

Sex ratio dynamics of the field population of the sugarcane rust mite *Abacarus sacchari* (Acari: Eriophyidae)

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Abstract: Sugarcane in southern Florida suffers from sugarcane rust mite (SRM) infestations, *Abacarus sacchari*, from summer to early fall. The mite's sex ratio is important in understanding the mite's demography and population dynamics. This is the first report on the sex ratio of the field population of the SRM. The objectives of the study were to determine the sex ratio of the SRM and the factors that affect any changes in the sex ratio. To determine the sex ratio, mites were sampled from a sugarcane canopy each month over a 12-month period and the sex ratio was expressed as the proportion of females to the total number of sexed mites. The population density and aerial mites were monitored weekly for 8 and 6 months, respectively. The total number of sexed mites from the sugarcane canopy was 27 941 mites, while 2 248 airborne mites were recorded. The result showed that the sex ratio of the SRM in the canopy was dynamic during the study, with a female bias more common than a male bias among the samples, which ultimately resulted in a slightly female bias. An obvious change from a female bias to a male bias occurred simultaneously with the increased mite density and dispersal. The factors affecting the oscillation of the sex ratio are discussed.

Keywords: field population; aerial mites; female bias; male bias

Sugarcane contributes to roughly 40–45% of the United States (US) sugar production and Florida accounts for more than 50% of the production or an equivalent of approximately 20% of the total sugar production in the country (Abadam 2022). Therefore, sugarcane is considered one of the most valuable crops in southern Florida. Sugarcane rust mite (SRM), *Abacarus sacchari*, is one of the pests associated with sugarcane. The mite was first discovered in southern Florida in 1982 and, since then, there have been no further reports on the mite until the

summer of 2007 (Beuzelin et al. 2022). The lack of reports could be due to the mite's microscopic size that made it unnoticeable; therefore, the symptoms produced by this mite were misidentified as foliar diseases which also commonly infest the sugarcane (Nuessly et al. 2015).

SRM is an eriophyid that is well known to be an arrhenotokous parthenogenetic and haplodiploid. The male is haploid, which develops from unfertilised eggs, while the female is diploid, which develops from fertilised eggs (Skoracka 2008; Michalska

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et al. 2009). The sex ratio in the haplodiploid arrhenotokous has been of particular interest because it determines the reproductive capacity and population growth (Vacante 2016). Moreover, it is important in mite demography to especially understand the population growth and population dynamics, as well as for practical purposes, such as population forecasting, pest management, and biological control programmes (Michalska & Mankowski 2006; Serra et al. 2007).

Sex ratio bias is common among arthropods living in patchy habitats (Walter & Proctor 2013), such as eriophyid ones, which are females that comprise 60–90% of the population (Bergh 2001; Ozman & Goolsby 2005; Michalska & Mankowski 2006; Druciarek et al. 2014). In such a situation, the female bias can accelerate the population growth and influence the population dynamics (Horowitz & Gerling 1992; Price et al. 2011; Gou et al. 2019; Kolk & Jebari 2022).

There is no information about the sex ratio which needs to be addressed and completed. The study aimed to estimate the sex ratio and the related factors that could influence this ratio in a sugarcane field. In this study, we examined the sex ratio of mature individuals of the population taken from a sugarcane field.

MATERIAL AND METHODS

Mite sampling. The study was conducted in a sugarcane field of various genotypes or cultivars at the Everglades Research and Education Center (EREC), the University of Florida, Florida, USA (26°39'27" N, 80°37'54" W). Samples were collected from the CP00-1372 sugarcane genotype within a commercial field with various genotypes released by the United States Department of Agriculture (USDA) Sugarcane Field Station at Canal Point, Florida, USA. The studied field was surrounded by various genotypes of commercial sugarcane fields.

The sex ratio was monitored using 30 plant samples taken monthly from April 2017 until March 2018. Only leaves showing mite infestation were sampled and transported to the laboratory for further mite collection and examination. The infested leaves were indicated by leaf chlorotic symptoms along with fine reddish-brown flecks on the midrib and lamina. Approximately 50–150 mites were picked from each leaf sample depending on its pop-

ulation density. Subsequently, the mites were transferred to glass slides with polyvinyl alcohol (PVA) (BioQuip Products Inc., Rancho Dominguez, CA, USA), and eventually covered with a glass cover. The mite preparation was conducted with an Olympus stereo zoom microscope SZ1145 (Olympus Corp., Japan). Meanwhile, the morphological characteristics and genitals of the mites were examined under a Nikon Optiphot phase contrast microscope (Nikon Corp., Japan) at 1 000 × magnification to distinguish between the male and female mites. The morphology of their genitalia was determined externally and internally. Finally, the mite sex ratio (SR) was expressed as the female proportion to the total mites. In addition, the length of 50 individual mites was measured with a micrometer mounted on the ocular of the microscope.

Along with the sex ratio observation, the mite density on the sugarcane canopy was monitored every two weeks from late June through early November 2017. Fifty plants were randomly chosen with a + 3 leaf position as the sample. A 1.9 cm × 7.62 cm piece of transparent tape was applied to the abaxial surface to recover the mites and then the tape was transferred to a glass slide. The population density was determined by counting the mites on the slides with a stereo-zoom microscope and the population was presented as a monthly aggregate.

The airborne mites were also observed using water pan traps from June through November 2017. Fifteen water pan traps were installed in the middle of the sugarcane field to monitor the airborne mites. The trap dimension was a 6 × 6 cm square with a depth of 9 cm filled with water and a few drops of liquid detergent to reduce the surface tension of the water. The traps were set in line within the sugarcane rows 50 cm above the ground. All the plant canopies above the traps were removed allowing the mites to fall freely from the air without any obstruction. Water was collected and examined weekly. The mites were extracted from the water and debris by sieving using two levels of sieves, that is, with a 2 mm and 50 µm filter. Subsequently, they were taken to the laboratory for counting, mounting, identifying, and sexing. The mounting and sexing utilised the procedure previously described and the population data were presented as monthly aggregates. The mite identification refers to the work of Amrine et al. (2003) and Navia et al. (2011).

The daily air temperature and humidity data were collected from the Florida Automated Weather

Network (FAWN) located at the EREC, University of Florida, USA. The temperature and humidity were measured at 2 m above the ground, which is around the height of the mature sugarcane canopy.

Statistical analysis. The SR of every sample was calculated and the plant status concerning the mite SR was determined statistically. The SR was expected to be balanced when it was not significantly different from 0.5 by the χ^2 -test ($P < 0.05$), otherwise, the SR was female- or male-biased. In addition, the aggregate sex ratio data of a given month was also tested for the SR status with the same procedure of the χ^2 -test.

A one-way analysis of variance (ANOVA) was performed to analyse the data of the monthly sex ratio. Linear and non-linear mixed effect models (lme package) were used to determine whether the sex ratios were different among the months. The data were transformed with Lambert transformation to fulfil the normality and homogeneity assumptions (Peterson 2021). The means were separated using the least significant difference (LSD) test at a 5% level of significance. Furthermore, the association between the SRM sex ratio and the climatic factors was also analysed using with Pearson's correlation. All the inferential analyses were performed in RStudio (version 1.4.1103).

RESULTS

The morphological appearances of the females and males were important in the mite sexing. The main characteristics of the females were the appearance of a genital cover flap (cv) and spermatheca (sp); while the males had none of these characteristics, instead they had testes (Figure 1). Additionally, internal features also often appeared in conjunction with the previous characteristics, including the egg(s) in females.

A total of 27 941 adults sampled from 360 plants in 12 months were successfully sexed. The rest of the mites could not be sexed due to their poor condition or were still in the immature stage; therefore, it was impossible to examine the genitalia. Generally, the male was significantly smaller than the female. However, this characteristic could not be used to determine the sex of the SRM because the length overlapped with the length of the males and females, which was 154 μm (120–180 μm) and 175 μm (140–200 μm), respectively.

The study showed that the sex ratio oscillation of the mite on the plant canopy occurred spatially and

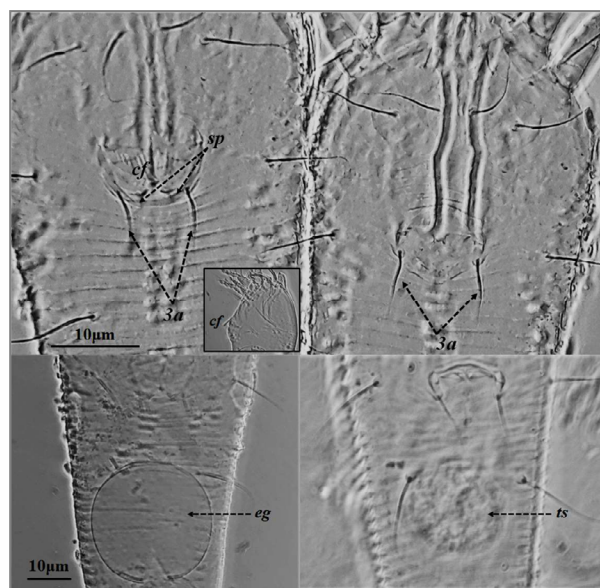


Figure 1. *Abacarus sacchari* internal and external genital characters for mite sexing (left) female and (right) male eg – egg, cf – cover flap, sp – spermatheca, ts – testis, 3a – 3a setae

temporally. Spatially, it was reflected in the variation from among the plants within a given sampling date, which ranged from a female bias to a male bias (Table 1, Figure 2). The wide range of the SR (0.183–0.943) meant that three statuses of the sex ratio coexisted. Furthermore, the skew toward females and a balance always occurred on every sampling date; while the male bias did not, especially in July, October and January. Accordingly, plants

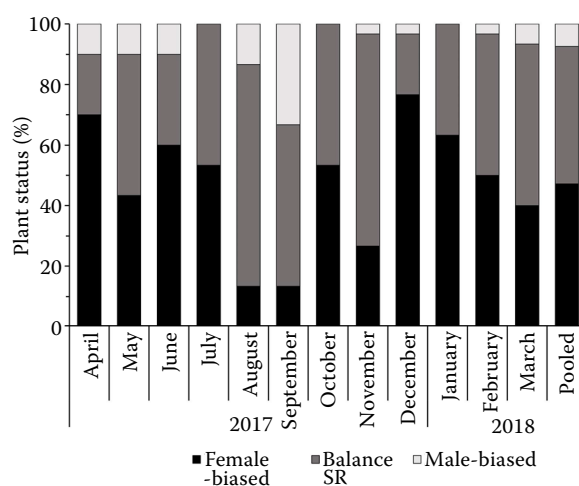


Figure 2. Status of the plant according to the sex ratio of the inhabited *Abacarus sacchari* population. The three statuses of the sex ratio co-existed in the monthly sampling ($n = 30$ samples)

Table 1. Sex ratio of *Abacarus sacchari* which determined based on each plant sampled

Sampling date	Number of mites sexed per sample (<i>n</i> = 30 plants)		Sex ratio (female proportion)	
	mean \pm SEM	range	mean \pm SEM	range
April 2017	93.2 \pm 4.8	37–144	0.644 \pm 0.026 ^{ab}	0.258–0.920
May 2017	43.7 \pm 1.3	28–58	0.575 \pm 0.032 ^{cd}	0.289–0.935
June 2017	67.6 \pm 1.6	50–86	0.640 \pm 0.030 ^{ab}	0.183–0.941
July 2017	65.0 \pm 2.0	43–101	0.620 \pm 0.018 ^{bc}	0.397–0.768
August 2017	74.9 \pm 1.5	64–100	0.494 \pm 0.020 ^{ef}	0.280–0.750
September 2017	83.9 \pm 2.1	54–109	0.462 \pm 0.021 ^f	0.226–0.642
October 2017	67.4 \pm 1.6	41–85	0.617 \pm 0.018 ^{bc}	0.409–0.806
November 2017	77.1 \pm 1.9	58–97	0.551 \pm 0.020 ^{de}	0.371–0.868
December 2017	91.7 \pm 2.4	66–112	0.693 \pm 0.026 ^a	0.347–0.943
January 2018	91.0 \pm 2.4	58–113	0.645 \pm 0.019 ^{ab}	0.457–0.857
February 2018	84.5 \pm 2.1	53–103	0.572 \pm 0.022 ^{cd}	0.264–0.784
March 2018	91.3 \pm 2.0	72–118	0.578 \pm 0.023 ^{cd}	0.314–0.812

The means of sex ratio followed by different letters are significantly different (LSD, $P < 0.05$); SEM – standard error of the mean

populated by the SRM female bias were more common and stronger than plants populated by the male bias. The percentages of the samples inhabited by the female-dominant, balanced and male-dominant SRM populations were 47%, 45% and 8%, respectively, for the whole sampling period.

Temporally, the sex ratio also fluctuated throughout the sampling dates, and it varied from a female bias, to a balance and a male bias (Figure 3). Again, the skew toward females was common during the

study, occurring 10 months out of the 12 months. Only once was the sex ratio balanced in August and the male bias occurred in September. All in all, the SR exhibited a slight female bias with the female proportion being 0.591. Furthermore, the patterns of mite population in the canopy and density in the air were similar, reaching their peaks in August.

Among the airborne mites collected, as many as 2 248 adults were successfully sexed, while 38 mites were still immature and several others were in poor condition and therefore could not be identified and or sexed. The genitalia examination showed that the

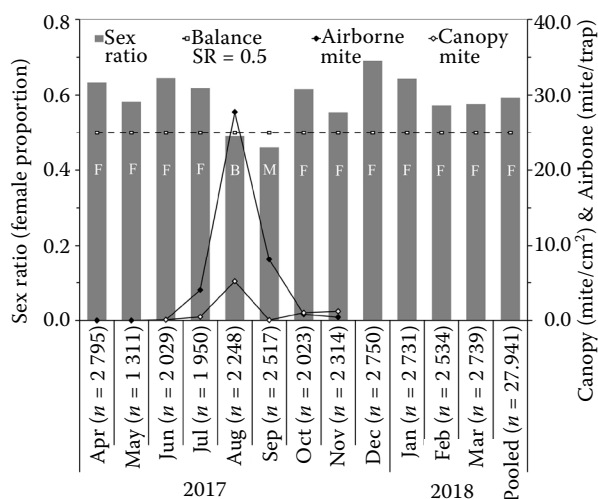


Figure 3. Dynamics of the *Abacarus sacchari* sex ratio (SR) in the plant canopy concerning the airborne and canopy mites

The letters indicate a female bias (F) if $SR > 0.5$; a balance (B) if $SR = 0.5$; and a male bias (M) if $SR < 0.5$ (χ^2 -test, $P < 0.05$); *n* – the number of mites sexed from the sugarcane canopy

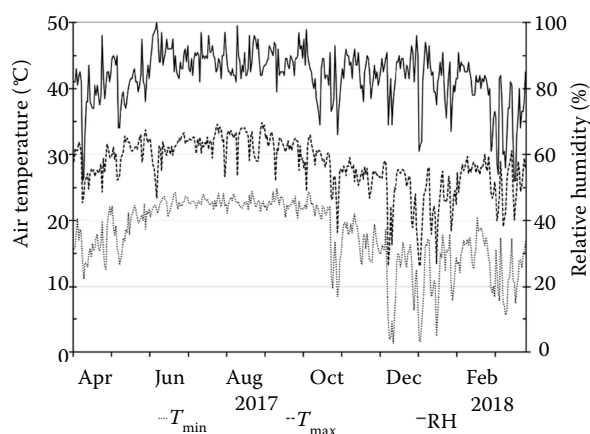


Figure 4. Daily oscillation in the air temperature and relative humidity at 2 m above the ground within the period of the mite sex ratio sampling

T_{\min} – minimum temperature; T_{\max} – maximum temperature; RH – relative humidity

Table 2. Pearson's correlation between the sex ratio and the monthly air temperature and humidity

	Coefficient correlation (<i>r</i>)	95% confidence interval		<i>t</i> _{0.05}	<i>df</i>	<i>P</i>
		lower	upper			
SR – <i>T</i> _{min}	–0.2680	–0.3614	–0.1693	–5.264	358	< 0.0000
SR – <i>T</i> _{max}	–0.2161	–0.3125	–0.1153	–4.188	358	< 0.0000
SR – RH	–0.0891	–0.1908	0.0143	–1.694	358	0.0912

SR – sex ratio; *T*_{min} – minimum temperature; *T*_{max} – maximum temperature; RH – relative humidity; *t*_{0.05} – *t*-test statistic with alpha = 0.05

airborne SRM was dominated by adults comprising about 98% of the total collected mites. Females predominated among those adults which comprised approximately 95.5%, resulting in a strong female bias sex ratio.

The daily air temperature and relative humidity oscillated during the study; however, it was more stable in the summer (Figure 4). The average maximum temperature was 28.1 °C reaching the highest at 34.8 °C, while the average minimum temperature was 17.5 °C reaching the lowest at 1.4 °C. The differences between the maximum and minimum daily temperature varied greatly from 1.9 °C to 20.5 °C. Furthermore, the minimum temperature was above 20 °C during the spring-summer and dropped below 10 °C in the fall and winter. Meanwhile, the average relative humidity was 83.2%, reaching the lowest at 52% and the highest at 100%. The association between the sex ratio and weather parameters is presented in Table 2. The temperature had weak and negative correlations with the sex ratio, even the relative humidity did not correlate.

DISCUSSION

The SRM population in southern Florida frequently experiences significant disruptions and catastrophic crashes due to pre-harvest burning practices followed by cane harvesting which cause habitat losses. Natural disasters from hurricanes and tropical storms that often strike Florida are another type of devastating disturbance, which also potentially reduces the mite population. When encountering such kinds of uncertainty, mites need a way to adapt to survive, using both a dispersal ability and reproduction strategy.

The success of the water pan traps collecting the SRM revealed that the mites were dispersed by air currents. The nonvolant dispersal by wind effectively covers a large area which has been recorded

among the eriophyid by Kiedrowicz et al. (2017); Laska et al. (2022), and Galvao et al. (2012), including the SRM. However, the lack of wings and visual ability makes landing random and undirected. The landing in the early colonisation was very low and resulted in a sparse population; consequently, encounters among individual mites were low. In addition, the low proportion of males (less than 5%) within the airborne mites lowered the chance of encounters, which eventually lowered the level of chance of mating availability (Liegeois et al. 2023). Arrhenotokous parthenogenesis combined with oedipal mating takes advantage in such a situation allowing a single female of eriophyid to start a new colony in a new habitat, even for the immature mites (McCulloch & Owen 2012; Filia et al. 2015; Ding et al. 2018). The colonisation of a new habitat takes place as follows: virgin females arrive in a new habitat and oviposit unfertilised eggs resulting in all male offspring, which then shifts the proportion of males. The male sons subsequently provide spermatophores for the mothers resulting in a higher female production (Farahi et al. 2018; Laska et al. 2022) and subsequently drives the sex ratio to a female bias. This reproduction strategy can explain the successful colonisation of the sugarcane field by the mites even after an ecologically catastrophic event due to the burning practices during the pre-harvest and cane harvest.

According to our results, the pooled sex ratio of the mite population from the sugarcane canopy was slightly female-biased, which is also common among free-living eriophyid (Skoracka & Kuczyski 2004). Although the females were dominant among the sampling dates and among the samples, three situations existed, that is, a female bias, a balance, and a male bias suggesting that the arrhenotokous parthenogenesis and sexual reproduction occur simultaneously. Otherwise, the parthenogenesis alternates with the sexual reproduction or cyclic parthenogenesis (Rouger et al. 2016).

The SR of the mite depends on the proportion of fertilised and unfertilised eggs. The more abundant number of females suggests that the mites developed from fertilised eggs were more common than those from the unfertilised ones and *vice versa*. If a greater proportion of eggs were fertilised, then the population would be skewed toward the female, otherwise, it would be biased toward the male.

The SRs of the airborne and canopy mites were both female-biased; however, the bias of the former was much stronger than that of the latter, indicating that the females were more likely to disperse than the males. Due to the large number of females leaving the plant canopy at the peak of the dispersal in August, reaching approximately 110 mites/trap/month, fewer females prevailed in the sugarcane canopy, and the males exceeded the number of females. Simultaneously, the status of the leaf sample with a female bias also decreased. Consequently, this situation resulted in a change in the sex ratio during the peak of the aerial dispersal. Bergh (2001) reported a similar finding on the airborne citrus rust mite, with the females reaching 100%.

Many of the airborne mites, as the early colonisers or foundresses, were strongly female biased and carried eggs indicating their readiness to reproduce, regardless of whether they were virgins or inseminated. Furthermore, among the collected airborne SRM, they had a large spheroid spermatheca, while some others were deflated. The spheroid shape indicates that the mites were inseminated, while the deflated ones showed uninseminated females (Michalska 2014; Lu et al. 2019). If the foundresses are inseminated, more female offspring will be produced; in contrast, if they are not inseminated, a male bias or even all male offspring of the population will follow.

The sex ratio showed oscillation between a female bias, a balance, and a male bias, indicating a cycle among the sex ratios in the populations. The explanation for the oscillation of the sex ratio is as follows: When the foundresses were virgin females without eggs, then eggs would be produced after their arrival in the new habitat to start a new generation without fertilisation; thus, the arrhenotokous parthenogenesis would produce all male offspring which would subsequently produce spermatophore for their mothers resulting in the female offspring. The cycles of the reproduction through the female insemination and non-insemination occurs repeatedly over generations and produces a stable or sustained oscillation in the sex ratio (Gardner 2014).

Sperm transfer in eriophyid occurs without courtship, instead the male deposits spermatophore on the substrate or host plant; then, the female picks up the spermatophore to obtain the sperm. In such a situation, the spermatophore is vulnerable to predation and abiotic threats such as water loss and solar radiation (Michalska 2016; Kuhsel et al. 2016). Accordingly, the spermatophore availability is affected, therefore, it may influence the sex ratio of the mite population.

Environmental factors, such as the air temperature and humidity, can affect the minute mite. The size of the SRM is microscopic; consequently, the surface-to-volume ratio is large, therefore increasing the risk of water loss. The amplitude of the daily temperature was up to 20 °C which might have a significant influence on the sex ratio; even though the temperature has a weak correlation with the sex ratio. As a fluctuation in the air temperature can cause variable water loss, the higher the temperature, the larger the water loss through respiratory activities (Kuhsel et al. 2016). The eriophyid spermatophores are exposed directly to such a situation because the male deposits them on the surface of the plant and the female will pick them up later. Exposure of the spermatophores to temperature fluctuations may kill the sperm. The sex proportion in the eriophyid results in fewer fertilised eggs; finally, it will increase the male proportion in the mite populations. However, no such situation occurred when the SR shifted to the male bias in August–September. Therefore, the female predominance of the airborne mites in this peaking season is more reasonable than the argument for the temperature impact. Accordingly, Michalska (2005) suggested that the high temperature and low humidity had a deleterious effect on the sperm.

Besides the oscillation, an extreme temperature situation, such as that occurs in southern Florida can affect the proportion of male and female offspring. The air temperature in this area can drop below 10 °C in the fall and winter, which is much lower than the annual average of 22.5 °C; thus, the female bias is stronger and the female population is more common among the plants in December and January. Simultaneously, sugarcane plants reduce their growth and photosynthesis reaching their maturity and are ready to be harvested; therefore, this becomes a significant disturbance for the SRM population. The Roy et al. (2003) study revealed that extreme temperatures could drive the popu-

lation into a female bias in the spider mite, *Tetranychus mcDanieli*, as an adaptative mechanism against deteriorating habitats.

This study supports Michalska and Mankowski's (2006) report that seasonal sex ratio oscillations were also recorded on three species of eriophyids inhabiting wild blackberry bushes. The seasonal oscillation occurs due to several factors, such as differential mortality and the preference of dispersal between sexes, spermatophore availability and viability, and climatic conditions (Michalska & Mankowski 2006).

In summary, the sex ratio of the SRM in the sugarcane field is dynamic starting with a female bias to male bias with the female bias being more common than the male bias. Mite dislodgement has a crucial effect on the sex ratio of the field population, on the other hand, the temperature has little impact on the shifting of the mite sex ratio.

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