and is referred to as sorghum aphid (SA) for the remainder of the work reported here.

This colony has been maintained at the USDA-ARS Stillwater, OK Laboratory by rearing on susceptible TX7000 sorghum seedlings in pots covered with sleeve cages in the greenhouse at temperatures ranging from 21 °C to 28 °C. The plants are grown under natural light supplemented by two T-8 fluorescent lights. New sorghum aphid colonies are transferred to new seedling plants every 2 weeks in the greenhouse to maintain viable colonies for experimentation.

2.3. Effect of Sorghum Aphid on Sudangrass Lines' Fitness

The five Sudangrass lines—Piper C0, Piper C1 lo, Piper C1 hi, Piper C2 lo, and Piper C2 hi—were tested in addition to two sorghum cultivars: the susceptible TX7000 and the sorghum-aphid-resistant TX2783. Three seeds of each line were sown in 8 L pots (filled with the same proportion of potting soil, fitting clay, and sand). After 1 week, thinning was performed, leaving one plant per pot. Plants were fertilized as above.

Forty days after planting, each plant was infested with 100 aphids using a camel hairbrush to transfer late instar nymphs and adult aphids from the colony to the plant leaves. After the initial infestation, the aphid population was allowed to proliferate naturally for 20 days. Control plants, which remained free of infestation, were kept in conditions identical to those of the infested plants. To prevent infestation, control plants were treated with 0.5 g of imidacloprid (Quali-PRO, Control Solutions Inc., Pasadena, TX, USA) per pot.

The physiological parameters evaluated were chlorophyll content (%), net photosynthetic rate (μmol CO_2 $m^{-2}{\cdot}s^{-1}$), and stomatal conductance (mol H_2O $m^{-2}{\cdot}s^{-1}$), following methods described by Paudyal et al. [18] and Carey et al. [19]. All the plants were evaluated before and after aphid infestation. The chlorophyll content (%), was evaluated using a SPAD model 502 chlorophyll meter (Minolta, Tokyo, Japan). This portable meter operates by absorbing light in the wavelength range of 430 to 750 nm as it traverses the surface of a leaf [20]. Each leaf was subjected to three separate readings, and the average SPAD value was subsequently calculated. Net photosynthetic rate (μmol CO_2 $m^{-2}{\cdot}s^{-1}$) and stomatal conductance (mol H_2O $m^{-2}{\cdot}s^{-1}$) were evaluated using a portable photosynthesis system (model LI-6400, LI-COR, Lincoln, NE, USA) at 1200 μmol photons $m^{-2}{\cdot}s^{-1}$ light intensity and a reference carbon dioxide of CO_2 of 400 ppm generated from a 12 g CO_2 cylinder.

2.4. Effect of Sorghum Aphid on Sudangrass Nitrate Concentration

Following the physiological evaluations, plant sap was extracted utilizing a commercial wheatgrass extractor (HWG800, Hamilton Beach Commercial, Richmond, VA, USA). The samples were submitted to the Soil, Water and Forage Analytical Laboratory of Oklahoma State University (Stillwater, OK, USA) where the nitrate concentration (ppm) was analyzed.

To separate the liquid from the solids, the samples were centrifugated using an LC-8™ Centrifuge (C3100, Growinglabs, Suwanee, GA, USA) at 3500 rpm for 5 min. After that, the solution was poured onto filter paper to collect filtrate. The nitrate (NO_3-N) was extracted with 1 M KCl solution and quantified by a Flow Injection Autoanalyzer (LACHAT, 1994—QuickChem Method 12-107-04-1-B—LACHAT Instrument, Milwaukee, WI, USA). The sample solutions were placed in Lachat tubes, and the analysis was made

with Omnion software 4.0 (Hach Company, Loveland, CO, USA) following the Lachat Method 12-107-04-1-B (Nitrate) adapted from Gavlak et al. [21].

2.5. Sorghum Aphid Demographics on Grasses Lines

The reproductive life-table demographics of the *S. sorghi* were compared among different grass lines. We tested all the Sudangrass lines—Piper C0, Piper C1 lo, Piper C1 hi, Piper C2 lo, and Piper C2 hi—in addition to sorghum cultivars: the susceptible TX7000 and the resistant TX2783 [22,23].

Two seeds of each entry were planted in cone-tainers™ (Model SC10, S7S greenhouse supply, Tangent, OR, USA) in a three-layer media of potting soil, fritted clay, and sand from bottom to top, respectively. When the plants reached the two-leaf stage (about 4–6 cm in height), the most vigorous plant was kept, whereas the other was removed. Each cone-tainer™ with an individual entry was considered 1 of 15 replicates, representing a total of 105 individual containers. Each cone-tainer™ was fitted with an 8 cm diameter Lexan sleeve that was 45 cm in height and ventilated with organdy cloth.

The cone-tainers™ were placed in a rack to hold them upright in a completely randomized design inside a growth chamber (Conviron®, Winnipeg, AB, Canada) set at 21 °C and 14:10 L:D photoperiod with lighting provided by seven TS 32W Ecolux® daylight fluorescent lamps (Fairfield, CT, USA) and four 60 W incandescent bulbs.

The remaining seedlings were infested by a single viviparous female, which was removed after 24 h. From these nymphs on each entry, a single, 24 h old nymph was selected to remain on replicates of each of the nine different sorghum entries. The development time to reproductive adult (d), net reproduction (M_d), longevity (L), and reproductive period (days in reproduction) were recorded. The intrinsic rate of increase (R_m) was calculated using the formula: $R_m = [0.738(\log_e M_d)]/d$ [24].

2.6. Statistical Analysis

The statistical analyses were conducted in the R computing environment, using the "AgroR" [25] and "ggplot2" packages for the graphs [26]. Before the SA infestation, we analyzed the measured parameters using a one-way ANOVA, treating the "Sudangrass/sorghum lines" as treatments. After the SA infestation, we used a two-way ANOVA to analyze the evaluated parameters, considering the factors of "Sudangrass/sorghum lines" and the "infestation".

Prior to both the one-way and two-way ANOVA, we conducted an exploratory data analysis to confirm the assumptions of residual normality [27] and variance homogeneity [28]. A post hoc analysis was conducted to identify the significant differences between treatment means, using the Tukey test at a significance level of $\alpha = 0.05$. Additionally, *t*-tests were performed to compare the effects of the infestation on the Sudangrass/sorghum lines.

We compared the demographic parameters, longevity (in days), fecundity, pre-reproductive period (d), days in reproduction (R_m), and number of progeny produced in an equal period d (M_d) using a one-way ANOVA, treating the 'Sudangrass/sorghum lines' as treatments.

3. Results

3.1. Effect of Sorghum Aphid on Sudangrass Physiology and Nitrate Concentration

The average nitrate concentration observed in the treatments varied from 18,450 ppm (Piper C1 hi infested) to 6752 ppm (Piper C1 hi check; Figure 1). Although these differences were not significant among the non-infested treatments ($F(6,18) = 0.71$, $p = 0.65$), differences were observed when there was SA infestation. When the effect of SA was compared within the line, a Student *t*-test showed that the values of nitrate concentration (ppm) in all the selected Sudangrass lines (Piper C1 lo, Piper-C1, high, Piper C2 lo, and Piper C2 hi) increased by SA infestations ($t(4.27) = -1.337$, $p = 0.02$; $t(5.99) = -4.24$, $p = 0.01$; $t(5.12) = -10.04$, $p < 0.001$; $t(5.81) = -2.53$, $p = 0.04$, respectively; Figure 1).

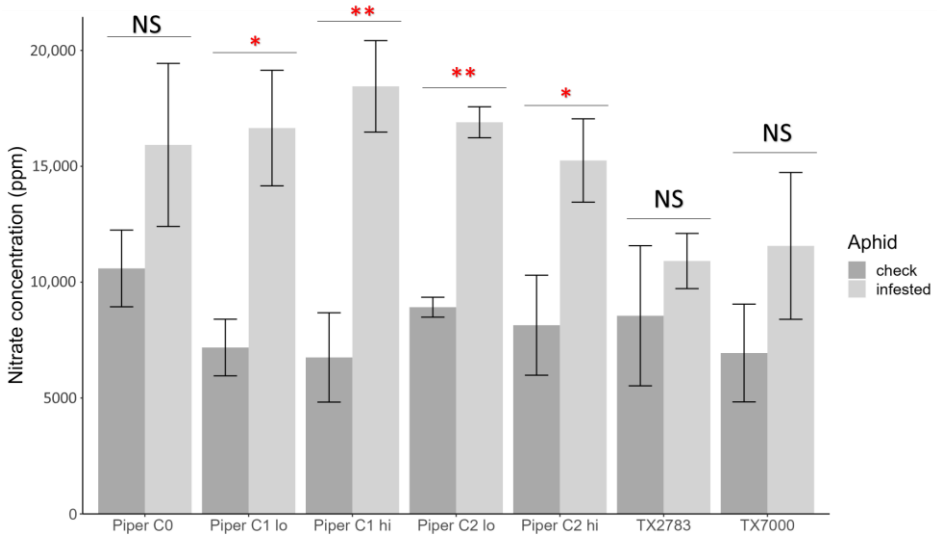


Figure 1. Nitrate concentration ± SE (ppm) of selected Sudangrass Piper lines (lo = low nitrate and hi = high nitrate) and susceptible (TX7000) and resistant (TX2783) sorghum genotypes for plants infested with sorghum aphid *Melanaphis sorghi* (Theobald) (Hemiptera: and check plants. NS = no significant difference according to a *t* test ($\alpha = 0.05$); * = significant difference ($p < 0.05$); ** = significant difference ($p < 0.01$).

The chlorophyll content (%) measured before infestation varied between 35.27 ± 3.11% for Piper C1 lo and 40.92 ± 19.00 for Piper C0, and there were no significant differences among the lines ($F(13,39) = 1.10$, $p = 0.39$). Similarly, after infestation, the chlorophyll contents were not significantly altered ($F(13,39) = 0.83$, $p = 0.62$, Table 1).

The photosynthetic rate (μmol CO_2 $m^{-2}{\cdot}s^{-1}$) and stomatal conductance (mol H_2O $m^{-2}{\cdot}s^{-1}$) were not significantly different between the treatments before infestation, with averages between 35.27 and 40.92 (μmol CO_2 $m^{-2}{\cdot}s^{-1}$) for the photosynthetic rate and between 0.042 and 0.048 (μmol CO_2 $m^{-2}{\cdot}s^{-1}$) for stomatal conductance. After infestation, however, a significant reduction in the photosynthetic rate ($F(13,39) = 35.17$, $p < 0.001$) and stomatal conductance ($F(13,39) = 18.70$, $p < 0.001$) occurred for plants exposed to aphids (Table 1).

3.2. Demographics of Sorghum Aphid on Grass Lines

Sorghum aphids lived significantly longer when feeding on the susceptible sorghum cultivar TX7000, lasting approximately 34 days, compared to only 18 days when feeding on the resistant cultivar TX2783. Aphids fed on Sudangrass lines exhibited intermediate lifespans ranging from 28 days when they were fed on the original 'Piper C0' line to 26 days on Piper C1 lo and 20 days on the remainder($F(6,66) = 223.82$; $p < 0.001$; Table 2).

Aphid fecundity followed the same pattern. Aphids fed on TX7000 showed the highest fecundity, producing approximately 150 offspring compared to 25 when fed on the resistant TX2783. Aphids fed on Piper C0 showed the second highest fecundity, with 97 offspring, followed by Piper C1 lo, Piper C2 lo, Piper C1 hi, and Piper C2 hi ($F(6,66) = 819.64$; $p < 0.001$; Table 2).

The females on TX7000 also demonstrated a higher number of days in reproduction (R_0), with 29 days, in contrast to the resistant TX2783, which had the lowest value, 11 days in reproduction (Table 2). The second-highest value was observed in Piper C0, followed by Piper C1 lo, Piper C2 lo, Piper C1 hi, and Piper C2 hi ($F(6,66) = 291.45$; $p < 0.001$; Table 2).

In the pre-reproductive period (d), two distinct groups were observed. The first group, which included TX7000, Piper C0, Piper C1 lo, and Piper C2 lo, spent approximately 6 days before producing the first progeny. The second group, composed of TX2783, Piper C1 hi, and Piper C2 hi, spent about 8 days to produce their first progeny ($F(6,66) = 1129.00$; $p < 0.001$; Table 2).

Table 1. Means ± SE of physiological parameters of selected Sudangrass lines and two sorghum genotypes.

	Chlorophyll Content [1] (%)	**Chlorophyll Content [2] (%)**		**Photosynthetic Rate [1] (mol H_2O $m^{-2}·s^{-1}$)**	**Photosynthetic Rate [2] (mol H_2O $m^{-2}·s^{-1}$)**		**Condutance [1] (mol H_2O $m^{-2}·s^{-1}$)**	**Condutance [2] (mol H_2O $m^{-2}·s^{-1}$)**	
Grass Lines	-	Check	Infested	-	Check	Infested	-	Check	Infested
Piper C0	40.92 ± 1.95	42.98 ± 1.82	40.99 ± 3.22	40.92 ± 1.95	11.61 ± 0.83	3.28 ± 1.63	0.047 ± 0.004	0.023 ± 0.003	0.014 ± 0.004
Piper C1 lo	35.27 ± 3.11	41.73 ± 2.36	37.59 ± 4.25	35.27 ± 3.11	8.35 ± 2.00	3.30 ± 1.17	0.046 ± 0.005	0.021 ± 0.003	0.012 ± 0.001
Piper C1 hi	38.65 ± 1.08	43.15 ± 1.29	35.93 ± 6.00	38.65 ± 1.08	13.92 ± 1.90	6.16 ± 3.24	0.042 ± 0.003	0.026 ± 0.002	0.018 ± 0.006
Piper C2 lo	35.78 ± 1.69	39.21 ± 2.19	33.93 ± 5.33	35.78 ± 1.69	13.08 ± 3.17	1.11 ± 0.54	0.048 ± 0.003	0.027 ± 0.005	0.010 ± 0.002
Piper C2 hi	38.42 ± 2.05	44.54 ± 1.51	37.06 ± 5.97	38.42 ± 2.05	10.83 ± 1.07	4.61 ± 2.30	0.043 ± 0.002	0.021 ± 0.001	0.015 ± 0.004
TX2783	35.64 ± 1.35	40.63 ± 0.26	39.76 ± 1.04	35.64 ± 1.35	9.11 ± 2.99	2.60 ± 1.39	0.047 ± 0.003	0.021 ± 0.002	0.013 ± 0.003
TX7000	37.29 ± 1.48	42.14 ± 2.13	40.44 ± 1.16	37.29 ± 1.48	8.65 ± 3.95	3.94 ± 3.29	0.043 ± 0.002	0.018 ± 0.005	0.013 ± 0.006
F [3]	1.10	0.57; 5.49; 0.33		1.29	0.83; 35.17; 0.59		0.43	0.63; 18.70; 0.50	
p [3]	0.39	0.78; 0.02; 0.91		0.25	0.55; >0.001; 0.16		1.01	0.70; >0.001; 0.80	
$DF_{residues}$	39	39		39	39		39	39	

[1] Parameters measured before sorghum aphid (SA) *Melanaphis sorghi* (Theobald) (Hemiptera: Aphididae) infestation. [2] Parameters measured after 20 days of 100 SA per plant infestation. [3] When two-way ANOVA values of F and *p* are for line, infestation, and interaction consecutively.

Table 2. Demographic parameters of sorghum aphid *Melanaphis sorghi* (Theobald) (Hemiptera: Aphididae) on Sudangrass selected lines and two sorghum genotypes. R_0 = net reproduction; M_d = reproductive period (days in reproduction), and R_m = Intrinsic rate of increase were calculated using the formula: $R_m = 0.738 (\log_e M_d)/d$ (Wyatt and White 1977). Means ± SE followed by different letters indicate significant differences based on Tukey's test $\alpha = 0.05$.

Grass Lines		**Longevity**	**Fertility**	**Pre-Reproductive Period (d)**	**R_0**	**M_d**	**R_m**
Piper C0		28.08 ± 0.29 b	97.00 ± 1.90 b	6.08 ± 0.08 b	23.00 ± 0.30 b	28.17 ± 1.08 b	0.49 ± 0.01 b
Piper C1 lo		25.67 ± 0.28 c	83.25 ± 1.24 c	6.00 ± 0.00 b	20.67 ± 0.28 c	28.08 ± 0.91 b	0.49 ± 0.01 b
Piper C1 hi		20.67 ± 0.19 d	29.00 ± 0.71 e	8.00 ± 0.00 a	13.67 ± 0.19 e	22.67 ± 0.72 c	0.33 ± 0.00 d
Piper C2 lo		20.42 ± 0.61 d	41.08 ± 1.42 d	6.00 ± 0.00 b	15.42 ± 0.61 d	21.67 ± 0.63 c	0.45 ± 0.01 c
Piper C2 hi		20.92 ± 0.23 d	31.33 ± 0.57 e	8.00 ± 0.00 a	13.92 ± 0.23 de	24.17 ± 0.24 bc	0.34 ± 0.00 d
TX2783		18.00 ± 0.35 e	25.17 ± 1.48 e	8.00 ± 0.00 a	11.00 ± 0.35 f	21.25 ± 1.12 c	0.32 ±0.01 d
TX7000		33.75 ± 0.49 a	149.92 ± 3.27 a	6.00 ±0.00 b	28.75 ± 0.49 a	37.58 ± 1.56 a	0.54 ± 0.01 a
Statistics	F	223.82	819.64	1129.00	291.45	34.70	294.08
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	$DF_{residues}$	66	66	66	66	66	66

As anticipated, the intrinsic rate of increase (R_m) was the highest in the susceptible TX7000 with 0.54, followed by Piper C0 and Piper C1 lo, both with 0.49, and Piper C2 lo with 0.45 ($F(6,66) = 294.08$; $p < 0.001$; Table 2).The lowest rates were observed in the resistant TX2783, Piper C1 hi, and Piper C2 hi, with values of 0.32, 0.33, and 0.34, respectively. No significant difference was observed among the latter group.

4. Discussion

There are increasing challenges that insect pests, including the SA, pose to sorghum and Sudangrass production in the US [15,16,29,30]. Because these crops are used as forage, it is extremely important to study the impact of SA not only on grain yield and forage productivity, but also on the nutritional changes in grass that can affect animal feeding. Our results clearly show that SA infestation increases the nitrate concentrations in the selected Sudangrass lines, compared to those in non-infested plants. To our knowledge, this is the first demonstration of the effect of aphids on the nitrate concentration in Sudangrasses.

An initial population of 100 aphids per plant, feeding and proliferating freely for 20 days, was sufficient to significantly increase the nitrate concentrations by more than 60%. Intriguingly, even though it was not statistically different, the nitrate concentrations in the sorghum genotypes were also higher under SA infestation, with increases of 22% and 40% in the TX2783 and TX7000 genotypes, respectively. The lack of significance was likely a result of a notable variation among replications, and future studies should be conducted to further characterize this observation.

Aphids feed exclusively on the phloem of plants; however, this sap is considered unbalanced for their dietary needs, mainly because it has a low concentration of essential amino acids and other nitrogen sources [31,32]. To circumvent this, aphids employ strategies that modify the physiology of their host plants, which results in systematic changes to the phloem concentrations of amino acids, particularly the essential ones [11,12]. Previous studies have shown that plants under aphid infestation increase rates of nitrogen acquisition and assimilation [7]. Thus, it is likely that SA has altered the physiology of Sudangrass and sorghum, increasing nitrate concentrations.

The nitrate concentration values above 10,000 ppm with aphid infestation found in this study are considered harmful for cattle and other ruminants [33]. However, caution should be used because of certain aspects and limitations of our experimental design. First, the Sudangrass and sorghum plants were planted and kept at a density of one plant per pot and experienced no competition for water or nutrients, whereas in fields, densities of 3.5–8 plants per m^2 are common [34]. Sudangrass growth and nitrate accumulation have a direct relationship with seed and fertilizer rates [35–37]. It is possible that the fertilization with ammonium nitrate in addition to other nutrient sources, could have allowed the plants to accumulate high levels of nitrates in response to aphid feeding.

Agricultural practices, including the use of nitrate fertilizers, can impact the quality of host plants and, consequently, influence the dynamics of aphid population [11]. In forage crops, excessive nitrogen fertilization, followed by plant stress, such as stand loss during subsequent cuttings, may lead to elevated levels of nitrate [38]. This was also observed in our results, which showed an increase in plant stress level resulted in a reduction in the photosynthetic rate and stomatal conductance in plants under SA infestation.

Interestingly, the nitrate concentration was similar among all the non-infested treatments, including the Piper C1 hi and Piper C2 hi that were selected for high nitrate levels. This might be a result of the inherent high variability of nitrate concentration in 'Piper' when subjected to accumulated nitrogen. Under field conditions, Sunaga et al. [39] reported variations up to 3.2-fold. However, when we compared within the same lines, between aphid-infested and control treatments, significant differences were observed, highlighting the effect of SA on nitrate expression. Additionally, this effect of nitrate expression was evident in SA demographics. Plants with higher nitrate had lower aphid infestations. Further research is needed to understand the impact of various nitrate and ammonia fer-

tilizer sources and doses on the initial nitrate levels in the selected Piper lines, and their implications on SA demographics.

In our experiment, feeding by increasing numbers of aphids resulted in a reduction in the photosynthetic rate and stomatal conductance, indicating that plants were affected and responding to herbivory. We also showed that plants with higher nitrate levels affected aphid demographics. The Piper Sudangrass line selections for high nitrate levels (Piper C1 hi and Piper C2 hi) drastically reduced the SA demographic parameters, as observed in longevity, pre-reproductive period, fecundity, days in reproduction, and intrinsic rate of increase. In most cases, these values were comparable to those observed for the resistant sorghum control TX2783 [40]. Excess nitrogen can cause cattle poisoning when the accumulation of nitrite leads to the conversion of hemoglobin to methemoglobin. Methemoglobin is unable to transport oxygen, thereby causing the animal to suffer from hypoxia [33,41]. Because insects do not transport oxygen in their hemolymph, aphids feeding on Piper C1 hi and Piper C2 hi have fitness reduced by other mechanisms, which should be further investigated.

5. Conclusions

Although the adoption of sorghum- and Sudangrass-resistant cultivars/hybrids is recommended for aphids' management [9,23,42], the selected lines, Piper C1 hi and Piper C2 hi, may not be the best option for SA management, even though they can reduce SA reproductive potential, because of the potential to harm livestock. Implementing good practices, such as adjusting planting dates and selecting hybrids resistant to SA, can reduce expenses while effectively managing SA. In some cases, this may even eliminate the need for insecticides [10,43,44]. However, when resistant cultivars are unavailable, insecticides still play a crucial role in SA management in order to avoid losses [8]. It is advisable to use field monitoring and adopt economic thresholds when considering insecticide applications [45], especially considering the significant impact of natural enemies on SA control [10].

It is also worth noting that balanced fertilization is extremely important for forage crop management. High doses of nitrogen fertilization in sorghum are associated with increased aphid infestation [8]. As observed in our experiments, excessive nitrogen can also elevate nitrate concentrations to toxic levels, especially when plants are exposed to herbivory. When farmers and ranchers suspect that forage has high nitrate levels, it is recommended to test the forage before livestock feeding to minimize the risk of poisoning [3,4].

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