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Within-plant distribution and rapid assessment of sugarcane rust mite population on sugarcane canopy

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Original research

ABSTRACT

Sugarcane rust mite (SRM), *Abacarus sacchari*, has been recognized in Florida since 1983. However, no detection technique had been developed yet, whereas a reliable, effective, and efficient technique is important in a mite management practice. The purposes of this study were to determine the within-plant distribution and to develop a sampling technique for SRM. The study was carried out in sugarcane fields naturally infested with the mite population. The mite distribution was identified with the visual direct counting technique and the imprinting technique. Subsequently, the effectiveness and reliability of both techniques were evaluated. The results showed that the within-plant distribution of mite populations was more concentrated in the middle canopy than in the upper or lower canopies. Furthermore, the mite sampling of the middle portion of the leaf could represent the entire mite population on the plant. More importantly, the imprinting technique was reliable, especially when the third or fourth (+3 or +4) fully expanded leaf was taken as sample, resulting in relative variation (RV) as low as 12.9% and 12.4%, respectively; therefore, the imprinting technique is a promising technique for population monitoring programs.

Keywords direct counting; imprinting technique; correlation; relative variation; reliable

Introduction

Sugarcane rust mite (SRM), *Abacarus sacchari*, was initially described by Channabasavanna from sugarcane in India in 1966. Subsequently, it was first discovered in Florida in 1983 and was described as its junior synonym, *A. officinari* Keifer (Hall 1988). Leaf chlorosis, along with orange to reddish flecks on infested leaf surfaces, appears in Florida from late summer to fall every year as an infestation result of this mite.

Although the mite has been discovered for decades, sampling protocols for SRM have not been developed or published yet. The microscopic sizes of females and males are only about 140–200 µm and 120–180 µm long, respectively, making mites' observation and detection very challenging and hindering the sampling program development. Consequently, SRM scouting will be laborious and time-consuming to be accurately quantified, especially when a large sample size must be taken, and mite populations are large to be counted, if it is needed.

Some reports regarding the eriophyoid within-plant distribution have been published yet, such as *Acaphylla theae* (Watt), *Acaphyllisa parindiae* Keifer, and *Calarus carinatus* (Green)

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of tea (Muraleedharan *et al.*, 1988), *Aceria sheldoni* (Ewing) on lemon tree (Walker *et al.* 1992), *Calacarus flagelliseta* Fletchmann, De Moraes et Barbosa on papaya canopy (Fournier *et al.* 2004). However, to our knowledge, there is no report on the distribution of SRM in the sugarcane canopy.

Within-plant distribution indicates the mite preference for plant organ positions which is important to be understood for developing sampling procedures to monitor mite population. Afterward, the sampling procedure is one of the important key elements in pest management practices, such as for decision-making in Integrated Pest Management (IPM), characterization of the nature of pest impact, and pesticide efficacy evaluation (Mo and Baker 2004; Mo *et al.* 2008; Brosius *et al.* 2010; Jandricic *et al.* 2014). In addition, sampling is also required in ecological studies such as mite demography and dispersal studies.

Generally, eriophyoid sampling can be classified into three techniques, namely direct visual counting, trapping, and extraction (Monfreda *et al.* 2010; Schreiner *et al.* 2014). Direct visual counting using hand lenses along with gridded transparent cards has been described by Allen (1976) and Fournier *et al.* (2004). Moreover, Santos *et al.* (2022) used funnel-shaped traps to collect airborne mites. Meanwhile, Siriwardena *et al.* (2005) utilized a washing technique to collect the coconut mite, *Aceria guerreronis* Keifer, then Monfreda *et al.* (2007) added to the washing technique a sieving step to extract mites from a sample. A different method using adhesive tape was proposed to recover eriophyoids from the plant material and showed adequate effectiveness and precision (Aghajanzadeh and Mallik 2007; Asbani and Amir 2010). Furthermore, Harvey and Martin (1988) and Lotfollahi *et al.* (2023) used sticky-band traps to observe movements of motile *Eriophyes tulipae* Keifer on wheat spikes and *A. angustifoliae* Denizhan *et al.* on Russian olive twigs, respectively. Eventually, reliability, practicality, and efficiency should be the main considerations in the mite sampling program development.

This study is carried out to determine the within-plant distribution patterns of SRM across sugarcane cultivars, to identify appropriate sampling units, and to develop a technique for SRM sampling. Thus, a practical and cost-effective sampling method can be established to estimate SRM populations in the sugarcane field and the information gap on the sampling protocol for the SRM could be filled.

Material and methods

The study was conducted in the sugarcane fields of the Everglades Research and Education Center (EREC), Institute of Food and Agricultural Sciences (IFAS), University of Florida, Belle Glade, Florida (26°39′27″ N, 80°37′54″ W), USA. Two observation techniques were used to study the within-plant distribution of mites, namely the visual direct counting and the imprinting technique.

Visual direct counting

The visual direct counting technique refers to mite counting directly from the leaf surface as described by Schreiner *et al.* (2014). As many as 16, 30, and 30 stalk samples were randomly chosen from commercial sugarcane fields of genotypes CP80-1743, CP88-1762, and CP96-1252, respectively, in the summer (May to July). Two to four stalks were taken from the field daily one after another and were brought to the laboratory; later, the stalks were placed in a bucket filled with water to prevent water loss.

Leaves of -2 downward to the maximum of +10 position were detached from their stalks and then each leaf was divided into four portions of equal length, that was, proximal, medial-1, medial-2, and distal. This procedure was employed one leaf after another starting from the lowest and moving upward. In this investigation, the Kuijper leaf nomenclature system for sugarcane was applied (van Dillewijn, 1952) in which the +1 leaf position was the top fully expanded leaf and known as the top visible dewlap (TVD).

The mite population on each leaf portion was examined under an Olympus SZ1145 stereo zoom microscope at 15–60x magnifications. Only the abaxial or lower leaf surface was examined because the preliminary study showed that more mites were found on the abaxial than the adaxial surface at the proportion of 60:40.

Imprinting technique

The imprinting technique refers to a technique employing force to squash and adhere mites onto materials (Southwood, 1978), such as transparent adhesive tapes in our study of having 1.91 cm width (Scotch®, 3M, St. Paul, Minneapolis, USA). Thirty plant samples were taken from the sugarcane fields of three genotypes, namely CP88-1762, CP89-2143, and CP00-1101. In the laboratory, each leaf was detached from the stalks and labeled with the nomenclature described previously. Mite counts started from the 0 leaf position until the lowest fresh leaves. Leaves -2 and -1 were not inspected because previous visual direct counting revealed that too few mites were found on these leaves. A 1.91 mm x 7.62 mm strip of adhesive tape was applied onto the abaxial surfaces of the proximal and medial portions. Subsequently, the tape was removed from the leaf and finally transferred onto microscope glass slides. The tapes needed to be divided into 5 mm x 5 mm square grids as the counting guidance using a sharp cutting blade. Finally, mites adhered to the tapes were counted using an Olympus SZ1145 stereo zoom microscope at 15–60x magnifications with light transmitted from below the slides to accentuate the mites. The mounted adhesive tapes with mites were kept at room temperature for about four years to be examined for the presence of the mites.

The mite recovery effectiveness of the imprinting technique was also evaluated. One hundred and fifty-seven leaf samples were examined using the mite counting protocol described previously. The number of mites adhered to adhesive tape and mites left on the leaf surface was counted. Eventually, the recovery rate was calculated as the number of mites on the adhesive tape divided by the total number of mites.

Sampling efficiency was evaluated by measuring the counting speed of mites on glass slides. As many as 158 glass slides with various mite densities taken from the previous observations were utilized in this evaluation. The mite density was recorded along with the time required for mite counting. Furthermore, observing the speed of the 93 blank slides (slides without mites) was also examined as a standard. This observation was performed at a fixed magnification of 15 times.

To estimate the area of the canopies, the leaf area was measured with the Li 3100C leaf area meter (Li-Cor Inc., Lincoln, Nebraska, USA). Five measurements were taken to five stalks of each genotype, namely CP88-1762, CP89-2143, and CP00-1101. Leaves from +1 to the lowest positions were detached from their stalks and measured using the leaf area meter.

Physiological measurement

The physiological status of each leaf was measured to find the appropriate leaf as the sampling unit. Twenty plants of the CP89-2143 genotype were taken from the field previously sprayed with acaricides to minimize the SRM population and ensure that the plants were free of SRM feeding symptoms. Measurements were taken using a LI-6400XT portable photosynthesis system equipped with a 2 cm^2 leaf chamber attachment (Li-Cor Inc., Lincoln, Nebraska, USA). The parameters consisted of net assimilation rate or photosynthetic rate (*A*), stomatal conductance (g_{sw}), intercellular CO_2 (*Ci*), and transpiration (*E*). During the measurements, the LED light source and CO_2 concentration were set to 1,500 µmol/m^2/s and 380 µL CO_2/L, respectively (Zhao *et al.*, 2011). The measurements started from -2 to +8 leaf positions. Later, the water use efficiency (WUE) was calculated from transpiration divided by the photosynthetic rate.

Statistical analysis

The reliability of sampling techniques was evaluated based on the relative variation (RV) value as described by Pedigo *et al.* (1972) which indicates the precision of a given sampling protocol (Buntin 1994) by measuring the spread of the mite population of the sample from its mean. The RV was calculated for each leaf position of each sample plant and then presented as the average of the samples.

Mite distributions within the plant canopy were analyzed using the *generalized mixed model* with *glmm.TMB* package. The leaf position and leaf portion were the fixed effects while the sample was the random effect. Due to many zero data, the Poisson distribution with zero inflation was chosen for all these mite population data analyzes. When the analysis of variance was significant, the Tukey post hoc test was used to compare mite populations between leaf portions and between leaves. Additional analysis was also performed to find the associations of mite densities among leaf portions using Spearman's correlation analyzes.

The photosynthetic rate was analyzed using a *linear mixed-effects* (*lme*) model within the *linear and nonlinear mixed-effects* (*nlme*) packages. The data were transformed with the *ordnorm* before being analyzed to fulfill assumptions of normality and homoscedasticity. The rest of the physiological data were analyzed using nonparametric statistics -Kruskal-Wallis- after several transformations failed to make the data meet the assumptions. The mite distributions and the plant physiology analysis described above were performed with R version 4.1.3. for Windows (R Core Team, 2022), and a significant level of $P=0.05$ was used for all inferential analysis. Lastly, the relationship between sugarcane rust mite density and counting speed with its graph was generated with Sigma Plot 13 (SystatSoftware 2014).

Results

Symptoms produced by SRM infestation consisted of fine reddish-brown flecks along with leaf chlorosis. However, the sugarcane genotypes responded differently to the SRM feeding activities such as CP89-2143 and CP00-1101 showed obvious symptoms, while CP80-1743, CP88-1762, and CP96-1252 did not show any symptom of mite feeding.

Visual direct counting

The minimum number of leaves per sample was 8 leaves consisting of -2 to +5 leaf positions, while the maximum was 13 leaves consisting of -2 to +10 leaf positions. However, the +9 and +10 leaves were excluded from the analysis because they had been shed off from most of the samples and these lower leaves had significant necrotic parts as well.

Mite examination in visual direct counting is based on living mites (Figure 1). Consequently, it was so demanding because of its microscopic size and mobility on leaf surfaces, which could potentially be miscounted. Furthermore, the unevenness of the leaf surface and the lack of contrast of mites with the leaf background color resulted in the longer time required for leaf examinations.

The leaf observations revealed that mite populations among plants and leaves varied greatly. The SRM populations per plant ranged from as low as 15 mites to as many as 23,408 mites with an average of 2,406 mites. Moreover, SRM populations per leaf ranged from 0–6,041 mites with an average of 228 mites. Along with the SRM populations, the leaves infested with mites varied within the range of 16–100% (Table 1). Leaves -2 and -1 were less infested, while leaves 0 downward to +8 were mostly infested by the SRM.

Sugarcane canopies can be vertically divided into three groups, namely top, middle, and lower canopies, representing -2 to +1, +2 to +5, and +6 to +8 leaves, respectively. General patterns of mite distributions within the canopy were influenced by the leaf positions on the stalks (Figure 2). The pooled data showed that leaves in the middle of the canopy had the

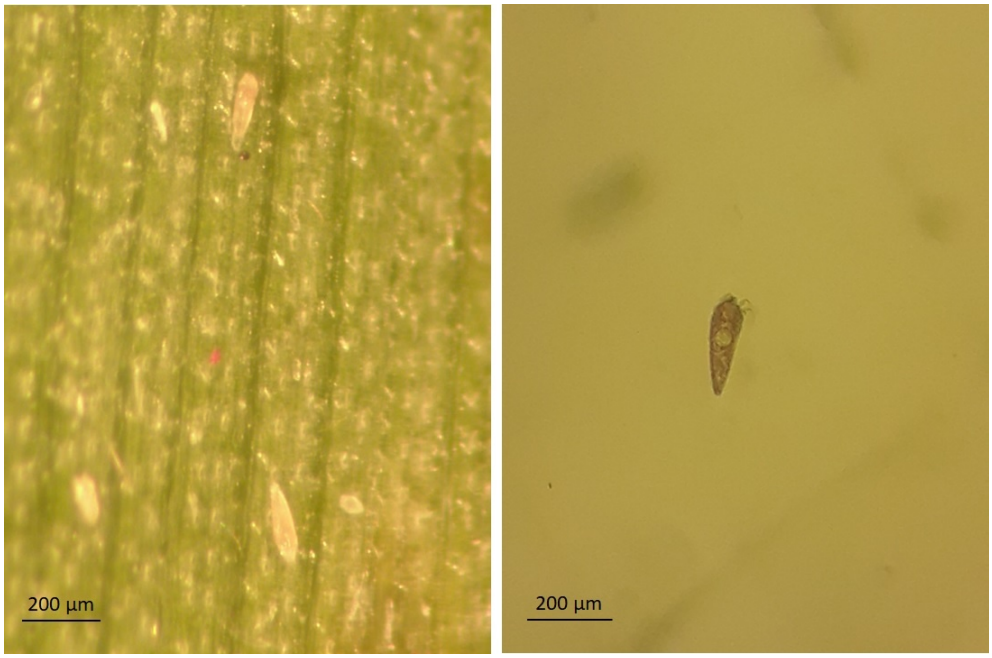

Figure 1 Mites appearances in visual direct count (left) and imprinting techniques (right).

highest number of SRM comprising almost 60% of the total mites on plants. In contrast, the upper and lower canopies were the lowest in the populations.

Mite distributions within leaf portions were similar in all leaves, regardless of the sugarcane cultivar or their position on stalks. Two medial portions were usually densely inhabited with mites, whereas the proximal and distal portions were less inhabited.

Imprinting technique

Leaves -2 and -1 were not observed in the imprinting technique because many leaves in the previous technique were without any mites. Furthermore, leaves +9 and +10 were observed but were not included in the analysis because they had been shed from many of the samples. The mite infestation rates were high between 69–100% (Table 1). Meanwhile, the mite density within the plant canopy varied greatly with an average of 2.0 mites/cm^2 and ranged from 0 to 35 mites/cm^2.

Table 1 Infestation rate and relative variation of the direct counting and imprinting technique for each leaf position

Leaf position	Direct counting technique						Imprinting technique			
	Infestation rate	RV (%)					Infestation	RV (%)		
	(%)	Proximal	Medial-1	Medial-2	Distal	Whole leaf	rate (%)	Proximal	Medial	Whole leaf
-2	16	91.2	69.9	57.1	66.9	59.3	-	-	-	-
-1	36	69.1	53.3	48.4	37.1	57.9	-	-	-	-
0	69.3	44.8	40.7	47.4	71.9	44.2	96.6	14.1	14.5	15.9
1	94.7	36.8	39.3	37.5	42.9	40	100	11.8	14	15.6
2	93.3	30.7	41.1	32.6	37.2	33.9	98.9	12.8	14.4	17.8
3	98.7	36.7	30.4	30.3	33.4	26.9	100	**10.4**	**9.5**	**12.9**
4	100	26.9	22.8	23.2	32.6	22.5	100	**8.6**	**10.1**	**12.4**
5	98.6	20.1	25.4	23.4	26.7	23.9	100	14.2	15.4	19.2
6	94.2	14.5	21.1	20	23.7	21.7	96.6	13.8	15.9	18.3
7	98	18.9	23.5	23.4	28.5	27.3	91	15.1	12	17
8	95.8	26.8	25.7	26.7	38.3	33.6	68.5	20.7	16	23.9

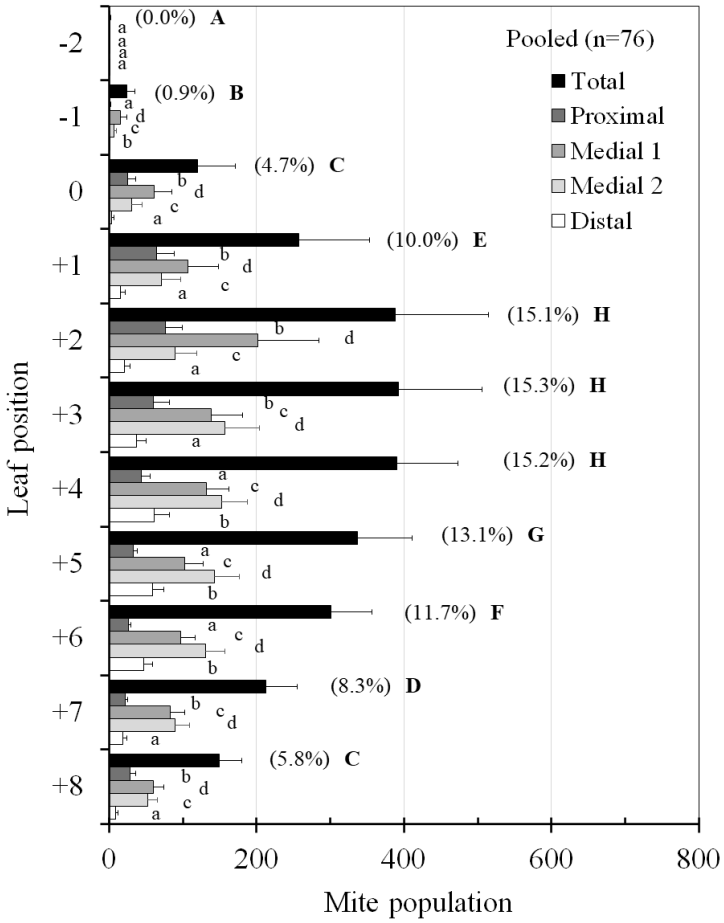

Figure 2 Within-plant distribution of sugarcane rust mite population based on direct counting on three sugarcane cultivars (mean ± SEM). The numbers within brackets are the proportions of mite populations within plants. Means across leaves with the same capital letters are not significantly different and means with the same lower letters on a given leaf position are not significantly different (Tukey, $P < 0.05$).

Like the visual direct counting, the pooled data of the imprinting technique also showed that the distribution patterns within plants were affected by the leaf positions (Figure 3). More mites were discovered in the middle canopy which was around 65% of mites than in the upper or lower canopies.

Sampling technique evaluation

Counting mites directly from fresh leaves was time-consuming and laborious. Only 2–4 plant samples per day (around 5 effective hrs) were examined in a day depending on the mite density and number of leaves per sample observed. The average number of leaves per sample was 10 leaves (-2 to +7 positions) and the average leaf area was 242 cm^2/leaf, thus it was equal to 2,420 cm^2/plant. If three sample plants were examined during ±5 hrs, then the average examination speed was 0.40 cm^2/s. When the average number of mites was 228 mites/leaf or equal to 0.94 mites/cm^2, thus, the counting speed was 0.38 mites/s. As the reference, the speed to observe the empty leaves was 0.25 s/cm^2.

The evaluation of the technique showed that the sticky tape could recover mites effectively from leaf samples on average 92% and within the range of 70–100%, while few mites remained

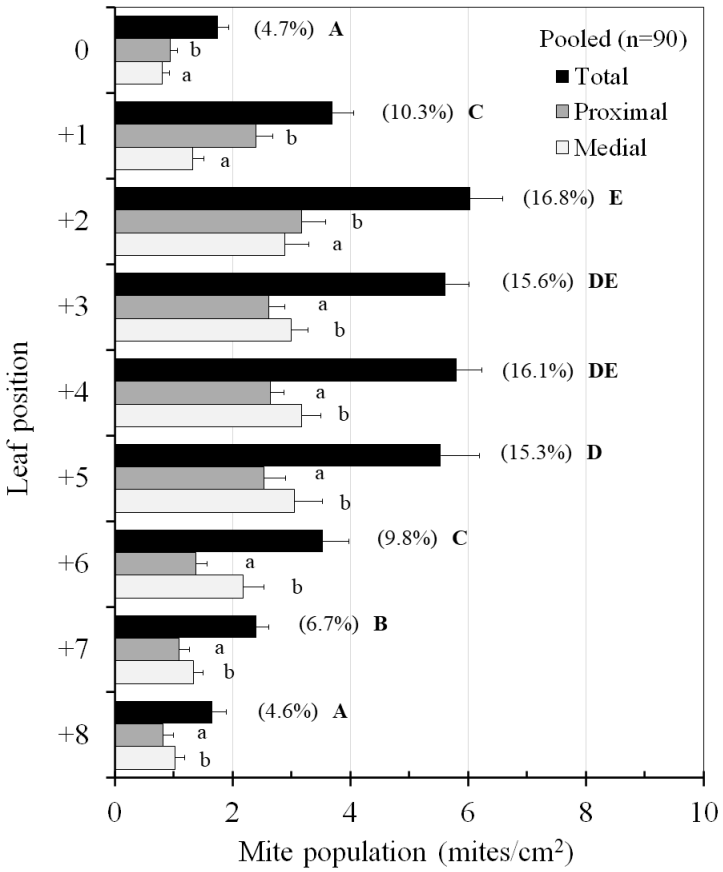

Figure 3 Within-plant distribution of sugarcane rust mite population based on the imprinting technique (mean ± SEM). The numbers within brackets are the proportions of mite populations within plants. Means across leaves with the same capital letters are not significantly different and means with the same lower letters on a given leaf position are not significantly different (Tukey, $P < 0.05$).

in leaf samples. The counting speed of mites on slides increased with mite density and leveled off at a speed average of 0.42 mites/s (Figure 4). As a comparison, the average scan speed for the empty slide (=slide without mites) was 4.04 ± 0.38 cm^2/s which was also the minimum period required for the examination of a slide.

All Spearman-rho correlation coefficients between mite densities throughout the canopy and mite densities in leaf portions from the direct counting technique showed positive correlations and the associations were moderate to very strong (Table 2). The strongest association was between the total number of mites in plants and the mean number of mites in medial-1 + medial-2. Furthermore, associations between mite populations on two adjacent portions were stronger than between mite populations of the two separated portions.

The associations between mite populations on entire plant and leaf portions in the imprinting technique were also moderate to very strong positives (Table 2). In addition, the association of the entire mites on canopy with the mites on medial portion was stronger than the proximal

Table 2 Spearman's rank correlation (ρ) between mite populations of leaf portions based on direct counting and imprinting technique.

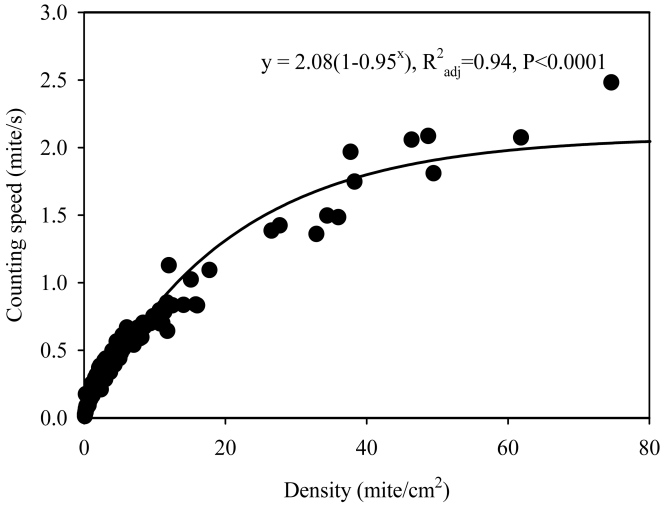

Figure 4 Relationship between sugarcane rust mite density and counting speed of the imprinting technique.

portion.

The RVs generated by direct counting were between 14.5–91.2% and none of them met the sampling requirement, regardless of leaf position (Table 1). On the other hand, the imprinting technique was more promising with the smaller RVs, especially for leaf +3 and +4 which was between 8.6–20.7%. The variation of the direct counting was larger than that of the imprinting technique as reflected by their RVs which were the larger the RVs, the larger the sample variations, and *vice versa*.

Physiological status

Analyses of variance revealed that the effect of leaf position on physiological parameters was not significant (Figure 5). The averages of net assimilation rate (A) or photosynthetic rate, stomatal conductance (g_{sw}), intercellular CO_2 (C_i), transpiration (E) and WUE were 15.85 µmol $CO_2/m^2/s$, 0.119 mol $H_2O/m^2/s$, 141.11 µmol CO_2/mol, 3.18 mmol $H_2O/m^2/s$ and 5.03 µmol CO_2/mmol H_2O, respectively. Furthermore, there were no obvious patterns in the parameters regarding the position of the leaves. Consequently, the physiological status of the leaf should not be considered in the SRM sampling.

Discussion

The visual direct counting technique and the imprinting technique reveal that the SRM populations are unevenly distributed throughout the sugarcane canopies. The mite is more populated in the middle canopy than in the upper and lower canopies because mites in the middle canopy have a longer and sufficient time to reproduce and build their populations. On the contrary, the leaves of the upper canopy are younger and have been exposed and colonized for a shorter time and are thereby less populated than the middle canopy.

The lower canopy has fewer mites because the lower leaves are less nutritious due to the natural aging process (Lewandowski and Kozak 2008). Moreover, the reduction in plant quality and changing physical structure may contribute to the lower population (Skoracka and Kuczyñski 2003, 2006). These situations cause more mites to flee or die in the lower canopy compared to expanding their numbers in the middle canopy. Another explanation is that mites'

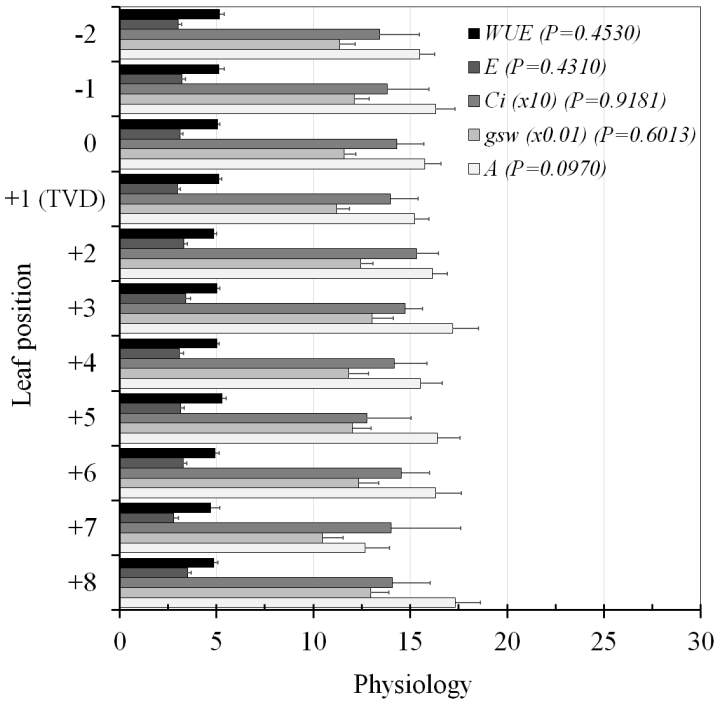

Figure 5 Physiological parameters of sugarcane canopy (mean±SEM). *A*=photosynthetic rate, g_{sw}=stomatal conductance, C_i=intercellular CO_2, *E*=transpiration, WUE=water use efficiency.

flee as an avoidance from intra-species competition due to high population density in the middle canopy which later becomes the low canopy as adding up leaves on the apical.

Another factor contributing to the unevenness of mite distribution is microclimatic variations of the canopy due to solar radiation penetration, temperature, humidity, and wind (Allen and McCoy 1979; Perring *et al.* 1996; de Lillo and Skoracka 2010). The adaxial leaf surfaces of the top canopy receive more solar radiation than the middle and low ones. Consequently, the temperature is higher in the upper, while the humidity is lower than that of the middle and low canopies. In such a situation, specific requirements and preferences of mites on the microclimate can result in an aggregated or uneven distribution across canopies.

Eriophyoids, including SRM, generally have a low ambulatory capacity that cannot support active long-distance dispersals. According to Asbani *et al.* (2023), SRM is less likely to move across leaf positions actively within a sugarcane canopy, consequently, it cannot facilitate the distribution evenly. Therefore, the dispersal of mites over a long distance such as movement between leaves in a canopy primarily relies on the wind instead of its ambulatory ability. Meanwhile, the aerial dispersal is undirected, thus the destination cannot be predicted.

The imprinting technique shows its superiority to visual direct counting for technical, statistical, and economic reasons. The imprinting technique reduces the size of the sampling unit from 242 cm^2 (average of one leaf area) into a unit of 14.5 cm^2 (7.62 cm x 1.9 cm) which is equal to a 94% area reduction. Consequently, the strength of the association decreases slightly, but it still has a very strong correlation. In return, the examination of the smaller sampling unit is fast and effortless, which eventually reduces its cost.

The imprinting technique has some benefits against direct counting. First, the mites under adhesive tape are preserved and long-lasting even after four years in storage still in good condition to be examined properly. Second, the speed in mite counting is also an important benefit since it reduces the time and labor required for sample examination. Third, the imprinting technique has less chance of miscounting the mites, thereby being more accurate

than direct visual counting. Fourth, the mite density resulting from the imprinting technique can be standardized and converted to a unit area of the leaf, such as in mites/cm^2, which allows comparisons among sampling dates or treatments easily. Last, it was superior to direct counting due to the absence of background distractions of leaf morphology (e.g., spinules, stomata, dust, and fungal hyphae) found on leaf surfaces; therefore, mites are easier to observe on the glass slides.

The value of RVs in this study is important in determining the reliability of the sampling. Small variations in a sampling will give very similar results on a repeated sample. Pedigo *et al.* (1972) and Kogan and Pitre (1980) suggested that an extensive sampling program requires an RV < 25, while an RV < 10 is required for an intensive sampling program. Based on these criteria, the variability in the imprinting technique of the SRM is acceptable and adequate only for an extensive sampling program. Furthermore, the direct visual sampling technique is inadequate for both extensive and intensive sampling programs.

Due to the physiological parameter not significantly affected by the leaf positions, this parameter should not be considered in the sampling development. Thus, the sampling unit determination should consider and rely merely on the vertical distribution of mites.

In summary, the imprinting technique with the proximal or middle portions of the +3 or +4 leaf positions as the sampling unit meets the requirements and is reliable. The technique is promising to be employed for the SRM monitoring program. Our suggestion for further study is that first, the time for sample preparation should be considered in the sampling evaluation, and later, the same leaves should be utilized for comparing the techniques.

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Contributions

The authors confirm their contribution to the paper as follows: study conception and design of the experiment: NA, RC, GN; data collection: NA; formal analysis: NA, interpretation of results: NA, RC, JB, HS, OL, GN; Writing original draft: NA, GN, Writing review & editing: NA, RC, JB, HS, OL, GN.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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