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# Evidence of host plant specialization among the U.S. sugarcane aphid (Hemiptera: Aphididae) genotypes

**Sulochana Paudyal[1], J. Scott Armstrong[2,*], Karen R. Harris-Shultz[3], Hongliang Wang[4], Kristopher L. Giles[1], Philippe C. Rott[5] and Mark E. Payton[6]**

[1]Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078, USA; [2]USDA-ARS, Wheat, Peanut and Other Field Crops Research Unit, 1301 N Western Street, Stillwater, OK, 74075, USA; [3]USDA-ARS, Crop Genetics and Breeding Research Unit, 115 Coastal Way, Tifton, GA 31793, USA;[4]USDA-ARS, Hard Winter Wheat Genetics Research, 4007 Throckmorton Hall, Manhattan, KS 66506, USA; [5]Plant Pathology Department, EREC, University of Florida, IFAS, Belle Glade, FL 33430, USA; [6]Department of Statistics, Oklahoma State University, 301 MSCS Building, Stillwater, OK 74078, USA.

## ABSTRACT

The sugarcane aphid (*Melanaphis sacchari* (Zehnter) (Hemiptera: Aphididae)) has become a serious pest of sorghum (*Sorghum bicolor* (L.) Moench) in the United States since it was detected in 2013. The sugarcane aphid was considered only a pest of sugarcane in Florida and Louisiana for over three decades before the 2013 outbreak. Recent studies suggest that the 2013 outbreak in sorghum was due to the introduction of a new genotype. Our scope for this study was to quantify phenotypic behaviors (host suitability as measured through life table statistics) and genetic diversity among sugarcane aphid clones collected from different hosts. We collected sugarcane aphid clones from sorghum (SoSCA), sugarcane (SuSCA), and Columbus grass (CoSCA) and determined biodemographic data and host suitability when offered five different hosts plants including, sugarcane, Columbus grass, Johnsongrass, and a resistant and susceptible grain sorghum. Sugarcane aphid clones collected from different hosts varied in performance among hosts plants. The survivorship and reproduction of the sugarcane-collected aphid clone (SuSCA) was significantly higher when offered sugarcane (>85%) as compared to other hosts and in contrast, there was negligible survival and reproduction when SoSCA and CoSCA were offered sugarcane as host. Genotyping of the aphid clones collected from various hosts with microsatellite markers indicated that SuSCA was a different genotype and belonged to the multilocus lineage MLL-D as compared to SoSCA and CoSCA which belonged to MLL-F. Our results suggest there exists two different host-specific biotypes of the sugarcane aphid within the United States.

**KEYWORDS:** *Melanaphis sacchari*, host-plant specialization, sugarcane aphid genotypes.

## INTRODUCTION

Phytophagous insect species that feed on different species of plants can lead to populations that become more specialized to different hosts over time [1-3]. Insects on different hosts may experience a diversity of environments, different sets of natural enemies, and different geographic locations which favors divergent selection [2, 4]. Additionally, host plant species have different nutritional compositions and secondary metabolites which can further place selection pressure on insects [5]. The term 'ecological speciation' has been used to describe this type of adaptation of a species to various ecological environments and plants as a result of ecologically

*Corresponding author: scott.armstrong@ars.usda.gov

based divergent selection [2, 6, 7]. Consequently, the evolutionary process of adaptation to different ecological environments can produce phenotypic and genetic differences among populations [7].

Phytophagous insects, especially aphids, are known as ecological specialists [1-2, 8, 9]. Pea aphid (*Acyrthrosiphon pisum* Harris) populations feeding on alfalfa and red clover, respectively, are known to be specialized on each of these hosts and show preference to the plant from which they have been collected [1]. These aphids have higher reproduction and survival rates on the host from which they were collected [2, 10]. Similarly, cotton-melon aphid (*Aphis gossypii* Glover) populations have a variable range in ability to reproduce and have host preferences among suitable host plants [6, 9, 11-12]. Several biotypes of greenbugs (*Schizaphis graminum* (Rondani)) and Russian wheat aphids (*Diuraphis noxia* (Mordvilko)) are distinguished on the basis of their reproductive behavior and the ability to damage various wheat genotypes [13-15].

The sugarcane aphid (*Melanaphis sacchari* (Zehntner)) is a relatively new pest of sorghum in the United States with distinct black cornicle (tailpipes), black tarsi, and black antennae [16, 17]. It has been reported to feed on sugarcane since 1977 in Florida [18-19] and 1999 in Louisiana [20]; however, yearly distribution of the aphid on sugarcane hasn't been described after these reports. Sugarcane aphid was not an economic threat from direct feeding on sugarcane, but a significant threat from being the vector of sugarcane yellow leaf virus [21, 22]. Since the outbreak of sugarcane aphid as a pest of sorghum in Texas in 2013, it has been rapidly expanding its geographic range [17, 23]. *Melanaphis sacchari* have 14 known suitable host plants worldwide which include *Cynodon dactylon* (L.), *Miscanthus sinensis* (L.), *Oryza sativa* (L.), *Panicum colonum*, *Panicum maximum*, *Paspalum sanguinale*, *Pennisetum* sp., *Saccharum officinarum*, *Setaria italic* (L.), *S. bicolor*, *S. halepense* (L.), *S. verticilliflorum* (Steud.), and *Zea mays* (L.) [24]. To date, the predominant biotype in the United States has a host range limited to *S. bicolor*, *S. halepense*, *Saccharum officinarum*, Sudan grass (*Sorghum drummondii*), and Columbus grass (*Sorghum almum*) [22, 25].

Genetic diversity has been examined worldwide and in the Americas for the sugarcane aphid. Nibouche *et al*. [26] collected sugarcane aphids from different geographic locations between 2007-2013 and documented five multilocus lineages (MLL) including, MLL-A from Africa, MLL-B from Australia, MLL-C from South America, the Caribbean, Reunion Island, and East Africa, MLL-D from the United States, and MLL-E from China. They also found host specialized lineages of sugarcane aphids collected from sugarcane and wild sorghum (*Sorghum bicolor* subsp. *verticilliflorum*) in Reunion Island, France. Harris-Shultz *et al*. [27] collected populations of *M. sacchari* from sorghum in 2015 from 17 different locations in the United States and concluded that these aphid populations are primarily one asexual clone. This asexual clone was attributed to a new lineage MLL-F and this has been a major pest to the sorghum industry in the United States since 2013 [28]. MLL-F is considered an invader to the Americas from Africa or Asia [28], and is genetically different from populations collected on sugarcane and Johnsongrass in 2007 from Louisiana and Hawaii [29]. For sugarcane aphid samples collected after 2013 in the continental United States, sugarcane aphids that were MLL-D were found only on sugarcane, but sugarcane aphid samples that were MLL-F were found on sugarcane, sorghum, and Johnsongrass [28]. Altogether, data from these studies suggested the occurrence of host-associated genotypes of the SCA in the United States.

In this study, host plant suitability of sugarcane aphids collected from three primary hosts, sugarcane (*Saccharum* spp.), sorghum (*Sorghum bicolor* (L.) Moench) and Columbus grass (*Sorghum almum*) were compared. Life table statistics were used to determine host utilization differences between sugarcane aphid clones collected from the aforementioned hosts and allowed to feed on sugarcane, susceptible and resistant sorghums, Johnsongrass, and Columbus grass. Additionally, the genetic diversity of the SCA clones collected from the respective host plants were compared using microsatellite markers and linked to past studies using mitochondrial cytochrome c oxidase subunit I gene (COI) sequencing.

## MATERIALS AND METHODS

### Aphid cultures

The sugarcane aphid clonal lineages were collected from three hosts including sorghum, sugarcane, and

Columbus grass. Sugarcane aphids feeding on sorghum were collected from near Bay City, Matagorda County, TX in August of 2013 and have since been maintained on susceptible sorghum RTx7000 in a greenhouse. The greenhouse is equipped with T6 fluorescent lighting (14:10 h (L: D) photoperiod) and temperatures were maintained between 21-31 °C. A Sugarcane aphid clone found feeding on sugarcane was collected from Belle Glade, FL (Palm Beach County) in March 2017 and maintained as previously described on susceptible sugarcane variety CP96-1252 as the host, whereas aphid clone found feeding on Columbus grass was collected in April 2018 from Belle Glade, FL and maintained on Columbus grass under the same greenhouse conditions. All sugarcane aphid populations were maintained on their respective host plants in 4.4-L pots each fitted with a 45-cm tall ×16-cm diameter cylinder of Lexan TM (SABIC Polymershapes, Tulsa, OK) covered at the top with organdy cloth for ventilation and to prevent aphids from escaping. The experiments were conducted between May 2017 and October 2018, using a randomized design.

### Host transfer experiments

Sugarcane aphids (SCA) were reared on their primary hosts including, sorghum (SoSCA), sugarcane (SuSCA), and Columbus grass (CoSCA) and transferred to sorghum (susceptible (KS 585) and resistant (AG1201)), sugarcane, Johnsongrass, and Columbus grass where life-table parameters and demographic statistics were compared. The susceptible sorghum germplasm (KS 585) was obtained from Chromatin Inc. and the resistant sorghum germplasm AG1201 [29] was obtained from Advanta Seeds Pty Ltd. Sugarcane stalk cuttings of cultivar CP96-1252 were obtained from University of Florida at Belle Glade.

### Life table demography

Sugarcane aphids and their respective host plants were maintained in growth chambers (25 ± 2 °C, 65 ± 5% RH, and 16:8 h (L: D) photoperiod). Two seeds of each sorghum genotype (susceptible and resistant), Columbus grass and Johnsongrass were planted in cone-tainers™ (model SC10, S7S Greenhouse Supply, Tangent, Oregon) with three layers of media that included potting soil, fritting clay, and sand (bottom to top respectively). When plants reached the three-leaf stage, they were thinned to one plant per cone-tainer™ and infested with one mature apterous *M. sacchari* female from sorghum (SoSCA), sugarcane (SuSCA), and Columbus grass (CoSCA) (8 replications for each host plant and each aphid). For sugarcane, single-bud cuttings were planted into the growth media in individual 4.4-L pots. Young shoots with three fully developed leaves were infested with one mature apterous aphid from each host plant: SoSCA, SuSCA, and CoSCA (8 replicates).

Following a 24 h settling period, the adult female and all nymphs were removed, with exception of one nymph. The experiment began with the one-day old nymphs and these were observed daily for their survival and reproduction. All newborn nymphs were removed from each host plant after counting every 24 h, and this process was continued until the founding female aphid died. Life table parameters including pre-reproductive period (d) (the time taken for the nymph to reach reproductive maturity), Md, the number of progeny produced for a time equivalent to d, reproductive period, total fecundity, average daily reproduction and longevity were recorded. The intrinsic rate of increase ($r_m$) was estimated using the formula developed by [30]: $r_m = 0.738 (\log_e M_d)/d$.

The three sugarcane aphid populations (SoSCA, SuSCA, and CoSCA) were observed for pre-reproductive period (d), reproductive period, longevity (d), number of nymphs per day, total fecundity, and intrinsic rate of increase ($r_m$). All variables were compared using PROC MIXED [31]. Sugarcane aphid clones, host plant species, and the aphid clones × host plant species interaction were considered as fixed effects. The eight host plants (8 replications) were used for each aphid clone which was considered as random effect. Means of all variables were separated using protected Fisher-type pairwise comparisons (DIFF option in a LSMEANS statement) using the Satterthwaite method for the degrees of freedom.

### Host plant differentiation

The population dynamics of sugarcane aphids from sorghum (SoSCA) and sugarcane (SuSCA) when reared on five different host plants (susceptible and resistant sorghum, sugarcane, Johnsongrass, and Columbus grass) were investigated in the greenhouse.

The greenhouse was equipped with T6 fluorescent lighting (14:10 (L: D) h photoperiod) and the temperature was maintained at 21-31 °C. Each host plant had 8 replications for each sugarcane aphid population (SoSCA, SuSCA, and CoSCA). Two seeds of each sorghum genotype (susceptible and resistant), Columbus grass and Johnsongrass were planted in cone-tainers™ with three layers of media, potting soil, fritting clay, and sand (bottom to top respectively) similar to the previous experiment. When sorghum plants were at the third-leaf stage, plants were thinned to one plant per cone-tainer™ and infested with 20 nymphal aphids. Sugarcane and Columbus grass were infested with 20 aphids from each host when the young shoots had two to three fully emerged leaves. The total number of aphids on each host entry was counted 48 h after infesting and every 48 h thereafter for 12 consecutive days.

Aphid counts were analyzed with mixed model analyses [31] following a square root transformation to correct for heterogeneous variances and the lack of normality of count response variables. A repeated measures model was utilized where, dpi (d post infestation) was used as a repeated factor. The covariances among the dpi values were modeled with an autoregressive period 1 correlation structure. Host plant species, dpi, and the host plant species × dpi interaction were considered as fixed effects. The eight host plants (8 replications) were used for each aphid clone which was considered as random effect.

**Sugarcane aphid taxonomy and genotyping**

For correct taxonomic identification, sugarcane aphid clonal colonies that were collected from grain sorghum from Texas (SoSCA), sugarcane (SuSCA) and Columbus grass (CoSCA) from Florida, were sent to Dr. Susan Halbert, Florida Department of Agriculture, and Drs. Gary Miller and Christopher Owen with the USDA-ARS Systematic Entomology Laboratory, Beltsville, MD.

For genotyping clones of the sugarcane aphid, SoSCA, CoSCA, and SuSCA were collected in 2-mL microcentrifuge tubes and stored at -80 °C until DNA extraction. For DNA extraction, four Zn-plated BBs (Daisy Outdoor Products, Rogers, AR) were added to each 2 mL tube. Aphid samples were ground to a fine powder using a vortex mixer. During the grinding process, the tubes containing aphids were continuously returned to liquid N to prevent thawing. The DNA was extracted using a GeneJET Plant Genomic DNA Purification kit (Thermo Fisher Scientific, Waltham, MA) and manufacturer recommendations were used except that aphids were used instead of plant tissue. DNA was quantified using a NanoDrop 2000c (Thermo Fisher Scientific).

Control sugarcane aphid DNA, named Bellfower1 and Brewer4, was obtained from a previous study [28] which consisted of aphid DNA from pooled aphid samples. A sample consisted of a 2-3 infested leaves from a location in a field of which all alive aphids were pooled into a microcentrifuge tube [27]. The sugarcane aphid sample Bellflower1, which was collected from sorghum in Tifton, GA, was used to represent the predominant aphid genotype (the super-clone) on sorghum and Johnsongrass in 2015, 2016, and 2017 [27, 32]. The sugarcane aphid sample Brewer4, collected from sorghum in Sinton, TX, was used to represent a sample containing the predominant genotype and another genotype. For comparison, Bellflower1 and Brewer4 were named S_2015Tifton_GA and S_2015Sinton_TX in the [33] dendrogram.

Aphid DNA containing microsatellites was amplified using nine previously published sugarcane aphid simple sequence repeat (SSR) markers (Molecular Ecology Resources Primer Development Consortium *et al*. 2010; Harris-Shultz *et al*. 2017). These nine markers that were amplified included CIRMsB09, CIRMsD02, CIRMsE01, MS4, MS5, MS9, MS11, MS14, and MS20 and were selected based on their ability to distinguish polymorphisms between the United States sugarcane aphid samples [27, 33].

Each SSR marker was amplified for each aphid DNA sample using a 10-µL reaction volume that consisted of 2 µL of 5X clear GoTaq reaction buffer (Promega, Madison, WI), 1 µL of 25 mM $MgCl_2$, 0.8 µL of 2.5 mM deoxynucleotide mix, 0.5 µL of M13-tagged forward primer at 1 µM, 2.0 µL of reverse primer at 1 µM, 1.8 µL of 1 µM M13 primer (M13-TGTAAAACGACGGCCAGT) fluorescently labeled with the IRDye 800 CW fluorophore (Eurofins MWG Operon, Huntsville, AL), 0.04 µL of GoTaq DNA polymerase (Promega), 0.86 µL of sterile water, and 1 µL of 2.5 ng $\mu L^{-1}$

of DNA. Thermocycler conditions were an initial denaturation at 94 °C for 3 min, 39 cycles of 94 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min and 10 s, and a final elongation step at 72 °C for 10 min. The thermocyclers used were a Gene Amp Polymerase Chain Reaction (PCR) System 9700 dual and single blocks (Applied Biosystems) and a T100 Thermal Cycler (Bio-Rad). The individual PCR products (2 µL) were combined with 5 µL of Blue Stop (LI-COR Biosciences, Lincoln, NE), and 0.35 µL of this mixture was loaded on a 6.5% (v/v) acrylamide gel using a LI-COR Biosciences 4300 DNA analyzer. Gel images were scored visually. For GenA1Ex 6.5 (Peakall and Smouse, 2006, 2012), the allele sizes generated for each marker for each sample were recorded and the data were treated as a diploid. This software was used to calculate genetic distance and to perform the Principle Coordinate Analysis using the option covariance-standardized. The Brewer4 sample, which was a mixture between a predominant genotype and another genotype, was divided into its respective genotypes. There was no missing data. The mitochondrial cytochrome c oxidase subunit I gene (COI) was sequenced from Bellflower1, SuSCA, and Brewer4. The primers used were the LCO1490 and HC02198 which were previously published (Folmer *et al*. 1994). Polymerase chain reactions (PCR) were set up using a proofreading enzyme in a 20 µL reaction volume. Each reaction consisted of 4 µL of 5X Phusion HF buffer, 1.6 µL of 2.5 mM dNTP mix, 1 µL of 10 µM of LCO1490, 1 µL of 10 µM of HC02198, 11.2 µL of water, 0.2 µL of Phusion DNA polymerase, and 1 µL of aphid DNA at the stock concentration (16-250 ng/µL). Thermocycler conditions were an initial denaturation of 98 °C for 30s, 34 cycles of 98 °C for 7s, 44 °C for 20s, and 72 °C for 30s, and a final extension at 72 °C for 7 min. The annealing temperature was increased to 55 °C for the Brewer4 sample due to the amplification of fungal DNA at 44 °C. Half of the reaction was separated on a 1% agarose gel that was stained with ethidium bromide to ensure a single PCR product of approximately 700 bp was present. The fragment was removed from the gel using a SafeXtractor (Thermo Fisher Scientific) and the PCR product was purified using a Wizard SV Gel and PCR Clean-Up System (Promega). The purified fragment was used for cloning into a pJET1.2 blunt Cloning Vector following the manufacturer recommendations of the Thermo Scientific CloneJET PCR Cloning Kit (Thermo Fisher Scientific). To reduce error, the Brewer4 COI PCR product was directly ligated into the vector using non-purified PCR product. The resulting plasmids were transformed into One Shot TOP10 chemically competent *Escherichia coli* cells (Thermo Fisher Scientific) and transferred to lysogeny broth (LB) agar plates supplemented with 100 µg/mL of ampicillin. Approximately 10 colonies per transformation, with the exception of Brewer 4 where 50 colonies were selected as this sample is a mixture of genotypes, were transferred using toothpicks to 15 mL centrifuge tubes containing 5 mL of LB broth supplemented with 100 µg/mL of ampicillin. The culture tubes were placed in a 37 °C incubator and were shook overnight. Plasmids were purified using a PureYield Plasmid Miniprep System (Promega) and 10 µL of plasmid at 60 ng/ µL were sent to Eurofins (Louisville, KY) for sequencing of the insert in both directions using the pJET1.2 forward and reverse sequencing primers which were provided by Eurofins. Sequences were trimmed manually and VecScreen (NCBI) was used to identify vector sequences that were removed. Sequences were aligned to reference sequences using Sequencher 4.10.1 (Gene Codes Corporation, Ann Arbor, MI).

Recently, Nibouche *et al*. [28] found that sugarcane aphid samples collected in the United States from 2013-2017 formed two lineages: MLL-F which includes COI genotypes H1 (the most frequent) and H6 and MLL-D which corresponds to COI genotype H3. Because we were unable to obtain DNA from their study for comparison to our samples due to limited sample volume (DNA extracted from single aphids), we sequenced the COI region of our aphids to link the two studies. Sequences of genotypes H1, H3, and H6 have been deposited by [26, 28] in GenBank under accession numbers KJ083125, KJ083208, and MG838280, respectively.

## RESULTS

### Life table demography

Clonal colonies of the sugarcane aphid that were maintained on their original host i.e. sorghum (SoSCA), sugarcane (SuSCA), and Columbus grass (CoSCA) varied widely in their survival, growth,

and reproduction when offered a variety of host plants (Table 1, 2, and 3; Fig. 1). Sugarcane aphids collected from sorghum and Columbus grass (SoSCA and CoSCA, respectively) produced >60 nymphs when sorghum (susceptible), Columbus grass, and Johnsongrass were provided as hosts; however, less than 5 aphids were produced when sugarcane was provided as a host (Fig. 1A and 1B). The SoSCA survived for only 9 d on sugarcane, while CoSCA survived for 20 d on sugarcane and both populations had negligible intrinsic rates of increase (Tables 1 and 3) when compared with the remaining host plants. Total fecundity of the SuSCA was highest for sugarcane and significantly lower for the other hosts (Fig. 1B). The total number of nymphs per day, pre-reproductive period, reproductive period, intrinsic rate of increase, and longevity of SuSCA for sorghum were significantly lower than those for sugarcane (Table 2). The total number of nymphs per day and pre-reproductive period of SuSCA for Columbus and Johnsongrass were similar to the number of nymphs for sugarcane; however the lifespan was shorter with < 21 d for both when compared with 34 d for sugarcane (34 d) (Table 1). The intrinsic rate of increase ($r_m$) value for SoSCA and CoSCA on sugarcane was significantly lower (≤ 0.03) than the $r_m$ value for the other hosts (≥0.19; Tables 1 and 3). For SuSCA, the $r_m$ value was highest on sugarcane (0.86) and lowest on resistant sorghum (0.00) (Table 2).

**Table 1.** Life table parameters of sugarcane aphids transferred from sorghum (SoSCA) to five host plants.

| Host transfer type | Number of nymphs per day | Pre-reproductive period (d) | Reproductive period (Rp) | Intrinsic rate of increase ($r_m$) | Longevity (L) |
|---|---|---|---|---|---|
| Sorghum – Sus. sorghum | 5.01 ± 0.28 a | 5.5 ± 0.4 a | 15.9 ± 0.8 ab | 0.48 ± 0.02 d | 28.1 ± 1.2 a |
| Sorghum – Res. Sorghum | 1.71 ± 0.33 b | 7.3 ± 1.1 a | 9.8 ± 2.5 b | 0.19 ± 0.03 b | 20.1 ± 3.2 b |
| Sorghum – Sugarcane | 0.06 ± 0.04 c | n/a | 2.6 ± 1.8 c | 0.00 ± 0.00 a | 9.0 ± 2.2 c |
| Sorghum – Johnsongrass | 4.92 ± 0.36 a | 5.5 ± 0.6 a | 18.5 ± 2.2 a | 0.45 ± 0.01 d | 30.3 ± 2.2 a |
| Sorghum – Columbus grass | 2.15 ± 0.21 b | 5.5 ± 0.3 a | 22.0 ± 1.1 a | 0.33 ± 0.02 c | 32.3 ± 1.2 a |
| | df = 4, 28; $F$ = 70.16; $P$ < 0.0001 | df = 4, 28; $F$ = 8.65; $P$ = 0.072 | df =4,28; $F$ = 21.10; $P$ < 0.0001 | df = 4,28; $F$ = 90.56; $P$ < 0.0001 | df = 4, 28; $F$ = 21.91; $P$ < 0.0001 |

Note: Data are Means ± SE. Statistical significance are based on One Way ANOVA. The value with n/a denotes: not available because aphid didn't reproduce (therefore no pre-reproductive period). Values in the same column followed by the same letters are not significantly different at $P < 0.05$ according to DIFF statement in the LSMEANS.

**Table 2.** Life table parameters of aphids transferred from sugarcane (SuSCA) to five host plants.

| Host transfer type | Number of nymphs per day | Pre-reproductive period (d) | Reproductive period (Rp) | Intrinsic rate of increase ($r_m$) | Longevity (L) |
|---|---|---|---|---|---|
| Sugarcane – Sus. sorghum | 0.4 ± 0.1 a | 4.3 ± 1.6 a | 2.9 ± 1.4 a | 0.07 ± 0.03 b | 11.1 ± 1.6 a |
| Sugarcane – Res. sorghum | 0.0 ± 0.0 a | n/a | 0.0 ± 0.0 a | 0.00 ± 0.00 a | 7.5 ± 1.1 a |
| Sugarcane – Sugarcane | 1.3 ± 0.2 b | 9.6 ± 0.7 b | 25.5 ± 3.3 c | 0.21 ± 0.38 c | 34.3 ± 2.4 c |
| Sugarcane – Johnsongrass | 1.1 ± 0.2 b | 8.3 ± 0.5 b | 10.5 ± 1.7 b | 0.19 ± 0.01 c | 20.9 ± 2.4 b |
| Sugarcane – Columbus grass | 1.0 ± 0.2 b | 7.2 ± 0.9 b | 10.0 ± 1.3 b | 0.17 ± 0.03 c | 20.6 ± 1.7 b |
| | df = 4,35; $F$=10.26; $P$ < 0.0001 | df = 4,35; $F$=17.25; $P$ < 0.0001 | df = 4,35; $F$=27.96; $P$ < 0.0001 | df = 4, 35; $F$=13.01; $P$ < 0.0001 | df = 4, 35; $F$=29.68; $P$ < 0.0001 |

Note: Data are Means ± SE. Statistical significance based on One Way ANOVA test. The value with n/a denotes: not available because aphid failed to reproduce (therefore no pre-reproductive period). Values in the same column followed by the same letters are not significantly different at $P < 0.05$ according to DIFF statement in the LSMEANS.

**Table 3.** Life table parameters of sugarcane aphids transferred from Columbus grass (CoSCA) to five host plants.

| Host transfer type | Number of nymphs per day | Pre-reproductive period (d) | Reproductive period (Rp) | Intrinsic rate of increase ($r_m$) | Longevity (L) |
|---|---|---|---|---|---|
| Columbus – Sus. sorghum | 3.7 ± 0.3 a | 5.5 ± 0.1 b | 17.9 ± 1.4 a | 0.43 ± 0.01 c | 31.7 ± 1.7 a |
| Columbus – Res. sorghum | 1.3 ± 0.1 b | 6.4 ± 0.4 b | 18.6 ± 2.3 a | 0.27 ± 0.02 b | 28.9 ± 1.6 a |
| Columbus – Sugarcane | 0.4 ± 0.1 c | 11.4 ± 2.1 a | 7.5 ± 2.1 b | 0.03 ± 0.03 a | 19.5 ± 2.0 b |
| Columbus – Johnsongrass | 3.5 ± 0.3 a | 5.4 ± 0.2 b | 15.8 ± 2.4 a | 0.37 ± 0.01 c | 29.5 ± 0.9 a |
| Columbus – Columbus | 3.4 ± 0.4 a | 5.0 ± 0.2 b | 21.6 ± 2.2 a | 0.41 ± 0.02 c | 33.0 ± 1.6 a |
| | df = 4,28; $F$ = 43.38; $P$ < 0.0001 | df = 4,35; $F$ = 7.74; $P$ = 0.0001 | df = 4,28; $F$ = 10.68; $P$ < 0.0001 | df = 4,35; $F$ = 61.8; $P$ < 0.0001 | df = 4,35; $F$ = 10.95; $P$ < 0.0001 |

Note: Data are Means ± SE. Statistical significance are based on One Way ANOVA test. The value with n/a denotes: not available because aphid failed to reproduce (therefore no pre-reproductive period). Values in the same column followed by the same letters are not significantly different at $P < 0.05$ according to DIFF statement in the LSMEANS.

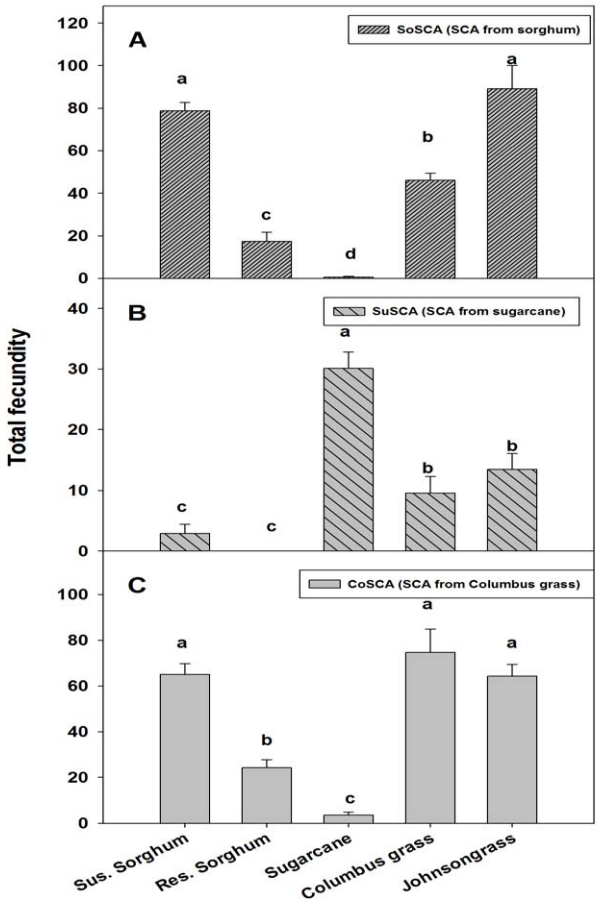


**Fig. 1.** Total fecundity (Mean ± SE) of aphids from sorghum (Fig. 1A, SoSCA), sugarcane (Fig. 1B, SuSCA), and Columbus grass (Fig. 1C, CoSCA) when transferred to resistant sorghum (Res.), susceptible sorghum (Sus.), sugarcane, Columbus grass and Johnsongrass. For each aphid, means (top of columns) with the same lowercase letters are not significantly different at $P > 0.05$. Differences among host plants were highly significant for the average number of nymphs per female of SoSCA (df = 4, 28; $F$ = 58.2; $P < 0.0001$), SuSCA (df = 4, 25; $F$ = 30.06; $P < 0.0001$), and CoSCA (df = 4, 35; $F$ =28.01; $P < 0.0001$).

### Host plant differentiation

The average reproductive capacity of SoSCA and SuSCA over 12 d on different host plants varied significantly (Figs. 2 and 3). Within 2 d after infestation, the SoSCA decreased on sugarcane to the point where there were no survivors on the plants (Fig. 2). The number of SoSCA increased with time on resistant sorghum and Columbus grass. However, the number of SoSCA on susceptible sorghum and Johnsongrass was close to zero when these host plants died (Fig. 2). The population size of SuSCA on sugarcane increased with time (Fig. 3), and although SuSCA survived on Johnsongrass and Columbus grass, it did not survive on sorghum (Fig. 3).

### Sugarcane aphid taxonomy and genotyping

SoSCA, CoSCA, and SuSCA aphid clonal lineages all were identified as *Melanaphis sacchari* (Zehnter) with similar antenna process terminalis and the hind tarsas [34]. Furthermore, they had similar measurement of each antennal segment, ultimate rostral segment, cauda, siphunculus, and the body length.

These aphid clonal lineages along with the control aphid samples, Bellfower1, that was the predominant clone found on the United States sorghum and Johnsongrass in 2015-2017 or a mixture of the predominant clone with another genotype (Brewer4) were genotyped using nine SSR markers. Among these samples only seven SSR markers were polymorphic (Table 4). Genotyping revealed the presence of three Multilocus Genotypes (MLG) (Table 4). The SoSCA, CoSCA, Bellflower1 (super-clone), and Brewer 4_A shared the same MLG (MLG1) (Fig. 4). Whereas the SuSCA and Brewer4_B samples each exhibited a unique genotype and formed MLG2 and MLG3, respectively.

Since SuSCA was a different MLG than CoSCA/ Bellflower1/SoSCA/Brewer4_A the COI region was sequenced from SuSCA, Bellflower1, and Brewer4. The COI haplotype of SuSCA was identical to the one of KJ093208 which is the H3 haplotype (Table 5). All sugarcane aphids with haplotype H3 are members of MLL-D (Nibouche *et al*. 2018). In contrast, the COI haplotype of Bellflower1 was identical to KJ083125, which is haplotype H1.

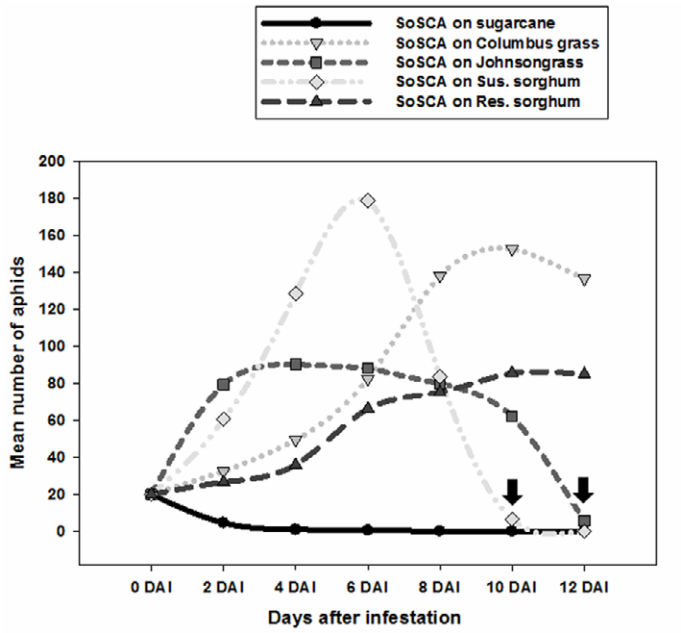


**Fig. 2.** Mean number of SoSCA aphids (aphids originally collected from and maintained on sorghum) during a 12-day time period after transfer to sugarcane, Columbus grass, Johnsongrass, and resistant and susceptible sorghum. Twenty adult aphids were transferred to each host plant when the new hosts reached a 3-leaf stage (sorghum, Columbus grass and sugarcane). The population size was counted starting at the second day after infestation and then every other day until the 12$^{th}$ day after infestation. Down arrow indicates that the plants (susceptible sorghum and Johnsongrass) were almost dead with a damage rating >8.

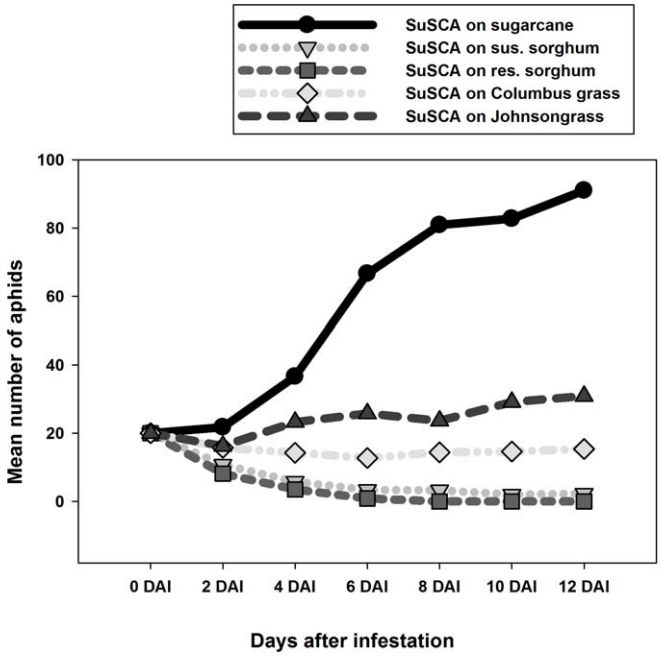


**Fig. 3.** Mean number of SuSCA aphids (aphids originally collected from and maintained on sugarcane) during a 12-day time period after transfer to sugarcane, Columbus grass, Johnsongrass, and resistant and susceptible sorghum. Twenty adult aphids were transferred to each host plant when the new hosts reached a 3-leaf stage (sorghum) or a 2 to 3-leaf stage (Columbus grass and sugarcane). The population size was counted starting at the second day after infestation and then every other day until the 12$^{th}$ day after infestation.

**Table 4.** Approximate allele sizes (in base pairs) for seven sugarcane aphid microsatellite primers that were polymorphic among five sugarcane aphid samples. Brewer4 was a mixture of genotypes and was divided into its respective genotypes.

| Aphid sample | Microsatellite primers | | | | | | | Multi Locus Genotype |
|---|---|---|---|---|---|---|---|---|
| | CIRMsB09 | CIRMsD02 | CIRMsE01 | MS4 | MS5 | MS9 | MS14 | |
| | ----------bp---------- | | | | | | | |
| SoSCA | 263, 263 | 238, 240 | 263, 263 | 191, 191 | 249, 249 | 133, 143 | 157, 157 | 1 |
| CoSCA | 263, 263 | 238, 240 | 263, 263 | 191, 191 | 249, 249 | 133, 143 | 157, 157 | 1 |
| SuSCA | 257, 259 | 244, 252 | 265, 265 | 191, 193 | 249, 251 | 133, 133 | 157, 157 | 2 |
| Bellflower1 | 263, 263 | 238, 240 | 263, 263 | 191, 191 | 249, 249 | 133, 143 | 157, 157 | 1 |
| Brewer4_A | 263, 263 | 238, 240 | 263, 263 | 191, 191 | 249, 249 | 133, 143 | 157, 157 | 1 |
| Brewer4_B | 263, 263 | 264, 292 | 263, 263 | 191, 191 | 249, 249 | 133, 143 | 335, 335 | 3 |

Sugarcane aphids belonging to COI haplotype H1 have been assigned to MLL-F, MLL-A, MLL-E, and MLL-B (Nibouche *et al*. 2018). However, Paudyal S *et al*. [29] have shown that sugarcane aphids collected in the United States that are haplotype H1 belong to MLL-F. The Bellflower1 and SuSCA COI sequences were submitted to DDBJ and have the GenBank accession numbers of LC424496 and LC424497, respectively. A total of 48 clones containing the Brewer4 COI region were obtained

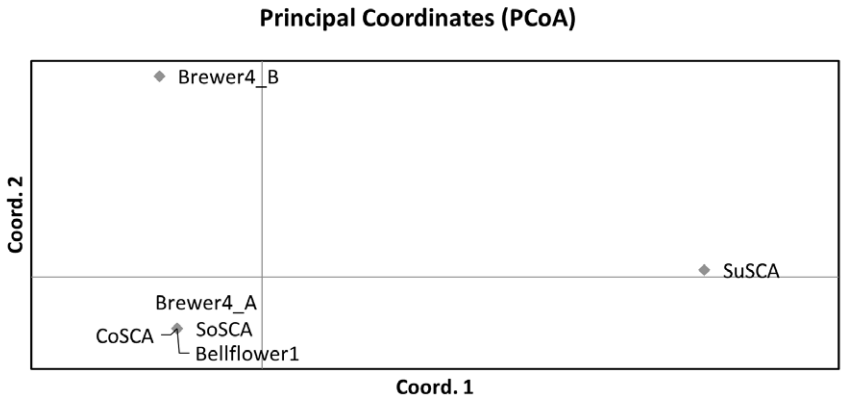


**Fig. 4.** Principal Coordinate Analysis (PCoA) of the sugarcane aphid samples SoSCA, SuSCA, CoSCA, and Brewer 4_A and 4_B. Brewer4_A, SoSCA, Bellflower1, and CoSCA belong to the same multilocus genotype. These labels were separated for ease of reading.

**Table 5.** Mitochondrial cytochrome c oxidase subunit I haplotype sequences from the sugarcane aphid (*Melanaphis sacchari*). Base pair numbers are based on [28].

| COI haplotype | GenBank/ Sample Name | ---------- Nucleotide Position (bp)------- | | |
|---|---|---|---|---|
| | | 294 | 343 | 531 |
| H1 | KJ083125 | G | A | C |
| H3 | KJ083208 | A | G | C |
| H6 | MG838280 | G | A | A |
| H1 | Bellflower1/ Brewer4 | G | A | C |
| H3 | SuSCA | A | G | C |

and sequenced. Among the 48 COI sequences, 46 were identical to the KJ083125 sequence (the H1 haplotype). One clone had a single nucleotide polymorphism (SNP) at bp position 316 (A→G) and another clone had a SNP at bp position 610 (T→A/T). These two SNPs could be artifacts due to the Phusion DNA polymerase which has a 1% error rate. A large number of clones were sequenced from the Brewer4 sample to ensure that the COI sequences from both genotypes (Brewer4_A, Brewer4_B) were examined.

## DISCUSSION

From maintaining over 30 clonal colonies of sugarcane aphids collected from sorghum, Johnsongrass, Columbus grass, from several locations within the U.S. at the USDA-ARS Laboratory in Stillwater, OK and from screening >1,000 sorghum genotypes and related sorghum species for host plant resistance to the sugarcane aphid, it became obvious to us that the clonal collection from sugarcane from Bell Grade, Florida responded differently than all others maintained based on phenotypic studies. This prompted us to further investigate the phenotypic and genotypic response of the sugarcane aphid clones collected from sugarcane in Florida, the sugarcane aphids collected from Columbus grass in Florida, and the initial colony collected from Texas sorghum in 2013. Host plant specialization is not a new phenomenon and has already been reported for several aphid species including sugarcane aphids [1, 6, 12, 26]. Host plant specialization between populations on alfalfa and clover was reported in Pea aphids (*A. pisum*) [1]. Five host races of *A. gossypii* have been reported to specialize on Cucurbitaceae, cotton, eggplant, potato and chili or sweet pepper [6]. Nibouche *et al.* [26] identified the existence of host plant specialization among the different multilocus genotypes of *M. sacchari* in Reunion Island, France.

Through phenotyping and host switching of sugarcane aphids collected from sugarcane, Columbus grass, and sorghum, then rearing on each host including Johnsongrass, we were able to show that the sugarcane aphids collected from and maintained on sugarcane (SuSCA) had significantly reduced survival and a longer reproductive period on

sorghum, Johnsongrass, and Columbus grass as compared to sugarcane. Sugarcane aphids collected from sorghum (SoSCA) had significantly reduced survival and reproduction (fewer numbers of nymphs per day, shorter reproductive period, and lower intrinsic rate of increase) when sugarcane was provided as a host as compared to the other hosts.

Based on our genotyping analysis, the clone collected from sugarcane (SuSCA) is clearly genetically different than the clones collected from sorghum (SoSCA) and Columbus grass (CoSCA). The MLL-F lineage of the sugarcane aphid from sorghum reported by [28] is the same MLL that we collected from sorghum and Columbus grass (MLG1). Furthermore, the sugarcane aphid clone collected from Florida from sugarcane (MLG2) corresponds to MLL-D reported by [28].

## CONCLUSION

This research corroborates the finding of [28] which suggests two apparent sugarcane aphid lineages (MLL-D and MLL-F) in the United States. Our study further suggests that these two genotypes are likely to be differentiated based on their host plant association. This suggests that the sugarcane aphid outbreak that started on sorghum in south Texas in 2013 was caused by the MLL-F type and not by the sugarcane type (MLL-D) that was present on sugarcane in Florida and Louisiana for over two decades [19-20]. Our results suggest there exists two different host-specific biotypes of the sugarcane aphid within the United States. The origin of the aphid MLL-F type that caused the epidemic outbreak in 2013 on sorghum in the United States remains to be determined.

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## CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

## REFERENCES

1. Ferrari, J., Godfray, H. C., Faulconbridge, A. S., Prior, K. and Via, S. 2006, Evolution, 60, 1574-1584.
2. Ferrari, J., Via, S. and Godfray, H. C. J. 2008, Evolution, 62, 2508-2524.
3. Jean, P. and Jean-Christophe, S. 2010, Ecol. Entomol., 35, 119-130.
4. Nosil, P. 2004, Proc. R. Soc. Lond. B., 271, 1521,1528.
5. Guerrieri, E. and Digilio, M. C. 2008, Journal of Plant Interactions, 3, 223-232.
6. Carletto, J., Lombaert, E., Chavigny, P., Brevault, T., Lapchin, L. and Vanlerberghe-Massutti, F. 2009, Mol. Ecol., 18, 2198-2212.
7. Nosil, P. 2012., Ecological Speciation. Oxford University Press Inc., New York. ISBN: 978-0-19-958710-0 (Hbk).
8. Via, S. 1991, Ecology, 72, 1420-1427.
9. Mokhtar A. M., Polgar, L. and Lucas. S. 1993, Critical issues in aphid biology, In: P. Kindlmann and A. F. G. Dixon (Eds.) Proceedings of the 4th International Symposium on Aphids, 89-94.
10. Via, S. 1999, Evolution, 53, 1446-1457.
11. Agarwala, B. K. and Choudhury, P. R. 2013, J. Insect Sci., 13, 34.
12. Wang L., Zhang, S., Luo, J-Y., Wang, C-Y., Lv, L-M., Zhu, X-Z., Li, C-H. and Cui, J-J. 2016, PLoS One, 6, 11, e0146345 doi: 10.1371/journal.pone.0146345
13. Wilhoit, L. R. and Mittler, T. E. 1991, Environ. Entomol.,20, 757-767.

14. Burd, J. D., Porter, D. R., Puterka, G. J., Haley, S. D. and Peairs, F. B. 2006, J. Econ. Entomol., 99, 1862-1866.
15. Puterka, G. J., Nicholson, S. J., Brown, M. J., Cooper, W. R., Peairs, F. B. and Randolph, T. L. 2014. J. Econ. Entomol., 107, 1274-1283. https://doi.org/10.1603/EC13408
16. Villanueva R. T., Brewer, M., Way, M. O., Biles, S., Sekula, D., Bynum, Ed., Swart, J., Crumley, C., Knutson, A., Porter, P., Parker, R., Odvody, G., Allen, C., Ragsdale, D., Rooney, W., Peterson, G., Kerns, D., Royer, T. and Armstrong, S. 2014, Texas A&M Agrilife Extension, Ento-035.
17. Bowling, R. D., Brewer, M. J., Kerns, D. L., Gordy, J., Seiter, N., Elliot, N. E., Buntin, G. D., Way, M. O., Royer, T. A., Biles, S. and Maxson, E. 2016, J. Integr. Pest Manag., 7, 1-13.
18. Mead, F. W. 1978, Cooperative Plant Pest Report, 3(34), 475.
19. Hall, D. G. 1987, J. Amer. Soc. Sugar Cane Techn., 7, 26-29.
20. White, W. H., Reagan, T. E. and Hall, D. G. 2001, Florida Entomologist, 84, 435-436.
21. Akbar, W., Showler, A. T., Reagan, T. E. and White, W. H. 2010, J. Econ. Entomol., 103, 1431-1437.
22. Armstrong, J. S., Rooney, W. L., Peterson, G. C., Villenueva, R. T., Brewer, M. J. and Sekula-Ortiz, D. 2015, J. Econ. Entomol., 108, 576-582.
23. Rodriguez-del-Bosque. L. A. and Teran, A. P. 2015, Southern Entomologist, 40, 433-434.
24. Singh, B. U., Padmaja, P. G. and Seetharama, N. 2004, Crop. Protection, 23,739-755.
25. Medina, R. F., Armstrong, S. J. and Harrison, K. 2016, Entomologia Experimentalis et Applicata, 162, 358-365.
26. Nibouche, S., Fartek, B., Mississippi, S., Delatte, H., Reynaud, B. and Costet, L. 2015, PLoS One, 9(8), e106067.
27. Harris-Shultz, K., Ni, X., Wadl, P. A., Wang, X., Wang, H., Huang, F. Fangneng Huang, Flanders, K., Seiter, N., Kerns, D., Meagher, R., Xue, Q., Reisig, D., Buntin, D., Cuevas, H. E., Brewer, M. and Yanget, X. 2017, Crop. Sci., 57, 2064-2072. doi:10.2135/cropsci2016.12.1010
28. Nibouche, S., Costet, L., Holt, J. R., Jacobson, A., Pekarcik, A., Sadeyen, J., Armstrong, J. S., Peterson, G. C., McLaren, N. and Medina, R. F. 2018, PLoS One, 13(4), e0196124.
29. Paudyal, S., Armstrong, J. S., Giles, K. L., Payton, M. E., Opit, G. P. and Limaje, A. 2019, J. Econ Entomol., pii: toz077. doi: 10.1093/jee/toz077.
30. Wyatt, I. J. and White, P. F. 1977, J. Appl. Ecol., 14, 757-766.
31. SAS Institute. 2010, SAS users guide, version 9.3. SAS Institute, Cary, NC.
32. Knoll, J. E., Harris-Shultz, K. R. and Ni, X. 2018, Update on sugarcane aphid genetics, sugarcane aphid management in sweet sorghum, and breeding for resistance. Adv. Sugar Crop Proc. Conv. Volume 2.
33. Harris-Shultz, K., Brewer, M., Wadl, P., Ni, X. and Wang, H. 2018, Crop. Sci., 58, 2533-2541. doi:10.2135/cropsci2018.03.0151
34. Blackman, R. L. and Eastop, V. F. 2000, Aphids on the World's Crops. An Identification and Information Guide. 2nd ed. John Wiley & Sons, Chichester, 197-199.