

The Role of Leaf Pubescence in Resistance to Yellow Sugarcane Aphids (YSA), *Sipha flava*, in Selected Sugarcane, *Saccharum officinarum* Varieties

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Abstract

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Screening for leaf pubescence in sugarcane (*Saccharum officinarum* L.) varieties against yellow sugarcane aphid (YSA) (*Sipha flava*) remains unexplored in the sugar industry of Zimbabwe. Field trials were undertaken to investigate the interaction between leaf pubescence and YSA. A 7*2*3 factorial complete randomized block design (CRBD) replicated four times was used, under two aphid treatments (Acetamiprid pesticide sprayed (control) and YSA infested). The first factor was varieties (96-1107, 00-1165, ZN 10, ZN 8, ZN 9, ZN 3L, and N14), aphid treatment was the second factor at two levels (sprayed and infested), and leaf position was the third factor (leaf position-1, leaf position-2 and leaf position-3). Quantitative data on trichome density (mm²) was measured using a light microscope, and aphids were physically counted to determine their number. The trichome density (mm²) and aphid number in the control varied greatly ($p < 0.001$), showing prominent variation depending on the leaf position ($p < 0.001$), and the variety ($p < 0.001$). In aphid-infested plots, ZN 10 had the lowest trichome density (30.9; 41.27; 56.72/mm²) for leaf-1, leaf-2, and leaf-3, respectively, whereas the sugarcane variety 00-1165 had the highest trichome density for all three leaf positions (105.7, 115.7 and 131/mm²), respectively. Linear regression analysis confirmed a significant ($p < 0.001$) negative correlation between the number of aphids for different varieties and trichome density. Resistance to YSA feeding on different sugarcane varieties is mediated by leaf pubescence, or dense trichomes. Farmers in the sugar industry should integrate pubescent (high-density trichome) sugarcane varieties into their existing YSA management strategies.

Key words: Leaf pubescence, trichomes, leaf position, sugarcane, *Sipha flava*.

Introduction

Yellow sugarcane aphid (YSA), *Sipha flava* is a piercing, sap-sucking pest that feeds primarily on the underside of leaves along the parallel veins of the sugarcane crop (Way *et al.*, 2015). This aphid is a major sugarcane pest causing yellowing and reddening of leaves, followed by early senescence of the leaves and stem death (Nuessly & Hentz, 2002; Wilson, 2019). According to Hall (2001), early-stage plant damage is a major factor in both yield and tillering loss in sugarcane. Studies of the Zimbabwe Sugar Association Experiment Station (ZSAES) has estimated annual yield losses of 5.4-7.2% in Zimbabwe during the period 2019-2023 due to infestation with *S. flava*.

Leaf pubescence plays an important role in plant growth, development and adaptation to the environment. It is defined as the presence of trichomes, which are ubiquitous in plants and comprise unicellular or multicellular outgrowths from the epidermis (Wood *et al.*, 2017; Xie *et al.*, 2020). In terms of biotic stress resistance, leaf pubescence can improve plant resistance to insects by influencing the preference and behavior of various insects (Nawab *et al.*, 2011), such as bird cherry oat aphid in wheat (Roberts & Foster, 1983). Roberts & Foster (1983) and de Correa *et al.* (2020) showed out that trichome density is ecologically significant as its indelibly affect insect feeding behavior. In support of this, Singh *et al.* (2021) pointed out that feeding

herbivores causes the subsequently formed leaves to produce higher trichome densities. This study aimed to assess the contribution of leaf position, accession (genotype) and treatment (sprayed and infested) on trichome density and aphid number. Furthermore, previous studies indicated that trichomes are usually more evident in young tissues than in older ones (Valkama *et al.*, 2004; McCall and Fordyce, 2010). Additionally, Brunissen *et al.* (2009) and Chandrasekhar *et al.* (2018) highlighted that defense mechanisms can also be dynamic and affected at different levels depending on herbivore attack, plant age, insect developmental stage, and genetic background of the crop.

Trichomes are the first plant structures that insects come into contact with during the initial stages of host acceptance (Moghadam *et al.*, 2013). Nevertheless, the effect of leaf pubescence on herbivorous insects such as polyphagous aphids can be positive or neutral (Moghadam *et al.*, 2013). Various studies (Gallun *et al.*, 1966; Roberts *et al.*, 1979; Roberts & Foster, 1983; Sosa, 1991; Webster *et al.*, 1994; White, 1990) have reported the contribution of pubescent wheat and sorghum cultivars in reducing the effects of aphids in general. However, hardly any study has determined the density of pubescence in sugarcane varieties against YSA in Zimbabwe. Moreover, the relationship between pubescence density and aphid infestation in sugarcane crop has been scarcely reported in the literature.

At present, the most widely employed YSA control approach registered for use in the Zimbabwe Sugar Industry (ZSI) is the chemical approach, utilizing neonicotinoids, Acetamiprid and Actara (thiamethoxam). Synthetic pesticide use over an extended period of time has a number of detrimental effects. Sugarcane growers are advised to switch to leaf pubescence (dense trichomes) sugarcane varieties in order to prevent the emergence of YSA resistant biotypes. Leaf pubescence resistance mechanism is a long-term, sustainable priority that is both environmentally benign and compatible with other integrated pest management (IPM) strategies. Variation of these mechanisms at individual parts within a plant may be one of the key elements of pest reproduction and feeding behavior (Jakobs & Müller, 2018). However, these variations have been reported in other aphid species and cereals at the expense of YSA. Therefore, the objective of this study is to investigate the role of leaf pubescence in mediating resistance to YSA in selected sugarcane varieties.

Materials and Methods

Study Site

The research was carried out during the 2023 season at the ZSAES which is owned by Tongaat Hullets in Chiredzi district, Masvingo Province in the southeast Lowveld. The ZSAES is found in agro-ecological region V which is characterized by very low and erratic rainfall of less than 500 mm per annum. ZSAES is situated on a 99 km peg along the Ngundu-Tanganda road. It is located 430 m above sea level at latitude of 20° 01' S and longitude 28° 38' E. Heavy rainfall is not usually experienced in summer which can help to wash YSA on leaves, thereby reducing infestations. The temperatures are high especially during the summer season, particularly in the months of September, October and November with an average temperature of 34°C. Low temperatures are recorded between May to July with the daily mean temperature of 25°C. These climatic conditions allow the YSA to exhibit two modes of reproduction; through non-mating (parthenogenetically) in warmer climates (Halbert *et al.*, 2013) and through mating, where live individuals are produced and females mate with males in cold winters as low temperatures induce oviparity (Nuessly, 2005; Way *et al.*, 2015). This has allowed the YSA to be a resident pest in the Lowveld.

Agronomic practices

All agronomic practices were done according to the sugarcane production manual (Clowes & Breakwell, 1998). For YSA treatment comparisons, Acetamiprid pesticide (300g/250 liters water) was applied after every two weeks in the control plots (Acetamiprid sprayed), whereas infested plots were not sprayed.

Leaf surface pubescence trials

Seven sugarcane varieties were selected by considering moderate level of resistance, susceptibility, cane yield and estimated recoverable crystal rate (ERC %) based on recommendations from the Plant Breeding and Entomology Departments. Two preleased varieties namely 00-1165 (low susceptibility) and 96-1107 (high susceptibility) were

selected. Moderately resistant varieties based on visual assessments (ZN 8, ZN 9 and ZN 3L) in addition to ZN 8 and ZN 9 that have the ability to recover from damage inflicted by YSA, were also selected. Lastly, ZN 10 and N14 were chosen despite being susceptible because of their high biomass and high ERC rate (%). The seven sugarcane varieties were examined for trichomes per mm² on the abaxial surfaces. Abaxial surfaces were used because YSA resides under the leaf during feeding. In support of this, Sosa (1991) suggested that YSA are more commonly found on the underside of leaves. Leaf sections were cleared of chlorophyll by treating the leaf sections with 80% ethanol at 85°C for 15 min, and rinsed with distilled water four times for three min each time at 45°C (Batyrrshina *et al.*, 2020; Singh *et al.*, 2021).

Experimental design

A 7×2×3 factorial complete randomized block design (CRBD) replicated four times was used, under two treatments of aphid infestation (Acetamiprid sprayed (control) and YSA infested). Variety was the first factor under seven levels (96-1107, 00-1165, ZN 10, ZN 8, ZN 9, ZN 3L and N14), second factor was aphid treatment under two levels (Acetamiprid sprayed (control)) and YSA infested), and leaf position was the third factor under three levels (leaf position-1, leaf position-2 and leaf position-3).

Data collection

Trichomes density- Trichome density was determined under a light microscope using the protocols by Batyrshina *et al.* (2020) and Singh *et al.* (2021). Non-glandular trichome density analysis was performed on the middle sections of leaf-1, leaf-2, and leaf-3, from untreated and aphid-infested plants. The tissue segment was placed on glass microscope slides facing the abaxial side, and the total number of non-glandular trichomes were counted (Batyrrshina *et al.*, 2020; Singh *et al.*, 2021). The numbers of trichomes were determined on an area covering 1 cm² with light microscope using the 10 × objective and 10 × ocular lenses following the protocol by Moghadam *et al.* (2013). The units used for measuring trichome density were number per square millimeters (mm²).

Aphids count- Aphids were physically counted once on each sampled leaf position when the sugarcane crop had reached the three-leaf stage after natural aphid infestation. Since resource allocation, developmental programming, epigenetic regulation, hormone regulation, and genetic predisposition are all at their peak during early growth stages, the three-leaf stage (one to two weeks following germination) was crucial. Furthermore, early determination paves the way for the coordinated and effective development of trichomes in sugarcane crops, which in turn supports their overall growth and survival. Moreover, the study was restricted to sugarcane of ≤ three months old. However, YSA are found at every growth stage of sugarcane if conditions are favorable and are reduced when the crop has reached physiological maturity.

Data analysis

A two-way analysis of variance (ANOVA) was used to test the effects of leaf position, genotype, aphid treatment, and their interaction (leaf position x genotype x aphid treatment).

Fishers Least Significant Difference (LSD) test ($p < 0.05$) was used in a one-way ANOVA to test for differences between genotypes. Using Fisher's least significant difference ($p < 0.05$), the statistical differences between the aphid-infested plants and the control (sprayed with Acetamiprid pesticide) were assessed for each sugarcane variety. General linear regression model was used to determine the correlation between aphid number and trichome density, trichome density and leaf position, and aphid number and leaf position.

Results

Effect of leaf position and genotype on aphid number and leaf pubescence (trichome density)

Aphid behavior on younger leaves (leaf-3) differed from that on older leaves (leaf-1), which led to the measurement of non-glandular trichomes as a physical defense. The constitutive density (per mm^2) of trichomes was highest in leaf 3 (90.11), followed by leaf 2 (75.74), and lowest in leaf 1 (62.1) (Figure 1), and an increase in trichome density resulted in reduced aphids number. ZN 10 variety scored the lowest trichome density (30.9, 41.27, 56.72/ mm^2) for leaf-1, leaf-2, and leaf-3, respectively, and the 00-1165 sugarcane variety recorded the highest trichome density (mm^2) across all three leaf positions (leaf-1, leaf-2, leaf-3) (105.7, 115.7, 131.0/ mm^2), respectively, in aphid-infested plots.

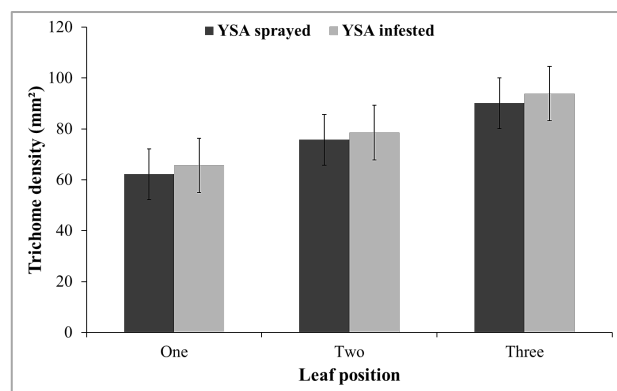


Figure 1. Trichome density on sprayed and infested leaf positions of sugarcane varieties.

The three leaf positions and sugarcane varieties showed significant differences ($p < 0.001$), on trichome density (Figure 2). ZN10 variety recorded the lowest trichome density of 20.97, 35.85, 50.97/ mm^2 , on leaf 1, leaf 2 and leaf 3, respectively, whereas the 00-1165 sugarcane variety recorded the highest trichome density across all leaf positions (93.07, 111.15, 124.9/ mm^2 , respectively).

The contribution of genotype, leaf position, and aphid treatment (Acetamiprid sprayed (control) and aphid-infested) was then determined by using a two-way ANOVA. The trichome density in the control plants showed notable variation depending on the leaf position ($p < 0.001$), the seven genotypes ($p < 0.001$). However, there was no significant interaction ($p > 0.05$) between genotype and leaf position. The trichomes density increased significantly by 3.75–5.66%

upon aphid feeding, based on the results of aphid infestation. The interaction between genotype and treatment of aphid infestation and the relationship between genotype and leaf position and treatment of aphid infestation were not statistically significant ($p > 0.05$). Ranking of resistance of sugarcane varieties to YSA was done according to leaf pubescence (trichome density) from low to high susceptibility as follows: 00-1165 > ZN 8 > ZN 9 > ZN 3L > 96-1107 > N14 > ZN 10.

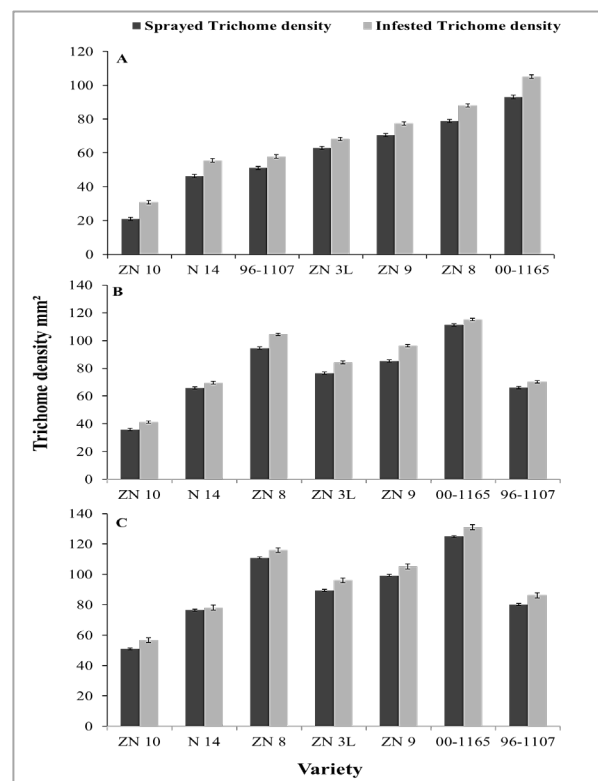


Figure 2. Trichome density at (A) leaf position one, (B) leaf position two, (C) leaf position three for tested sugarcane varieties.

Effect of leaf pubescence (trichome density) on the YSA number on sugarcane varieties

Aphid counts on leaf-1, leaf-2, and leaf-3 showed significant differences ($p < 0.001$) amongst the seven sugarcane varieties (Figure 3). Leaf position had a significant impact ($p < 0.001$) on the number of aphids per unit of leaf area, as demonstrated by a two-way ANOVA. Furthermore, the results indicated that there were significant differences ($p < 0.001$) amongst the different sugarcane varieties in terms of aphid number. In the control (Acetamiprid sprayed plants) aphid treatment, ZN 10 variety had the highest aphid number (46.5, 39.8 and 37.5) for leaf-1, leaf-2, leaf-3, respectively, whereas 00-1165 sugarcane variety had lower aphid number across three leaf positions of 16.5, 12.5, and 6.5, respectively. In aphid infested treatments, ZN 10 variety had the highest aphid population across the three leaf positions (156, 97, 42, respectively), whereas 00-1165 variety had the least number of aphids of 53.0, 32.5 and 16.5 for leaf-1, leaf-2, and leaf-3, respectively. There was also no significant interaction ($p > 0.05$) between genotype, aphid number and

leaf position. Overall, this indicates that there is an impact of genetic background and leaf position on the dynamics of the aphid population in sugarcane.

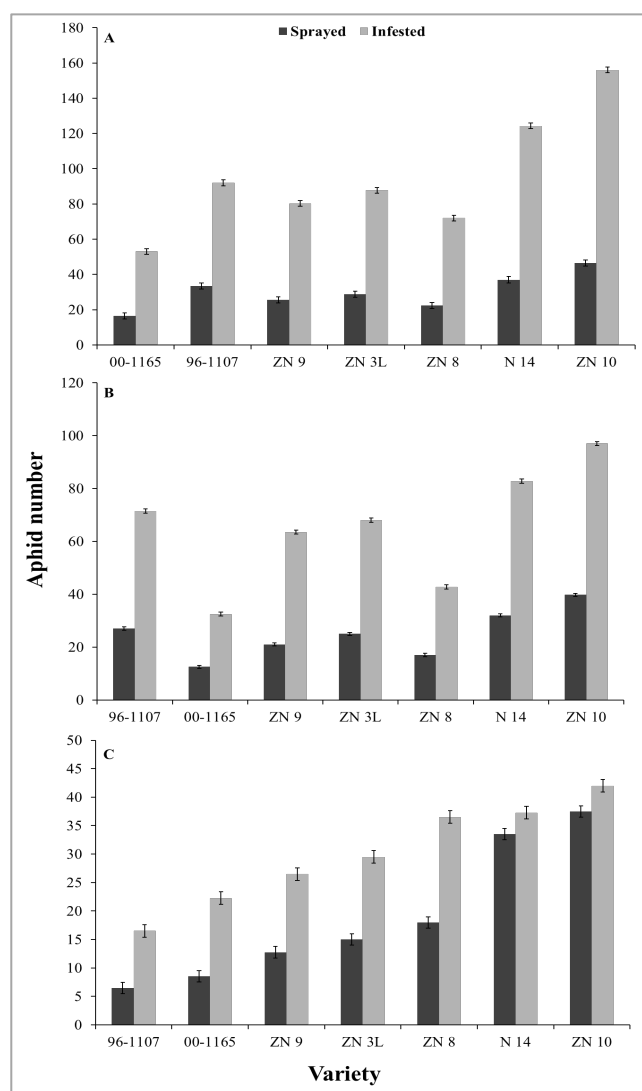


Figure 3. Effect of trichome density on YSA number at (A) leaf position one, (B) leaf position two, (C) leaf position three for tested sugarcane varieties.

Relationship between the number of YSA present on plants and the trichome density of sugarcane cultivars

The results of the linear regression analysis showed a significant strong negative correlation between the number of aphids and trichome density after natural infestation on various sugarcane varieties (Figure 4, $Y = -0.64X + 96.6$, $p < 0.001$, $r = 0.52$).

Relationship between trichomes density and leaf position of sugarcane cultivars

The linear regression analysis demonstrated a significant weak positive correlation between the trichome density for various sugarcane cultivars and leaf position after natural infestation (Figure 5, $Y = 6.33X + 50.97$, $p < 0.001$, $r = 0.45$).

Relationship between the number of YSA and leaf position of different sugarcane cultivars

Linear regression analysis confirmed a significant weak negative correlation between the number of aphids for various sugarcane varieties and trichome density after natural infestation (Figure 6, $Y = -24.8X + 82.11$, $p < 0.001$, $r = 0.48$).

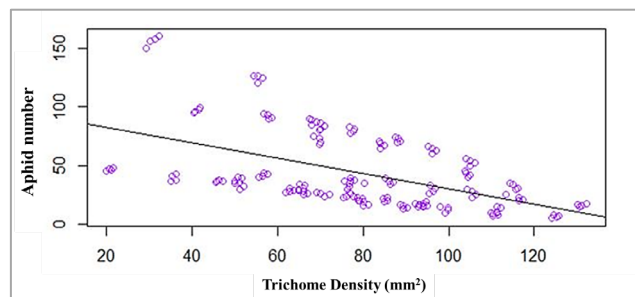


Figure 4. Relationship between aphid number and trichome density (number/mm²) on different sugarcane varieties.

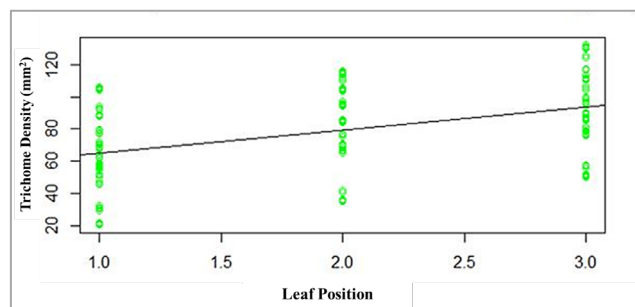


Figure 5. Relationship between leaf position and trichome density for different sugarcane varieties.

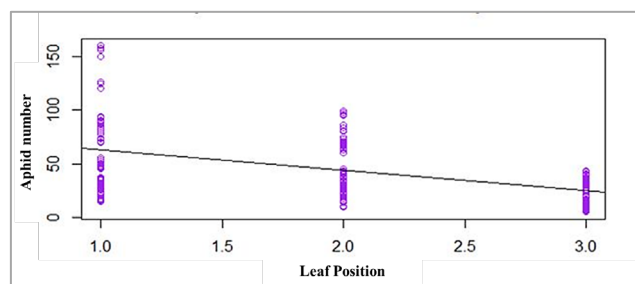


Figure 6. Relationship between leaf position and aphid number on tested sugarcane varieties.

Discussion

The results obtained from the present study indicated that leaf pubescence (trichome density) differed significantly among sugarcane varieties and that aphid infestation resulted in increased trichome density. Additionally, significant differences were obtained for all three leaf positions (leaf-1, leaf-2 and leaf-3). The 00-1165 sugarcane variety had the highest trichome density and low aphid number when

compared with the susceptible ZN 10 genotype. Results of variation in trichome density on different leaf positions confirmed the increase in the trichomes density on newly sprouting leaves (Traw & Bergelson, 2003). Additionally, YSA-infested sugarcane relatively increased trichome density as an induced defense strategy to reduce the damage caused by YSA. It has been reported earlier that environmental conditions and biotic stresses influence trichome density (Nawab *et al.*, 2011). This study supports the hypothesis that the physical barrier created by non-glandular trichomes in sugarcane can restrict insect movement and prevent phloem feeders from inserting their stylets as supported by earlier studies (Sato and Kudoh, 2015; Singh *et al.*, 2021). Moreover, the findings of this study showed that trichomes density differed according to leaf position. These results are consistent with those of Gyan *et al.* (2020) and Singh *et al.* (2021) who emphasized how different leaf positions, development stages, genetic backgrounds, and even insect attack can affect trichome density. Furthermore, results corroborate the findings of Sosa (1991) who reported that leaf surface pubescence may be a key morphological trait for resistance to YSA. In contrast to this, Starks & Merkle (1977) reported that some wheat cultivars with pubescence may be slightly resistant to greenbugs, but the resistance is most likely not caused by pubescence. However, Roberts & Foster (1983) demonstrated that dense leaf pubescence in wheat confers resistance to the grain aphid, *Rhopalosiphum padi* L.

The trichome density of the sugarcane varieties was found to be between 20.97 and $\pm 121/\text{mm}^2$, which is comparable to the results obtained by Ferreira *et al.* (2007). This confirms a variation of trichome density among different sugarcane varieties which might be genetically controlled. In support of these findings, comparable patterns of trichomes presence were observed on the leaves of other cereals such as barley and wheat (Correa *et al.*, 2020; Batyrshina *et al.*, 2020). Furthermore, trichome density was significantly negatively correlated with aphid number. This relationship might be a result of other factors which influence trichome density. Results obtained in this study concurred the findings of Batyrshina *et al.* (2020) who suggested that aphid number and constitutive levels of trichome density are inversely correlated. Similar results were also reported by Amin *et al.* (2017) who indicated a significant negative correlation between the number of

aphids for various cotton cultivars and trichome density after natural infestation. Additionally, Batyrshina *et al.* (2020) reported similar results in wheat pertaining to reduced aphid reproduction. Furthermore, a significant weak positive correlation was observed between trichome density and leaf position. This might be an indication that there are other external variables acting on the leaf resulting in variation in trichome density which requires further studies. Older leaves (leaf-1) had low trichome density when compared to younger leaves (leaf-3). This study findings validate research conducted by Singh *et al.* (2021) who noted that feeding of herbivores increases leaves trichome's density that are subsequently formed after feeding. In support of this, Chandrasekhar *et al.* (2018) pointed out that, depending on the plant's age, developmental stage, and genetic make-up, defense mechanisms can also be dynamic and induced in response to herbivore attack (Gyan *et al.*, 2020; McCall and Fordyce, 2010; Singh *et al.*, 2021; Valkama *et al.*, 2004). The functioning of non-glandular trichomes, or leaf pubescence, as a partial defense mechanism against YSA on sugarcane leaves was highlighted in this study at early stages of growth. These results will open the door for breeding YSA resistance sugarcane varieties as a long-term, environmentally friendly, and sustainable solution.

It can be concluded from this study that leaf pubescence (trichome density) differs among the sugarcane varieties in relation to leaf position and aphid infestation. On different sugarcane varieties, resistance to YSA feeding is mediated by leaf pubescence, or dense trichomes. Level of resistance of sugarcane varieties to YSA was ranked from low to high susceptibility based on trichome density as follows: 00-1165 > ZN 8 > ZN 9 > ZN 3L > 96-1107 > N14 > ZN 10. Farmers in the sugar industry should use pubescent (high dense trichome) sugarcane varieties in order to curb YSA infestations. There is need to integrate leaf pubescence in existing integrated pest management (IPM) strategies as it confers resistance to YSA in sugarcane.

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المخلص

ساكادزو، ن.، م. موبفوما، م.س. موكانغا، أ.ر.س. مابفيني ور. موسونديري. 2026. دور التغطية الوبرية للأوراق في مقاومة حشرة من القصب الأصفر (*Sipha flava*) في أصناف مختارة من قصب السكر (*Saccharum officinarum* L.). مجلة وقاية النبات العربية، 44(1):55-61.

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لا تزال دراسة التغطية الوبرية للأوراق في أصناف قصب السكر (*Saccharum officinarum* L.) كآلية مقاومة لحشرة من القصب الأصفر (*Sipha flava*) غير مستكشفة في صناعة السكر في زيمبابوي. أجريت تجارب حقلية بهدف دراسة التفاعل بين التغطية الوبرية للأوراق ومن القصب الأصفر. تم استخدام تصميم تجريبي عشوائي كامل (CRBD) بعامل ثلاثي (7 × 2 × 3) بأربعة مكررات، تحت معاملتين لحشرات المن (رش بمبيد "Alice" كشاهد، والإصابة بمن القصب الأصفر). كان العامل الأول هو الصنف، وشمل سبعة أصناف: 00-1165، ZN 8، ZN 9، ZN 3L، و N14؛ والعامل الثاني هو المعاملة بالمن (مرشوشة ومصابة)؛ أما العامل الثالث فهو موضع الورقة (الموضع 1، الموضع 2، الموضع 3). تم قياس الكثافة العددية للزوائد الوبرية (trichomes) بوحدة mm^2 باستخدام مجهر ضوئي، كما تم عد حشرات المن يدوياً. أظهرت النتائج اختلافاً كبيراً في كثافة الزوائد الوبرية وعدد حشرات المن في معاملة الشاهد ($p < 0.001$)، وتبين وجود اختلاف

واضح حسب موضع الورقة ($p < 0.001$) والصنف النباتي ($p < 0.001$). في القطع المصابة بالمن، سجل الصنف ZN 10 أقل كثافة للزوائد الوبرية (30.9، 41.27، 56.72 مم² للورقة 1، 2، 3، على التوالي)، بينما سجل الصنف 00-1165 أعلى كثافة في المواضع الثلاث للأوراق (105.7، 115.7، 131 مم²، على التوالي). أكد تحليل الانحدار الخطي وجود علاقة عكسية ذات دلالة إحصائية ($p < 0.001$) بين كثافة الزوائد الوبرية وعدد حشرات المن في الأصناف المختلفة. أشارت النتائج إلى أن مقاومة أصناف قصب السكر لتغذية من القصب الأصفر تتم بوساطة التغطية الوبرية للأوراق أو الكثافة العالية للزوائد الوبرية. وعليه، يُوصى بدمج أصناف قصب السكر ذات التغطية الوبرية الكثيفة في استراتيجيات مكافحة المتكاملة لحشرة من القصب الأصفر في قطاع صناعة السكر.

كلمات مفتاحية: التغطية الوبرية للأوراق، الزوائد الوبرية، موضع الورقة، قصب السكر، *Sipha flava*.

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