



Host-Plant Resistance Mechanisms (HPRs) of Commercial Sugarcane (*Saccharum officinarum* L.) Varieties in Response to Yellow Sugarcane Aphid (YSA) (*Sipha flava* Forbes) Herbivory under Irrigated Cropping Systems

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Certification page

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Abstract

Worldwide, the well-known and problematic Yellow Sugarcane Aphid (YSA) (*Sipha flava*) plagues sugarcane (*Saccharum officinarum* (L.)). The study's objective was to classify and document commercial sugarcane accessions host-plant resistance mechanisms according to their morphology, physiology, phytochemistry, and biochemistry in response to YSA herbivory. First, a multidisciplinary systematic literature review (SLR) was conducted to comprehend the host plant resistance mechanisms (HPRs) of different sugarcane accessions in response to YSA herbivory. Second, seven sugarcane cultivars were subjected to factorial experiments utilizing a natural aphid infestation, with the following four objectives in mind; explore leaf pubescence that mediates resistance to YSA, assess physiological response as a tolerance mechanism to YSA herbivory, evaluate biochemical resistance in retort to YSA feeding, carryout phytochemical profiling of metabolites that confer resistance to YSA feeding, and reconnoiter plant resistant index (PRI) of tested sugarcane germplasm in response to YSA incursion. The results of the SLR revealed a bias towards *S. flava* in sugarcane, with most studies being skewed in favor of other crops, insect arthropods, and other aphid species. These results demonstrated the paucity of knowledge regarding the previously specified SLR objectives in sugarcane. The majority of the research focused on comprehending the host plant resistance mechanisms induced by sugarcane aphid (SCA). Therefore, studying YSA was novel and crucial for controlling this pest of sugarcane. The results of leaf pubescence (trichome density) varied significantly ($p < 0.05$) among the sugarcane accessions in terms of leaf position and aphid number. Leaf pubescence resistance was ranked from high to low based on the trichome density: 00-1165 > ZN 8 > ZN 9 > ZN 3L > 96-1107 > N14 > ZN 10. Information on physiological tolerance was gathered using SPAD and CIRAS-3 instruments. The content of chlorophyll and the gas exchange responses of the sugarcane accessions under investigation showed significant ($p < 0.05$) tolerance variations. In addition, the sugarcane accessions; 96-1107, N14, and ZN 10 were the most susceptible to the physiological damage caused by YSA. The findings showed that in susceptible sugarcane accessions, YSA decreased biochemical parameters (total protein, total soluble sugars, and total chlorophyll content). Furthermore, the evaluated biochemical properties of sugarcane accessions were either completely retained or partially decreased on resistant (00-1165) and moderately resistant (ZN 9, ZN 8, and ZN 3L) sugarcane accessions. The synthesis of high phenol and flavonoid content in sugarcane accessions was significantly ($p < 0.05$) stimulated by YSA feeding.

Additionally, there was a significant ($p < 0.05$) positive correlation between the following: the percentage change in flavonoid content and the number of aphids, and the percentage change in phenol content. Three mechanisms of resistance; antixenosis, tolerance, and antibiosis were used to evaluate PRI. 00-1165 sugarcane accession is only moderately resistant, according to PRI reading of 37.6, when compared to other accessions that exhibit tolerance, antixenosis, and antibiosis traits. Furthermore, accessions such as ZN 8, ZN 3L, and ZN 9 have relatively >5 PRI in comparison to the susceptible check (ZN 10). Leaf pubescence and leaf position reduced YSA number among the sugarcane varieties. Among the sugarcane accessions, leaf pubescence and leaf position decreased the number of YSA. Additionally, in susceptible sugarcane accessions, YSA reduced the biochemical parameters that were examined. Furthermore, sensitive varieties' chlorophyll content and gas exchange responses were reduced by YSA, but tolerant varieties were able to maintain or compensate for these losses. Moreover, YSA increased the production of flavonoids and phenols in sugarcane accessions, resulting in induced resistance. The 00-1165 sugarcane accession exhibited a moderate expression of the three resistance mechanisms that were studied: tolerance, antibiosis, and antixenosis. Furthermore, antixenosis and tolerance resistance mechanisms were demonstrated by ZN 8, ZN 9, and ZN 3L. The susceptible accessions; N14, ZN 10, and 96-1107 PRI were unable to fall within the three resistance mechanisms. ZN 8 and ZN 9 sugarcane accessions showed low resistance to YSA stress, while 00-1165 exhibited medium resistance as shown by its aphid quantity ratio (AQR), which fell between 0.30-0.60. Moreover, low YSA sensitivity was observed in ZN 3L and 96-1107. N14 had a medium sensitivity as well. Finally, cultivar ZN 10 can be regarded as a very susceptible accession due to its AQR which was more than 1.50. For the Zimbabwean sugar sector, the SLR study on the previously described research objectives was required in order to incorporate resistance into the current Integrated YSA management tactics. Sugarcane growers should update their YSA management programs to include pubescent (high-density trichome) accessions. Growers of sugarcane should use biochemically tolerant YSA accessions in their crop management plans to maintain or compensate for total soluble sugars, according to the study's findings. The physiologically compensating and sustaining behavior of YSA tolerant accessions with respect to gas exchange responses and chlorophyll content should also be taken into consideration by farmers. Since seven sugarcane accessions were used, there is a need for ongoing screening of the industry's sugarcane germplasm for PRI based on antixenosis,

tolerance, and antibiosis. Molecular breeding approaches must be used to isolate the genes of high phenol and flavonoid tolerant varieties for YSA integrated management. As this study indicates, host plant resistance is a promising sustainable management option for controlling *S. flava*; thus, it needs to be incorporated into the current integrated pest management strategies (IPM) because there is an overreliance on chemical method.

Dedication

This thesis is dedicated to my loving wife Marble Musingarimi Sakadzo for her unconditional love and support that has enabled me to complete my Doctor of Philosophy Degree (Ph.D.) studies. Her support continues to ignite my enthusiasm in scientific research. I owe a debt of gratitude to my whole Sakadzo family for their love and support. I would have loved to include a long list of people in this dedication, including my friends, who have always been a huge source of support, but the page is too small to accommodate all of their names.

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List of acronyms

AAP	Annual Action Programme
AQR	Aphid quantity ratio
CRBD	Complete Randomised Block Design
ERC	Estimated Recoverable Crystal
FAOSTAT	Food Agriculture Organisation and Statistics
GCMS	Gas Chromatography Mass Spectrometry
GDP	Gross Domestic Product
HPRs	Host Plant Resistance Mechanisms
IPM	Integrated Pest Management
LSD	Least Significance Difference
P.hd	Doctor of Philosophy
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analysis
SCA	Sugarcane Aphid
SLR	Systematic Literature Review
SPAD	Handheld Spectrophotometry Device
TCH	Total Cane per Hectare
USDA	United States Department of Agriculture
YSA	Yellow Sugarcane Aphid
ZSAES	Zimbabwe Sugar Association Experiment Station
ZSI	Zimbabwe Sugar Industry

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CHAPTER ONE: Introduction

1.1 Background information

Sugarcane (*Saccharum officinarum* L.) is a very important crop which provides sugar, ethanol and other by-products worldwide (Clowes and Breakwell, 1998; Esterhuzein, 2012; Mary and Sujata, 2016; Shabani *et al.*, 2020). Sugarcane from Zimbabwe is exported to other countries (Chandiposha, 2013) and its production is expected to expand by 0.8 % per year throughout the perspective period to reach 1 924 Mt by 2031 (+168 Mt). Brazil and India are expected to contribute 58 % of the increase in world output volume (52 % and 19 %, respectively) (OECD-FAO, 2022). Post forecasts on sugar cane production in Zimbabwe is expected to increase by 1 percent to 3.5 million metric tonnes (MT) in the marketing year of 2023/24 (MY) based on normal weather conditions, availability of irrigation water and increased area planted (Moobi and Woody, 2023).

Hess *et al.* (2016) highlighted the importance of sugarcane on livelihood enhancement and economic development. FAOSTAT (2018) pointed out that the bulk (80 %) of the sugar comes from sugarcane whereas the remaining (20 %) comes from sugar beets (*Beta vulgaris*). The Gross Domestic Product (GDP) contribution to the economy of Zimbabwe is approximately 1.4% (Annual Action Programme, 2009; Scoones *et al.*, 2017). The sugar sector faces a number of obstacles despite these beneficial contributions (Shabani *et al.*, 2020). The production of sugarcane is hampered by pests and diseases, which lowers yield quantity and quality (Hussnain *et al.*, 1997). According to Ahad *et al.* (2016), there are about 1500 different types of insects that infest sugarcane, such as aphids, mealy bugs, scale insects, stem borers, and shoot borers. Stem and root borers have been identified as economically significant pests in the sugarcane industry worldwide (Ahad *et al.*, 2016).

Aphids are also problematic pests of sugarcane; they are serious polyphagous herbivorous pests and are of major concern in many agricultural and ornamental crops worldwide (Hinson, 2017). They belong to the Superfamily Aphidoidea (Ferreira de Souza, 2018). Blackman and Eastop (2000) and Dixon (2012) averred that these aphids exhibit polymorphism (apterous (wingless) or alate (winged)) under different environmental conditions. The pest is widespread; with

approximately 4700 species known (Blackman and Eastop, 2007). Aphid species extract substantial amounts of plant sap, secreting huge amounts of honeydew, and spreading a wide range of viral diseases (Quisenberry and Ni, 2007; Bayoumay *et al.*, 2016). Hinson (2017) postulated that aphids are characterized as phytotoxic and non-phytotoxic based on the extent of damage to plant tissue directly.

At least ten different species of aphids have been identified by Blackman and Eastop (2000) and Akbar (2009) to be affecting sugarcane in different parts of the world. The two most common aphids in Zimbabwe include the sugarcane aphid (SCA) (*Melanaphis sacchari*) and the Yellow Sugarcane Aphid (YSA) (*Sipha flava*). Currently, YSA is the prevalent insect pest causing economic losses in the sugar industry. The first record of YSA in Zimbabwe was in 2014 (Zimbabwe Sugar Association Experiment Station (ZSAES), unpublished data). Several authors (Tripathi, 1995; White *et al.*, 2001; Joshi and Viraktamath, 2004; Akbar *et al.*, 2010; Srikanth *et al.*, 2008; Ferreira de Souza, 2018) have also highlighted that SCA, cane woolly aphids (*Ceratovacuna lanigera*), and YSA are a threat to sugarcane fields worldwide.

Sikuka (2021) indicated that the main pests of concern in Zimbabwe's sugar industry include sugarcane stalk borer (*Eldana saccharina*), YSA and Black Maize Beetle (*Heteronychus sanctae-helenae*). There have been several successful options for the management of black maize beetles and sugarcane stalk borers, leaving attention to YSA. Several authors (Reagan, 1994; Hentz *et al.*, 2004; Nuessly, 2005; Nuessly *et al.*, 2010; Wilson, 2019) reported losses from 6 % up to 19 % initiated by chlorophyll loss as a result of YSA feeding. Madiope *et al.* (2021) showed that the quantification of yield losses in field trials was impossible because of patchy, unpredictable insect infestation. During, an outbreak in Puerto Rico in 1964, Gaud *et al.* (1965) calculated losses of almost a million US dollars as a result of damage by YSA. According to ZSAES's unpublished report, Zimbabwe's yield estimations losses from YSA in terms of percentage total cane per hectare (TCH) for the previous 2023 season was 5.4 %.

There are several control measures for YSA including chemical, cultural, and physical strategies. Currently, chemical control is the dominant method used by several farmers in Zimbabwe (Hall, 2001; Wilson *et al.*, 2013; Mutonyi and Babikha, 2019; Wilson, 2019; Mutongi *et al.*, 2021; Dumont *et al.*, 2023). Posey *et al.* (2001) reported that several insecticides are effective against

YSA and SCA. These insecticides were tested against SCA and YSA in United States of America (Akbar *et al.*, 2009). In the sugar industry of Zimbabwe, Allice 20 SP acetamiprid, a neonicotinoid and Actara have been registered for use (ZSAES, unpublished). However, this method is costly and unsustainable as it kills YSA, beneficial insects (Akbar, 2009), and natural enemies (ladybird beetles, earwigs, hover flies, ants, and wasps); hence, there is a need for alternatives, such as the use of host plant resistance mechanisms (HPRs).

Host plant resistance is the least disruptive and effective integrated pest management (IPM) strategy which can help to raise economic thresholds (ETs) and perhaps eliminate or postpone the need for insecticides. Painter (1951) and Smith (2005) outlined three main types of plant resistance, viz; tolerance, antibiosis and antixenosis. Antixenosis indicates distinct plant genotypes' preferences for eating or different ways of colonizing an area while antibiosis can alter the biology of insects and reduce their chances of surviving. Furthermore, plants that possess tolerance are able to fend off pest attacks (Painter, 1951). However, a variety of resistance categories can typically have an impact on pest populations (Hill, 2004; van Emden, 2017; Paudyal, 2019).

Dumont *et al.* (2023) stated that cultivar resistance is a very good option for long-term pest control. Resistance is characterized as constitutive and induced. To date, little work has been published on the physiological variation of the sugarcane germplasm to YSA. Some studies have been focussing on SCA in sorghum (Akbar, 2009; Mbulwe, 2017; Paudyal, 2019), a bias towards YSA and sugarcane crop. In other areas, White (1990) reported large variability in YSA fecundity and feeding preference among the six tested cultivars. The limitation of the aforementioned study is that there was no comprehensive use of modern phenotypic approaches that are not labor intensive such as using handheld spectrometry (SPAD), CIRAS-3 portable photosystem, atomic absorption spectrometer (AAS) and gas chromatography mass spectrometry (GC-MS). Furthermore, Akbar *et al.* (2010) used three sugarcane varieties and found that the duration and fecundity of YSA were variety dependent. This calls for continuous screening of sugarcane varieties due to evolution characteristics of aphids. Additionally, Nuessly *et al.* (2010) indicated a substantial variability in aphid resistance in the germplasm studied and reported a wide range of sensitivity to YSA feeding among 34 selected sugarcane varieties from the breeding program in Florida. Therefore, the study seeks to document commercial sugarcane

varieties according to three host-plant resistance mechanisms (antixenosis, antibiosis, and tolerance) based on morphology, physiology, and biochemistry response to YSA herbivory in irrigated cropping systems. Irrigation cropping system provide a conducive environment for aphids to persist because they can survive and reproduce on alternative hosts, such as weeds, or other crops within the irrigation even when the primary crop is not present.

1.2 Problem statement

Since the recent appearance of YSA in 2014 in sugarcane fields of Zimbabwe, there has not been any investigation into the potential heterogeneity in host plant resistance mechanisms seen in local materials. The identification of YSA-resistant sugarcane germplasm has advanced significantly, but breeding efforts require interdisciplinary understanding of the mechanisms behind YSA resistance. Understanding the mechanisms underlying resistance is essential to making the best use of the genetic resistance varieties that are currently accessible and to further our knowledge of plant–insect interactions for the development of novel insect control strategies.

Although the responses of plants to insect herbivory have been the subject of several researches, little is known about the biochemical and physiological response of the insect (Peterson and Higley, 2001). There has been little research on the mechanisms behind plant responses (Macedo et al., 2003). Currently, there is a glaring paucity of knowledge regarding the processes underlying the commercial sugarcane cultivars' host plant resistance to YSA intrusion in Zimbabwe's sugar sector. Moreover, leaf pubescence effects, phytochemical profiling of metabolites, biochemical response and physiological response of sugarcane varieties in response to *S. flava* herbivory is not known. Literature review studies by Wilson (2019) stressed the need to explore the growth and yield of \leq three months sugarcane in the presence of *S. flava* infestation. This calls the need for such studies for early yield predictions, hence, exposing a research gap for this study to address. The relationships between irrigated cropping systems and aphids have not been fully explored to document aphid-sugarcane interactions. Furthermore, the bulk of the studies on YSA have been done outside Zimbabwe in a different environment set up from the Lowveld conditions.

1.3 Justification of the study

In order to effectively regulate YSA in the sugar industry, pertinent data regarding host plant resistance mechanisms need to be produced. Since aphids can change their appearance at any time, reducing YSA damage requires the development of IPM solutions. As a result, continuous research is needed to develop sugarcane that is long-term resistant to YSA. This is so because plant resistance to insects is a financially viable pest control method; it relies on the plant's own self-defense mechanisms (Serba and Michaud, 2019). Furthermore, HPR is another low-cost and ecologically friendly method of controlling insect pests. Thus, this strategy promotes the growth of beneficial organisms like natural enemies by delaying the emergence of pesticide resistance in insect populations (Serba and Michaud, 2019). It is appropriate to find the extra sources of resistance in order to maintain and retain sugarcane resistance to YSA by building YSA resistant banks. The study's conclusions also intend to raise sugarcane productivity by incorporating resistant and tolerant sugarcane genes into the existing genotypes. Improving breeding through genetic identification of genes linked to sugarcane resistance to YSA is an arsenal for combating the current threat under irrigated cropping systems. The relationships between irrigated cropping systems and aphids are complex and multifaceted. Irrigation provides a consistent water supply which can lead to increase in aphid populations, promotes plant growth which attracts aphids, impact aphid populations in relation to crop type grown, support diverse range of aphid species etc. Therefore, the objective of the study is to categorize commercial sugarcane varieties according to three host-plant resistance mechanisms (antixenosis, antibiosis, and tolerance) based on morphology, physiology, and biochemistry response to YSA herbivory under irrigated cropping systems.

1.4 Objectives

1.4.1 Major objective

The main objective of this thesis was to:

Categorize commercial sugarcane varieties according to three host-plant resistance mechanisms (antixenosis, antibiosis, and tolerance) based on morphology, physiology, and biochemistry response to YSA herbivory in irrigated cropping systems.

1.4.2 Specific objectives

The specific objectives of this dissertation were to:

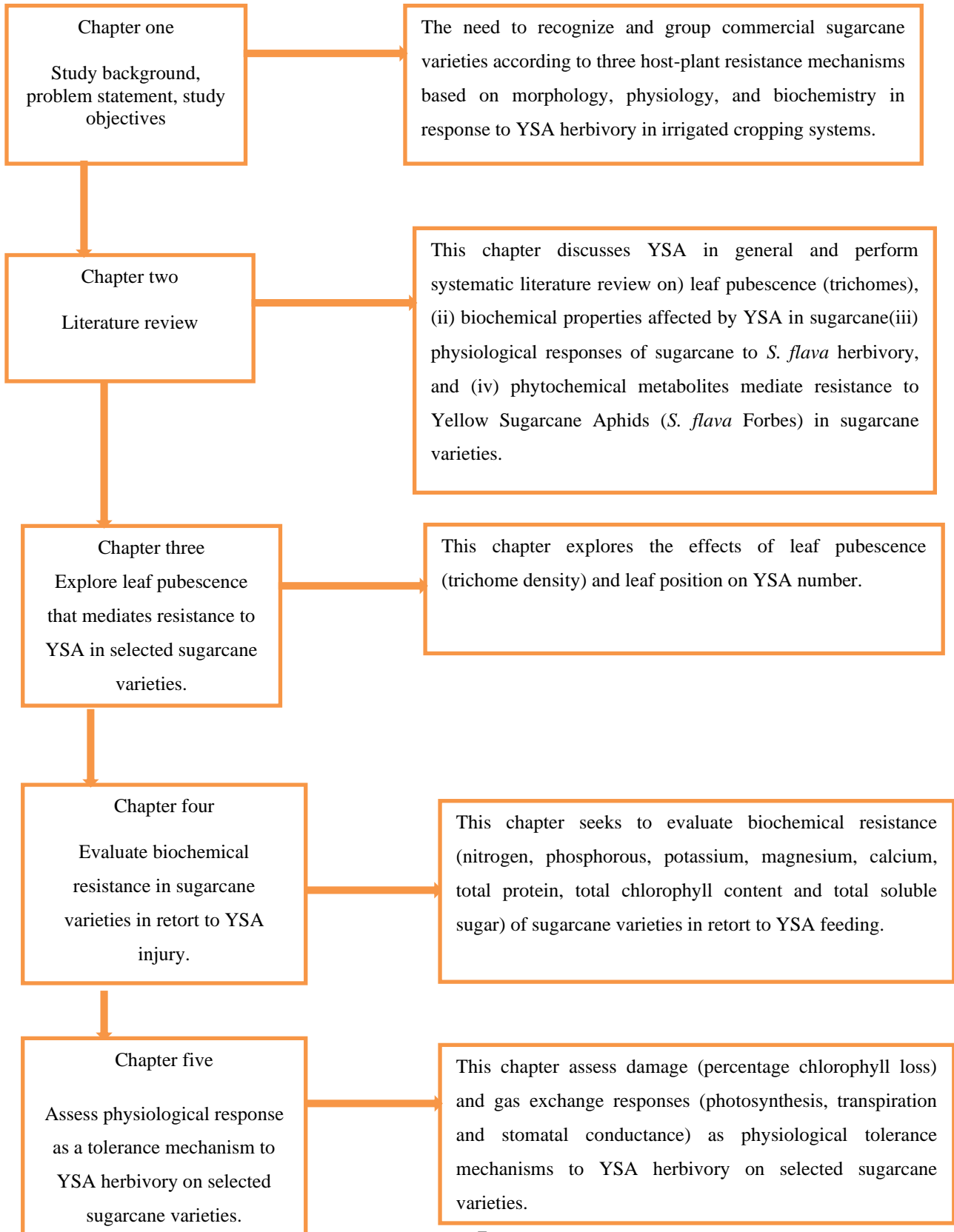
- i. Determine leaf pubescence that mediates resistance to YSA in selected sugarcane varieties.
- ii. Evaluate biochemical resistance in sugarcane varieties in retort to YSA injury.
- iii. Assess physiological response as a tolerance mechanism to YSA herbivory on selected sugarcane varieties.
- iv. Perform phytochemical profiling of secondary metabolites in sugarcane that confer resistance to YSA feeding.
- v. Reconnoiter plant resistant index (PRI) of tested sugarcane germplasm in response to YSA incursion.

1.5 Hypotheses

- i. H₀: Leaf pubescence does not mediate resistance to YSA in selected sugarcane varieties.
- ii. H₀: There is no physiological response as a tolerance mechanism to YSA feeding in selected sugarcane varieties.
- iii. H₀: There is no biochemical resistance in sugarcane varieties in retort to YSA feeding.
- iv. H₀: Secondary metabolites in sugarcane do not confer resistance to YSA feeding among the sugarcane varieties.
- v. H₀: Plant resistant indexes (PRI) of tested sugarcane germplasm do not vary in response to YSA incursion.

1.6 Thesis layout

The thesis comprises nine chapters (Figure 1).



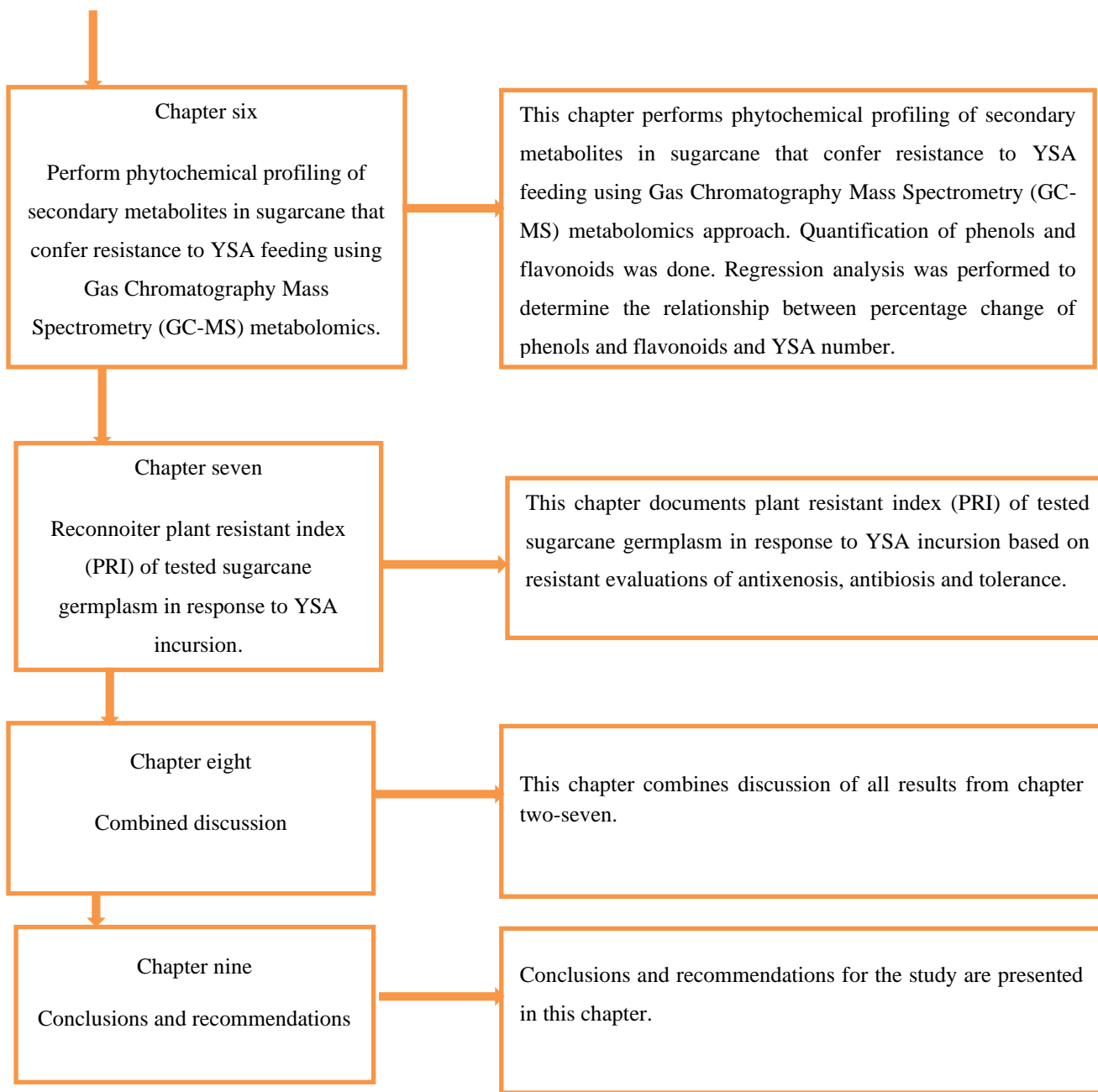


Figure 1: Thesis layout

References

- Ahad, A., Ferdaus, R. R., Ahsan, R., Hoque, M., and Islam, A. N. 2016. Survey of Major Insect Pests, Uses of Management Practices and Other Related Information of Sugarcane (*Saccharum officinarum* L.) Growers of the Northern Region of Bangladesh. *American Journal of Life Science*. 3(6): 408–411. <https://doi.org/10.11648/j.ajls.20150306.15>
- Akbar, W. 2009. Categorization and identification of mechanisms of sugarcane resistance to the sugarcane aphid (Hemiptera: Aphididae). Louisiana State University and Agricultural and Mechanical College.
- Akbar, W., Showler, A., Reagan, T., and White, W. 2010. Categorizing sugarcane cultivar resistance to the sugarcane aphid and yellow sugarcane aphid (Hemiptera: Aphididae). *Journal of Economic Entomology*. 103: 1431–1437.
- Bayoumay, M. H., Perumal, R., and Michaud, J. P. 2016. Comparative life histories of greenbugs and sugarcane aphids (Hemiptera: Aphididae) infesting susceptible and resistant sorghums. *Journal of Economic Entomology*. 109: 385-391.
- Blackman, R. L., and Eastop, V. F. 2007. “Taxonomic issues,” in *Aphids as Crop Pests*. Eds. van Emden, H. F. and Harrington, R. (Trowbridge, UK: CABI), 1–29.
- Blackman, R. L., and Eastop, V. F. 2000. *Aphids on the world's crops: an identification and information guide*, 2nd edn. Wiley, New York, p 2, 475 p.
- Chandiposha, M. 2013. Potential impact of climate change in sugarcane and mitigation strategies in Zimbabwe. *African Journal of Agricultural Research*. 8(3): 2814-2818.
- Clowes, M. J., and Breakwell, W. L. 1998. *Zimbabwe Sugarcane Production Manual*. Zimbabwe Sugar Association, Chiredzi, Zimbabwe.
- Dixon, A. F. 2012. *Aphid ecology an optimization approach*. Springer Science and Business Media.
- Dumont, T., Marodon, J., Nibouche, S., Hoarau, J. Y., and Barau, L. 2023. Genetic variability of sugarcane varieties for resistance to *Sipha flava*. In *Proceedings of the International Society of Sugar Cane Technologists*. 31: 628-636.

Esterhuzein, D. 2012. The report focuses on the production and usage of sugar in Zimbabwe. USDA Foreign Agricultural Service, United States of America.

FAOSTAT. 2018. Sugarcane. <http://www.fao.org/faostat/en/#search/Sugar%20cane>. Accessed on 5 March 2022

Ferreira de Souza, M. 2018. "Impact of Temperature, Plant Species, and Sorghum Cultivar on the Population Dynamics of *Melanaphis sacchari*". 2018. *LSU Doctoral Dissertations*. 4652. <https://digitalcommons.lsu.edu/gradschooldissertations/4652>

Gaud, S. M., Martorell, L. F., and Robles, R. B. 1965. Notes on the biology and control of the yellow aphid of sugarcane, *Sipha flava* (Forbes), in Puerto Rico. *Proc Int Soc Sug Cane Technol*. 12: 1307-1320.

Hall, D. G. 2001. Notes on the yellow sugarcane aphid *Sipha flava* (Homoptera: Aphididae) and the lady beetle *Diomus terminatus* (Coleoptera: Coccinellidae) in Florida. *J Am Soc Sug Cane Technol*. 21: 21-29.

Hentz, M., and Nuessly, G. 2004. Development, longevity, and fecundity of *Sipha flava* (Homoptera: Aphididae) feeding on *Sorghum bicolor*. *Environmental entomology*. 33(3): 546-553.

Hess, T. M., Sumberg, J., Biggs, T., Georgescu, M., Haro-Monteagudo, D., Jewitt, G., Osdogan, M., Marshall, M., Thenkabali, P., Daccache, A., Marin, F., and Knox, J. W. 2016. A sweet deal? Sugarcane, water and agricultural transformation in Sub-Saharan Africa. *Global Environmental Change*. 39: 181–194.

Hill, C. B., Li, Y., and Hartman, G. L. 2004. Resistance to the soybean aphid in soybean germplasm. *J. Econ. Entomol.* 97(3): 1071–1077.

Hinson, P. O. 2017. Effect of temperature on the development of sugarcane aphid, *Melanaphis sacchari*, on sorghum. A Thesis Submitted in Partial Fulfilment of the Requirement for the Degree Masters of Science in West Texas A & M University Canyon, Texas, USA.

Hussnain, Z., Naheed, A., and Rizwana, S. 1997. Biocontrol of Insect Pests of Sugarcane (*Saccharum sp.*). Jhang, PAKISTAN. insecticide test, 2000. *Arthro. Manage. Tests*. 26: F108.

Joshi, S., and Viraktamath, C. A. 2004. The sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner (Hemiptera: Aphididae): its biology, pest status and control. *Current science*. 87 (3): 307-316.

Macedo, T. B. 2003. Physiological responses of plants to piercing-sucking arthropods. The University of Nebraska-Lincoln.

Madiope, K. W., Keeping, M. G., and Fourie, D. V. 2021. Quantifying yield loss to yellow sugarcane aphid in potted sugarcane. Short non-refereed paper. Conference Paper in South African Journal of Education, August 2021.

Mary, J., and Sujata, M. 2016. An economic analysis of area, production, yield and export of Sugarcane in India. *International Journal of Research in Management, Social Science and Technology*: 2939(16): 1–17.

Mbulwe, L. 2017. Evaluating host-plant resistance against sugarcane aphid (*Melanaphis sacchari* (Zehntner)) in sorghum (*Sorghum bicolor* (L.) Moench). A Dissertation Submitted to the Office of Graduate and Professional Studies of Texas A & M University in partial fulfilment of the requirements for the degree of doctor of philosophy.

Moobi, M., and Woody, K. 2022. United States Department of Agriculture, Foreign Agricultural Services. Zimbabwe Sugar Annual Report. 10 May 2023. <https://apps.fas.usda.gov/newgainapi/api/Report/DownloadReportByFileName?fileName=Zimbabwe%20AnnualReport2023-0002.pdf>

Mutongi, W., Mabveni, A. R. S., and Makaza, K. 2021. The efficacy of *Datura ferox* (fierce thorn apple) leaf extracts against the Yellow Sugarcane Aphid (*Sipha flava*) in the Zimbabwe Sugar Industry. In *Proceedings of the 1st International Electronic Conference on Entomology, 2021 July* (pp. 1-15).

Mutonyi, J., and Babikha, S. J. 2019. The occurrence of sugarcane yellow aphid (*Sipha flava* (Homoptera: Aphididae) in Kakamega North Sub-County, Kenya. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*. 12 (6): 34-38.

Nuessly, G. S. 2005. Featured Creatures: Yellow Sugarcane Aphid; publication #EENY-354; University of Florida: Gainesville, FL, USA; Available online:

<http://entnemdept.ufl.edu/creatures/field/bugs/yellowsugarcaneaphid.htm> (accessed on 16 January 2022).

Nuessly, G. S., Hentz, M. G., and Gilbert, R. A. 2010. Susceptibility of stage IV canal point (CP) sugarcane clones to yellow sugarcane aphid (*Sipha flava* (Forbes) feeding damage. *J Am Soc Sug Cane Technol.* 30: 104-114.

OECD-FAO. 2022. OECD-FAO Agricultural Outlook 2022-2031. <https://www.oecd-ilibrary.org/agriculture-and-food/oecd-fao-agricultural-outlook-2022-2031f1b0b29c-en>

Painter, R. H. 1951. Insect resistance in crop plants. The McMillan Co. New York, pp. 520.

Paudyal, S. 2019. Host plant resistance to the sugarcane aphid (Hemiptera: Aphididae). A Dissertation Submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfillment of the requirements for the Degree of Doctor of philosophy.

Peterson. R. K. D., and Higley. L. G. 2001. Illuminating the black box: the relationship between injury and yield, pp. 1–12. *In* R.K.D. Peterson and L. G. Higley [eds], Biotic stress and yield loss. CRC, Boca Raton, FL.

Posey, F. R., Reagan, T. E., and Coburn, G. E. 2001. Sugarcane aphid control-small plot insecticide test, 2000. *Arthro. Manage. Tests.* 26: F108.

Quisenberry, S. S., and Ni, X. 2007. 13 Feeding Injury. Aphids as crop pests. 331.

Reagan, T. E. 1994. Pest Status, Biology and control measures of sugar cane sap suckers in the North American sugar growing regions. pp 123-124 *In*: AJM Carnegie and DE Conlong (Eds), *Biology, Pest Status and Control Measure Relationships of Sugar Cane Insect Pests.*

Serba, D. D., and Michaud, J. P. 2019. Sugarcane Aphid Resistance in Pearl Millet. *Kansas Agricultural Experiment Station Research Reports.* 5(6): 23.

Scoones, I., Mavedzenge, B., and Murimbarimba, F. 2017. Sugar, People and Politics in Zimbabwe's Lowveld. *Journal of Southern African Studies.* 43(3): 567-584, doi: 10.1080/03057070.2016.1187972

- Shabani, A. A., Venkataramana, P., and Mbega, E. R. 2020. Infestation Status and Farmers' Knowledge on Yellow Sugarcane Aphid (*Sipha flava*) in Tanzania. *International Journal of Biosciences*. 17 (6): 227-240.
- Smith, C. M. 2005. Plant resistance to arthropods – molecular and conventional approaches. Springer, The Netherlands. 423 pp.
- Srikanth, N. M. J., Asokan, B. S. S., and Goud, N. K. Y. 2008. Assessment of woolly aphid impact on growth, yield and quality parameters of sugarcane. *Sugar Technology*. 10(2): 143–149.
- Tripathi, G. M. 1995. Record of parasite and predator complex of sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner in Nagaland. *Indian Sugar*. 44: 883–885.
- Sikuka, W. 2021. United States Department of Agriculture, Foreign Agricultural Services. Zimbabwe Sugar Annual Report. 15 April 2021. <https://www.fas.usda.gov/data/zimbabwe-sugar-annual-5>
- van Emden, H. F. 2017. Host-plant resistance, pp. 515–527. In H. F. van Emden and R. Harrington (eds.), *Aphid as a Crop Pest*. CAB International, Boston, MA.
- White, W. H. 1990. Yellow Sugarcane Aphid (Homoptera: Aphididae) resistance mechanisms in selected sugarcane cultivars. *J Econ Ent*. 83(5): 2111-2114.
- White, W. H., Reagan, T. E., and Hall, D. G. 2001. *Melanaphis sacchari* (Homoptera: Aphididae), A sugarcane Pest New to Louisiana. *Florida Entomologists*. 84(3): 435–436.
- Wilson, B. E. 2019. Hemipteran Pests of Sugarcane in North America. *Insects*. 10 (107): 1-15. doi:10.3390/insects10040107
- Wilson, B. E., VanWeelden, M. T., Beuzelin, J. M., and Mészáros, A. 2013. Assessment of insecticides against a complex of hemipteran pests in sugarcane. *Arthrop. Manag. Tests*. 38: F73.

CHAPTER TWO: Review of literature

A Multi-disciplinary Approach to Understanding Host Plant Resistance Mechanisms (HPRs) of sugarcane varieties to yellow sugarcane aphid (YSA) (*Sipha flava*) herbivory: A systematic methodical review.

Abstract

The Yellow Sugarcane Aphid (YSA) (*Sipha flava*) has become a threat to sugarcane fields worldwide because of its pre-harvest damage and stunting of the plant, which results in huge economic losses. We conducted a systematic literature search to understand HPRs of sugarcane to YSA from Google Scholar (<https://scholar.google.com>), SCOPUS (<https://www.scopus.com>), Web of Science (<https://www.webofknowledge.com>) and Sciencedirect (<https://sciencedirect.com>) and selected 992 papers from year 1884 up to 2023. The hunt for literature commenced in 1884, the year Forbes identified YSA, in order to fully capitalize on the necessity of this investigation. The search parameters included the following terms ("yellow sugarcane aphid" OR "*Sipha flava*") AND ("Host plant resistance mechanism") AND ("sugarcane" "*Saccharum officinarum*"). Book chapters, reviews, and meta-analyses were not included in the analyses. Other prerequisites for suitability for inclusion in the review were studies including both SCA and YSA studies involving cereal grasses or sugarcane. Seventy-eight articles were included in the study. The study aimed to assemble literature on *S. flava* to address the four review physiology objectives: (i) leaf pubescence (trichomes) that mediate resistance to YSA in sugarcane varieties (ii) biochemical properties affected by *S. flava* in sugarcane (iii) physiological responses of sugarcane to *S. flava* herbivory (iv) phytochemical profiling of metabolites that confer resistance to *S. flava* in sugarcane. Most of the work was biased towards other; aphid species, insect arthropods and crops other than *S. flava* in sugarcane. Using YSA resistant sugarcane varieties in combination with other management practices is a promising management strategy for *S. flava* control. Therefore, there is a need for studies that address the aforementioned research objectives to incorporate resistance into existing Integrated YSA management strategies. By integrating these disciplines, researchers can gain a comprehensive understanding of the complex mechanisms underlying sugarcane resistance to YSA, ultimately leading to development of more resilient and sustainable sugarcane varieties.

Key words: Host plant resistance, leaf pubescence, trichomes, arthropods, *S. flava*.

2.1 Introduction

It is the essence of this Systematic Literature Review (SLR) to understand the intraspecific variations in the morphological, physiological, and biochemical nature of sugarcane species multi-disciplinary, which will aid in Yellow Sugarcane Aphid-resistant breeding strategies for developing resistant varieties. This review addresses whether there are any variations with regards to:

- i) leaf pubescence (trichomes),
- ii) biochemical properties affected by *S. flava* in sugarcane,
- iii) physiological responses of sugarcane to *S. flava* herbivory, and
- iv) phytochemical metabolites mediate resistance to YSA in sugarcane varieties.

This review is of paramount importance in Zimbabwe and other African sugar producing countries, in that, it seeks to integrate host plant resistance in existing breeding strategies to curb YSA challenge. Furthermore, in most African countries, there is an over reliance on chemical use, resulting in resurgence and aphid resistance biotypes. Moreover, for example, in Zimbabwe, the out-growers scheme has more than 1000 farmers, hence indicating their interest in enhancing sugarcane production. However, most of these farmers repeatedly use synthetic chemicals due to the ability of the sugarcane to ratoon. This has exacerbated the effects of climate change and environmental pollution arising from the use of pyrethroids, organophosphates and neocotinoids to manage aphids. Some of these sugarcane smallholder farmers in Africa are poorly resourced, hence, purchasing of insecticides becomes costly to them. Therefore, this review is of great significance, since it identifies the gaps in literature which need to be addressed by this study. Findings will aid agricultural policy makers and farmers at large, as it seeks to develop a permanent solution of sugarcane varietal resistance to YSA. By and large, the best alternative practices are reviewed so that farmers are sensitized with sustainable options so as to overcome YSA, thus, reducing the cyclic application of synthetic insecticides.

2.2 Nomenclature of yellow sugarcane aphid

Yellow sugarcane aphid, also referred to as the *S. flava*, and was first identified by Forbes in 1884 in Illinois under the name *Sorghum aphid*. Later, Forbes (1884) placed the pest in the genus Chaitophorus, and Davis (1909) positioned it in the genus *Sipha*. This genus contains 12 species of grass feeders, at least four of which have originated in America and northern Mexico

(Hernández-Castellano and Pérez Hidalgo, 2014). The remaining eight species are found in the sub-genus *Rungsia*. Characteristics of this genus include a short, triangular last rostral segment, compound eyes (not stalked), five segmented antennae, and stump-shaped, possibly obtuse. According to theories propounded by Wieczorek (2010), Blackman and Eastop (2006), the body length of *Sipha* is double that of its width, and its apterous (wingless) individuals have rounded anal plates, spiky bristles, and are highly pigmented and strongly sclerotized. Although a few have been reported in the Cyperaceae family, the *Sipha* species described are thought to be both holocyclic and anholocyclic, growing on host plants of the grass family. Ten species have been reported from Europe, for both *Sipha* (three species), and *Rungsia* (seven species) (Holman, 2009; Wieczorek, 2010; Nieto nafría *et al.*, 2013). Only one species (*S. flava*) has been identified in African countries like Zimbabwe (Adbelmajid, 2008; Way *et al.*, 2015; Conlong and Way, 2014a; Way *et al.*, 2014; Mutonyi and Babikha, 2019; January *et al.*, 2020). In the Iberian Peninsula, the following three species have been identified: *Sipha (Rungsia) maydis* Passerini Kaltenbach, *Sipha (Rungsia) elegans* Del Guercio, and *Sipha (S.) glyceriae* (Nieto nafría and Mier durante, 1998).

2.3 Taxonomy of *S. flava*

YSA taxonomy is stated in Table 2.1 below.

Table 2.1: Taxonomy of YSA

Kingdom:	Insecta
Phylum:	Arthropoda
Class:	Insecta
Order:	Hemiptera
Suborder:	Stemorrhyncha
Family:	Aphididae
Genus:	<i>Sipha</i>
Species:	<i>S. flava</i>

2.4 Geographical distribution of yellow sugarcane aphid

Sorghum-growing temperate and subtropical areas of North America are thought to be the YSA's source of origin (Reagan, 1994; Blackman and Eastop, 2006; Wilson, 2019). Wieczorek (2010) and Blackman and Eastop (1984, 2006) noted that this pest moved from South and Central America to the Caribbean and Hawaiian Islands, where it became a serious pest with significant economic implications (Kindler and Dalrymple, 1999). High temperatures experienced in the Lowveld of Zimbabwe have made YSA to be a resident pest in the sugar industry. Furthermore, Wieczorek (2010) discovered that *S. flava* is widespread in the Neotropical area and was initially discovered in Europe in 1979 (Sousa-Silva and Ilharco, 1995; Coeur d'acier *et al.*, 2010; Hernández-Castellano and Pérez Hidalgo, 2014). The earliest reports of this pest in Africa date back to November 2006, and they came from Morocco in North Africa (Adbelmajid, 2008). It then made its way to southern Africa (South Africa) in May 2013 (Conlong and Way, 2014a; Way *et al.*, 2015). Later on, it subsequently spread to other regions that produced sugarcane, including Zambia, Zimbabwe, Malawi, Swaziland, Kenya in 2016 (Mutonyi and Babikha, 2019), and Tanzania in 2019 (January *et al.*, 2020). Figure 2.1 shows worldwide geographical distribution of YSA while Table 2.2 shows first record of YSA in African countries.

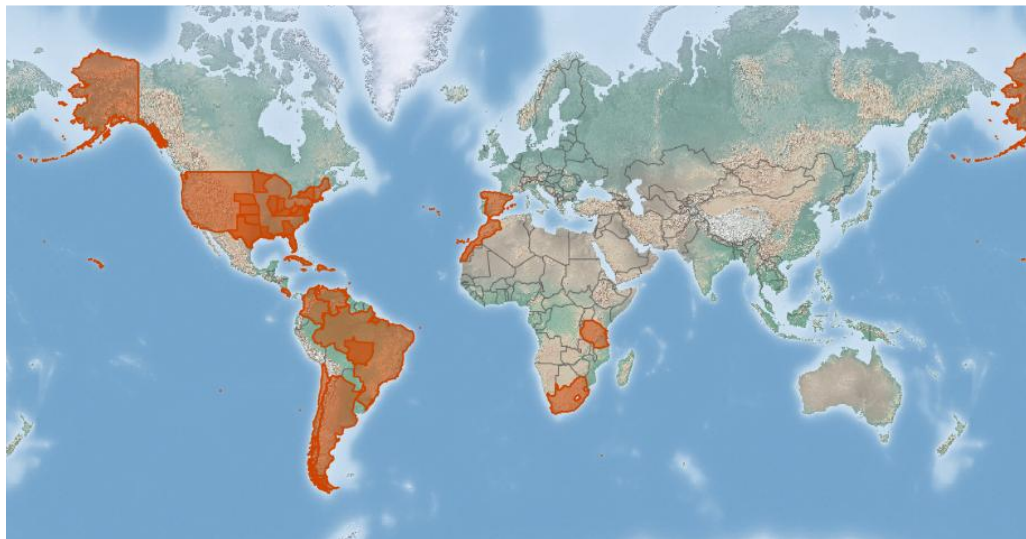


Figure 2.1: Geographical distribution of *S. flava* indicated by the area marked in red.
Source: CABI (2021)

<https://plantwisepiusknowledgebank.org/doi/10.1079/PWKB.Species.50170>

Table 2.2: First record of *S. flava* in African countries

Country	Year reported	References
Morocco	2006	Adbelmajid, 2008
South Africa	2013	Conlong and Way, 2014; Way <i>et al.</i> , 2014, 2015 Zimbabwe Sugar Association Experiment Station (ZSAES), unpublished;
Zimbabwe	2014	Way <i>et al.</i> , 2014
Swaziland	After 2013	Conlong and Way, 2014; Way <i>et al.</i> , 2014
Malawi	After 2013	Conlong and Way, 2014; Way <i>et al.</i> , 2014
Zambia	After 2013	Conlong and Way, 2014; Way <i>et al.</i> , 2014
Kenya	2016	KARLO-SRI, 2018; Mutonyi and Babikha, 2019, Shabani <i>et al.</i> , 2020
Tanzania	2019	January <i>et al.</i> , 2020

A synthesis made by authors based on literature mentioned in the table: Source: This study

2.5 Description, biology and ecology of yellow sugarcane aphid

Wilson (2019) opined that YSA is light in color, less than 2 mm in diameter and have short, stiff hair covering their head, thorax, and abdomen. The size of siphuncular cornicles is smaller than that of other aphid species. The vivid yellow abdomen of apterous (wingless) species can be used to identify alate (winged) types. Blackman and Eastop (2006) indicated that wingless are straw yellow to bright yellow or perhaps green. They have some knobbed cauda, many thorn-like hairs arranged in three rows on the back and antennae (Wieczorek, 2010; Hernández-Castellano, and Pérez Hidalgo, 2014).

Two modes of reproduction are exhibited by YSA: non-mating (parthenogenetically) in warmer areas (Halbert *et al.*, 2013) and mating, in which live individuals are generated and females mate with males in the winter when low temperatures cause oviparity (Nuessly, 2005, Way *et al.*, 2015). Way *et al.* (2015) emphasized that there are four instar stages in the maturation of nymphs into adults. Hentz and Nuessly (2004) found that on sorghum, one to five nymphs are formed daily for eight to 15 days, after which they mature into adults. On sugarcane, it takes between 18 to 22 days for nymphs to mature into adults. A close association between YSA and SCA has

been reported by Nuessly (2005). Figure 2.2 shows YSA colonies feeding on the underside of sugarcane.



Figure 2.2: Colonies of *S. flava*, nymphs and adults underside yellowing/reddening of sugarcane leaves caused by *S. flava* feeding. Source (This study)

2.6 Damage to sugarcane and crop economic losses

YSA is a piercing and sap sucking pest that feeds preferentially on the bottom of leaves along the parallel veins of the sugarcane crop (Way *et al.*, 2014). Honeydew is produced, which promotes the development of sooty mould (Nuessly and Hentz, 2002a). However, in Zimbabwe, the presence of sooty mould during feeding has not been recorded. Reports by Nuessly and Hentz (2002a) and Wilson (2019) indicates that heavy infestation results in leaves turning yellow to red followed by leaf senescence and death of the entire stem. According to Mutonyi and Babikha (2019), temperature and host plants have an impact on the color change of leaves from yellow to red. Early-stage plant growth damages include those that have a major effect on sugarcane growth and tillering loss (Hall, 2001). Reagan (1994), Hentz *et al.* (2004), Nuessly (2005), Nuessly *et al.* (2010), and Wilson (2019) hypothesized that feeding by YSA can cause chlorosis losses of up to 6-19 % when the crop is less than three months of age. Gaud *et al.* (1965) reported an estimated loss of one million US dollars due to an outbreak of YSA in Puerto Rico in 1964. However, the statistics mentioned above are outdated, indicating the necessity for more recent research.

Madiope *et al.* (2021) noted that the unpredictable and patchy insect infestation made it impossible to evaluate yield losses in the field trials conducted in 2014 in the KwaZulu-Natal, South Africa. Furthermore, Madiope *et al.* (2021) provided evidence of the detrimental effects of YSA on southern African sugarcane as sucrose losses of up to 19 % were observed. Reports indicate that, YSA is a vector of the sugarcane mosaic potyvirus (Blackman and Eastop, 2000). However, the disease has not been reported in Zimbabwe. Figure 2.3 shows healthy sugarcane free from YSA infestation while Figure 2.4 shows sugarcane crop damaged by YSA.



Figure 2.3: A healthy young sugarcane crop free from *S. flava* infestation. Source (This study)



Figure 2.4: Damage to sugarcane crop in field, by *S. flava*. Source (This study)

2.7 Host plant range of the yellow sugarcane aphid

YSA is believed to be from sorghum (*Sorghum bicolor* L.) in Illinois, as reported by Forbes in 1884 (Forbes, 1884; Reagan, 1994). Worldwide, hosts for *S. flava* include crops and some cereal pasture grasses (Blackman and Eastop, 1984; Nuessly, 2005; Way et al., 2014). Further reports were in Commeliaceae (Kindler and Dalrymple, 1999). YSA was collected from *S. officinarum*, *S. bicolor*, and *Echinochloa colona* (L.) Link., *Tragus berteronianus* Schult., *D. ciliaris* (Retz.) Koeler (Way et al., 2014). The host range in Zimbabwe only confirmed sugarcane, sorghum, maize (*Zea mays* L.) and guinea grass (*Panicum maximum* Jacq) (Figure 2.5), therefore, the need to research on other host plants as the pest has become established.



Figure 2.5: Guinea grass (*Panicum maximum*) in front and sugarcane crop behind. Source (This study)

2.8 Management options for YSA

2.8.1 Chemical

Numerous discussions have been sparked by the use of synthetic pesticides, including those of the disruption of natural enemies, the resurgence of pests, and pollution. Several pesticides have been approved for use against YSA and SCA (Posey *et al.*, 2001). Wilson (2013), also recommended products, such as flupyridifuron, sulfoxaflor, and imidacloprid. Moreover, Wilson (2019) highlighted that certain pyrethroids are available for the management of YSA although Mutonyi and Babikha (2019) signposted that pyrethroids have the potential to disturb natural enemy populations, leading to increased infestations in the weeks that follow application. In Zimbabwe, Allice and Actara are currently recommended for use in the sugar industry. Continued use of organophosphate, pyrethroid, and neonicotinoid class insecticides can cause aphids to become resistant, although no studies have been conducted in Zimbabwe's sugar industry. However, Wilson (2019) emphasized the lack of evidence for insecticides in yield improvement. This calls for an integrated strategy that takes host plant resistance into account.

2.8.2 Biological

This is an approach of pest management that makes use of pathogens, parasitoids, or natural predators. Mbulwe (2017) attested to the method's mediocre success because so few farmers had

adopted it. According to Way *et al.* (2015), *S. flava* populations in South Africa are being lowered by the presence of natural enemies (ladybird species, hover flies, spiders, earwigs and ants). The application of biological agents and natural enemies to monitor the dynamics of the YSA population is currently understudied in Zimbabwe. This necessitates research on the main natural predators in fields infested with aphids. Aphid-infested areas in the sugarcane sector have been discovered to host fire ants and lady bird species (Coccinellidae) (ZSAES, unpublished). Figure 2.6a and 2.6b shows ladybird beetles, Figure 2.7 shows ladybird larva, and Figure 2.8 shows fire ants.

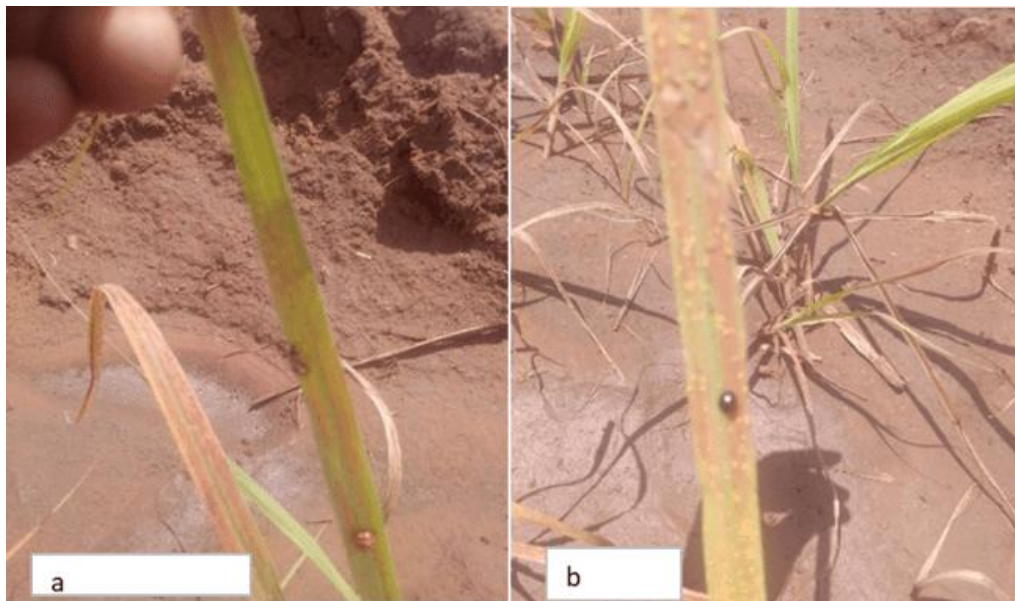


Figure 2.6: (a) Yellow and black speckled ladybird beetle on leaves infested by Yellow Sugarcane Aphids, *S. flava* (Forbes), on sugarcane (b) Black ladybird beetle on leaves infested by Yellow Sugarcane Aphids, *S. flava* (Forbes), on sugarcane. Source (This study).



Figure 2.7: Ladybird beetle larva. Source (This study).



Figure 2.8: Ants. Source (This study).

2.8.3 Cultural

Field hygiene and weed control are two crucial elements of cultural YSA management. It has been demonstrated that eliminating volunteer crops and burying residues can reduce aphid populations (Abawi and Widmer, 2000). Aphid numbers have been reported to decrease when robust, aphid-resistant cultivars are planted (Buntin, 2009).

2.8.4 Host plant resistance

Heritable characteristics of a plant that influence the amount of damage an insect causes is referred to as host plant resistance (Painter, 1951). It may be induced by eating insects or constitutive (Figure 2.9) or induced (Figure 2.10).

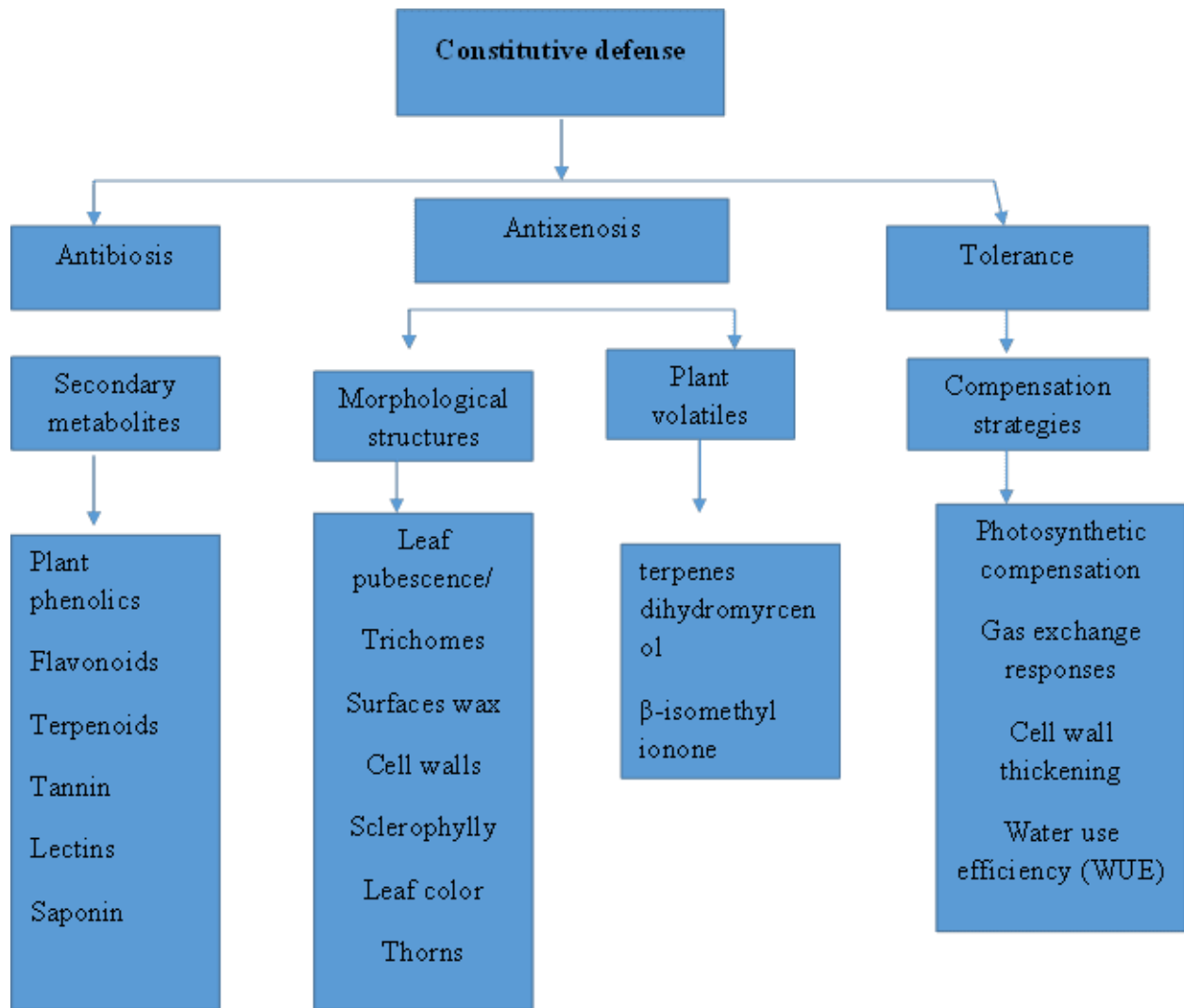


Figure 2.9: Constitutive defense in response to aphids in sugarcane

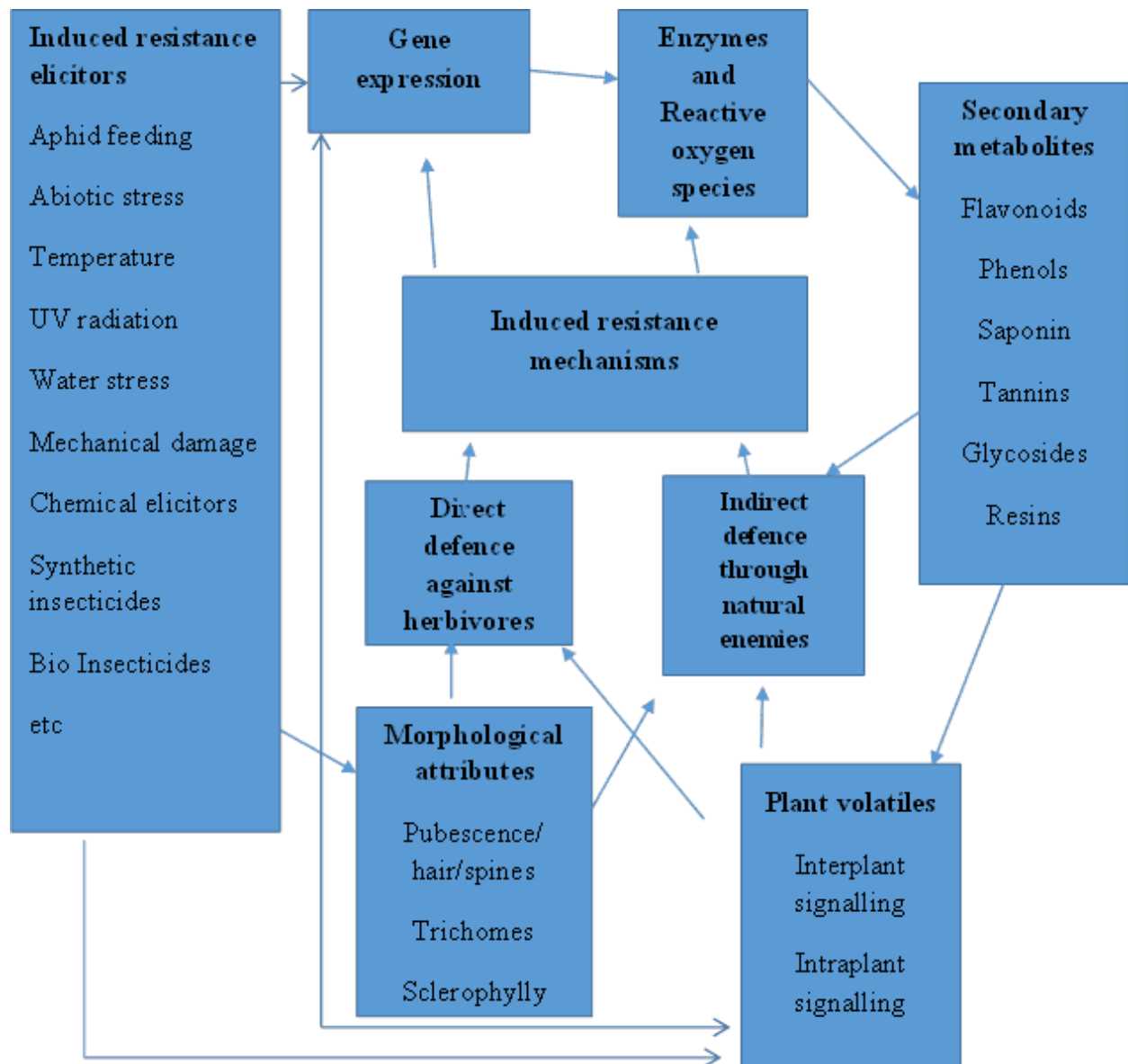


Figure 2.10: Mechanism of induced resistance in sugarcane. Source: Modified from War et al. (2012).

Three distinct mechanisms of plant resistance: tolerance, antixenosis, and antibiosis have been documented (Painter, 1951). According to several studies (Gallun *et al.*, 1966; Roberts *et al.*, 1979; Roberts and Foster, 1983, Sosa, 1990; White, 1990; Nuessly, 2005; Nuessly *et al.*, 2010), sugarcane varieties that are pubescent (trichome dense) provide resistance against damage from *S. flava*, a concept known as antixenosis. Furthermore, when *S. flava* feeds on resistant cultivars, less chlorophyll is lost (Akbar *et al.*, 2010). Conclusions were made by Webster *et al.* (1994) that pubescent wheat cultivars reduced YSA and greenbug (*Schizaphis graminum*) reproduction and preference. Resistance to YSA in sugarcane may result from structural or biochemical traits.

High nutritious sorghum cultivars have been observed to attract aphid populations' more than lower nutritional varieties (Akbar *et al.*, 2011). Moreover, it has been observed that plants with greater nitrogen levels are more vulnerable to outbreaks of aphids (Hsieh, 1988). Alternatively, cyanogenic glucosides (CNGlcs) and other phenolic compounds found in plants such as sorghum have been proposed to induce defense against insect pests (Kahn *et al.*, 1997; Fürstenberg-Hägg *et al.*, 2013). Physical barriers such as wax and trichomes are associated with antixenosis (nonpreference) (Singh *et al.*, 2004). Host-plant resistance is another important strategy for pest management in addition to chemical, biological, and cultural control (Peters and Starks, 1990; Knutson *et al.*, 2016; Mbulwe, 2017).

In contrast to the earlier discussions, Starks and Merkle (1977) observed that leaf pubescence might not consistently serve as a deterrent to insect pests and all aphid species (Webster *et al.*, 1994). However, the sugar industry has not assessed the role of gas exchange, secondary metabolites, biochemical processes, and leaf trichomes in response to feeding by *S. flava*. Therefore, there is a need for studies that can fill such gaps as this can add more knowledge on the categorization of host plant resistance mechanisms to YSA herbivory. The suggested model for incorporating host plant resistance into IPM is shown in Figure 2.11.

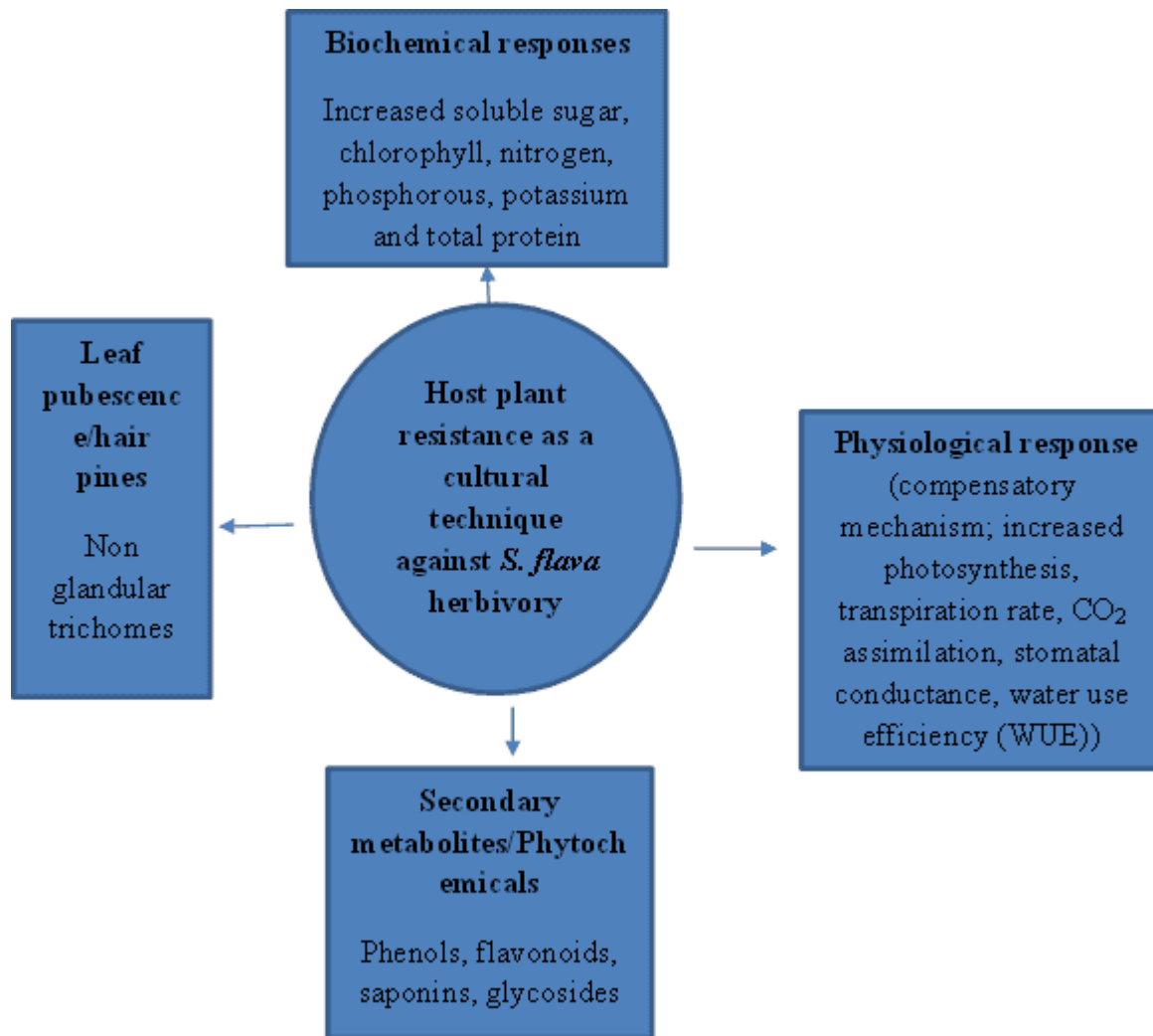


Figure 2.11: Proposed model for host plant resistance as a cultural control technique.
Source (This study)

2.9 Materials and methods

2.9.1 Selection of studies

A SLR was performed according to the PRISMA 2020 updated guidelines for systematic reviews (Page *et al.*, 2020). Primary studies examining host plant resistance mechanisms of cereals/grasses to yellow sugarcane cane cultivars were retrieved from the following databases: Google Scholar (<https://scholar.google.com>), SCOPUS (<https://www.scopus.com>), Web of Science (<https://www.webofknowledge.com>) and Scimedirect (<https://sciencedirect.com>). Candidate publications for systematic literature review were selected in two phases. First potential publications in the selected databases from August 2023 to November 2023 were selected based on primary search. The search parameters included the following terms ("yellow sugarcane aphid" OR "*Sipha flava*") AND ("Host plant resistance mechanism") AND ("sugarcane" "*Saccharum officinarum*"). "The searches were performed within **Topic** criteria that search similarities in the **Article Title, abstract, keyword and keyword plus** using the above-mentioned restriction ranges.

Reverse snowballing was used to perform a secondary search in which papers from the study reference list were chosen from the initial search (Alfayez *et al.*, 2020; Amarathunga *et al.*, 2021; Raharjana *et al.*, 2021). For the primary search, a set of keywords and phrases were defined to cover the objective of the SLR. The fundamental search query was then created by combining the chosen keywords using "AND" and "OR" links. The primary study articles were limited to the years 1884–2023. Book chapters, reviews, and meta-analyses were not included in the analyses. The following objectives guided the meticulous screening process for the papers:

- (i) phytochemical profiling of metabolites that confer resistance to *S. flava* in sugarcane;
- (ii) biochemical properties affected by *S. flava* in sugarcane;
- (iii) physiological responses of sugarcane to *S. flava* herbivory; and
- (iv) leaf pubescence (trichomes) that mediate resistance to YSA in sugarcane varieties.

Other prerequisites for suitability for inclusion in the review were studies including both SCA and YSA studies involving cereal grasses or sugarcane.

2.10 Results

2.10.1 Host plant resistance mechanism of grasses/ cereals to yellow sugarcane aphids

Seventy-eight full texts articles were selected for the review after removal of duplicate and disallowed articles (Figure 2.12 and Table 2.3). The three mechanisms of resistance namely tolerance, antixenosis, and antibiosis have been directed to the insect, whereas tolerance has been directed to the response of the plant to insect damage.

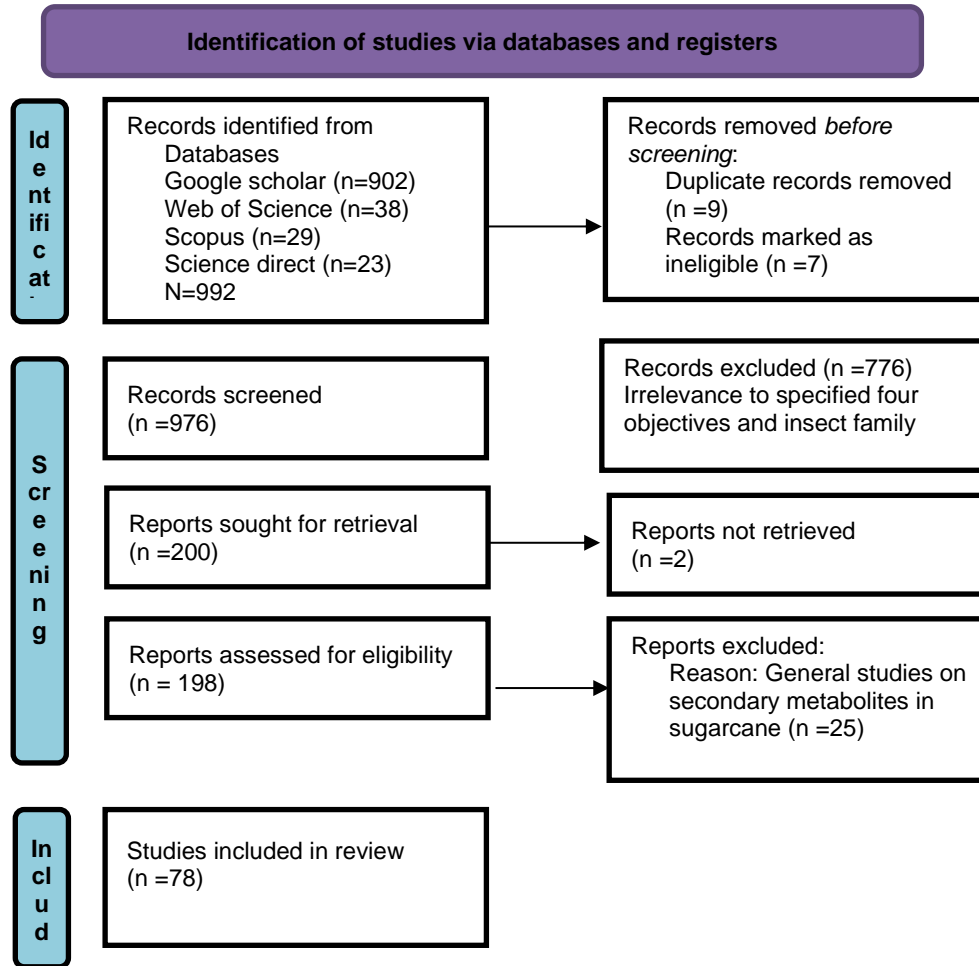


Figure 2.12: PRISMA 2020 flow diagram: Adapted and modified (Page *et al.*, 2020).

Table 2.3: Studies that have mentioned host plant resistance mechanisms in response to aphid herbivory

Host plant	Aphid species	Topic	References
Sugarcane	<i>Sipha flava</i>	Categorization and identification of mechanisms of sugarcane resistance to the sugarcane aphid (Hemiptera: Aphididae)	Akbar (2009)
Sugarcane	<i>Melanaphis sacchari</i> , <i>Sipha flava</i>	Evaluation of aphid resistance among sugarcane cultivars in Louisiana	Akbar <i>et al.</i> , 2011
Sugarcane	<i>Melanaphis sacchari</i> , <i>Sipha flava</i>	Categorizing sugarcane cultivar resistance to the sugarcane aphid and yellow sugarcane aphid (Hemiptera: Aphididae)	Akbar <i>et al.</i> , 2010
Sorghum	<i>Melanaphis sacchari</i>	Sugarcane aphid (Hemiptera: Aphididae): host range and sorghum resistance including cross-resistance from greenbug sources	Armstrong <i>et al.</i> , 2015
Sorghum	<i>Melanaphis sacchari</i>	Bio-ecology and management of the sorghum aphid, <i>Melanaphis sacchari</i> .	Balikai (2001)
Sorghum	<i>Sipha flava</i>	Relationships of yellow sugarcane aphid (Homoptera: Aphididae) density to sorghum damage	Breen and Teetes (1986)
Turfgrass	greenbug, <i>Schizaphis graminum</i> (Rondani); bird cherry-oat aphid, <i>Rhopalosiphum padi</i> (L.), <i>Sipha flava</i>	Enhanced resistance to three species of aphids (Homoptera: Aphididae) in Acremonium endophyte-infected turfgrasses	Breen (1993)
Switch grass	greenbug, biotypes E, I, and Florida; Russian wheat aphid, <i>Diuraphis noxia</i> (Kurdj.), biotype 2; bird-cherry oat aphid, <i>Rhopalosiphum padi</i> (L.); corn leaf aphid, <i>R. maidis</i> (Fitch); English grain aphid, <i>Sitobion avenae</i> (F.); and yellow sugarcane aphid, <i>Sipha</i>	Establishment and host effects of cereal aphids on switchgrass (<i>Panicum virgatum</i> L.) cultivars ¹	Burd <i>et al.</i> , 2012

<i>flava</i> (Forbes)			
Sorghum	<i>Melanaphis sacchari</i>	Identification of Grain Sorghum Resistance to Sugarcane Aphid and Field Test Response by Hymenoptera to Honeydew	Caballero (2020)
Sorghum	<i>Sipha flava</i>	Feeding by the aphid <i>Sipha flava</i> produces a reddish spot on leaves of Sorghum halepense: an induced defense	Costa-Arbulú <i>et al.</i> , 2001
Sorghum	<i>Schizaphis graminum</i>	Mechanisms of resistance and their interactions in twelve sources of resistance to biotype E greenbug (Homoptera: Aphididae) in sorghum	Dixon <i>et al.</i> , 1990
Sugarcane	<i>Sipha flava</i>	Genetic variability of sugarcane varieties for resistance to <i>Sipha flava</i> .	Dumont <i>et al.</i> , 2023
Sorghum	<i>Melanaphis sacchari</i>	Impact of Temperature, Plant Species, and Sorghum Cultivar on the Population Dynamics of <i>Melanaphis sacchari</i>	Ferreira de Souza (2018)
<i>Aegilops tauschii</i>	<i>Schizaphis graminum</i>	Categories of resistance to greenbug (Homoptera: Aphididae) biotype 1 in <i>Aegilops tauschii</i> germplasm	Flinn <i>et al.</i> , 2001
Wheat	<i>Diuraphis noxia</i>	Physiological and biochemical responses of resistant and susceptible wheat to injury by Russian wheat aphid.	Franzen <i>et al.</i> , 2007
Wheat	Cereal aphid (<i>Sitobion avenae</i>)	Dimboa glucoside, a wheat chemical defense, affects host acceptance and suitability of <i>Sitobion avenae</i> to the cereal aphid parasitoid <i>Aphidius rhopalosiphi</i> .	Fuentes-Contreras <i>et al.</i> , 1998
Sorghum	<i>Melanaphis sacchari</i>	Elevated production of	Shankar

		reactive oxygen species is related to host plant resistance to sugarcane aphid in sorghum.	and Yinghua, 2021
Sorghum	<i>Schizaphis graminum</i>	Sorghum germplasm tolerant to greenbug (Homoptera: Aphididae) feeding damage as measured by reduced chlorophyll loss	Girma <i>et al.</i> , 1998
Johnson grass (<i>Sorghum halepense</i>)	<i>Sipha flava</i>	Host plant changes produced by the aphid <i>Sipha flava</i> : consequences for aphid feeding behaviour and growth.	Gonzales <i>et al.</i> , 2002
Sorghum	<i>Melanaphis sacchari</i>	Development of economic thresholds for sugarcane aphid (Hemiptera: Aphididae) in susceptible grain sorghum hybrids	Gordy <i>et al.</i> , 2019
Sorghum	<i>Melanaphis sacchari</i>	Molecular and Physiological Mechanisms Underlying Sorghum-Sugarcane Aphid Interactions	Grover, 2021
Barley	<i>Diuraphis noxia</i>	Physiological responses of resistant and susceptible barley, <i>Hordeum vulgare</i> to the Russian wheat aphid, <i>Diuraphis noxia</i> (Mordvilko)	Gutsche <i>et al.</i> , 2009
Wheat	<i>Diuraphis noxia</i>	Physiological and growth tolerance in wheat to Russian wheat aphid (Homoptera: Aphididae) injury	Haile <i>et al.</i> , 1999
Wheat	<i>Diuraphis noxia</i>	Comparison of chlorophyll and carotenoid concentrations among Russian wheat aphid (Homoptera: Aphididae)- infested wheat isolines	Heng-Moss <i>et al.</i> , 2003
Switch grass	<i>Sitobion avenae</i>	Analysis of Cereal	Koch

		Aphid Feeding Behavior and Transcriptional Responses Underlying Switchgrass-Aphid Interactions	(2017)
Switch grass	<i>Sitobion avenae</i>	Characterization of Cereal Aphid Resistance in Tetraploid Switchgrass Populations (<i>Panicum virgatum</i> L.)	Koch (2013)
Switch grass	<i>Schizaphis graminum</i> , <i>Sipha flava</i>	Categories of resistance to greenbug and yellow sugarcane aphid (Hemiptera: Aphididae) in three tetraploid switchgrass populations	Koch <i>et al.</i> , 2014a
Switch grass	<i>Schizaphis graminum</i> , <i>Sipha flava</i>	Evaluation of greenbug and yellow sugarcane aphid feeding behavior on resistant and susceptible switchgrass cultivars	Koch <i>et al.</i> , 2018
Switch grass	<i>Sipha flava</i> , <i>Schizaphis graminum</i> (Rondani) (biotype I), <i>Rhopalosiphum padi</i> (L.), and <i>Diuraphis noxia</i> (Mordvilko)	Evaluation of tetraploid switchgrass (Poales: Poaceae) populations for host suitability and differential resistance to four cereal aphids	Koch <i>et al.</i> , 2014b
Switch grass	<i>Schizaphis graminum</i>	Characterization of greenbug feeding behavior and aphid (Hemiptera: Aphididae) host preference in relation to resistant and susceptible tetraploid switchgrass populations	Koch <i>et al.</i> , 2015
Switch grass	<i>Schizaphis graminum</i> , <i>Sipha flava</i>	Aphid-responsive defense networks in hybrid switchgrass.	Koch <i>et al.</i> , 2020
Switch grass	<i>Schizaphis graminum</i>	Divergent switchgrass cultivars modify cereal aphid transcriptomes	Koch <i>et al.</i> , 2019
Wheat	<i>Diuraphis noxia</i>	Resistance categories of synthetic hexaploid wheat resistant to the Russian wheat aphid (<i>Diuraphis noxia</i>).	Lage <i>et al.</i> , 2004
Sorghum	<i>Melanaphis sacchari</i>	Antibiosis and	Limaje <i>et</i>

		Tolerance Discovered in USDA-ARS Sorghums Resistant to the Sugarcane Aphid <i>Melanaphis sacchari</i> (Hemiptera: Aphididae)	<i>al.</i> , 2017
Wheat	<i>Diuraphis noxia</i>	Light activation of Russian wheat-elicited physiological responses in susceptible wheat	Macedo <i>et al.</i> , 2003b
Sorghum	<i>Melanaphis sacchari</i>	Evaluating host-plant resistance against sugarcane aphid (<i>Melanaphis sacchari</i> (Zehntner)) in sorghum (<i>Sorghum bicolor</i> (L.) Moench).	Mbulwe (2017)
Wheat	<i>Sipha flava</i>	Resistance of wheat to the yellow sugarcane aphid (Homoptera: Aphididae)	Merkle and Starks (1985)
Barley	<i>Diuraphis noxia</i>	Physiological effects of Russian wheat aphid (Homoptera: Aphididae) on resistant and susceptible barley	Miller <i>et al.</i> , 1994
Kikuyu	<i>Sipha flava</i>	Resistance to yellow sugarcane aphid: Screening kikuyu and other grasses	Miyasaka <i>et al.</i> , 2007a
Kikuyu	<i>Sipha flava</i>	Effects of nitrogen and potassium in kikuyu grass on feeding by yellow sugarcane aphid	Miyasaka <i>et al.</i> , 2007b
Wheat	<i>Schizaphis graminum</i>	Resistance of wheat cultivars and lines to <i>Schizaphis graminum</i> (Hemiptera: Aphididae) under laboratory conditions	Mojahed <i>et al.</i> , 2013
Sorghum	<i>Schizaphis graminum</i>	Molecular mapping of tolerance to greenbug feeding damage in sorghum	Nagaraj, 2004
Sorghum	<i>Schizaphis graminum</i>	Relationship between chlorophyll loss and photosynthetic rate in greenbug (Homoptera: Aphididae) damaged sorghum	Nagaraj, 2002

Wheat	<i>Diuraphis noxia, Rhopalosiphum padi</i>	Dynamic change in photosynthetic pigments and chlorophyll degradation elicited by cereal aphid feeding	Ni <i>et al.</i> , 2002
Wheat, Peas, Cabbage	<i>Acyrtosiphon pisum</i> Harris, <i>Rhopalosiphum padi</i> , <i>Myzus persicae</i>	Effect of Aphid Foraging on the Intensity of Photosynthesis and Transpiration of Selected Crop Plants in Its Early Stages of Growing	Nietupski <i>et al.</i> , 2022
Sugarcane	<i>Sipha flava</i>	Susceptibility of stage IV canal point (CP) sugarcane clones to yellow sugarcane aphid (<i>Sipha flava</i> (Forbes)) feeding damage	Nuessly <i>et al.</i> , 2010
Digitaria	<i>Sipha flava</i>	Resistance in Digitaria to the yellow sugarcane aphid, <i>Sipha flava</i> (Forbes).	Oakes and Ratcliffe (1976)
Sugarcane	<i>Melanaphis sacchari</i>	Host plant resistance to the sugarcane aphid (Hemiptera: Aphididae)	Paudyal, 2019
Switch grass	<i>Sitobion avenae</i>	Differential defense responses of upland and lowland switchgrass cultivars to a cereal aphid pest	Pingault <i>et al.</i> , 2020
Switch grass	<i>Schizaphis graminum</i> , <i>Sipha flava</i>	Biochemical, physiological, and anatomical insights into aphid-bioenergy switchgrass interactions	Prochaska (2015)
23 Non cultivated grass	<i>Diuraphis noxia</i>	Russian wheat aphid, <i>Diuraphis noxia</i> (Kurdjomov), ecology and reproduction on five non cultivated grass hosts in high elevation environments.	Pucherelli <i>et al.</i> , 2010
Wheat	<i>Diuraphis noxia</i>	Feeding damage of Russian wheat aphid on resistant and susceptible wheat genotypes.	Rafi <i>et al.</i> , 1997
Digitaria	<i>Sipha flava</i>	Yellow sugarcane aphid resistance in selected	Ratchliffe and Oakes

		Digitaria germplasm	(1982)
Wheat	<i>Rhopalosiphum padi</i>	Resistance of wheat lines to <i>Rhopalosiphum padi</i> (Hemiptera: Aphididae) under laboratory conditions.	Razmjou <i>et al.</i> , 2012
Sorghum	<i>Schizaphis graminum</i>	Importance and quantification of plant tolerance in crop pest management programs for aphids: greenbug resistance in sorghum	Reese <i>et al.</i> , 1994
Wheat	<i>Rhopalosiphum padi</i>	Effect of leaf pubescence in wheat on the bird cherry oat aphid (Homoptera: Aphididae)	Roberts and Foster (1983)
Wheat	<i>Schizaphis graminum</i>	Drought/greenbug interactions: photosynthesis of greenbug resistant and susceptible wheat	Ryan <i>et al.</i> , 1987
Sorghum	<i>Melanaphis sacchari</i>	Elevated production of reactive oxygen species is related to host plant resistance to sugarcane aphid in sorghum	Shankar and Yinghua (2021)
Sugarcane	<i>Sipha flava</i>	Pubescence in sugarcane as an obstacle to yellow sugarcane aphid establishment	Sosa, 1990
Sorghum, rice, sweetpotato, <i>Ipomea batatas</i> (L.), maize, Johnsongrass, wheat	<i>Melanaphis sacchari</i>	Detailed characterization of <i>Melanaphis sacchari</i> (Hemiptera: Aphididae) feeding behavior on different host plants	Souza and Davis (2020a)
Sorghum	<i>Melanaphis sacchari</i>	Characterizing host plant resistance to <i>Melanaphis sacchari</i> (Hemiptera: Aphididae) in selected sorghum plant introductions	Souza and Davis (2021)
Maize, Oats, Rye, Triticale, Barley, Pearl millet, Wheat	<i>Schizaphis graminum</i> , <i>Sipha flava</i>	Yellow sugarcane aphid: plant resistance in cereal crops	Starks and Mirkes (1979)
Wheat	<i>Schizaphis graminum</i>	Low level resistance in wheat to greenbug	Starks and Merkes (1977)

Sorghum	<i>Melanaphis sacchari</i>	Rapid data analytics to relate sugarcane aphid [(<i>Melanaphis sacchari</i> (Zehntner)] population and damage on sorghum (<i>Sorghum bicolor</i> (L.) Moench)	Uchimiya and Knoll (2019)
Wheat	<i>Diuraphis</i> , ; biotype E <i>Schizaphis graminum</i> ; <i>Rhopalosiphum padi</i> , <i>Sipha flava</i>	Reactions of four aphid species on a Russian wheat aphid resistant wheat.	Webster and Porter (2000)
Wheat	<i>Schizaphis graminum</i> , <i>Sipha flava</i>	Leaf pubescence effects in wheat on yellow sugarcane aphids and greenbugs (Homoptera: Aphididae)	Webster <i>et al.</i> , 1994
Sorghum	<i>Sipha flava</i>	Yellow sugarcane aphid (Homoptera: Aphididae): Detection and mechanisms of resistance among Ethiopian sorghum lines	Webster (1990)
Wheat	<i>Diuraphis noxia</i>	Plant resistance studies with <i>Diuraphis noxia</i> (Homoptera: Aphididae), a new United States wheat pest	Webster <i>et al.</i> , 1987
Sugarcane	<i>Sipha flava</i>	Yellow Sugarcane Aphid (Homoptera: Aphididae) resistance mechanisms in selected sugarcane cultivars	White (1990)

Source: This study

2.10.2 Leaf pubescence (trichomes) that mediate resistance to YSA aphids in sugarcane varieties

The results from the four databases yielded seven research articles for inclusion. Further review resulted in six papers being selected for use in SLR. The results obtained indicated that two papers mentioned antixenosis (leaf pubescence/trichomes) in sugarcane in the presence of YSA. However, one study evaluated the effects of trichome density in wheat on YSA and greenbugs, indicating a scarcity of studies on sugarcane. These studies did not incorporate the effect of leaf position on trichome density hence indicating a research gap. One paper evaluated the use of electrical penetration graph (EPG) on feeding behavior of greenbug and YSA feeding behavior in switchgrass. This shows scarcity of such information in sugarcane in the presence of YSA.

The SLR conclusively showcased that leaf pubescence as influenced by leaf position and delayed penetration of the stylet due to surface resistance is an important morphological trait contributing to delay feeding in sugarcane varieties. However, the combination of trichomes and the EPG technique is currently an understudied area in Zimbabwe. The SLR points out that some studies where the EPG technique was used in determining varietal surface resistance in sugarcane and other cereals was directed towards other aphid species such as sugarcane aphids. Moreover, the SLR found that 100 % of the studies on resistance categories and mechanisms in sorghum and sugarcane focused primarily on sugarcane aphids. Table 2.4 indicates studies in which leaf pubescence/trichomes were mentioned as a defense mechanism against insect herbivores in sugarcane. However, under these studies the EPG technique was not incorporated to simultaneously conclude the contribution of trichomes, leaf position and delayed feeding conferred by surface resistance in response to YSA infestation.

Table 2.4: Studies in which leaf pubescence/trichomes was mentioned to be a defense mechanism against insect herbivores in sugarcane

Plant species	Arthropod affected	Order	References
Sugarcane	<i>Aleurolobus</i>		
	<i>barodensis</i>	Homoptera	Agarwal, 1969
	<i>Diatraea saccharalis</i>	Lepidoptera	Sosa, 1990
	<i>Melanaspis glomerata</i>	Heteroptera	Agarwal, 1969
	<i>Scirpophaga ivella</i>	Lepidoptera	Verma and Marthur, 1950
			Gallun <i>et al.</i> , 1966;
			Roberts <i>et al.</i> , 1979;
			Roberts and Foster,
			1983; Sosa, 1990;
			White, 1990; Nuessly,
			2005; Nuessly <i>et al.</i> ,
			2010
		Hemiptera	

Adapted and Modified: Source: Peter *et al.* (1995)

2.10.3 Biochemical properties affected by *S. flava* in sugar cane

A search of all available literature across the four databases yielded five articles for inclusion. Further review resulted in two articles being selected for the SLR. Two studies indicated that biochemical parameters were affected by YSA. This indicates that data on biochemical properties affected by YSA are scarce in sugarcane crops, thus prompting the performance of such studies in the sugar industry. This review highlighted that studies have focused on other grass species and SCA in sugarcane. Biochemical results showed that soluble sugar, nitrogen, and total protein contents were significantly reduced in sugarcane in the presence of other insects not specifically YSA.

2.10.4 Physiological responses of sugarcane to *S. flava* herbivory

A thorough search of the four databases yielded 51 articles for inclusion. Further review resulted in 23 articles being selected for the SLR. The SLR findings indicated that physiological parameters of interest in sugarcane include photosynthesis, transpiration, chlorophyll content, and gas exchange responses. The SLR found that the bulk of the studies concentrated on physiological responses (photosynthetic rate, transpiration rate, stomatal conductivity, respiration, and chlorophyll content) of plants to aphids and other pests in general; hence, little effort has been directed towards YSA.

2.10.5 Phytochemical profiling of metabolites that confer resistance to *S. flava* in sugarcane

Twenty-nine articles were included, further review resulted in three articles being selected. The SLR findings highlighted the presence of phenols and flavonoids in sugarcane and its by-products. Additionally, increase in phenol and flavonoids in sugarcane were reported in other insect pests and aphid species at the expense of YSA. Furthermore, SLR postulated a change in plant chemistry in the presence of foraging insects, which demonstrates the scarcity of such information in sugarcane. Therefore, the core aim of this study is to fill this research gap.

2.11 Discussion

2.11.1 Host plant resistance mechanism of grasses/ cereals to yellow sugarcane aphids

SLR results support the existence of three mechanisms of host plant resistance to insect damage (Painter, 1951; Horber, 1980; Dent, 2000; Schoonhoven *et al.*, 2005; Smith, 2005; Stout, 2013). However, Leybourne and Aradottir (2022) grouped the mechanisms of resistance into four categories (physical, chemical, nutritional, and molecular) based on their review. The findings of the SLR point out that there is little available research on antibiosis (biochemical and secondary

metabolites), as most of these studies concentrated on the life history parameters of aphids on tolerance, and existing studies have focused on chlorophyll loss rather than considering in-depth studies on physiological gas exchange responses of sugarcane to SCA (Akbar, 2009; Prochaska, 2015; Mbulwe, 2017; Paudyal, 2019). The review noted that several studies have focused on antixenosis (preference), where aphids in choice or no choice experiments were attracted to different hosts rather than digging on the first line of defense (leaf pubescence).

Host plant resistance mechanisms may be induced by feeding insects or constitutive (resistance expressed by well-defined morphological and chemical deterrents to pest infestations that are constantly occurring. Balikai (2001) emphasized that insects depend on intersecting mechanisms as well as morphological and biochemical factors. Antixenosis is a mechanism, whereby aphids do not prefer certain genotypes, as influenced by physical barriers, whereas antibiosis is a biochemical reaction (Painter, 1951; Smith, 2005; Mbulwe, 2017). Kogan (1994) specified other components of antixenosis which stimulate, suppress or deter pests on host. Several authors (Gallun *et al.*, 1966; Webster *et al.*, 1987; Dixon *et al.*, 1990; Webster *et al.*, 1994; Flinn *et al.*, 2001; Andarge and Westhuizen, 2004; Mbulwe, 2017) documented the importance of antixenosis. SLR on antibiosis identified definitions that concentrated mainly on insect physiology rather than considering secondary metabolites and biochemical responses. These results concur with the findings of Painter (1951), who identified antibiosis as an adverse effect of feeding-resistant plants on insects. In support of this perspective, Dixon's (1998) definition of antibiosis concentrated much on the insect, a bias towards plant chemicals. Additionally, Smith (2005) reported reduced fecundity as an overall result of antibiosis.

Numerous authors investigated different resistance mechanisms of cereals/ grasses/ sugarcane to aphids (Akbar, 2009; Akbar *et al.*, 2010, 2011; Webster and Porter, 2000; Webster, 1990; Uchimiya and Knoll, 2019; Starks and Mirkes, 1979, Souza and Davis, 2020a, 2020b, 2021; Reese *et al.*, 1994; Razmjou *et al.*, 2012; Ratcliffe and Orkes, 1982; Pucherelli *et al.*, 2010; Pingault *et al.*, 2020; Oakes and Ratcliffe, 1976; Mojahed *et al.*, 2013; Nagaraj, 2004; Merkle and Starks, 1985; Gonzalez *et al.*, 2002, Girma *et al.*, 1998; Ferreira de Souza, 2018; Costa-Arbulu *et al.*, 2001; Caballero, 2020; Burd *et al.*, 2012; Breen, 1993; Breen and Teetes, 1986; Minamoto *et al.*, 2007a, 2007b; Lage *et al.*, 2004; Koch, 2013, Koch *et al.*, 2014a, 2015, 2017, 2018, 2019, 2020; Dumont *et al.*, 2023). Tolerance has been defined as the ability of a plant to

grow in the presence of the pest (Painter, 1951; Smith *et al.*, 2012). Their definition lacked physiological compensatory behavior (photosynthesis and transpiration), which requires in-depth study to separate the tolerance mechanisms to the audience. Therefore, these mechanisms require an integrated multidisciplinary approach to understand the host plant resistance mechanisms of commercial sugarcane varieties to YSA. Findings from this review pointed out that the contributions of morphological, chemical, and physiological aspects have not been fully addressed in sugarcane in response to YSA infestation. This indicates a research gap that this study seeks to address.

2.11.2 Leaf pubescence (trichomes) that mediate resistance to YSA in sugarcane varieties

The results revealed the rarity of the current literature on commercial sugarcane varieties worldwide on the contribution of leaf pubescence in conferring resistance to YSA. Some studies (Levin, 1973; Glas *et al.*, 2012; Dalin *et al.*, 2008) have pointed out the importance of trichomes in plant resistance. SLR indicates that surface resistance (leaf pubescence) is an important morphological trait to control *S. flava*. These results concur with the findings of various authors (Gallun *et al.*, 1966; Roberts *et al.*, 1979; Roberts and Foster, 1983, Sosa, 1990, 1991; White 1989, 1990; Nuessly, 2005; Nuessly *et al.*, 2010) which conclusively indicated that sugarcane varieties with antixenosis properties affect the feeding behavior of *S. flava*. In support of this, van Helden and Tjallingii (1993) highlighted that physical barriers influence the penetration rate of aphid stylets. Additionally, Wang *et al.* (2004) highlighted that aphids must overcome host physical defenses (trichomes and waxes) before they can insert their stylets into the host. However, SLR findings showed paucity of studies on EPG in modifying YSA feeding behavior in sugarcane. Moreover, some authors (Fartek *et al.*, 2012; Akbar *et al.*, 2014; Tetreault *et al.*, 2019; Gyan *et al.*, 2020 and Singh *et al.*, 2021) assessed the potential surface resistance of cereal crops to SCA. In support of this, Gyan *et al.* (2020) observed similar findings in terf (*Eragrostis tef*) accessions a bias towards YSA. Tetreault *et al.* (2019) suggested that feeding patterns are influenced by morphological attributes and phytochemicals. Singh *et al.* (2021) through the use of EPG investigated the contribution of trichome density and in leaf position in affecting feeding time of aphids in wheat. Additionally, Cardona *et al.* (2023) pointed out that morphological barrier affects SCA infestation in sorghum. The use of EPG has been biased to grasses such as terf, barley, wheat, sorghum and sugarcane and these studies were directed towards sugarcane

aphid. Therefore, follow-up studies are needed to incorporate this morphological attribute into sugarcane breeding programs to manage YSA.

2.11.3 Biochemical properties affected by *S. flava* in sugar cane

Findings from the SLR indicated that crops respond differently to biochemical parameters in response to insect infestations. However, this study documented a rarity in terms of addressing the effects of YSA on sugarcane biochemical parameters. Currently, there is no independent published data on the effects of YSA on sugarcane biochemical parameters, except for switchgrass. The SLR showed that sucking pests negatively affected soluble sugar, chlorophyll, nitrogen, total protein, and phosphorous content. In support of this finding, Saeedi and Ziaee (2020) indicated that whitefly (*Neomaskellia Andropogonis*) in sugarcane reduces the aforementioned biochemical parameters. In contrast, Prochaska (2015) reported no differences in protein content in YSA control and infested treatments in switchgrass. Additionally, defense hormones, were not affected by the presence of YSA (Koch *et al.*, 2020).

2.11.4 Physiological responses of sugarcane to *S. flava* herbivory

The results obtained foregrounded that most studies were directed to other aphid species and cereals at the expense of sugarcane and YSA. Furthermore, the SLR results underscore that the general physiological parameters of interest in sugarcane include photosynthesis, transpiration, stomata conductance and chlorophyll content responses. In support of the effects of aphids on physiological parameters, several authors (Ryan *et al.*, 1987; Meyer and Whitlow, 1992; Larson, 1998; Haile *et al.*, 1999; Macedo *et al.*, 2003a; Diaz-Montano *et al.*, 2007; Pierson *et al.*, 2011; Paudyal, 2019) avows to this. Additionally, numerous studies have reported the effects of aphids on the physiological responses of several crops (Miller *et al.*, 1994; Haile *et al.*, 1999; Haile and Higley, 2003; Macedo *et al.*, 2003a and b; Deol *et al.*, 2001; Frazen *et al.*, 2007; Nagaraj *et al.*, 2002; Retuerto *et al.*, 2004; Diaz-Montano *et al.*, 2007; Gutsche *et al.*, 2009; Pierson *et al.*, 2011; Limaje *et al.*, 2017; Paudyal, 2019).

Numerous studies (Burd and Elliott, 1996; Rafi *et al.*, 1997; Ni *et al.*, 2002; Macedo *et al.*, 2003b; Franzen *et al.*, 2007) have documented the effects of Russian wheat aphid (*Diuraphis noxia*) on chlorophyll losses in both resistant and susceptible wheat varieties. In the presence of greenbug, Ryan *et al.* (1987) observed decreased photosynthesis in susceptible wheat varieties. Additionally, some authors (Dedryver *et al.*, 2010; Frier *et al.*, 2012; Tomczyk and Wrblewska,

2013; Thanakitpipattana *et al.*, 2022) indicated that these phytophagous insects influence the photosynthesis of host plants and transpiration (Nietupski *et al.*, 2022). These results underline the necessity of conducting research on YSA in sugarcane

The SLR amplifies that previous studies have provided physiological insights in wheat, sorghum, and other grasses; with a bias on YSA in sugarcane. Grover (2021) investigated the molecular and physiological mechanisms underlying sorghum–SCA interactions. Agitating the need for such studies in sugarcane, Melo *et al.* (2018) concluded that sugarcane genotypes that maintain chlorophyll, as well as stomatal conductance and photosynthesis were resistant to spittlebug (frog hopper) (*Mahanarva fimbriolata*) damage.

2.11.5 Phytochemical profiling of metabolites that confer resistance to *S. flava* in sugarcane

SLR emphasizes the presence of phytochemicals in sugarcane and its by-products especially phenols and flavonoids. However, there is still a research gap regarding the phytochemical varietal response of sugarcane varieties to YSA. In support of the existence of defense mechanisms, Godshall and Legendre (1988) and Legendre (1988) identified phenols in sugarcane and its by-products. Furthermore, the results of the SLR indicate alterations in plant chemistry in the presence of foraging insects. Several authors (Colombo *et al.*, 2005, 2006, 2008; Schoonhoven *et al.*, 2005; Duarte-Almeida; Feng *et al.*, 2014; Singh *et al.*, 2014; Pinheiro *et al.*, 2017; Rajendran *et al.*, 2017; Koch *et al.*, 2018; Soukoulis and Tzia; 2018; Ali *et al.*, 2019; Salgado, 2020; Kerdchan *et al.*, 2020; Kerdchan *et al.*, 2020; Ni *et al.*, 2021; Srihanam *et al.*, 2021; Rao *et al.*, 2022; Shafiq-atikah *et al.*, 2022) confirmed existence of secondary metabolites in cane. Some authors (Feng *et al.*, 2014; Kraphankhieo and Srihanam, 2016; Naowaset and Srihanam, 2017) have indicated that phytochemicals in sugarcane vary depending on the variety and geographical area in which the crop is planted. However, Nutt *et al.* (2004) and Silva *et al.* (2005) reported changes in total phenols in sugarcane in the presence of whitegrubs and frog hopper nymphs, respectively. Additionally, Malekshah *et al.* (2022) reported that *Sesamia nonagrioides* was affected by physicochemical properties of sugarcane. This calls for further investigations against YSA in sugarcane. In support of the contribution of secondary metabolites in conferring resistance, Silva *et al.* (2005) pointed out that sugarcane leaves attacked by the root-sucking frog hopper (spittlebug) (*Mahanarva fimbriolata* Stal.) recorded a significant increase in total phenol content. Furthermore, phenol content in sugarcane was significantly

increased or decreased due to feeding of the white pit *Antitrogus parvulus* Britton in cane roots (Nutt *et al.*, 2004). Additionally, Akbar *et al.* (2009) pointed out that there is a possibility of changes in phenolic compounds in sugarcane owing to insect feeding. In support of this, Niemeyer (1990) concluded that phenols have stimulating or repellent effects on aphid colonization as they act as antifeedants. Fuentes-Contreras and Niemeyer (1998) showed that wheat cultivars high in hydroxamic acid (DIMBOA glucoside) had reduced mean relative growth rate and body size. Zhang *et al.* (2022) reported that host plants could induce signal transduction in response to insect damage. Therefore, the SLR outlines that aphids can be affected positively or negatively by phenols calling for further research.

2.12 Limitations of the SLR and future research

Prerequisites for studies to be considered in the review were limited to: (a) studies including both SCA and YSA in sugarcane (b) studies involving cereal grasses and, (c) original research papers. The study was too broad, as it encompassed four objectives that need to be narrowed down in a stand-alone SLR. Most of the literature addressed other continents, a bias towards African and Zimbabwean conditions. Therefore, there is need to extend the tentacles of research to these areas in order to build comprehensive literature on YSA in sugarcane growing areas.

2.13 Conclusion

Conclusions were made on highlighted research questions as follows:

2.13.1 Leaf pubescence (trichomes) that mediate resistance to YSA in sugarcane varieties

Leaf pubescence is an important morphological trait contributing to YSA resistance in sugarcane varieties. However, there is scant information on studies that mention surface resistance of leaf pubescence, leaf position in combination with the use of EPG that monitors feeding behavior of YSA feeding behavior in sugarcane.

2.13.2 Biochemical properties affected by *S. flava* in sugar cane

The SLR pointed out that different sugarcane varieties respond differently to biochemical properties in the presence of YSA, as highlighted in two studies. Furthermore, biochemical properties of interest that were significantly reduced in sugarcane in the presence of other insects included soluble sugar, nitrogen, total protein, and chlorophyll content.

2.13.3 Physiological responses of sugarcane to *S. flava* herbivory

The SLR results showed that physiological parameters of interest in sugarcane include photosynthesis, transpiration, chlorophyll content, and gas exchange responses. However, the bulk of the studies have been directed to other aphid species in general; hence, little efforts have been devoted to YSA.

2.13.4 Phytochemical profiling of metabolites that confer resistance to *S. flava* in sugarcane

The SLR findings stressed the presence of the two most important phytochemicals (phenols and flavonoids) involved in sugarcane defense mechanisms. Furthermore, the SLR postulated a change in plant chemistry (induced defense) in the presence of other foraging insects such as aphids, but not specifically YSA in sugarcane.

Additionally, the SLR is the first to explore host plant resistance mechanisms multi-disciplinary by integrating plant breeding, entomology, and biochemistry in YSA management. However, most of the studies were limited to YSA in sorghum, wheat and switchgrass.

References

- Abawi, G.S., and Widmer, T. L. 2000. Impact of soil health management practices on soil borne pathogens, nematodes and root diseases of vegetable crops. *Applied Soil Ecology*. 15:37-47.
- Abdelmajid, N. 2008. Première apparition de *Sipha flava* (Homoptera, Aphididae) sur la canne à sucre au Maroc. - Bull. OEPP/EPPO Bull. 38: 220-222.
- Agarwal, R. A. 1969. Morphological characteristics of sugarcane and insect resistance. *Entomological Experimentalis et Applicata*. 12: 767-776.
- Ahad, A., Ferdous, R. R., Ahsan, R., Hoque, M., and Islam, A. N. 2016. Survey of Major Insect Pests, Uses of Management Practices and Other Related Information of Sugarcane (*Saccharum officinarum* L.) Growers of the Northern Region of Bangladesh. *American Journal of Life Science*. 3(6): 408–411. <https://doi.org/10.11648/j.ajls.20150306.15>
- Akar, Z., and Burnaz, N. A. 2019. A new colorimetric method for CUPRAC assay with using of TLC plate. *LWT*. 112: 108212.8.
- Akbar, W. 2009. Categorization and indentification of mechanisms of sugarcane resistance to the sugarcane aphid (Hemiptera: Aphididae). Louisiana State University and Agricultural and Mechanical College.

- Akbar, W., Showler, A. T., Reagan, T. E., Davis, J. A., and Beuzelin, J. M. 2014. Feeding by sugarcane aphid, *Melanaphis sacchari*, on sugarcane cultivars with differential susceptibility and potential mechanism of resistance. *Entomologia Experimentalis et Applicata*. 150(1): 32-44.
- Akbar, W., Showler, A., Beuzelin, J., Reagan, T., and Gravois, K. 2011. Evaluation of aphid resistance among sugarcane cultivars in Louisiana. *Annals of the Entomological Society of America*. 104: 699–704.
- Akbar, W., Showler, A., Reagan, T., and White, W. 2010. Categorizing sugarcane cultivar resistance to the sugarcane aphid and yellow sugarcane aphid (Hemiptera: Aphididae). *Journal of Economic Entomology*. 103: 1431–1437.
- Aldea, M., Hamilton, J. G., Resti J. P., Zangerl, A. R., Berenbaum, M. R., and DeLucia, H. 2005. Indirect effects of insect herbivory on leaf gas exchange in soybean. *Plant, Cell and Environment*. 28: 402–411. doi: 10.1111/j.1365-3040.2005.01279.x
- Alfayez, R., Alwehaibi, W., Winn, R., Venson, E., and Boehm, B. 2020. A systematic literature review of technical debt prioritization. In *Proceedings of the 3rd international conference on technical debt* (pp. 1-10).
- Ali, S. E., El Gedaily, R. A., Mocan, A., Farag, M. A and El-Seedi, H. R. 2019. Profiling Metabolites and Biological Activities of Sugarcane (*Saccharum officinarum* Linn.) Juice and Its Product Molasses via a Multiplex Metabolomics Approach. *Molecules*. 24: 934. doi:10.3390/molecules24050934
- Amarathunga, D. C., Grundy, J., Parry, H., and Dorin, A. 2021. Methods of insect image capture and classification: A Systematic literature review. *Smart Agricultural Technology*. 1: 100023.
- Andarge, A., and Van Der Westhuizen. M. C. 2004. Mechanisms of resistance of lentil *Lens culinaris* Medikus, genotypes to the pea aphid *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae). *Int. J. Tropical Insect Sci.* 24: 249-254.
- Armstrong, J. S., Rooney, W. L., Peterson, G. C., Villeneuve, R. T., Brewer, M. J., and Sekula-Ortiz, D. 2015. Sugarcane aphid (Hemiptera: Aphididae): host range and sorghum resistance including cross-resistance from greenbug sources. *J. Econ. Entomol.* 108: 576-582.

- Armstrong, J. S., Mbulwe, L., Sekula-Ortiz, D., Villanueva, R. T., and Rooney, W. L. 2016. Resistance to *Melanaphis sacchari* (Hemiptera: Aphididae) in forage and grain Sorghums. *Journal of Economic Entomology*. 110:259-265.
- Balikai, R. A. 2001. Bio-ecology and management of the sorghum aphid, *Melanaphis sacchari*. Ph.D. thesis, University of Agricultural Sciences, Dharwad, Karnataka, India
- Bayoumay, M. H., Perumal, R., and Michaud, J. P. 2016. Comparative life histories of greenbugs and sugarcane aphids (Hemiptera: Aphididae) infesting susceptible and resistant sorghums. *Journal of Economic Entomology*. 109: 385-391.
- Blackman, R. L., and Eastop, V. F. 2007. "Taxonomic issues," in *Aphids as Crop Pests*. Eds. van Emden, H. F. and Harrington, R. (Trowbridge, UK: CABI), 1–29.
- Blackman, R. L., and Eastop, V. F. 1984. *Aphids on the World's Crops—An Identification Guide*; John Wiley and Sons: New York, NY, USA.
- Blackman, R. L., and Eastop, V. F. 2000. *Aphids on the world's crops: an identification and information guide*, 2nd edn. Wiley, New York, p 2, 475 p.
- Breen, J. P., and Teetes, G. L. 1986. Relationships of yellow sugarcane aphid (Homoptera: Aphididae) density to sorghum damage. *Journal of Economic Entomology*. 79: 1106–1110.
- Breen, J. P. 1993. Enhanced resistance to three species of aphids (Homoptera: Aphididae) in *Acremonium* endophyte-infected turfgrasses. *Journal of Economic Entomology*. 86: 1279–1286.
- Buntin, D. G. Sorghum insect pests and their management. University of Georgia Cooperative Extension. University of Georgia Georgia. <http://athenaeum.libs.uga.edu/bitstream/handle/10724/12154/B1283.pdf?sequence=1>
- Burd, J. D., and Elliott, N. C. 1996. Changes in chlorophyll a fluorescence induction kinetics in cereals infested with Russian wheat aphid. *J. Econ. Entomol.* 89: 1332-1337.
- Burd, J. D., Prasifka, J. R., and Bradshaw, J. D. 2012. Establishment and host effects of cereal aphids on switchgrass (*Panicum virgatum* L.) cultivars¹. *Southwestern Entomologist*. 37: 115–122.

Caballero, M. A. 2020. Identification of Grain Sorghum Resistance to Sugarcane Aphid and Field Test Response by Hymenoptera to Honeydew.

CABI. 2021. *Sipha flava* (yellow sugarcane aphid). Technical fact sheet. <https://plantwiseplusknowledgebank.org/doi/10.1079/PWKB.Species.50170>

Capinera, J. L. 1981. Some effects of infestation by bean aphid, *Aphis fabae* Scopoli, on carbohydrate and protein levels in sugar beet plants, and procedures for estimating economic injury levels. *Zeitschrift für angewandte Entomologie*. 92: 374–384.

Cardona, J.B., Grover, S., Bowman, M. J., Busta, L., Kundu, P., Koch, K. G., and Louis, J. 2023. Sugars and cuticular waxes impact sugarcane aphid (*Melanaphis sacchari*) colonization on different developmental stages of sorghum. *Plant Science*. 330: 111646.

Coeur d'acier, A., Pérez hidalgo, N., and Petrovi - obradovi, O. 2010. Aphids (Hemiptera: Aphididae), Chapter 9.2. In: Alien terrestrial arthropods of Europe, Roques et al. Eds.- *BioRisk* 4(1): 435-474

Colombo, R, Lancas, F. M., and Yariwake, J. H. 2006. Determination of flavonoids in cultivated sugarcane leaves, bagasse, juice and in transgenic sugarcane by liquid chromatography-UV detection. *J. Chromatogr. A*. 1103: 118–124.

Colombo, R., Yariwake, J. H., McCullagh, M. 2008. Study of C- and O-glycosylflavones in sugarcane extracts using liquid chromatography: Exact mass measurement mass spectrometry. *J. Braz. Chem. Soc*. 19: 483–490.

Colombo, R., Yariwake, J. H., Queiroz, E. F., Ndjoko, K., and Hostettmann, K. 2005. On-line identification of sugarcane (*Saccharum officinarum* L.) methoxyflavones by liquid chromatography-UV detection using post-column derivatization and liquid chromatography-mass spectrometry. *J. Chromatogr. A*, 1082: 51–59.

Conlong, D., and Way, M. 2014. New Pest Alert, Yellow Sugarcane Aphid. *Links*. 23 (2): e.13

Costa-Arbulú, C., Gianoli, E., Gonzáles, W. L., and Niemeyer, H. M. 2001. Feeding by the aphid *Sipha flava* produces a reddish spot on leaves of *Sorghum halepense*: an induced defense. *Journal of Chemical Ecology*. 27: 273–283.

- Dalin, P., Agren, J., Björkman, C., Huttunen, P., and Kärkkäinen, K. 2008. Induced plant resistance to herbivory. A. Schaller (Ed.). pp. 89-105. Springer Science.
- Davis, J. J. 1909. Biological studies on three species of Aphididae. Washington DC. US Dept. Agriculture.
- Delaney, K. J., and Higley, L. G. 2006. An insect countermeasure impacts plant physiology: midrib vein cutting, defoliation and leaf photosynthesis. *Plant, Cell and Environment*. 29: 1245–1258.
- Dent, D. 2000. Insect pest management, 2nd Edition, CABI Bioscience, UK.
- Deol, G. S., Reese, J. C., Gill, B. S., Wilde, G. E., and Campbell, L. R. 2001. Comparative chlorophyll losses in susceptible wheat leaves fed by Russian wheat aphids or greenbugs (Homoptera: Aphididae). *J. Kans. Entomol. Soc.* 74: 192–198.
- Diaz-Montano, J., Reese, J. C., Schapaugh, W. T., and Campbell, L. R. 2007. Chlorophyll loss caused by soybean aphid (Hemiptera: Aphididae) feeding on Soybean. *J. Econ. Entomol.* 100: 1657–1662.
- Dixon, A. F. 2012. Aphid ecology an optimization approach. Springer Science and Business Media.
- Dixon, A. F. G. 1998. Aphid ecology an optimization approach. Springer Science and Business Media, London. p. 1-7.
- Dixon, A. G. O., Bramel-Cox, P. J., Reese, J. C., and Harvey, T. L. 1990. Mechanisms of resistance and their interactions in twelve sources of resistance to biotype E greenbug (Homoptera: Aphididae) in sorghum. *J. Econ. Entomol.* 83: 234-240.
- Dixon, A. F. 2012. Aphid ecology an optimization approach. Springer Science & Business Media.
- Duarte-Almeida, J. M., Negri, G., Salatino, A., de Carvalho, J. E., and Lajolo, F. M. 2007. Antiproliferative and antioxidant activities of a tricin acylated glycoside from sugarcane (*Saccharum officinarum* L.) juice. *Phytochemistry*. 68(8): 1165-1171. doi:10.1016/j.phytochem.2007.01.015
- Duarte-Almeida, J. M., Negri, G., Salatino, A., de Carvalho, J. E., and Lajolo, F. M. 2007.

Antiproliferative and antioxidant activities of a tricin acylated glycoside from sugarcane (*Saccharum officinarum* L.) juice. *Phytochemistry*. 68(8): 1165-1171. doi:10.1016/j.phytochem.2007.01.015

Dumont, T., Marodon, J., Nibouche, S., Hoarau, J. Y., and Barau, L. 2023. Genetic variability of sugarcane varieties for resistance to *Sipha flava*. In *Proceedings of the International Society of Sugar Cane Technologists*. 31: 628-636.

Fartek, B., Nibouche, S., Turpin, P., Costet, L., and Reynaud, B. 2012. Resistance to *Melanaphis sacchari* in the sugarcane cultivar R 365. *Entomologia Experimentalis et Applicata*. 144(3): 270-278.

Feng, S., Luo, Z., Zhang, Y., Zhong, Z., and Lu, B. 2014. Phytochemical contents and antioxidant capacities of different parts of two sugarcanes (*Saccharum officinarum* L.) cultivars. *Food Chemistry*. 151: 452-458. doi:10.1016/j.foodchem.2013.11.057

Ferreira de Souza, M. 2018. "Impact of Temperature, Plant Species, and Sorghum Cultivar on the Population Dynamics of *Melanaphis sacchari*". 2018. *LSU Doctoral Dissertations*. 4652. <https://digitalcommons.lsu.edu/gradschooldissertations/4652>

Flinn, M., Smith, C. M., Reese, J. H., and Gill, G. 2001. Categories of resistance to greenbug (Homoptera: Aphididae) biotype 1 in *Aegilops tauschii* germplasm. *J. Econ. Entomol.* 94: 558-563.

Forbes, S. A. 1884. Recent Observations. Plant-Lice – Aphides. Thirteenth Report of the State Entomologist on the Noxious and Beneficial Insects of the State of Illinois, May 31, 203 pp.

Franzen, L. D., Gutsche, A. R., Heng-Moss, T. M., Higley, L. G., Sarath, G., and Burd, J. D. 2007. Physiological and Biochemical Responses of Resistant and Susceptible Wheat to Injury by Russian wheat aphid. *J. Econ. Entomol.* 100: 1692–1703.

Frier, J. P. D., Hernández, C. V. S., and Tiessen, A. 2012. "Friend or Foe? Exploring the Factors that Determine the Difference between Positive and Negative Effects on Photosynthesis in Response to Insect Herbivory", in *Artificial Photosynthesis*; Najafpour, M.M., Ed.; InTech: Rijeka, Croatia, 2012.

- Fuentes-Contreras, E., and Niemeyer, H. M. 1998. Dimboa glucoside, a wheat chemical defense, affects host acceptance and suitability of *Sitobion avenae* to the cereal aphid parasitoid *Aphidius rhopalosiphi*. *J. Chem. Ecol.* 24: 371-381.
- Fürstenberg-Hägg, J., Zagrobelny, M., and Bak, S. 2013. Plant defense against insect herbivores. *International Journal of Molecular Sciences.* 14:10242-10297.
- Gallun, R. L., Ruppel, R., and Everson, E. H. 1966. Resistance of small grains to the cereal leaf beetle. *J. Econ. Entomol.* 59: 827-829.
- Gaud, S. M., Martorell, L. F., and Robles, R. B. 1965. Notes on the biology and control of the yellow aphid of sugarcane, *Sipha flava* (Forbes), in Puerto Rico. *Proc Int Soc Sug Cane Technol.* 12: 1307-1320.
- Geetha, T. S., and Geetha, N. 2014. Phytochemical screening, quantitative analysis of primary and secondary metabolites of *Cymbopogon citratus* (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu. *International Journal of pharmtech research.* 6(2): 521-529.
- Girma, M., Kofoid, K. D and Reese, J. C. 1998. Sorghum germplasm tolerant to greenbug (Homoptera: Aphididae) feeding damage as measured by reduced chlorophyll loss. *Journal of the Kansas Entomological Society.* 108–115.
- Giron, D., Dubreuil, G., Bennett, A., Dedeine, F., Dicke, M., Dyer, L.A., et al., 2018. Promises and challenges in insect-plant interactions. *Entomol. Exp. Appl.* 166, 319–343. doi: 10.1111/eea.12679
- Glas, J. J., Schimmel, B. C. J., Alba, J. M., Escobar-Bravo, R., Schuurink, R. C., and Kant, M. R. 2012. Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. *International Journal of Molecular Sciences.* 13: 17077-17103.
- Godshall, M. A., and Legendre. B. L. 1988. Phenolic content of maturing sugar cane. *Internat. Sugar J.* 90: 16-19.
- Gomez, S. K., Oosterhuis, D. M., Hendrix, D. L., Johnson, D. R., and Steinkraus. D. C. 2006. Diurnal pattern of aphid feeding and its effect on cotton leaf physiology. *Environ. Exper. Bot.* 55: 77–86.

- Gonzales, W., Ramirez, C., Olea, N., and Niemeyer, H. 2002. Host plant changes produced by the aphid *Sipha flava*: consequences for aphid feeding behaviour and growth. *Entomologia Experimentalis et Applicata*. 103: 107–113.
- Gordy, J. W., Brewer, M. J., Bowling, R. D., Buntin, G. D., Seiter, N. J., Kerns, D. L., Reay-Jones, F. P. F., and Way, M. O. 2019. Development of economic thresholds for sugarcane aphid (Hemiptera: Aphididae) in susceptible grain sorghum hybrids. *J. Econ. Entomol.* toz028. <https://doi.org/10.1093/jee/toz028>
- Grover, S. 2021. Molecular and Physiological Mechanisms Underlying Sorghum-Sugarcane Aphid Interactions (Doctoral dissertation, The University of Nebraska-Lincoln).
- Gutsche, A. R., Heng-Moss, T. M., Higley, L. G., Sarath, G., and Mornhinweg, D. W. 2009. Physiological responses of resistant and susceptible barley, *Hordeum vulgare* to the Russian wheat aphid, *Diuraphis noxia* (Mordvilko). *Arthropod-Plant Interactions*. 3: 233–240.
- Gyan, N. M., Yaakov, B., Weinblum, N., Singh, A., Cna'ani, A., Ben-Zeev, S., Saranga, Y., and Tzin, V. 2020. Variation Between Three Eragrostis tef Accessions in Defense Responses to *Rhopalosiphum padi* Aphid Infestation. *Front. Plant Sci.* 11: 598483. doi: 10.3389/fpls.2020.598483
- Haile, F. J., and Higley, L. G. 2003. Changes in soybean gas-exchange after moisture stress and spider mite injury. *Environ. Entomol.* 32: 433–440. doi: 10.1603/0046-225X-32.3.433
- Haile, F. J., Higley, L. G., Ni, X., and Quisenberry, S. S. 1999. Physiological and growth tolerance in wheat to Russian wheat aphid (Homoptera: Aphididae) injury. *Environ. Entomol.* 28: 787–794.
- Halbert, S. E., Miller, G. L., and AMES, L. M. 2013. *The genus Sipha Passerini (Hemiptera: Aphididae) in North America*. - Ins. Mundi, Paper 831.
- Hall, D. G. 2001. Notes on the yellow sugarcane aphid *Sipha flava* (Homoptera: Aphididae) and the lady beetle *Diomus terminatus* (Coleoptera: Coccinellidae) in Florida. *J Am Soc Sug Cane Technol.* 21: 21-29.

- Hall, D. G., and Bennett, F. D. 1994. Biological control and IPM of sugarcane pests in Florida, pp. 287-325. In D. Rosen, F. D. Bennett, and J. L. Capinera [eds.], Pest management in the subtropics, biological control – a Florida perspective. Intercept Ltd., Andover, UK.
- Heng-Moss, T. M., Ni, X., Macedo, T., Markwell, J. P., Baxendale, F. P., Quisenberry, S. S., and Tolmay, V. 2003. Comparison of chlorophyll and carotenoid concentrations among Russian wheat aphid (Homoptera: Aphididae) infested wheat isolines. *J. Econ. Entomol.* 96: 475- 481.
- Hentz, M., and Nuessly, G. 2004. Development, longevity, and fecundity of *Sipha flava* (Homoptera: Aphididae) feeding on *Sorghum bicolor*. *Environmental Entomology*. 33(3): 546-553.
- Hernández-Castellano, C., and Pérez Hidalgo, N. 2014. First record of the yellow sugarcane aphid *Sipha flava* (Forbes) (Hemiptera Aphididae) in the European continent.
- Hess, T. M., Sumberg, J., Biggs, T., Georgescu, M., Haro-Monteagudo, D., Jewitt, G., Osdogan, M., Marshall, M., Thenkabali, P., Daccache, A., Marin, F., and Knox, J. W. 2016. A sweet deal? Sugarcane, water and agricultural transformation in Sub-Saharan Africa. *Global Environmental Change*. 39: 181–194.
- Hill, C. B., Li, Y., and Hartman, G. L. 2004. Resistance to the soybean aphid in soybean germplasm. *J. Econ. Entomol.* 97(3): 1071–1077.
- Hinson, P. O. 2017. Effect of temperature on the development of sugarcane aphid, *Melanaphis sacchari*, on sorghum. A Thesis Submitted in Partial Fulfilment of the Requirement for the Degree Masters of Science in West Texas A and M University Canyon, Texas, USA.
- Hoad, S. P., Marzoli A., Grace, A., and Jeffree C. E. 1998. Response of leaf surfaces and gas exchange to wind stress and acid mist in birch (*Betula pubescens*). *Trees – Structure and Function*. 13: 1–12.
- Holman, J. 2009. *Host plant catalog of aphids. Palaearctic Region*. - Springer Science. Business Media B.V., 1216 pp.
- Horber, E. 1980. Types and classification of resistance. In *Breeding Plants Resistant to Insects* (Edited by Maxwell, F. G. and Jennings, P. R.), pp. 15–21. Wiley, New York.

- Hsieh, J. S. 1988. Cyanogenesis and aphid resistance in sorghum. *Memoirs of the Faculty of Agriculture, National Taiwan University*. 28:81-87.
- Hunter, M. D. 2001. Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Agricultural and Forest Entomology*. 3: 153–159.
- Hussain, A., Razaq, M., Zaka, S. M., Shahzad, W., and Mahmood, K. 2015. Effect of Aphid Infestation on Photosynthesis, Growth and Yield of *Brassica carinata* A. Braun. *Pakistan J. Zool.* 47(5): 1335-1340.
- Hussnain, Z., Naheed, A., and Rizwana, S. 1997. Biocontrol of Insect Pests of Sugarcane (*Saccharum sp.*). Jhang, PAKISTAN. insecticide test, 2000. *Arthro. Manage. Tests*. 26: F108.
- January, B., Yusufu, A., and Urassa, F. 2020. First report of yellow sugarcane aphid *Sipha flava* Forbes (Homoptera: Aphididae) in Tanzania. *Journal of Plant Protection Research*. 60(3).
- Joshi, S., and Viraktamath, C. A. 2004. The sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner (Hemiptera: Aphididae): its biology, pest status and control. *Current Science*. 87 (3): 307-316.
- Kahn, R. A., Bak, S., Svendsen, I., Halkier, B. A., and Moller, B. L. 1997. Isolation and reconstitution of cytochrome P450ox and in vitro reconstitution of the entire biosynthetic pathway of the cyanogenic glucoside dhurrin from sorghum. *Plant Physiology*. 115:1661-1670.
- KARLO-SRI. 2018. First Incidence of Yellow sugarcane aphids in Kenya. KARLO- Sugar Research Institute P.O. Box 44-40100, Kisumu Kenya.
- Kerdchan, K., Kotsaeng, N., and Srihanam, P. 2020. Oxidative Compounds Screening in the Extracts of Sugarcane (*Saccharum officinarum* L.) Planted in Maha Sarakham Province, Thailand. *Asian journal of plant science*. 19(4): 390-397.
- Kindler, S. D., and Dalrymple, R. L. 1999. Relative Susceptibility of Cereals and Pasture Grasses to the Yellow Sugarcane Aphid (Homomptera: Aphididae). *J. Agr. Urban. Entomol*, 16(2): 113-122.

- Knutson, A., Bowling, R., Brewer, M. J. Bynum, E., and Porter, P. 2016. The sugarcane aphid: Management guidelines for grain and forage sorghum in Texas. Texas A&M AgriLife Extension and Research. Texas A and M University. <http://ccag.tamu.edu/sorghum-insect-pests/>
- Koch, K. G. 2017. Analysis of Cereal Aphid Feeding Behavior and Transcriptional Responses Underlying Switchgrass-Aphid Interactions. The University of Nebraska-Lincoln.
- Koch, K. G., Bradshaw, J. D., Heng-Moss, T. M., and Sarath, G. 2014a. Categories of resistance to greenbug and yellow sugarcane aphid (Hemiptera: Aphididae) in three tetraploid switchgrass populations. *BioEnergy Research*. 7: 909–918.
- Koch, K. G., Donze-Reiner, T., Baird, L. M., Louis, J., Amundsen, K., Sarath, G., Bradshaw, J. D., and Heng-Moss, T. 2018. Evaluation of greenbug and yellow sugarcane aphid feeding behavior on resistant and susceptible switchgrass cultivars. *BioEnergy Research*. 11: 480–490.
- Koch, K. G., Fithian, R., Heng-Moss, T. M., Bradshaw, J. D., Sarath, G., and Spilker, C. 2014b. Evaluation of tetraploid switchgrass (Poales: Poaceae) populations for host suitability and differential resistance to four cereal aphids. *Journal of Economic Entomology*. 107: 424–431.
- Koch, K. G., Palmer, N. A., Donze-Reiner, T., Scully, E. D., Seravalli, J., Amundsen, K., and Sarath, G. (2020). Aphid-responsive defense networks in hybrid switchgrass. *Frontiers in Plant Science*. 11: 1145.
- Koch, K. G., Palmer, N., Stamm, M., Bradshaw, J. D., Blankenship, E., Baird, L. M., Sarath, G., and Heng-Moss, T. M. 2015. Characterization of greenbug feeding behavior and aphid (Hemiptera: Aphididae) host preference in relation to resistant and susceptible tetraploid switchgrass populations. *Bioenergy Research*. 8: 165–174.
- Koch, K. G., Scully, E. D., Palmer, N. A., Geib, S. M., Sarath, G., Heng-Moss, T., and Bradshaw, J. D. 2019. Divergent switchgrass cultivars modify cereal aphid transcriptomes. *Journal of Economic Entomology*. 112: 1887–1901.
- Koch, K. G. 2013. Characterization of Cereal Aphid Resistance in Tetraploid Switchgrass Populations (*Panicum virgatum* L.).

- Kogan, M. 1994. Plant resistance in pest management. In: Metcalf, R.L and Luckmann, W.H. (Eds.) Introduction to insect pest management, 3rd Ed., John Wiley & Sons, Inc.
- Kraphankhieo, W., and Srihanam, P. 2016. Investigation of phytochemical and antioxidant activity of different parts of sugarcane planted in Buriram province. In *Proceedings of the 12th Mahasarakham University Research Conference* (pp. 728-743).
- Lage, J., Skovmand, B., and Andersen, S. 2004. Resistance categories of synthetic hexaploid wheats resistant to the Russian wheat aphid (*Diuraphis noxia*). *Euphytica*. 136: 291–296.
- Larson, K. C. 1998. The impact of two gall-forming arthropods on the photosynthetic rates on their hosts. *Oecologia*. 115: 161–166.
- Legendre, B. L. 1988. Chapter 12 - Varietal Differences in the Chemical Composition of Sugarcane, in: Clarke, M.A., Godshall, M.A. (Eds.), Sugar Series. *Elsevier*, pp. 176–185. <https://doi.org/10.1016/B978-0-444-43020-5.50018-7>.
- Levin, A. D. 1973. The role of trichomes in plant defense. *The Quarterly Review of Biology*. 48: 3-15.
- Leybourne, D. J., and Aradottir, G. I. 2022. Common resistance mechanisms are deployed by plants against sap-feeding herbivorous insects: insights from a meta-analysis and systematic review. *Scientific Reports*. 12:17836. <https://doi.org/10.1038/s41598-022-20741-3>
- Limaje, A., Armstrong, S., Hoback, W., Zarrabi, A., Chad, H., Paudyal, S., and Burke. J. 2017. Antibiosis and Tolerance Discovered in USDA-ARS Sorghums Resistant to the Sugarcane Aphid *Melanaphis sacchari* (Hemiptera: Aphididae). *J. Entomol. Sci.* 53(2): 230–241.
- Macedo, T. B. 2003. Physiological responses of plants to piercing-sucking arthropods. The University of Nebraska-Lincoln.
- Macedo, T. B., Bastos, C. S., Highley, L. G., Ostlie, K. R., and Madhavan. S. 2003a. Photosynthetic responses of soybean-to-soybean aphid (Homoptera: Aphididae) injury. *J. Econ. Entomol.* 96: 188–193.
- Macedo, T. B., Highley, L. G., Ni, X., and Quisenberry. S. S. 2003b. Light activation of Russian wheat-elicited physiological responses in susceptible wheat. *J. Econ. Entomol.* 96(1): 194–201.

Madiope, K. W., Keeping, M. G., and Fourie, D. V. 2021. Quantifying yield loss to yellow sugarcane aphid in potted sugarcane. Short non-refereed paper. Conference Paper in South African Journal of Education, August 2021.

Mbulwe, L. 2017. Evaluating host-plant resistance against sugarcane aphid (*Melanaphis sacchari* (Zehntner)) in sorghum (*Sorghum bicolor* (L.) Moench). A Dissertation Submitted to the Office of Graduate and Professional Studies of Texas A & M University in partial fulfillment of the requirements for the degree of Doctor of philosophy.

Melo, C. G., Tomaz, A. C., Soares, B. O., Kuki, K. N., Peternelli, L. A., and Barbosa, M. P. 2018. Anatomical, morphological, and physiological responses of two sugarcane genotypes of contrasting susceptibility to *Mahanarva fimbriolata* (Hemiptera: Cercopidae). *Bulletin of Entomological Research*. 108(4): 556-564.

Merkle, O., and Starks, K. 1985. Resistance of wheat to the yellow sugarcane aphid (Homoptera: Aphididae). *Journal of Economic Entomology*. 78: 127–128.

Meyer, G. A., and Whitlow, T. H. 1992. Effects of leaf and sap feeding insects on photosynthetic rate of goldenrod. *Oecologia*. 92: 480–489.

Miller, H., Porter, D. R., Burd, J. D., Mornhinweg, D. W., and Burton, R. L. 1994. Physiological effects of Russian wheat aphid (Homoptera: Aphididae) on resistant and susceptible barley. *J. Econ. Entomol.* 87: 493–499.

Miyasaka, S. C., Hansen, J. D., and Fukumoto, G. K. 2007a. Resistance to yellow sugarcane aphid: Screening kikuyu and other grasses. *Crop Protection*. 26: 503–510. <https://doi.org/10.1016/j.cropro.2006.04.022>

Miyasaka, S. C., Hansen, J. D., McDonald, T. G., and Fukumoto, G. K. 2007b. Effects of nitrogen and potassium in kikuyu grass on feeding by yellow sugarcane aphid. *Crop Protection*. 26: 511–517. <https://doi.org/10.1016/j.cropro.2006.04.023>

Mojahed, S., Razmjou, J., Golizadeh, A., and Naseri, B. 2013. Resistance of wheat cultivars and lines to *Schizaphis graminum* (Hemiptera: Aphididae) under laboratory conditions. *Applied Entomology and Zoology*. 48: 39–45.

- Mortazavi Malekshah, S. A., Naseri, B., Ranjbar Aghdam, H., Razmjou, J., Fathi, S. A. A., Ebadollahi, A., and Changbunjong, T. 2022. Physicochemical Properties of Sugarcane Cultivars Affected Life History and Population Growth Parameters of *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae). *Insects*. 13(10): 901.
- Mutongi, W., Mabveni, A. R. S., and Makaza, K. 2021. The efficacy of *Datura ferox* (fierce thorn apple) leaf extracts against the Yellow Sugarcane Aphid (*Sipha flava*) in the Zimbabwe Sugar Industry. In *Proceedings of the 1st International Electronic Conference on Entomology, 2021 July* (pp. 1-15).
- Mutonyi, J., and Babikha, S. J. 2019. The occurrence of sugarcane yellow aphid (*Sipha flava*) (Homoptera: Aphididae) in Kakamega North Sub-County, Kenya. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*. 12 (6): 34-38.
- Naessens, E., Dubreuil, G., Giordanengo, P., Baron, O. L., and Minet-Kebdani, N. 2015. A secreted MIF cytokine enables aphid feeding and represses plant immune responses. *Curr. Biol.* 25: 1898–1903. doi: 10.1016/j.cub.2015.05.047
- Nagaraj, N. 2004. Molecular mapping of tolerance to greenbug feeding damage in sorghum. Kansas State University.
- Nagaraj, N., Reese, J. C., Kirkham, M. B., Kofoid, K., Campbell, L. R., and Loughin, T. M. 2002. Relationship between chlorophyll loss and photosynthetic rate in greenbug (Homoptera: Aphididae) damaged sorghum. *Journal of Kansas Entomological Society*. 75: 101–109.
- Naowaset, D., and Srihanam, P. 2017. Phytochemical contents and antioxidant activity of partially purified sugarcane extract by silica gel column. In *Proceedings of the 13th Mahasarakham University Research Conference* (pp. 444-453).
- Ni, X., Quisenberry, S. S., Heng-Moss, T., Markwell, J., Higley, L., Baxendale, F., Sarath, G., and Klucas, R. 2002. Dynamic change in photosynthetic pigments and chlorophyll degradation elicited by cereal aphid feeding. *Entomol. Exp. Appl.* 105: 43:53.
- Ni, Y., Chen, H., Liu, D., Zeng, L., Chen, P., and Liu, C. 2021. Discovery of genes involved in anthocyanin biosynthesis from the rind and pith of three sugarcane varieties using integrated metabolic profiling and RNA-seq analysis. *BMC Plant Biology*. 21(1): 1-15.

- Nieto nafría, J. M., and Mier durante, M. P. 1998. *Hemiptera, Aphididae I*. In: Fauna Ibérica, vol. 11, Ramos M.A. et al. Eds., Museo Nacional de Ciencias Naturales, CSIC, Madrid, 424 pp. (in Spanish).
- Nieto nafría, J. M., Andreev, A. V., Binazzi, A., Mier durante, M. P., Pérez hidalgo, N., Rakauskas, R., and Stekolschchikov, A. V. 2013. *Aphidoidea*. - Fauna Europaea version 2.6.2. Web service available online at <http://www.faunaeur.org>. [Accessed March 2022]
- Nietupski, M., Ludwiczak, E., Olszewski, J., Gabry´s, B., and Kordan, B. 2022. Effect of Aphid Foraging on the Intensity of Photosynthesis and Transpiration of Selected Crop Plants in Its Early Stages of Growing. *Agronomy*. 12: 2370. <https://doi.org/10.3390/agronomy12102370>
- Nuessly, G. S., and Hentz, M. G. 2002a. Feeding effects of yellow sugarcane aphid on sugarcane. *Journal of the American Society of Sugar Cane Technology*. 22: 126-127.
- Nuessly, G. S. 2005. Featured Creatures: Yellow Sugarcane Aphid; publication #EENY-354; University of Florida: Gainesville, FL, USA; Available online: <http://entnemdept.ufl.edu/creatures/field/bugs/yellowsugarcaneaphid.htm> (accessed on 16 January 2022).
- Nuessly, G. S., Cherry, R. H., and Sandhu, H. 2015. Miscellaneous insect pests of Florida sugarcane. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, EDIS; 2015.
- Nuessly, G. S., Hentz, M. G., and Gilbert, R. A. 2010. Susceptibility of stage IV canal point (CP) sugarcane clones to yellow sugarcane aphid (*Sipha flava* (Forbes)) feeding damage. *J Am Soc Sug Cane Technol*. 30: 104-114.
- Nutt, K. A., O'Shea, M. G., and Allsopp, P. G. 2004. Feeding by sugarcane whitegrubs induces changes in the types and amounts of phenolics in the roots of sugarcane. *Env. and Exper. Botany*. 51: 155-165.
- Oakes, A., and Ratcliffe, R. 1976. Resistance in *Digitaria* to the yellow sugar-cane aphid, *Sipha flave* (Forbes). *Tropical Agriculture*. 53: 15–24.

- Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al., 2020. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372: n71. doi: 10.1136/bmj. n71
- Painter, R. H. 1951. Insect resistance in crop plants. The McMillan Co. New York, pp. 520.
- Paudyal, S. 2019. Host plant resistance to the sugarcane aphid (Hemiptera: Aphididae). A Dissertation Submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfillment of the requirements for the Degree of Doctor of philosophy.
- Peter, A. J., Shanower, T. G., and Romeis, J. 1995. The role of plant trichomes in insect resistance: a selective review. *Phytophaga*. 7: 41-63.
- Peters, D. C., and Starks, K. J. 1990. Pest management systems for sorghum insects. *CRC Hand Book of Pest Management in Agriculture*. 3:549-562.
- Peterson, R. K. D. 2001. Photosynthesis, yield loss, and injury guilds. In *Biotic Stress and Yield Loss*, pp. 83–98. Eds R. K. D. Peterson., and L. G. Higley. Boca Raton, FL: CRC Press. doi: 10.1201/9781420040753
- Peterson, R. K. D., and Higley, L. G. 1993. Arthropod injury and plant gas exchange: current understandings and approaches for synthesis. *Trends in Agricultural Sciences: Entomology*, 1, 93–100.
- Peterson, R. K. D., Shannon, C. L., and Lenssen, A. W. 2004. Photosynthetic responses of Legume species to leaf-mass consumption injury. *Environmental Entomology*. 33: 450–456.
- Peterson, R. K. D., and L. G. Higley. 2001. Illuminating the black box: the relationship between injury and yield, pp. 1–12. In R. K. D. Peterson and L. G. Higley [eds], *Biotic stress and yield loss*. CRC, Boca Raton, FL.
- Pierson, L. M., Heng-Moss, T. M., Hunt, T. E., Reese, J. 2011. Physiological responses of resistant and susceptible reproductive stage soybean to soybean aphid (*Aphis glycines* Matsumura) feeding. *Arthropod-Plant Interaction*. 5: 49–58.

Pincebourde, S., and Ngao, J. 2021. The Impact of Phloem Feeding Insects on Leaf Ecophysiology Varies with Leaf Age. *Front. Plant Sci.* 12: 625689. doi: 10.3389/fpls.2021.625689

Pincebourde, S., Frak, E., Sinoquet, H., Regnard, J. L., and Casas, J. 2006. Herbivory mitigation through increased water-use efficiency in a leaf-mining moth-apple tree relationship. *Plant Cell Environ.* 29: 2238–2247. doi: 10.1111/j.1365-3040.2006.01598.x

Pingault, L., Palmer, N. A., Koch, K. G., Heng-Moss, T., Bradshaw, J. D., Seravalli, J., and Sarath, G. 2020. Differential defense responses of upland and lowland switchgrass cultivars to a cereal aphid pest. *International Journal of Molecular Sciences.* 21(21): 7966.

Pinheiro, F. G. C., Soares, A. K. L., Santaella, S. T., Alexandre e Silva, L. M., Canuto, K. M., Cáceres, C. A., Rosa, M. D. F., Feitosa, J. P. D. A., Leitão, R. C. 2017. Optimization of the acetosolv extraction of lignin from sugarcane bagasse for phenolic resin production. *Ind. Crops Prod.* 96: 80–90.

Posey, F. R., Reagan, T. E., and Coburn, G. E. 2001. Sugarcane aphid control-small plot insecticide test, 2000. *Arthro. Manage. Tests.* 26: F108.

Prochaska, T. J. 2015. *Biochemical, physiological, and anatomical insights into aphid-bioenergy switchgrass interactions.* The University of Nebraska-Lincoln.

Pucherelli, S. F., Peairs, F. B., Cranshaw, W., and Hansen, N. 2010. Russian wheat aphid, *Diuraphis noxia* (Kurdjomov), ecology and reproduction on five non cultivated grass hosts in high elevation environments.

Quisenberry, S. S., and Ni, X. 2007. 13 Feeding Injury. Aphids as crop pests. 331.

Rafi, M. M., Zemetra, R. S., and Quisenberry, S. S. 1997. Feeding damage of Russian wheat aphid on resistant and susceptible wheat genotypes. *Cereal Res, Commun.* 25: 63-68.

Raharjana, I. K., Siahaan, D., and Fatichah, C. 2021. User stories and natural language processing: A systematic literature review. *IEEE access.* 9: 53811-53826.

- Rajendran, P., Bharathidasan, R., and Sureka, I. 2017. Phytochemical Screening GC-MS and FT-IR Analysis of Sugarcane Juice. *Int J Pharma Res Health Sci.* 5 (6): 1962-67 doi:10.21276/ijprhs.2017.06.12
- Rao, M. J., Duan, M., Yang, M., Li, M., and Wang, L. 2022. Sugarcane rind secondary metabolites and their antioxidant activities in eleven cultivated sugarcane varieties. *Sugar Tech.* 24(5): 1570-1582.
- Ratcliffe, R., and Oakes, A. 1982. Yellow sugarcane aphid resistance in selected *Digitaria* germplasm. *Journal of Economic Entomology.* 75: 308–314.
- Razmjou, J., Mohamadi, P., Golizadeh, A., Hasanpour, M., and Naseri, B. 2012. Resistance of wheat lines to *Rhopalosiphum padi* (Hemiptera: Aphididae) under laboratory conditions. *Journal of Economic Entomology.* 105: 592–597.
- Reagan, T. E. 1994. Pest Status, Biology and control measures of sugar cane sap suckers in the North American sugar growing regions. pp 123-124 In: AJM Carnegie and DE Conlong (Eds), *Biology, Pest Status and Control Measure Relationships of Sugar Cane Insect Pests.*
- Reese, J. C., Schwenke, J. R., Lamont, P. S., and Zehr, D. D. 1994. Importance and quantification of plant tolerance in crop pest management programs for aphids: greenbug resistance in sorghum. *J. Agric. Entomol.* 11: 255–270.
- Retuerto, R., Fernandez-Lema, B., Roiloa, R., and Obeso, J. R. 2004. Increased photosynthetic performance in holly trees infested by scale insects. *Funct. Ecol.* 18: 664–669. doi: 10.1111/j.0269-8463.2004.00889.x
- Roberts, J. J., and Foster, J. E. 1983. Effect of leaf pubescence in wheat on the bird cherry oat aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 76: 1320-1322.
- Roberts, J. J., Gallun, R. L., Patterson, F. L., and Foster, J. E. 1979. Effects of wheat leaf pubescence on the Hessian fly. *J. Econ. Entomol.* 72: 211-214.
- Ryan, J. D., Johnson, R. C., Eikenbary, R. D and Dorschner, K. W. 1987. Drought/greenbug interactions: photosynthesis of greenbug resistant and susceptible wheat. *Crop Sci.* 27(2): 283–288.

- Saeedi, Z., and Ziaee, M. 2020. Biochemical responses of two sugarcane varieties to whitefly (*Neomaskellia andropogonis*) Infestation and its control by a new butenolide insecticide, flupyradifurone. *Agriculture and Forestry*. 66 (1): 69-81.
- Salgado, L. D. 2020. Characterization of Resistance to Two Crambid Stem Borers Among Louisiana Sugarcane Cultivars. Louisiana State University and Agricultural and Mechanical College.
- Schimmel, B. C. J., Ataide, L. M. S., Chafi, R., Villarroel, C. A., and Alba, J. M. 2017. Overcompensation of herbivore reproduction through hypersuppression of plant defenses in response to competition. *New Phytol*. 214: 1688–1701. doi: 10.1111/nph.14543
- Schoonhoven, L. M., van Loon, J. J. A., and Dicke, M. 2005. *Insect-plant biology*. 2nd ed. Oxford, UK: Oxford University Press.
- Scoones, I., Mavedzenge, B., and Murimbarimba. 2017. Sugar, People and Politics in Zimbabwe's Lowveld. *Journal of Southern African Studies*. 43(3): 567-584, doi: 10.1080/03057070.2016.1187972
- Shabani, A. A., Venkataramana, P., and Mbega, E. R. 2020. Infestation Status and Farmers' Knowledge on Yellow Sugarcane Aphid (*Sipha flava*) in Tanzania. *International Journal of Biosciences*. 17 (6): 227-240.
- Shafiq-atikah, M. K., Mahyudin, N. A., Abas, F., Nur-syifa', J., and Nor-khaizura, M. A. R. 2022. The proximate composition and metabolite profiling of sugarcane (*Saccharum officinarum*) molasses. *Malays Appl. Biol*. 51(2): 63-68. <https://doi.org/10.55230/mabjournal>
- Shankar, P., and Yinghua, H. 2021. Elevated production of reactive oxygen species is related to host plant resistance to sugarcane aphid in sorghum. *Plant Signaling and Behavior*. 16 (2): 1849523, DOI: 10.1080/15592324.2020.1849523
- Shannag, H. K. 2007. Effect of black bean aphid, *Aphis fabae*, on transpiration, stomatal conductance and crude protein content of faba bean. *Ann Appl Biol*. 151: 183–188.

- Silva, R. J. N. da., Guimaraes, E. R. Garcia, J. F. Botelho, P. S. M., Ferro, M. I. T., Mutton, M. A., and Mutton, M. J. R. 2005. Infestation of froghopper nymphs changes the amounts of total phenolics in sugarcane. *Sci. Agric. (Piracicaba, Braz.)*. 62: 543-546.
- Singh, A., Dilkes, B., Sela, H., and Tzin, V. 2021. The Effectiveness of Physical and Chemical Defense Responses of Wild Emmer Wheat Against Aphids Depends on Leaf Position and Genotype. *Front. Plant Sci.* 12: 667820. doi: 10.3389/fpls.2021.667820
- Singh, A., Lal, U. R., Mukhtar, H. M., Singh, P. S., Shah, G., and Dhawan, R. K. 2014. Phytochemical profile of sugarcane and its potential health aspects. *Pharmacognosy Reviews*. 9(17): 45-54.
- Singh, B. U., Padmaja, P. G., and Seetharama, N. 2004. Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: A review. *Crop Protection*. 23:739-755.
- Singh, J., Singh, M., Chandra, P., Rao, G. P., and Singh. H. N. 1993. Biochemical studies on resistance to red rot in sugarcane. *Sugar Cane*. 6: 16-19.
- Smith, C. M. 2005. Plant resistance to arthropods: Molecular and conventional approaches. Springer Science and Business Media, Dordrecht. p. 19-122.
- Smith, C., and Clement, S. 2012. Molecular bases of plant resistance to arthropods. *Annual Review of Entomology*. 57: 309-28.
- Sosa, O. Jr. 1990. Pubescence in sugarcane as an obstacle to yellow sugarcane aphid establishment. *Sugar Y Azuca*. 85: 30.
- Sosa, O., Jr. 1991. Yellow sugarcane aphid (Homoptera: Aphididae) antixenosis in sugarcane. *Sugar Cane*. 2: 9-10.
- Soukoulis, C., and Tzia, C. 2018. Grape, raisin and sugarcane molasses as potential partial sucrose substitutes in chocolate ice cream: A feasibility study. *International Dairy Journal*. 76: 18-29. <https://doi.org/10.1016/j.idairyj.2017.08.004>

Sousa-Silva C. R., and Ilharco, F. A. 1995. Afídeos do Brasil e suas plantas hospedeiras (lista preliminar). - Universidade Federal de São Carlos Edufscar-Editora da Universidade, São Carlos, 85 pp.

Souza, M. A. 2018. Effects of temperature on sugarcane aphid, *Melanaphis sacchari*, life history on three different host plants. Thesis submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfilment of the requirements for the Degree of Master of science December, 2018.

Souza, M., and Davis, J. 2020a. Detailed characterization of *Melanaphis sacchari* (Hemiptera: Aphididae) feeding behavior on different host plants. *Environmental Entomology*. 4: 683–691.

Souza, M., and Davis, J. 2020b. Potential population growth of *Melanaphis sacchari* (Zehntner)(Hemiptera: Aphididae) under six constant temperatures on grain sorghum (*Sorghum bicolor* L.). *Florida Entomologis*. 103: 116–123.

Souza, M., and Davis, J. 2021. Characterizing host plant resistance to *Melanaphis sacchari* (Hemiptera: Aphididae) in selected sorghum plant introductions. *Journal of Economic Entomology*. 114: 959–969.

Srihanam, P., Thonpho, A., Sanarat, P., and Motham, P. 2021. Phytochemicals and antioxidant activity in sugarcane (*Saccharum officinarum* L.) bagasse extracts. *Suan Sunandha Science and Technology Journal*. 8(2): 26-35.

Srikanth, N. M. J., Asokan, B. S. S., and Goud, N. K. Y. 2008. Assessment of woolly aphid impact on growth, yield and quality parameters of sugarcane. *Sugar Technology*. 10(2): 143–149.

Starks, K. J., and Merkle. O. G. 1977. Low level resistance in wheat to greenbug. *J. Econ. Entomol*. 70: 305-306.

Starks, K. J., and Mirkes, K. 1979. Yellow sugarcane aphid: plant resistance in cereal crops. *Journal of Economic Entomology*. 72: 486–488.

Stout, M. J. 2013. Re-evaluating the conceptual framework for applied research on host-plant resistance. *Insect Science*. 20: 263–272.

- Tang, J. Y., Zielinski, R. E., Zangerl, A. R., Crofts, A. R., Berenbaum, M. R., and DeLucia, E. H. 2006. The differential effects of herbivory by first and fourth instars of *Trichoplusia ni* (Lepidoptera: Noctuidae) on photosynthesis in *Arabidopsis thaliana*. *J. Exp. Bot.* 57: 527–536. doi: 10.1093/jxb/erj032
- Tetreault, H. M., Grover, S., Scully, E. D., Gries, T., Palmer, N. A., Sarath, G., and Sattler, S. E. 2019. Global responses of resistant and susceptible sorghum (*Sorghum bicolor*) to sugarcane aphid (*Melanaphis sacchari*). *Frontiers in plant science.* 10: 145.
- Tomczyk, A., and Wróblewska, J. 2013. Wpływ równoczesnego z' erowania róż' nych gatunków mszyc na intensywnos' c' fotosyntezy porzeczki czarnej i borówki wysokiej. *Prog. Plant Prot. Post. W Ochr. Ro' sl.* 53: 721–724.
- Tripathi, G. M. 1995. Record of parasite and predator complex of sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner in Nagaland. *Indian Sugar.* 44: 883–885.
- Trumble, J. T., Kolodny-Hirsch, D. M., and Ting, I. P. 1993. Plant compensation for arthropod herbivory. *Annu. Rev. Entomo.* 38, 93–119. doi: 10.1146/ annurev.en.38.010193.000521
- Uchimiya, M., and Knoll, J. E. 2019. Rapid data analytics to relate sugarcane aphid [*Melanaphis sacchari* (Zehntner)] population and damage on sorghum (*Sorghum bicolor* (L.) Moench). *Scientific Reports.* 9: 370.
- van Emden, H. F. 2017. Host-plant resistance, pp. 515–527. In H. F. van Emden and R. Harrington (eds.), *Aphid as a Crop Pest*. CAB International, Boston, MA.
- Velikova, V., Salerno, G., Frati, F., Peri, E., Conti, E., Colazza, S., et al. 2010. Influence of feeding and oviposition by phytophagous pentatomids on photosynthesis of herbaceous plants. *J. Chem. Ecol.* 36: 629–641. doi: 10.1007/ s10886-010-9801-7
- Venna, S. C., and Mnthur, S. 1950. The epidermal characters of sugarcane leaf in relation to insect pests. *Indian Journal of Agricultural Science.* 20: 387-389.
- War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., and Sharma, H. C. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signaling and Behavior.* 7(10): 1306-1320.

- Warrington, S., Cottam, D. A., and Whittaker, J. B. 1989. Effects of insect damage on photosynthesis, transpiration and SO₂ uptake by sycamore. *Oecologia*. 80: 136–139.
- Way, M. J., Conlong D. E., Martin, L. A., McFarlane, S. A., Stranack, R., Keeping M. G., and Rutherford, R. S. 2015. First record of yellow sugarcane aphid, *Sipha flava* (Homoptera: Aphididae), in the South African sugarcane industry. *Proceeding of the South African Sugar Technology Association*. 87: 53–57.
- Way, M. J., De ConLong, D. C., Martin, L. A., McFarlane, S. A., Stranack, R., Keeping, M. G., and Rutherford, R. S. 2014. First record of yellow sugarcane aphid, *Sipha flava* (Homoptera: Aphididae), in the South African sugarcane industry.
- Webster, J. A. 1990. Yellow sugarcane aphid (Homoptera: Aphididae): Detection and mechanisms of resistance among Ethiopian sorghum lines. *Journal of Economic Entomology*. 83: 1053–1057.
- Webster, J. A., Inayatullah, C., Hamissou, M., and Mirkes, K. A. 1994. Leaf pubescence effects in wheat on yellow sugarcane aphids and greenbugs (Homoptera: Aphididae). *J. Econ. Entomol.* 87. 231-240.
- Webster, J. A., Starks, K. J., and Burton, R. L. 1987. Plant resistance studies with *Diuraphis noxia* (Homoptera: Aphididae), a new United States wheat pest. *J. Econ. Entomol.* 80: 944-949.
- Webster, J., and Porter, D. 2000. Reactions of four aphid species on a Russian wheat aphid resistant wheat. *Southwestern Entomologist*. 25: 83–90.
- Welter, S. C. 1989. “Arthropod impact on plant gas exchange,” in *Insect-Plant Interactions*, ed. E. A. Bernays (Boca Raton, FL, USA: CRC Press), 135–150. doi: 10.1201/9780429290916-5
- White, W. H. 1990. Yellow Sugarcane Aphid (Homoptera: Aphididae) resistance mechanisms in selected sugarcane cultivars. *J Econ Ent.* 83(5): 2111-2114.
- White, W. H., Reagan, T. E., and Hall, D. G. 2001. *Melanaphis sacchari* (Homoptera: Aphididae), A sugarcane Pest New to Louisiana. *Florida Entomologists*. 84(3): 435–436.
- Wilson, B. E. 2019. Hemipteran Pests of Sugarcane in North America. *Insects*. 10 (107): 1-15. doi:10.3390/insects10040107

Wilson, B. E., VanWeelden, M. T., Beuzelin, J. M., and Mészáros, A. 2013. Assessment of insecticides against a complex of hemipteran pests in sugarcane. *Arthrop. Manag. Tests*. 38: F73.

Wood, C. W., Tracy, P. W., Reeves, D. W., and Edmisten, K. L. 1992. Determination of cotton nitrogen status with a handheld chlorophyll meter, *J. Plant Nutr.* 15(9): 1435–1448. doi: 10.1080/01904169209364409

Zangerl, A. R., Hamilton, J. G., Miller, T. J., Crofts, A. R., Oxborough, K., Berenbaum, M. R., et al. 2002. Impact of folivory on photosynthesis is greater than the sum of its holes. *Proc. Nat. Acad. Sci U.S.A.* 99: 1088–1091. doi: 10.1073/pnas.022647099

Zhang, W., Yu, L., Han, B., Liu, K., and Shao, X., 2022. Mycorrhizal inoculation enhances nutrient absorption and induces insect-resistant defense of *Elymus nutans*. *Frontiers in Plant Science*. 13: 898969.

CHAPTER THREE

Leaf Pubescence that Mediates Resistance to Yellow Sugarcane Aphids (YSA) (*Sipha flava* Forbes) (Hemiptera: Aphididae) in selected sugarcane (*Saccharum officinarum* L.) varieties

Abstract

The presence of leaf pubescence in sugarcane cultivars improves the control of yellow sugarcane aphid (YSA) (*Sipha flava*). The objective of the study was to determine the effect of leaf pubescence (trichome density) on YSA infestation in sugarcane. A 7 x 2 x 3 factorial design in a complete randomized block design (CRBD) replicated four times was used, under two treatments of aphid infestation: uninfested (control); and infested. Variety was the first factor under seven levels (96-1107, 00-1165, ZN 10, ZN 8, ZN 9, ZN 3L, and N14) and leaf position was the second factor under three levels (leaf position-1, leaf position-2 and leaf position-3). Quantitative data on trichome density (mm^2) was measured using a light microscope. The trichome density (mm^2) and aphid number in the control (un-infested) plants varied greatly ($p < 0.001$), showing prominent variations depending on the leaf position ($p < 0.001$), and the seven genotypes ($p < 0.001$). The trichome density (mm^2) and aphid number in the control (un-infested) plants varied greatly ($p < 0.001$), showing prominent variations depending on the leaf position ($p < 0.001$), the seven genotypes ($p < 0.001$). 00-1165 sugarcane variety recorded the highest constitutive trichome density across all leaf positions (leaf-1; leaf-2; leaf-3) (93.07; 111.15; 124.9), whereas the ZN10 variety recorded the lowest trichome density (leaf-1, leaf-2, leaf-3) (20.97; 35.85; 50.97), respectively. In aphid-infested treatments, ZN 10 recorded the highest aphid population across the three leaf positions (leaf-1, leaf-2, leaf-3) (156; 97; 42) respectively, whilst 00-1165 recorded the least (leaf-1; leaf-2; leaf-3) (53; 32.5; 16.5) respectively. Linear regression analysis confirmed a significant ($p < 0.001$) negative correlation between; the number of aphids for various cultivars and trichome density ($r = 0.52$), trichome density and leaf position ($r = 0.45$). Leaf pubescence and leaf position reduced YSA number among the sugarcane varieties. Farmers in the sugar industry should integrate pubescent (high-density trichome) sugarcane varieties into their existing YSA management strategies.

Keywords: leaf pubescent, trichomes, resistance, leaf position, IPM

3.1 Introduction

YSA 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). Nuessly and Hentz (2002a) and Wilson (2019) reported that severe infestation and continuous feeding on young plants can cause yellowing to reddening of the leaves, which is followed by early senescence of the leaves and stem death. Madiope *et al.* (2021) observed that the biomass yield of dry leaves and stems of sugarcane was significantly reduced. According to Hall (2001), early-stage plant damage is a major factor in both yield and tillering loss in sugarcane. Reagan (1994), Henzt *et al.* (2004), Nuessly (2005), Nuessly *et al.* (2010), and Wilson (2019) proposed that feeding by YSA can cause chlorosis losses which significantly affects yield.

Biological, chemical, and cultural strategies are among the management approaches for YSA. The most common method used in the Zimbabwe Sugar Industry (ZSI) at the moment is the chemical approach, which uses Allice (ACETA) a neonicotinoid that has been registered for use. Long-term use of synthetic pesticides is thought to frequently lead to several negative outcomes, including increased pesticide residues, resistant pest genotype emergence, harmful impacts on beneficial insects and humans, and secondary pest outbreaks (Macharia *et al.*, 2009; Yalcin *et al.*, 2015). Mutonyi and Babikha (2019) suggested that pyrethroid application is likely to disturb natural enemy populations resulting in increased infestations in the weeks that follow. In support of this, Ehrlich *et al.* (1993) pointed out those large quantities of synthetic pesticides cause do not break down easily in the environment causing pollution. Growers should implement integrated pest management (IPM) techniques in response to the detrimental effects of synthetic pesticides by adopting leaf pubescence sugarcane varieties to control YSA.

Leaf pubescence is an adaptive morphological trait and a common trait of angiosperms (Solereder, 1908; Ehleringer *et al.*, 1976). 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; Xi *et al.*, 2020). In terms of biotic stress resistance, leaf pubescence can improve plant resistance to insects by influencing the preferences and behavior of various insects (Nawab *et al.*, 2011), such as bird cherry-oat aphids in wheat (Roberts and

Foster, 1983). The degree and density of leaf hairiness is associated with variations in the degree of resistance or susceptibility to different insect pests (Mergher *et al.*, 1997; Konyspaevna, 2012). In addition, pubescence can serve as a barrier against infection by foliar pathogens (Lai *et al.*, 2000).

Many plant species possess defense mechanisms to protect them from herbivore attacks (Moghadam *et al.*, 2013). There are two types of defense mechanisms; chemical (toxic compounds and other secondary metabolites) and physical (tissue roughness, surface waxes, and trichomes) (Schoonhoven *et al.*, 2005). Some authors (Shufran *et al.*, 1997; Khan *et al.*, 2000; Moghadam *et al.*, 2013) have pointed out that trichomes are the first line of defence that insects come into contact with. In addition, these are important morphological barriers that prevent herbivorous insects from oviposition (Baur *et al.*, 1991; Handley *et al.*, 2005), foraging (Eisner *et al.*, 1998; Lam and Pedigo, 2001), and movement (Ramalho *et al.*, 1984; Eisner *et al.*, 1998). Nevertheless, the effect of leaf pubescence on herbivorous insects such as polyphagous aphids can be positive or neutral (Moghadam *et al.*, 2013). Woodman and Fernandes (1991); and Lovinger *et al.* (2000) pointed out that some herbivores are protected from natural enemies by trichomes. Contrary to this, some studies in cotton (McAuslane, 1996; Srinivasana and Uthamasa, 2005; Oriani and Vendramim, 2010) have shown that dense pubescence (hairy cotton cultivars) is associated with higher herbivore oviposition and feeding rates. This requires more studies to conclusively build concrete literature on the effects of leaf trichomes on herbivory insects in sugarcane accessions. Various studies (Gallun *et al.*, 1966; Roberts *et al.*, 1979; Roberts and Foster, 1983; Sosa, 1990; White, 1990; Webster *et al.*, 1994; Nuessly, 2005; Nuessly *et al.*, 2010) have reported the contribution of pubescent wheat and sorghum cultivars in reducing the effects of aphids in general; few YSA reports in sugarcane and conditions were outside Zimbabwe. However, hardly any study has determined the density of pubescence in sugarcane plants against YSA in Zimbabwe. Moreover, the relationship between pubescence density and aphid infestation numbers for sugarcane crops has been scarcely reported in the literature.

Oguchi *et al.* (2018) indicated that some trichomes have a secretory function and can secrete a variety of chemicals such as terpenoids and phenylpropanoids are important in plant-animal or

plant-fungus interactions (Pandey *et al.*, 2017). Trichomes exist in two forms; glandular and non-glandular hence there is a need for studies that can fully exploit their contributions in sugarcane. Glandular trichomes are used for the storage and secretion of exudate, whereas non-glandular trichomes, which are specialized epidermal hair-like structures, can influence aphid movement and reproductive rate (Riddick and Simmons, 2014). Studies by several authors (Leybourne *et al.*, 2019; de Correa *et al.*, 2020; Gyan *et al.*, 2020) have confirmed the presence of non-glandular trichomes on leaf surfaces of young wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and tef (*Eragrostis tef*) plants, which confer resistance to hemipteran herbivores. In support of this, Roberts and Foster (1983); and de Correa *et al.* (2020) showed that trichome density is ecologically significant as it inducibly affects insect feeding behaviours and limits their performance in wheat and barley crops. These significances are worthy of exploration in future research. In support of this view, Singh *et al.* (2021) pointed out that studies have shown that feeding herbivores causes the subsequently formed leaves to produce higher trichome densities. It is the essence of this study to conclusively concur with such findings assessing the contribution of leaf position, accession (genotype), and treatment (sprayed and infested) on trichome density and aphid number. Further, literature has indicated that trichomes are usually more evident in young tissues than in older ones (Valkama *et al.*, 2004; McCall and Fordyce, 2010).

Mitchell *et al.* (2016) reported that plants have developed physical and chemical defense strategies to control plant-aphid interactions and ensure plant survival and fitness. Constitutive defense mechanisms are present in plant tissues as prophylactic strategies to prepare for upcoming adverse conditions (Mertens *et al.*, 2021). Several authors (Howe and Jander, 2008; Brunissen *et al.*, 2009; Chandrasekhar *et al.*, 2018; Batyrshina *et al.*, 2020a) have highlighted that defense mechanisms can also be dynamic and induced upon herbivore attack, depending on the plant age, developmental stage, and genetic background of the crop. Variation of these mechanisms at individual parts within a plant may be one of the key elements of pest reproduction and feeding behavior (Awmack and Leather, 2002; Karley *et al.*, 2002; Jakobs and Müller, 2018). Liu *et al.* (2021) highlighted that the relationship between defense mechanisms, their defensive variation, and their effectiveness in response to an aphid infestation has not been fully explored. Such reports have not been mentioned indicating the absence of such studies in

the sugarcane industries of Africa and Zimbabwe, hence a research gap. In addition, these variations have been reported in other aphid species and cereals at the expense of YSA. Currently, pesticide application remains the only means to control YSA, but aphid resurgence is being witnessed year in and year out. Moreover, continuous use of this method will result in aphid-resistant biotypes in sugarcane; hence, the use of resistant pubescent sugarcane varieties would be the ideal method of managing this pest. Therefore, the objective of this study was to investigate Leaf Pubescence that Mediates Resistance to Yellow Sugarcane Aphids (*S. flava* Forbes) (Hemiptera: Aphididae) in selected sugarcane (*S. officinarum* L.) varieties.

3.2 Materials and methods

3.2.1 Study Site description

The research was carried out at the Zimbabwe Sugar Association Experiment Station (ZSAES) which is owned by Tongaat Hullets in Chiredzi district, Masvingo Province in the southeast Lowveld during the 2023 season. 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 the 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 very high especially during the summer season, particularly in September, October, and November with an average temperature of 34 °C. Low temperatures are recorded between May to July with a daily mean temperature of 25 °C. These climatic conditions allow the YSA to exhibit two modes of reproduction; non-mating (parthenogenetically) in warmer climates (Halbert *et al.*, 2013) and mating, where live individuals are produced and females mate with males in cold winters as low temperatures induce oviparity (Nuessly, 2005, Way *et al.*, 2014). This has allowed the YSA to be a resident pest in the Lowveld.

3.2.2 Agronomic practices

The land for leaf pubescence experiments was heavily disced to combine stool destruction. Using a tractor-drawn tine ripper, a 500 mm deep rip was performed to break the soil pan and increase root penetration. Then, to improve soil sett contact and break up larger soil clods, the land was fine-tilted with a fine disc harrow. The ridge construction was carried out at a standard gradient

of 1:250, resulting in a 1:200 furrow gradient. To restore soil nutrients and promote early crop growth, a single Super Phosphate was sprayed at a rate of 400 kg/ha in furrows.

After the land was irrigated, planting took place when the moisture content reached 90 mm (TAM-90). Other agronomic procedures were carried out in all treatments when required; except a factor that was being studied (Clowes and Breakwell, 1998). Planting was done on the 24th of February, 2023. Net plot size and gross plot size of 3307.5 m² and 1890 m² were used respectively. The area was divided into blocks to cater for variation in gradient. Each experimental block (plot) consisted of four rows 15 meters long with an inter-row spacing of 1.5 meters and in-row spacing of 0.5 meters at random locations in each field. This spacing translated to a density of 13333 plants per hectare. The remaining area was allocated to discard and path. The application of fertilizer was done based on the soil analysis. Single superphosphate fertilizer (superphosphate triple/300 kg per hectare) was applied in the furrows before planting. Furthermore, potassium was applied as Muriate of Potash (MOP) at 60 kg/ha after four weeks from emergence. Newly planted crop requires 120 -140 kg/ha which was applied as Ammonium Nitrate (AN) or Urea and split applied as 60 kg/ha four weeks after emergence and the other 80kg/ha at eight weeks. Sugarcane sets were sprinkled with 500 ml Bayfidan diluted in 5 liters to control termites according to the label. Later on, sugarcane sets were dipped in 60 ml/60 liters water Shavit (triadimanol) to control smut disease. Each cane variety was planted as set (60-70 cm and free from stem borer infestation). The absence of; feeding marks on funnel leaves, dead hearts, and holes in stems was the method used to certify sets free from stem borers. The sets used were dissected to verify the presence of stem borers. After planting the seed cane sets, all furrows were treated with atrazine and Sencor herbicides (2 + 2 kg per hectare) post-emergence herbicides based on local recommendations to suppress annual weeds. During plant growth, manual weeding was performed in experimental plots.

3.2.3 Leaf surface pubescence experiments

The seven sugarcane varieties were selected by considering moderate level of resistance, susceptibility, cane yield and estimated recoverable crystal (ERC %) based on recommendations from the Plant Breeding and Entomology Departments. Two preleased varieties namely; 00-1165 (low susceptible) and 96-1107 (highly susceptible) were selected. YSA moderate resistant sugarcane varieties based on visual assessments; ZN 8, ZN 9 and ZN 3L, hence ZN 8 and ZN 9

have the ability to recover from damage inflicted by YSA. Lastly, ZN 10 and N14 susceptible sugarcane varieties were chosen because of their high biomass and high ERC %. These sugarcane varieties are leading in terms of area under production in both out-grower farmers and estates. 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) suggests 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.*, 2020b; Singh *et al.*, 2021).

3.2.4 Experimental design

A 7 × 2 × 3 factorial design in a complete randomized block design (CRBD) replicated four times was used, under two treatments of aphid infestation (un-infested (control) and infested). Variety was the first factor with seven levels (96-1107, 00-1165, ZN 10, ZN 8, ZN 9, ZN 3L, and N14) and leaf position was the second factor under three levels (leaf position-1, leaf position-2 and leaf position-3). Leaf position refers to the arrangement of leaves on a sugarcane stem. It was determined two to three weeks after germination. Table 3.1 shows treatment combinations.

Table 3.1 Treatment combinations

Varieties	Aphid treatment	Leaf position
ZN 10	uninfested (control)	one
ZN 9	Infested	two
ZN 8		three
ZN 3L		
N14		
96-1107		
00-1165		

3.2.5 Data collection

Trichome density was determined on a light microscope using the protocols by Batyrrshina *et al.* (2020b) and Singh *et al.* (2021).

3.2.5.1 Trichome density

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. From each leaf, 1 cm² was excised, cleaned

with 80 % ethanol at 85 °C for 15 minutes, and rinsed with distilled water four times for three min each time at 45 °C. The tissue segment was placed on glass microscope slides facing the abaxial side, and the total number of non-glandular trichomes was counted (Batyrshtina *et al.*, 2020b; Singh *et al.*, 2021). The numbers of trichomes were determined on an area covering 1 cm² with a 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 square millimeters (mm²). Figure 3.1 shows the sugarcane crop leaf positions where trichomes were determined.

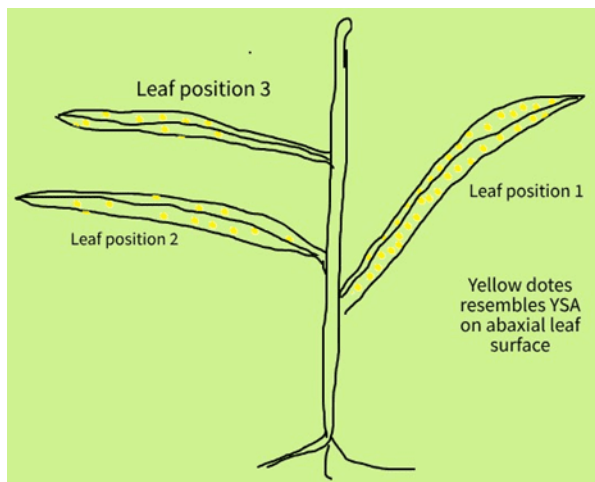


Figure 3.1: Animated diagram of leaf position in sugarcane crop at three-leaf stage

3.2.5.2 Aphid number

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 \leq three months old. However, YSA are found at every growth stage of sugarcane if conditions are favorable and becomes reduced when the crop has reached physiological maturity.

3.2.6 Data analysis

A two-way analysis of variance (ANOVA) was used to test the effects of leaf position, accession, aphid treatment, and their interaction (leaf position x accession x aphid treatment). Fishers Least Significance Difference (LSD) test ($p < 0.05$) was used in a one-way ANOVA to test for differences between the accessions. Using Fisher's least significance difference ($p < 0.05$), the statistical differences between the aphid-infested plants and the control (sprayed with Alice 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, aphid number and leaf position.

3.3 Results

3.3.1 Effect of leaf position and genotype (variety or accession) on aphid and leaf pubescence (trichome density)

Aphid performance 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 (mm^2) of trichomes was highest in leaf 3 (90.11), followed by leaf 2 (75.74), and lowest in leaf 1 (62.1), as shown in **Figure 3.2**. An increase in trichome density resulted in reduced aphid number, as shown in **Figure 3.2**. Leaf-3 (93.87) was most affected, followed by leaf-2 (78.58) and leaf-1 (65.71). ZN 10 scored the lowest trichome density (mm^2) (30.9; 41.27; 56.72) for leaf-1; leaf-2; 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) respectively on aphid-infested plots.

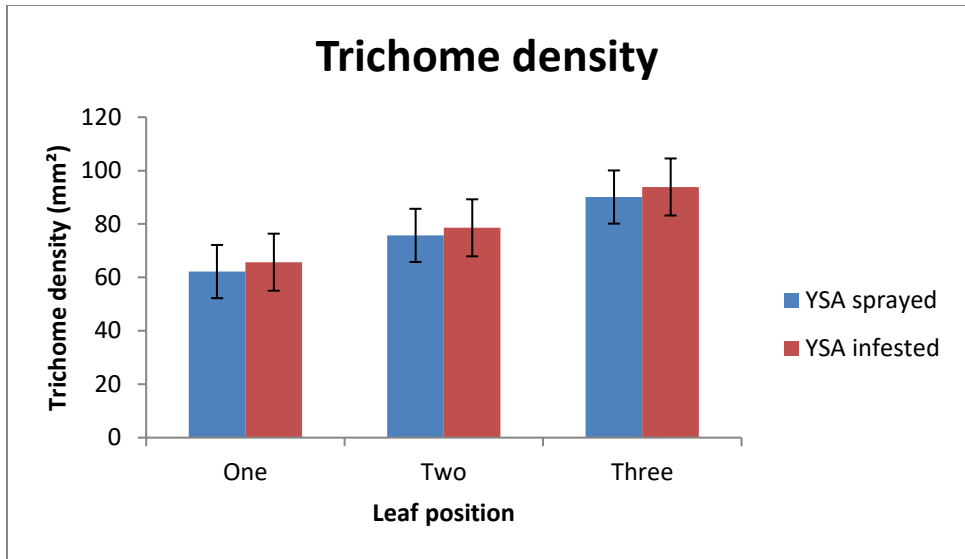


Figure 3.2: Trichome density on sprayed and infested leaf positions of sugarcane varieties

The three leaf positions and sugarcane varieties showed significant differences ($p < 0.001$), on trichome density according to the results (**Figure 3.3**). ZN10 recorded the lowest trichome density (mm²) (leaf-1, leaf-2, leaf-3) (20.97; 35.85; 50.97) respectively, while the 00-1165 sugarcane variety recorded the highest trichome density (mm²) across all leaf positions (leaf-1; leaf-2; leaf-3) (93.07; 111.15; 124.9) respectively.

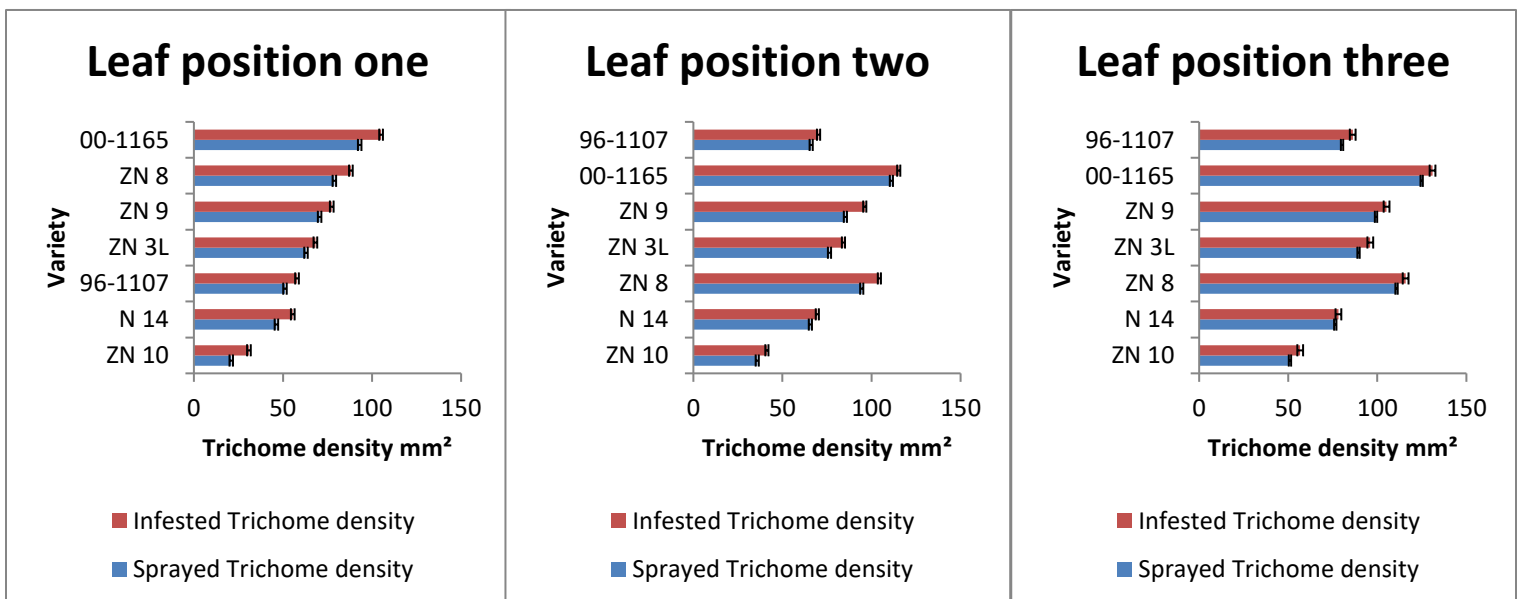


Figure 3.3: Trichome density at three leaf positions for tested varieties

The contribution of each parameter: genotype (accession), leaf position (leaf-1-3), and aphid treatment (Alice sprayed (control) and aphid-infested) was then determined by using a two-way ANOVA. The trichome density in the control plants varied greatly, showing notable variations depending on the leaf position ($p < 0.001$), the seven accessions ($p < 0.001$). However, there was no significant interaction ($p > 0.05$) between variety (accession) and leaf position. The trichomes increased significantly by 3.75–5.66 % upon aphid feeding, according to the results of the aphid infestation. The interaction between accessions and treatment of aphid infestation and the relationship between accession and leaf position and treatment of aphid infestation were not statistically significant ($p > 0.05$). Lastly, aphid treatment had no effect on leaf position. Ranking of resistance of sugarcane varieties to YSA was done according to leaf pubescence (trichome density) from low to high susceptibility: 00-1165 > ZN 8 > ZN 9 > ZN 3L > 96-1107 > N14 > ZN 10.

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

High leaf pubescence conferred resistance to YSA. Aphid counts on leaf-1, leaf-2, and leaf-3 showed significant differences ($p < 0.001$) amongst the seven sugarcane varieties as shown in **Figure 3.4**. 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 (Alice sprayed plants) aphid treatment, ZN 10 recorded the highest aphid number (46.5; 39.8; 37.5) for leaf-1; leaf-2; leaf-3 respectively whereas 00-1165 sugarcane variety realized lower aphid number across three leaf positions being 16.5; 12.5; 6.5 respectively. In aphid infested treatments, ZN 10 recorded the highest aphid population across the three leaf positions) (156; 97; 42) respectively whereas 00-1165 recorded the least number of aphid (53; 32.5; 16.5) for leaf-1; leaf-2; leaf-3 respectively. There was also no significant interaction ($p > 0.05$) between accession on the aphid number and leaf position. Overall, this indicates that the genetic background and leaf position impact on the dynamics of the aphid population in sugarcane. This could be the result of different defense strategy magnitudes.

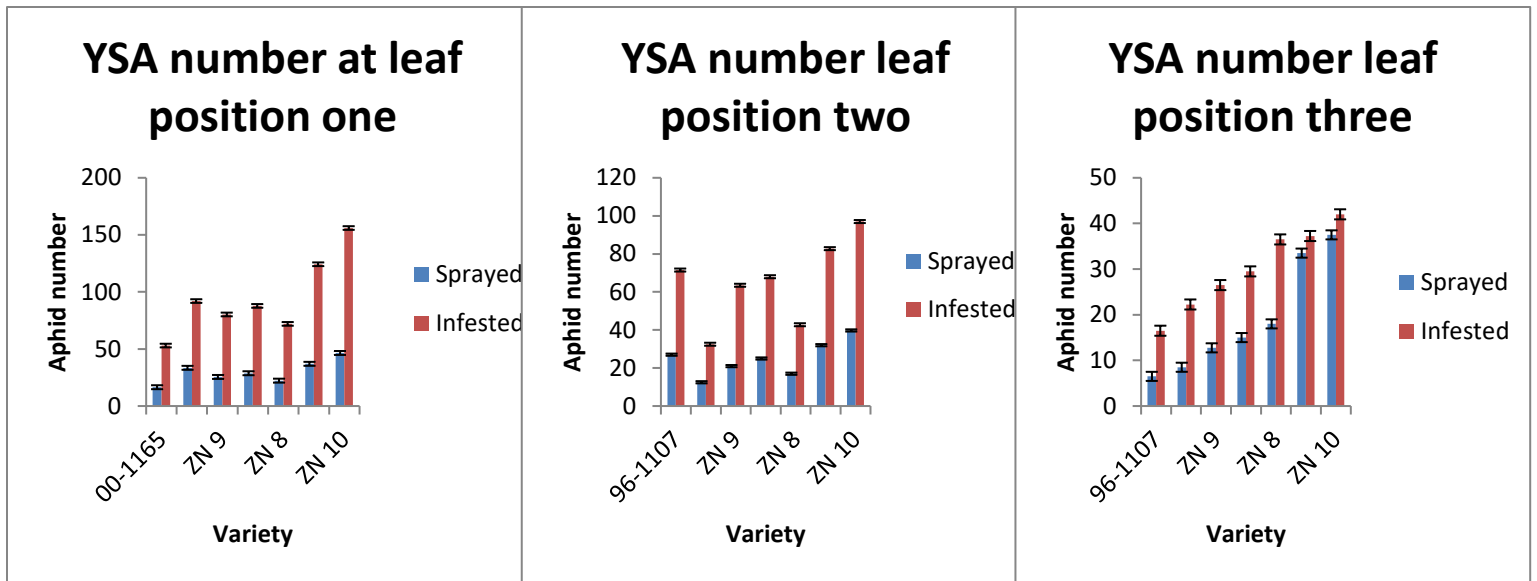


Figure 3.4: Effect of trichome density on YSA number at three leaf positions for tested varieties

3.3.3 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 varieties (Figure 3.5, $Y = -0.64X + 96.6$, $p < 0.001$, $r = 0.52$).

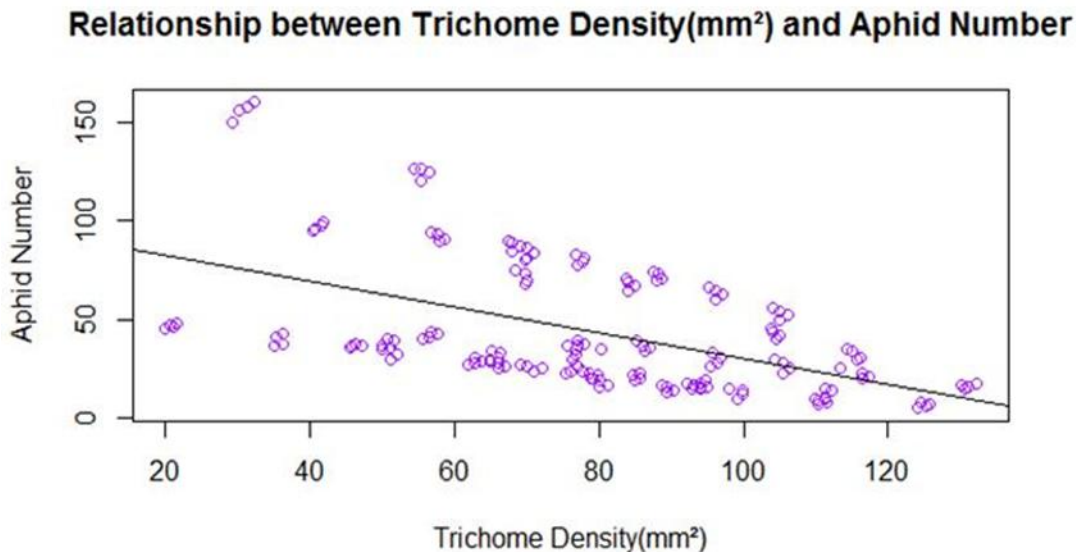


Figure 3.5: Relationship between aphid number and trichome density on different sugarcane varieties

3.3.4 Relationship between trichomes density and leaf position of sugarcane cultivars

The linear regression analysis demonstrated a significant weak positive correlation between the number of trichome density for various cultivars and leaf position after natural infestation (Figure 3.6, $Y = 6.33X + 50.97$, $p < 0.001$, $r = 0.45$).

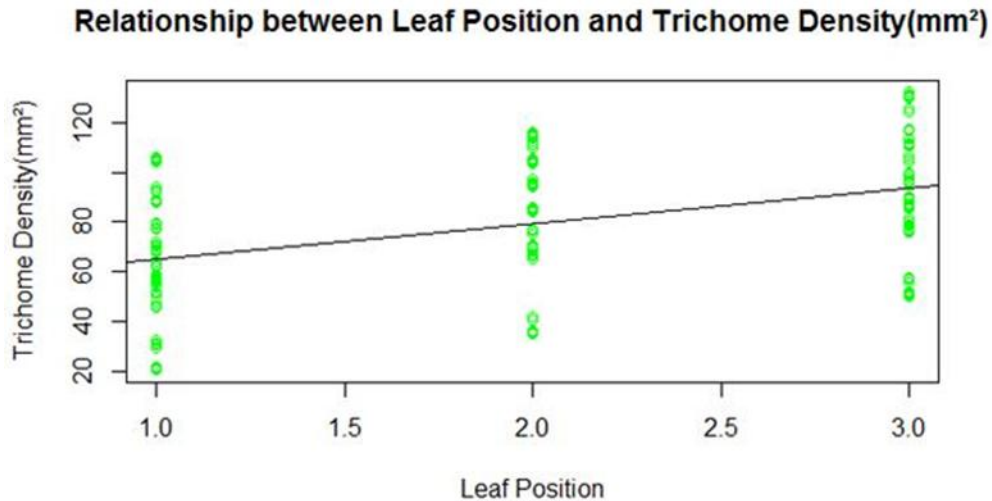


Figure 3.6: Relationship between leaf position and trichome density for sugarcane varieties

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

Findings of the linear regression analysis confirmed a significant weak negative correlation between the number of aphids for various varieties and trichome density after natural infestation (Figure 3.7, $Y = -24.8X + 82.11$, $p < 0.001$, $r = 0.48$).

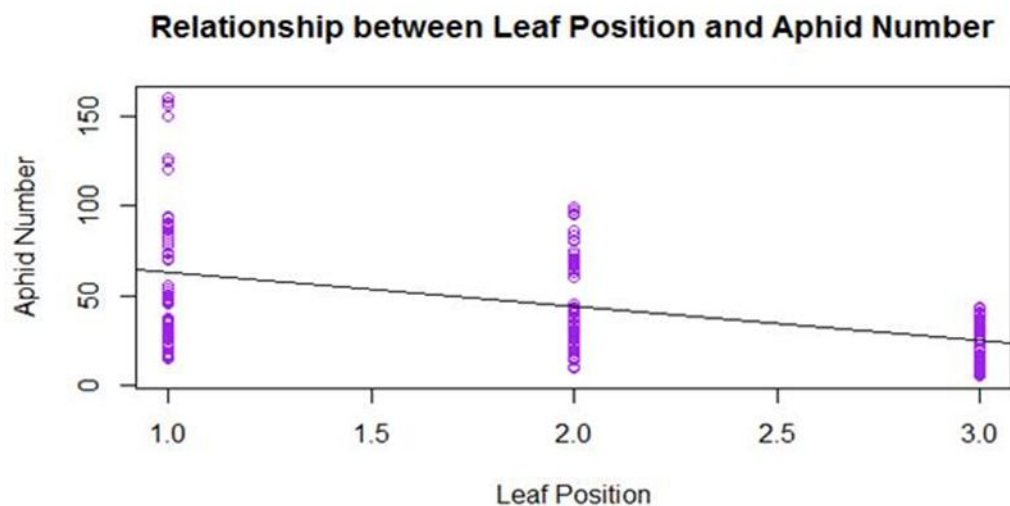


Figure 3.7: Relationship between leaf position and aphid number on tested sugarcane varieties

3.10 Discussion

The results obtained indicated that leaf pubescence (trichome density) differed significantly among sugarcane varieties and that trichome density increased in response to an increase in aphid number. This shows that stressed plants tend to increase defense strategies to reduce the risks of biotic and abiotic conditions. These results agree with findings by Boughalleb *et al.* (2011) who observed that the anatomical parameters of leaves indicate that drought stress caused an increase in the thickness of the palisade mesophyll and upper epidermis, as well as a rise in the number of stomata and trichomes. In particular, in developing organs, the presence of non-glandular trichomes on leaf surfaces offers adaptive advantages under stressful conditions by combining mechanical, biochemical, and physical protection. Karabourniotis *et al.* (2020) emphasized that, in the never-ending search for more stress-tolerant plant characteristics, trichomes constitute a significant plant characteristic that should be considered. In this study, stress might have contributed to increased trichome density; hence, this requires a multinomial regression to make valid conclusions about the effects of environmental conditions on YSA. Some studies have reported the positive contribution of trichomes in that they reduce the heat stress load across the leaf surface by reducing radiation absorbance (Ehleringer *et al.*, 1976;

Vogelmann, 1993). Ehleringer (1984) highlighted that, because of the increased relative humidity near the leaf surface, there is less water potential difference between the leaf tissue and the surrounding air layer, which reduces transpiration water loss. Wellso *et al.* (1982) demonstrated that the trichome density was positively associated with leaf length and negatively associated with soil moisture. Furthermore, temperature and the trichome profiles of the first seedling leaf had a positive correlation (Wellso *et al.*, 1982). These aforementioned studies show that stress contributes positively to the realization of high trichome densities in plants which might be a similar case in the current study. This explains why there was no interaction between leaf position and sugarcane accession because other external factors contribute to trichome density.

This study showed that sugarcane leaves are covered with non-glandular trichomes, structures that resemble epidermal hair-like. Furthermore, the sugarcane varieties' trichome density fell within the range of 20.97 to $\pm 122 \text{ mm}^2$ which was realized by Ferreira *et al.* (2007). This confirms a variation of trichome density among different sugarcane accessions found in different localities. Comparable patterns of trichome existence were observed on the leaves of other cereals such as barley and wheat (Leybourne *et al.*, 2019; Correa *et al.*, 2020; Batyrshina *et al.*, 2020b). These results corroborate the findings of Sosa (1991) who reported that leaf surface pubescence may be a key morphological trait for resistance to YSA. He also noted that pubescent sugarcane clones were resistant to YSA. However, Roberts and Foster (1983) study demonstrated that dense leaf pubescence in wheat confers resistance to the grain aphid; *Rhopalosiphum padi* L. Periodic scouting was performed to monitor aphid populations after spraying of controls. From the visual assessments at leaf stage three in this study, the aphid population was not within the economic injury and threshold levels. This procedure confirmed, a visual evaluation of the damage caused by aphid feeding on the plant which was preserved as damage ratings (Smith *et al.*, 1997). The damage rating system that Heng-Moss *et al.* (2002) proposed was implemented for YSA damage ratings. The assessments proved that trichome density during the early stages of growth minimized aphid infestations among the sugarcane varieties.

Results of this study support the hypothesis that the physical barrier created by non-glandular trichomes in sugarcane can restrict insect movement and prevent phloem feeders from probing (Handley *et al.*, 2005; Sato and Kudoh, 2015; Singh *et al.*, 2021). Moreover, the findings showed that trichomes differed according to leaf position. These results are consistent with those of Leybourne *et al.* (2019); Gyan *et al.* (2020) and Singh *et al.* (2021) who emphasized how different leaf positions, development stages, genetic backgrounds, and even insect damage can affect trichome density. Results aligns with the optimal defense theory (McCall and Fordyce, 2010), which states that defenses are directed toward younger leaves. In all three leaf positions (leaf-1, leaf-2, and leaf-3), the 00-1165 accession had the highest trichome density and aphid number when compared to the susceptible ZN 10 accession, indicating the combined effects of leaf position and genotype. Feeding by aphids might have inflicted stress on the crop resulting in increased trichome density. Similar results have been reported on effects of environmental conditions and biotic stresses on trichome density (Nawab *et al.*, 2011).

Furthermore, trichome density was negatively correlated with aphid number showing that denser trichomes in sugarcane interferes with YSA feeding. This requires further studies where an electrical penetration graph (EPG) is to be used to monitor how trichomes interfere with aphid feeding as reported by Gyan *et al.* (2020) and Singh *et al.* (2021). Moreover, the study confirms results by Batyrshina *et al.* (2020b) who suggested that aphid number and constitutive levels of trichome density are inversely correlated and on newly developing leaves (Traw and Bergelson, 2003). However, dense trichomes might have delayed the penetration of stylets during YSA feeding.

In line with findings on delayed stylet penetration, phloem feeders might have their stylet insertion interrupted by non-glandular trichomes on the leaf surface as reported by Handley *et al.* (2005) and Sato and Kudoh (2015). The results of feeding behavior highlight the possibility that the high number of trichomes on leaf-3 may have prolonged the time it took for aphids to penetrate the leaf mesophyll. This resulted in a negative correlation between aphids and trichome density; and aphid number and leaf position. Similar results were also tabled by Amin *et al.* (2017) who reported a negative correlation between the number of aphids for various cotton cultivars and trichome density after natural infestation. This explains that an increase in trichome

density amongst the varieties resulted in a reduction of aphid populations. Additionally, Batyrshina *et al.* (2020b) reported related results in wheat about reduced aphid reproduction. Furthermore, a weak positive correlation was observed between trichome density and leaf position. This might be an indication that other external variables were 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). These findings validate the research that was conducted by Ebisui *et al.* (1998); Cambier *et al.* (2000); Nomura *et al.* (2005, 2008) who observed that during development toward the juvenile stage, trichome levels tend to decrease and are typically highest in young seedlings. This affirms the study by Singh *et al.* (2021) who noted that research has demonstrated that feeding herbivores results in higher trichome densities in the leaves that are subsequently formed. Numerous studies (Howe and Jander, 2008; Brunissen *et al.*, 2009; Chandrasekhar *et al.*, 2018; Batyrshina *et al.*, 2020a) pointed out that, depending on the plant's age, developmental stage, and genetic makeup, defense mechanisms can also be dynamic and induced in response to herbivore attack.

Moreover, leaf position affected trichome density. These findings concur with results by Valkama *et al.* (2004) and McCall and Fordyce (2010) who indicated that trichomes are usually more evident in young tissues than in older ones. Additionally, studies by Gyan *et al.* (2020) and Singh *et al.* (2021) confirmed similar results on the contribution of leaf position on trichome density secretion in response to aphid herbivory. This indicates the functioning of non-glandular trichomes (leaf pubescence) as a partial defense mechanism on sugarcane leaves.

3.11 Conclusion

Leaf pubescence (trichome density) differs among the sugarcane varieties regarding leaf position and aphid infestation, as the level of resistance was ranked from high to low: 00-1165 > ZN 8 > ZN 9 > ZN 3L > 96-1107 > N14 > ZN 10. Breeding sugarcane varieties with desirable trichome density can be a valuable strategy for developing YSA-resistant varieties.

References

- Amin, M. R., Afrin, R., Alam, M. Z., Hossain, M. M., and Kwon, Y. J. 2017. Effect of leaf trichomes and meteorological parameters on population dynamics of aphid and jassid in cotton. *Bangladesh Journal of Agricultural Research*. 42(1): 13-25.
- Awmack, C. S., and Leather, S. R. 2002. Host plant quality and fecundity in herbivorous insects. *Annu. Rev. Entomol.* 47: 817–844.
- Batyrshina, Z. S., Cna'ani, A., Rozenberg, T., Seifan, M., and Tzin, V. 2020a. The combined impacts of wheat spatial position and phenology on cereal aphid abundance. *PeerJ*. 8:e9142. doi: 10.7717/peerj.9142
- Batyrshina, Z. S., Yaakov, B., Shavit, R., Singh, A and., Tzin, V. 2020b. Comparative transcriptomic and metabolic analysis of wild and domesticated wheat genotypes reveals differences in chemical and physical defense responses against aphids. *BMC Plant Biol.* 20:19. doi: 10.1186/s12870-019-2214-z
- Baur, R., Binder, S., and Benz G. 1991. Nonglandular leaf trichomes as short-term inducible defense of the gray Alder, *Alnus incana* (L), against the chrysomelid beetle, *Agelastica alni* L. *Oecologia*. 87: 219-226.
- Berlyn, G. P., and Miksche. J. P. 1976. Botanical microtechnique and cytochemistry, pp. 126-128. Iowa State University Press, Ames.
- Boughalleb, F., and Hajlaoui, H. 2011. Physiological and anatomical changes induced by drought in two olive cultivars (cv Zalmati and Chemlali). *Acta Physiologiae Plantarum*. 33: 53-65.
- Brunissen, L., Cherqui, A., Pelletier, Y., Vincent, C., and Giordanengo, P. 2009. Host-plant mediated interactions between two aphid species. *Entomol. Exp. Appl.* 132: 30–38. doi: 10.1111/j.1570-7458.2009.00862.x
- Cambier, V., Hance, T., and de Hoffmann, E. 2001. Effects of 1,4-benzoxazin- 3-one derivatives from maize on survival and fecundity of *Metopolophium dirhodum* (Walker) on artificial diet. *J. Chem. Ecol.* 27:359.

- Chandrasekhar, K., Shavit, R., Distelfeld, A., Christensen, S and Tzin, V. 2018. Exploring the metabolic variation between domesticated and wild tetraploid wheat genotypes in response to corn leaf aphid infestation. *Plant Signal. Behav.* 13:e1486148. doi: 10.1080/15592324.2018.1486148
- Clowes, M. J., and Breakwell, W. L. 1998. Zimbabwe sugarcane production manual. Zimbabwe Sugar Association Experiment Station, Chiredzi, Zimbabwe.
- Correa, L. J., Maciel, O. V. B., Bucker-Neto, L., Pilati, L., Morozini, A. M., Faria, M. V., *et al.* 2020. A comprehensive analysis of wheat resistance to *Rhopalosiphum padi* (Hemiptera: Aphididae) in Brazilian wheat cultivars. *J. Econ. Entomol.* 113:1493–1503. doi: 10.1093/jee/toaa059
- de Correa, L. J., Maciel, O. V. B., Bucker-Neto, L., Pilati, L., Morozini, A. M., Faria, M. V., *et al.* 2020. A comprehensive analysis of wheat resistance to *Rhopalosiphum padi* (Hemiptera: Aphididae) in Brazilian wheat cultivars. *J. Econ. Entomol.* 113, 1493–1503. doi: 10.1093/jee/toaa059
- Ebisui, K., Ishihara, A., Hirai, N., and Iwamura, H. 1998. Occurrence of 2,4- Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and a β -Glucosidase Specific for Its Glucoside in Maize seedlings. *Z. Naturforsch.* 53: 793–798. doi:10.1515/znc-1998-9-1003
- Ehleringer, J. 1984. Ecology and ecophysiology of leaf pubescence in North American desert plants. *Plant trichomes.* 113-132.
- Ehleringer, J., Björkman, O., and Mooney, H. A. 1976. Leaf pubescence: effects on absorptance and photosynthesis in a desert shrub. *Science.* 192(4237): 376-377.
- Ehrlich, P. R., Ehrlich, A. H., and Daily, G. C. 199). Food security, population and environment. *Population and development review.* 1-32.
- Eisner, T., Eisner, M., and Hoebcke, E. R. 1998. When defense backfires: Detrimental effect of a plant's protective trichomes on an insect beneficial to the plant. *Proceedings of the National Academy of Sciences.* 95: 4410-4414.

- Ferreira, E.A., Ventrella, M.C., Santos, J.B., Barbosa, M.H.P., Silva, A.A., Procópio, S.O and Silva, E.A. M. (2007). Leaf blade quantitative anatomy of sugarcane cultivars and clones. *Planta Daninha*. 25: 25-34.
- Gallun, R. L., Ruppel, R., and Everson, E. H. 1966. Resistance of small grains to the cereal leaf beetle. *J. Econ. Entomo.* 59: 827-829.
- Gaud, S. M., Martorell, L. F and Robles, R. B. 1965. Notes on the biology and control of the yellow aphid of sugarcane, *Sipha flava* (Forbes), in Puerto Rico. *Proc Int Soc Sug Cane Technol.* 12: 1307-1320.
- Gyan N. M., Yaakov, B., Weinblum, N., Singh, A., Cna'ani, A., Ben-Zeev, S., Saranga, Y and., Tzin, V. 2020. Variation Between Three Eragrostis tef Accessions in Defense Responses to *Rhopalosiphum padi* Aphid Infestation. *Front. Plant Sci.* 11:598483. doi: 10.3389/fpls.2020.598483
- Halbert, S. E., Miller, G. L., and AMES, L. M. 2013. *The genus Sipha Passerini (Hemiptera: Aphididae) in North America.*- Ins. Mundi, Paper 831.
- Hall, D. G. 2001. Notes on the yellow sugarcane aphid *Sipha flava* (Homoptera: Aphididae) and the lady beetle *Diomus terminatus* (Coleoptera: Coccinellidae) in Florida. *J Am Soc Sug Cane Technol.* 21: 21-29.
- Handley, R., Ekbom, B., and Agren, J. 2005. Variation in trichome density and resistance against a specialist insect herbivore in natural populations of *Arabidopsis thaliana*. *Ecological Entomology.* 30: 284-292. doi: 10.1111/j.0307-6946. 2005.00699.x
- Heng-Moss, T. M., Baxendale, F. P., Riordan, T. P., and Foster, J. E. 2002. Evaluation of buffalograss germplasm for resistance to *Blissus occiduus* (Hemiptera: Lygaeidae). *J Econ Entomol.* 95(5):1054–1058. doi:10.1603/0022-0493-95.5.1054
- Howe, G. A., and Jander, G. 2008. Plant immunity to insect herbivores. *Annu. Rev. Plant Biol.* 59: 41–66. doi: 10.1146/annurev.arplant.59.032607.092825
- Jakobs, R., and Müller, C. 2018. Effects of intraspecific and intra-individual differences in plant quality on preference and performance of monophagous aphid species. *Oecologia.* 186: 173–184. doi: 10.1007/s00442-017-3998-x

- Karabourniotis, G., Liakopoulos, G., Nikolopoulos, D and Bresta, P. 2020. Protective and defensive roles of non-glandular trichomes against multiple stresses: structure–function coordination. *Journal of Forestry Research*. 31(1): 1-12.
- Karley, A. J., Douglas, A. E., and Parker, W. E. 2002. Amino acid composition and nutritional quality of potato leaf phloem sap for aphids. *J. Exp. Biol.* 205: 3009–3018. doi: 10.1242/jeb.205.19.3009
- Khan, M. M., Kundu, R., and Alam, M. Z. 2000. Impact of trichome density on the infestation of *Aphis gossypii* Glover and incidence of virus disease in ashgourd *Benincasa hispida* (Thunb.). *International Journal of Pest Management*. 46: 201-204.
- Konyspaevna, S. K. 2012. Spring wheat resistance against cereal leaf beetle (*Oulema melanopus* Z.) in relation to leaf pubescence. *Aust. J. Basic Appl. Sci.* 6: 515–518.
- Lai, A., Cianciolo, V., Chiavarini, S., and Sonnino, A. 2000. Effects of glandular trichomes on the development of *Phytophthora infestans* infection in potato (*S. tuberosum*). *Euphytica*. 114: 165–174.
- Lam, W. K. F., and Pedigo, L. P. 2001. Effect of trichome density on soybean pod feeding by adult bean leaf beetles (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*. 94: 1459-1463.
- Leake, A. R. 2003. Integrated pest management for conservation agriculture. *Conservation agriculture: environment, farmers experiences, innovations, socio-economy, policy*. 271-279.
- Leybourne, D. J., Valentine, T. A., Robertson, J. A. H., Pérez-Fernández, E., Main, A. M., Karley, A. J., et al. 2019. Defence gene expression and phloem quality contribute to mesophyll and phloem resistance to aphids in wild barley. *J. Exp. Bot.* 70: 4011–4026. doi: 10.1093/jxb/erz163
- Liu, Z., Wang, Q., Wan, H., Yang, F., Li, S., Yang, M., Luo, J., Zheng, J., Deng, Q., Long, H., et al. 2021. Identification and Validation of a Novel Major QTL Controlling Leaf Pubescence in the Chinese Wheat Landrace ‘Baimaomai’. *Agronomy*. 11: 2237. <https://doi.org/10.3390/agronomy11112237>

- Lovinger, A., Liewehr, D., and Lamp, W. O. 2000. Glandular trichomes on alfalfa impede searching behavior of the potato leafhopper parasitoid. *Biological Control*. 18(3): 187-192.
- Macharia, I. N., Mithöfer, D., and Waibel, H. 2009. Potential environmental impacts of pesticides use in the vegetable sub-sector in Kenya. *African Journal of Horticultural Science*. 2:138-151.
- McAuslane, H. J. 1996. Influence of leaf pubescence on ovipositional preference of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on soybean. *Environmental Entomology*. 25: 834-841.
- McCall, A. C., and Fordyce, J. A. 2010. Can optimal defence theory be used to predict the distribution of plant chemical defences? *J. Ecol.* 98: 985–992. doi: 10.1111/j.1365-2745.2010.01693.x
- Mergher, R. L., Smith, C. W and Smith, W. J. 1997. Preference of *Gossypium* genotypes to *Bemisia argentifolii* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 90: 1046–1052.
- Mertens, D., Boege, K., Kessler, A., Koricheva, J., Thaler, J. S., Whiteman, N. K., et al. 2021. Predictability of biotic stress structures plant defense evolution. *Trends Ecol. Evo.* 36: 444–456. doi: 10.1016/j.tree.2020.12.009
- Mitchell, C., Brennan, R. M., Graham, J., and Karley, A. J. 2016. Plant defense against herbivorous pests: exploiting resistance and tolerance traits for sustainable crop protection. *Front. Plant Sc.* 7:1132. doi: 10.3389/fpls.2016. 01132
- Moghadam, S. G., Hosseini, M and Awal, M. M. 2013. Does leaf pubescence of wheat affect host selection and life table parameters of *Sipha maydis* (Hemiptera: Aphididae)? *J. Crop Pro.*, 2 (1): 81-92.
- Mutonyi, J., and Babikha, S. J. 2019. The occurrence of sugarcane yellow aphid (*Sipha flava* (Homoptera: Aphididae) in Kakamega North Sub-County, Kenya. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*. 12 (6): 34-38.
- Nawab, N. N., Khan, I. A., Khan, A. A., and Amjad, M. 2011. Characterization and inheritance of cotton leaf pubescence. *Pak. J. Bot.* 43: 649–658.

- Nomura, T., Ishihara, A., Yanagita, R. C., Endo, T. R., and Iwamura, H. 2005. Three genomes differentially contribute to the biosynthesis of benzoxazinones in hexaploid wheat. *Proc. Natl. Acad. Sci. U S A.* 102: 16490–16495. doi: 10.1073/pnas.0505156102
- Nomura, T., Nasuda, S., Kawaura, K., Ogihara, Y., Kato, N., Sato, F., et al. 2008. Structures of the three homoeologous loci of wheat benzoxazinone biosynthetic genes TaBx3 and TaBx4 and characterization of their promoter sequences. *Theor. Appl. Genet.* 116. 373–381. doi: 10.1007/s00122-007-0675-671
- Nuessly, G. S., and Hentz, M. G. 2002a. Feeding effects of yellow sugarcane aphid on sugarcane. *Journal of the American Society of Sugar Cane Technology.* 22: 126-127.
- Nuessly, G. S. 2005. Featured Creatures: Yellow Sugarcane Aphid; publication #EENY-354; University of Florida: Gainesville, FL, USA; Available online: http://entnemdept.ufl.edu/creatures/field/bugs/yellow_sugarcane_aphid.htm (accessed on 16 January 2022).
- Nuessly, G. S., Hentz, M. G., and Gilbert, R. A. 2010. Susceptibility of stage IV canal point (CP) sugarcane clones to yellow sugarcane aphid (*Sipha flava* (Forbes)) feeding damage. *J Am Soc Sug Cane Technol.* 30: 104-114.
- Oguchi, R., Onoda, Y., Terashima, I., and Tholen, D. 2018. Leaf anatomy and function of chapter 5. In *The Leaf: A Platform for Performing Photosynthesis, Advances in Photosynthesis and Respiration Including Bioenergy and Related Processes*, 2nd ed.; Adams, W.W., III, Terashima, I., Eds.; Springer Nature Switzerland AG: Gewerbestrasse, Switzerland. 44: 98–128.
- Oriani, M. A. D .G., and Vendramim, J. D. 2010. Influence of trichomes on attractiveness and ovipositional preference of *Bemisia tabaci* (Genn.) B Biotype (Hemiptera: Aleyrodidae) on tomato Genotypes. *Neotropical Entomology.* 39: 1002-1007.
- Pandey, P., Irulappan, V., Bagavathiannan, M. V., and Senthil-kumar, M. 2017. Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. *Front. Plant Sci.* 8: 537.
- Ramvalho, F. S., Parrott, W. L., Jenkins, J. N., and McCarty, J. C. 1984. Effects of cotton leaf trichomes on the mobility of newly hatched tobacco budworms (Lepidoptera: Noctuidae). *Journal of Economic Entomology.* 77: 619-621.

- Riddick, E. W., and Simmons, A. M. 2014. Do plant trichomes cause more harm than good to predatory insects? *Pest Manag. Sci.* 70: 1655–1665. doi: 10.1002/ps.3772
- Roberts, J. J., and Foster, J. E. 1983. Effect of leaf pubescence in wheat on the bird cherry oat aphid (Homoptera: Aphidae). *J. Econ. Entomol.* 76: 1320–1322. doi: 10.1093/jee/76.6.1320
- Roberts, J. J., Gallun, R. L., Patterson, F. L., and Foster, J. E. 1979. Effects of wheat leaf pubescence on the Hessian fly. *J. Econ. Entomol.* 72: 211-214.
- Sato, Y., and Kudoh, H. 2015. Tests of associational defence provided by hairy plants for glabrous plants of *Arabidopsis halleri* subsp. *gemmaifera* against insect herbivores. *Ecol. Entomol.* 40: 269–279. doi: 10.1111/een.12179
- Shufran, R. A., Wilde, G. E., and Sloderbeck, P. E. 1997. Response of three greenbug (Homoptera: Aphididae) strains to five organophosphorous and two carbamate insecticides. *Journal of Economic Entomology.* 90: 283-286.
- Singh, A., Dilkes, B., Sela, H., and Tzin, V. 2021. The Effectiveness of Physical and Chemical Defense Responses of Wild Emmer Wheat Against Aphids Depends on Leaf Position and Genotype. *Front. Plant Sci.* 12:667820. doi: 10.3389/fpls.2021.667820
- Smith, C. M., Khan, Z. R., and Pathak, M. D. 1993. Techniques for evaluating insect resistance in crop plants. CRC, Boca Raton
- Solereider, H. 1908. Systematic anatomy of dicotyledons: A handbook for laboratories of pure and applied botany. *Natur.*, 79: 211–212.
- Sosa, O. Jr. 1990. Pubescence in sugarcane as an obstacle to yellow sugarcane aphid establishment. *Sugar Y Azuca.* 85: 30.
- Srinivasana, R., and Uthamasa, S. 2005. Trichome density and antibiosis affect resistance of tomato to fruit borer and whitefly under laboratory conditions. *Journal of Vegetable Science.* 11: 3-17.
- Traw, M. B., and Bergelson, J. 2003. Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*. *Plant Physiol.* 133: 1367–1375. doi: 10.1104/pp.103.027086

- Valkama, E., Salminen, J. P., Koricheva, J., and Pihlaja, K. 2004. Changes in leaf trichomes and epicuticular flavonoids during leaf development in three birch taxa. *Ann. Bot.* 94: 233–242. doi: 10.1093/aob/mch131
- Vogelmann, T. C. 1993. Plant tissue optics. *Annual review of plant biology.* 44(1): 231-251.
- Way, M. J., Conlong D. E., Martin, L. A., McFarlane, S. A., Stranack, R., Keeping M. G., and Rutherford R. S. 2014. First record of yellow sugarcane aphid, *Sipha flava* (Homoptera: Aphididae), in the South African sugarcane industry. *Proceeding of the South African Sugar Technology Association.* 87: 53–57.
- Webster, J. A., Inayatullah, C., Hamissou, M., and Mirkes, K. A. 1994. Leaf pubescence effects in wheat on yellow sugarcane aphids and greenbugs (Homoptera: Aphididae). *J. Econ. Entomol.* 87 (1): 231-240.
- Wellso, S. G., and Hoxie, R. P. 1982. The Influence of Environment on the Expression of Trichomes in Wheat 1, *Crop Science.* 22(4): 879-886.
- White, W. H. 1990. Yellow Sugarcane Aphid (Homoptera: Aphididae) resistance mechanisms in selected sugarcane cultivars. *J Econ Ent.* 83(5): 2111-2114.
- Wilson, B. E. 2019. Hemipteran Pests of Sugarcane in North America. *Insects.* 10 (107): 1-15. doi:10.3390/insects10040107
- Wood, W., Gore, J., Catchot, A., Cook, D., Dodds, D., and Krutz, J. 2017. Effect of leaf pubescence on tarnished plant bug (Hemiptera: Miridae) ability to cause damage and yield loss in cotton. *J. Cotton Sci.* 21: 122–127.
- Woodman, R. L., and Fernandes, G. W. 1991. Differential mechanical defense - Herbivory, evapotranspiration, and leaf-hairs. *Oikos.* 60: 11-19.
- Xie, Y., Yu, X., Jiang, S., Xiao, K., Wang, Y., Li, L., Wang, F., He, W., Cai, Q., Xie, H., et al. 2020. OsGL6, a conserved AP2 domain protein, promotes leaf trichome initiation in rice. *Biochem. Biophys. Res. Commun.* 522: 448–455.

Yalçın, S. S., Örün, E., Yalçın, S., and Aykut, O. 2015. Organochlorine pesticide residues in breast milk and maternal psychopathologies and infant growth from suburban area of Ankara, Turkey. *International journal of environmental health research*. 25 (4):364-372.

CHAPTER FOUR

Biochemical resistance in commercial sugarcane (*S. officinarum*) cultivars in retort to Yellow Sugarcane Aphid (*S. flava*) injury

Abstract

Under conditions of natural infestation, the biochemical influence of YSA feeding was ascertained. With seven sugarcane cultivars (00-1165, ZN 3L, ZN 8, ZN 9, 96-1107, N14, and ZN 10) under two treatments of aphid infestation (un-infested (control) and infested), a 7×2 factorial in a complete randomized block design (CRBD) replicated four times was utilized. Results showed that there were significant differences ($p < 0.05$) among the sugarcane varieties in leaf nitrogen content and protein content. ZN 9 sugarcane variety recorded the highest leaf nitrogen content (2.49) which was a 32.5 % increase in response to aphid infestation in summer. Moreover, the least leaf nitrogen leaf content (2.13) which constitutes a 16 % decrease in nitrogen leaf content in response to Yellow Sugarcane Aphid (*S. flava*) incursion was obtained on ZN 10. Nevertheless, there were no significant differences ($p > 0.05$) in the phosphorous, potassium content, magnesium, and calcium among the sugarcane varieties in aphid-infested plots. Outcomes of the regression analysis revealed a highly significant ($p < 0.001$) positive correlation ($r = 0.90$) between aphid number and percentage chlorophyll change. Moreover, highly significant differences ($p < 0.001$) were recorded on total soluble sugar content in control and YSA-infested plots. In aphid-infested plots, 00-1165 sugarcane variety recorded the highest (25.39) soluble sugar content while N14 scored the lowest (8.13). Regression results showed a strong highly significant ($p < 0.001$) positive correlation ($r = 0.80$) between aphid number and total soluble sugars. YSA significantly reduced the tested biochemical parameters in susceptible sugarcane varieties. Farmers should add YSA biochemical tolerant varieties into their management programs.

Keywords: sugarcane, biochemical, susceptible, tolerant, YSA

4.1 Introduction

Ten aphid species have been reported to be infesting sugarcane (*Sacharum officinarum* L.) have been documented worldwide (Blackman and Eastop, 2000; Akbar, 2009). Among them is yellow sugarcane aphid (YSA) (*Sipha flava*). Since its introduction into Zimbabwe's sugarcane industry in 2014, YSA has grown to be a significant economic pest (ZSAES, unpublished). It has been described as a sap-sucking, piercing pest that prefers to feed along parallel veins on the underside of sugarcane leaves (Way *et al.*, 2014). In sugarcane, early-stage damage results in considerable yield and tiller losses (Hall, 2001).

Prevalent management options for YSA include chemical, biological, and cultural approaches. Currently, the sugar industry is using the chemical control option; therefore, other approaches, such as biochemical resistance require to be incorporated into existing integrated pest management (IPM) strategies. Biochemical compensation in response to YSA has been reported in switch grass (Koch, 2015) and sugarcane aphid (SCA) in sorghum (Akbar, 2009), with a bias towards sugarcane crops. Moreover, the biochemical reactions of two sugarcane cultivars to whiteflies (*Neomaskellia andropogonis*) were investigated by Saeedi and Ziaee (2020). Their findings demonstrated that the levels of soluble sugars, nitrogen, and total protein were markedly decreased, hence the need for such studies on YSA.

In a study on the resistance of various sorghum genotypes to sorghum aphids and their relationship to the physical features of sorghum, Paudyal *et al.* (2019) discovered that leaf sugar concentration and chlorophyll content were important factors contributing to aphid resistance. It has been observed that leaves with high levels of sugar, chlorophyll, nitrogen, and potassium are less attractive to aphids, and leaves with high levels of phosphorus, potassium, and polyphenols are also less attractive to aphids (Tao *et al.*, 2020).

Zhang *et al.* (2022) indicated that one or more genes might be responsible for controlling a plant's resistance to aphids. Furthermore, other researchers (Dogimont *et al.*, 2010; Porras Mitzy *et al.*, 2018) have pointed out that aphid resistance in many plant species is usually regulated by dominant and single genes (Du *et al.*, 2009, Xanying and Shiqiang, 2004) whereas recessive genes have been reported previously (Nkongolo *et al.*, 1991; Catena and Glogoza, 2004).

Furthermore, it has been documented that feeding by aphids have caused a significant increase in total protein content when compared to the control (Ni *et al.*, 2001). Wilson *et al.* (2011) reported an increase in nitrogen and nitrogen reductase in aphid infested leaves relative to the control treatment. Other researchers (Syvertsen *et al.*, 2003; Urban *et al.*, 2004; Pincebourde and Ngao, 2021) have highlighted that increased leaf nitrogen content supports the sink hypothesis in leaves attacked by apple green aphids. However, Prochaska (2015) documented no significant differences in total protein content between aphid-infested and aphid-free control treatments in switchgrass. This calls for such studies in the sugar industry to document the biochemical responses of tolerant and susceptible sugarcane genotypes, where YSA has become a resident pest.

According to Mithfer and Boland (2012), there have been over million years of documented coevolution between plants and insects, which has led to the evolution of a broad range of traditional defence mechanisms that thwart and elude different insect attacks. Several authors (Howe and Schaller 2008; Jaouannet *et al.*, 2014; Nalam *et al.*, 2019) have noted that the majority of plants have developed physical and chemical barriers as part of their defense mechanisms against aphids. Moreover, plants use both chemical and physical defence mechanisms known as host plant resistance to protect themselves from pest infestation (Mithfer and Boland, 2012; Marimuthu and Smith, 2012). The breeding and cultivation of aphid-resistant cultivars is a crucial strategy and task in managing aphids (Dogimont *et al.*, 2010). Zhang *et al.* (2022) demonstrated that host plant resistance influences herbivore growth and development. In support of this, Du *et al.* (2004) highlighted that herbivores have different chemical compositions and contents, which affect how their natural enemies feed. Additionally, Zhang *et al.* (2022) highlighted that host plants can trigger signal transduction in response to insect damage.

Biochemical responses to insect damage may be governed by phytohormones, such as jasmonic acid (JA) and salicylic acid (SA) (Robert-Seilaniantz *et al.*, 2011) which control the various signaling pathways that help plants defend them against insect pests. De Vos *et al.* (2005) and Bidart-Bouzat and Kliebenstein (2011) reported that plants display different defense mechanisms in response to insects. In support of this, Zhang *et al.* (2013) and Nalam *et al.* (2019) revealed that most phloem-feeding insects trigger defense reactions mediated by SA; hence, several defense genes, including those that encode proteins linked to pathogenesis-related proteins (PR),

are regulated by the SA pathway. Furthermore, Martinez De Ilarduya *et al.* (2003) concluded that potato aphids (*Macrosiphum euphorbiae*) stimulated the expression of PR proteins when feeding on tomato plants. Protease inhibitors (PIN) and wound-induced proteins (WIP) have also been reported as examples of JA-defense-responsive defence genes (Wang and Wu, 2013). Some studies have shown that JA provides resistance against piercing and sucking insects (Zhu-Salzman *et al.*, 2004; Sun *et al.*, 2017).

Douglas *et al.* (2006) and Foyer *et al.* (2016) indicated that aphids consume the phloem, and their diet is high in sugar and low in nitrogen, requiring insects to consume enormous amounts of food to obtain sufficient nitrogen. During probing activity, aphids produce a rapidly-gelling "sheath saliva" around the stylets that is rich in conjugated carbohydrates, phospholipids, pectinases, phenoloxidasases, and β -glucosidasases (Miles, 1999). However, it is unclear whether plant hosts can recognize the components of the aphid sheath (Bak *et al.*, 2013). Aphids release "watery saliva" at puncture sites and feeding places, in addition to quickly gelling sheath saliva (Tjallingii, 2006). Watery saliva enzymes obstruct the healing of feeding-related injury by preventing the penetration of tissues to initiate the plant wound response (Will *et al.*, 2009). However, aphid saliva also contains substances that function as elicitors to trigger plant defense mechanisms in plants (Miles, 1999; de Vos and Jander, 2009).

The continuous use of chemical pesticides has led to imbalances in the biotic system and the development of insect pest resistance. Additionally, there is a decline in biodiversity, particularly in terms of parasites and predators, which compounds the problem of pest resurgence and secondary pest outbreaks, while also creating issues of environmental degradation, contamination of the food chain, and health hazards for humans and animals (Kumar *et al.*, 2018). Consequently, it has become necessary to switch from a unilateral chemical approach to IPM. The most important tactic in IPM is the selection of resistant cultivars, because they are inexpensive, adaptable, environmentally friendly, and useful for controlling diseases and insect pests (Kumar *et al.*, 2019). Therefore, a multifaceted strategy that incorporates chemical, cultural, and host plant resistance is required. The primary threat to various ecosystems is intensified agriculture through monoculture farming, which provides a perfect environment for economically significant pests to develop, multiply, and damage. For pest management, conservation of biological control is becoming increasingly crucial to prevent negative

consequences from the use of agricultural inputs. It is important to reduce the "green bridge" of volunteer and alternating grass-host species that aphids rely on to thrive from one season to the next. Therefore, it is essential to address pest management using comprehensive and sustainable strategies such as biochemical tolerance. Understanding how YSA influences the biochemical characteristics of sugarcane may aid in explaining the biochemical processes underlying tolerance. Therefore, the specific objective was to assess *S. flava* damage to some biochemical properties of seven sugar cane (*S. officinarum*) cultivars.

4.2 Materials and Methods

On February 24, 2023, a field experiment was conducted at the Zimbabwe Sugar Association Experiment Station (ZSAES) (Figure 4.1). On September 22, 2023, to commemorate the start of the second season, sugarcane was cut back so as to analyze the effect of the plant's biochemical traits, providing some resistance against YSA infestation of the sugarcane crop 90 days after the natural YSA infestation. Based on suggestions from the departments of plant breeding and entomology, a moderate degree of resistance, susceptibility, prior cane yield, and Estimated Recoverable Crystal (ERC %) were taken into consideration while choosing the experimental material (varieties). There were two pre-leased varieties: 96-1107 (very susceptible) and 00-1165 (low susceptibility). For each biochemical trait, four replicates, including infested and control (un-infested) leaves, of seven sugarcane cultivars were collected. The leaves were placed in plastic bags and taken to the laboratory for oven drying at 70 °C for 24 h. The samples that had been dried in the oven were cooled, pulverized in a Willey grinding mill using a 1 mm screen, and then moved to a bow mill for additional grinding. The graphic sequence of the actions involved in foliar sample preparation is depicted in the Figure 4.2.



Figure 4.1: Field planting and established cutback. Source (This study)

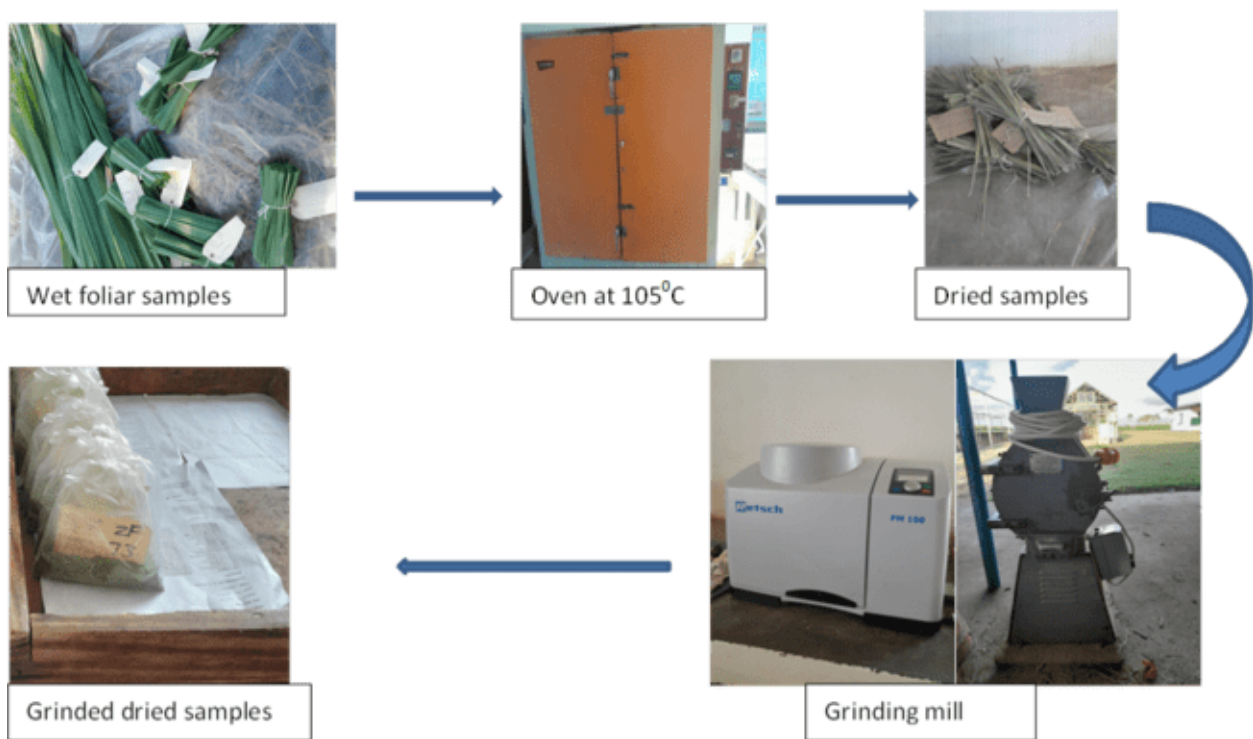


Figure 4.2: Pictorial sequence of procedures involved in foliar preparation. Source (This study)

4.2.1 Experimental design

The study employed a complete randomized block design (CRBD) with four replications using a 7×2 factorial design. The first factor under seven levels (00-1165, 96-1107, ZN 10, ZN 8, ZN 9, ZN 3L, and N14) was genotype, whereas the second factor under two levels (un-infested (control) and infested) was aphid treatment.

4.2.2 Data collection

Data on aphid number, nitrogen (N), phosphorous (P), potassium (P), calcium (Ca), magnesium (Mg), total protein content (TPC), total chlorophyll content (TCC), and total soluble sugars (TSS) were collected.

4.2.2.1 Aphid number

Weekly aphid assessments were conducted on five randomly chosen primary tillers from both aphid-infested and control (un-infested/sprayed) plots (Figure 4.3). The tillers were randomly marked with a string of 1m from each other within the same row. Throughout the trial, measurements were taken with these tillers for easier tracking of the changes. Aphids were physically counted on all leaves of each tiller 90 days after natural YSA infestation during foliar sample collection to correlate aphid numbers with soluble sugar, percentage nitrogen change, and total chlorophyll content.



Figure 4.3: Aphid assessments and counting. Source (This study)

4.2.2.2 Total chlorophyll content (TCC)

The methods described by Lichtenthaler (1987) and Saeedi and Ziaee (2020) were used to measure the chlorophyll content of five randomly selected plants of each type. After weighing 0.1 g the fresh leaf tissue, 10 ml of Merck 80 % acetone was poured into the leaves. After rubbing the leaves, 5 mL acetone was added, resulting in a total volume of 15 mL (Figure 4.4). The sample was then centrifuged at 4000 rpm for 20 minutes (SIGMA-D-37520 Osterode,

USA). The supernatant was extracted to quantify total chlorophyll. Total chlorophyll content was estimated using a UV 2100 spectrophotometer (Chemito Instruments Pvt. Ltd., Braunschweig, Germany). An 80 % acetone control was used (blank). Pigments were calculated as mg/g of the moisture weight of the plants.



Figure 4.4: Chlorophyll removal on dissected seven sugarcane leaves. Source (This study)

4.2.2.3 Nitrogen (N) and total protein content (TPC)

Steam distillation and the Kjeldahl digestion methods were used to determine the nitrogen content of both infested and un-infested leaves. A foliar sample weighing 0.25 g was placed in a Pyrex tube and weighed. In a Pyrex tube, 5 ml of concentrated sulfuric acid (H_2SO_4) and 3 ml of hydrogen peroxide (H_2O_2) were combined. Using a Kegel heating isomantle, the mixture was heated to 360 °C for one hour. The mixture was allowed to cool for 30 min. Then, 20 ml deionized water was added, and the mixture was allowed to cool for half an hour. The mixture was then topped off with deionized water and filtered into a 100 ml volumetric flask. After 20 ml of the digested solution (100 ml was pipetted into a 50 ml volumetric flask), the nitrogen content was determined and distilled into a 100 ml conical container containing 5 ml of bromocresol green and methyl red indicator with H_3BO_3 after 20 ml of a 10M sodium hydroxide (NaOH) solution was added. Finally, 0.00714M H_2SO_4 was used to titrate the distillate and the results were computed. Crude protein content was calculated by increasing the nitrogen content by a plant factor (6.25) (Balmain and Smith, 1993).

4.2.2.4 Phosphorus (P)

Ten milliliters of the 100 ml wet digested solution was pipetted into a 50 ml volumetric flask. After adding 2 ml of ammonium metavanadate and shaking the mixture, an appropriate amount of deionized water was added. After incubating the mixture for 30 min to develop its color, a UV 2100 spectrophotometer (Chemito Instruments Pvt. Ltd., Braunschweig, Germany) was used to read the results at 420 nm.

4.2.2.5 Potassium (K)

From a 100 ml wet digested solution, 10 ml of the solution was pipetted into 50 ml volumetric flasks. Subsequently, 5 ml of lanthanum nitrate and 5 ml of ethylenediaminetetraacetic acid (EDTA) were added. Deionized water was added to the solution at an appropriate concentration. The results were read using an Atomic Absorption Spectrometer (AAS) (Figure 4.5) with a potassium lamp.



Figure 4.5: Atomic Absorption Spectrometer used in measuring potassium, calcium, and magnesium. Source (This study)

4.2.2.6 Calcium (Ca)

Ten milliliters of the solution was pipetted from 100 ml wet digested solution into 50 ml volumetric flasks. Lanthanum nitrate (5 ml) and ethylenediaminetetraacetic acid (EDTA) 5 ml) were added. The solution was then topped with deionized water. The results were recorded on an Atomic Absorption Spectrometer (AAS) using a calcium lamp.

4.2.2.7 Magnesium

The same procedure was performed on other ions, except that a magnesium lamp was used. Ten milliliters of the solution was pipetted from 100 ml wet digested solution into 50 ml volumetric flasks. Then, 5 ml Lanthanum Nitrate and 5 ml Ethylin acid (EDTA) were added to the volumetric flask. The solution was then topped with deionized water. The results were recorded on an Atomic Absorption Spectrometer (AAS) using a magnesium lamp.

4.2.2.8 Total soluble sugar (TSS)

The method described by Dubois *et al.* (1956) with modifications was used to estimate the total amount of soluble sugars. First, 0.02 grams the dried extract were weighed. Next, ethanol extract

(0.2 ml) was taken into a volumetric flask and 1.8 ml of distilled water and 0.25 ml of 80 % phenol were added. Deionized water was added to the mark. The sample was shaken, and 5 ml of concentrated H₂ SO₄ was added immediately and allowed to stand for 30 min. Filtration of the samples was performed with Whatman filter paper number 90 to remove excess dirt, which can interfere with reading on the spectrophotometer. Using Spectronic-20, the percent absorbance at 490 nm was measured using a blank without the sugar extract. The sample values and absorbance scale were corrected for the mean of five reaction blanks of glucose, and one with deionized water was used to determine the soluble sugar content using a UV 2100 spectrophotometer (Chemito Instruments Pvt. Ltd., Braunschweig, Germany) at a wavelength of 490 nm. Figure 4.6 shows the phenol–sulfuric acid dehydration procedure. A standard curve of glucose concentration versus absorbance was used to calculate the total soluble sugar concentration in the YSA control and infested samples (Figure 4.7).

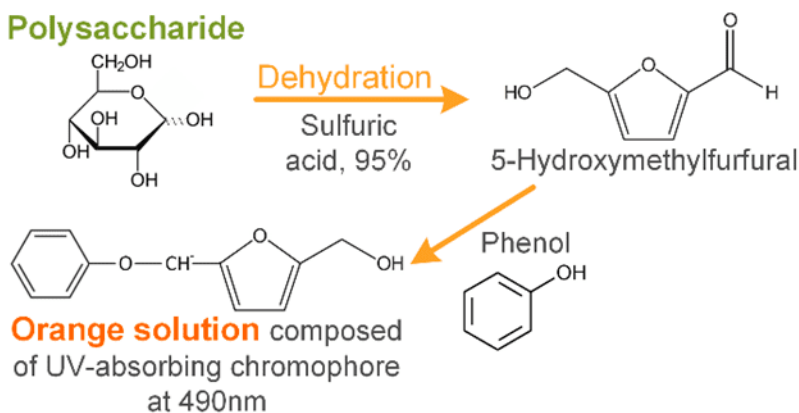


Figure 4.6: Schematic diagram of phenol sulphuric acid for soluble sugar determination. Adapted from Ussatto and Roberto (2004)

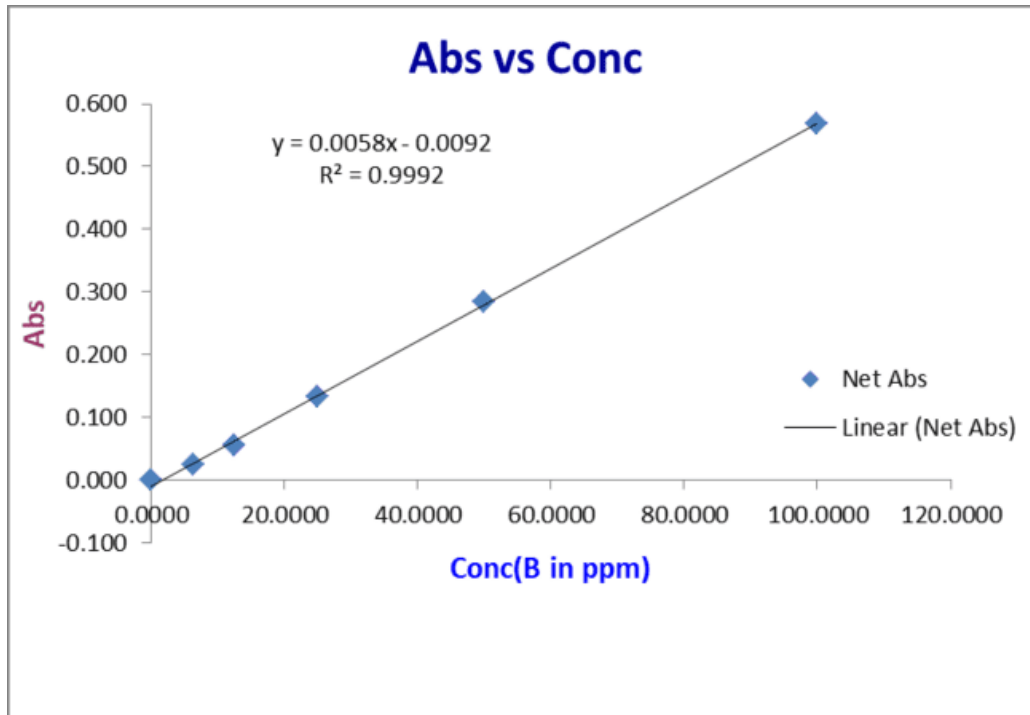


Figure 4.7: Glucose absorbance calibration curve to determine total soluble sugars. Source (This study)



Figure 4.8: Glucose standards to the left and prepared soluble sugars for YSA control and infested treatments. Source (This study)

4.3 Data analysis

The GenStat 18th edition was used to examine variations in total protein content, total chlorophyll content, total soluble carbohydrates, and nitrogen, phosphorus, potassium, calcium, and magnesium for each measurement. A 7 (sugarcane genotypes) \times 2 (YSA-infested and control (sprayed)) replicated four times was used to analyze the parameters. Fisher's Protected Least Significance test was used to separate the means at 5 %. The relationships between aphid number and percentage nitrogen change, aphid number, and total soluble sugars were determined using regression analysis. A regression analysis was used to incorporate the unique contribution of each sugarcane variety to biochemical resistance. Aphids are known to induce biochemical tolerance in certain genotypes under test if their number has a beneficial impact on the nitrogen content and percentage of nitrogen change. The decrease in nitrogen concentration indicates aphid feeding, which tampers with the nutritional components. Moreover, crop physiology is significantly affected by the link between soluble sugar content and aphid population. Additionally, the regression analysis was legitimate because it aimed to demonstrate whether there was a strong or weak correlation to effectively conclude that YSA feeding influences biochemical resistance in sugarcane.

4.4 Results

4.4.1 Aphid number

The relationship between variety and aphid treatment (infested and uninfested) was significantly interacting ($p < 0.001$). Significant differences ($p < 0.001$) were noted in YSA infested and YSA uninfested treatments for winter and summer seasons. In winter, ZN 10 recorded the highest (159) aphid number while 00-1165 scored the least (14) in the control treatment and infested treatments (230, 8) respectively. Summer results indicated that sugarcane variety ZN 10 had the highest aphid number (219) in the aphid control, whereas 00-1165 had the lowest (74). Additionally, in YSA-infested plots, ZN 10 sugarcane variety had the highest aphid number (886) whereas 00-1165 had the lowest (366) (Figure 4.9).

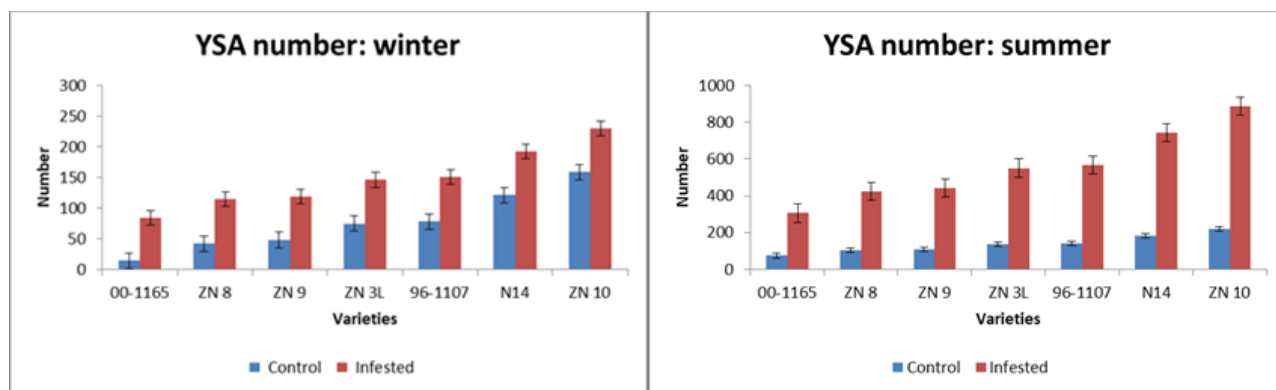


Figure 4.9: Aphid number between YSA control and infested

4.4.2 Effects of YSA on foliar nutrient composition of commercial sugarcane varieties

4.4.2.1 Nitrogen

There were significant differences ($p < 0.05$) in leaf nitrogen content among the sugarcane varieties in the control treatment in both winter and summer seasons. In winter, ZN 10 recorded the highest (2.5) nitrogen content while ZN recorded the least (1.89) in the control treatment. In aphid infested treatments, ZN 9 variety recorded the highest (2.4) nitrogen while N14 recorded the lowest (2.02). Summer results showed that the highest leaf nitrogen content (2.54) was recorded in the ZN 10 sugarcane variety, whereas the lowest (1.88) was observed in ZN 9 in the control treatment. However, there were no significant differences ($p > 0.05$) in leaf nitrogen content as well as a percentage change in nitrogen among the sugarcane varieties in aphid infested plots. In the aphid-infested plots, the ZN 9 sugarcane accession had the highest leaf nitrogen content (2.49), which was a 32.5 % increase in response to aphid infestation as compared to the 29.5 % of winter. Moreover, the lowest leaf nitrogen content (2.130), which constitutes a 16 % decrease in leaf nitrogen content in response to YSA incursion, was obtained on ZN 10 (Figure 4.10).

4.4.2.2 Phosphorous

Winter results indicated that ZN 10 variety recorded the highest phosphorous content (0.28) while ZN 3L recorded the lowest (0.20) in YSA control and infested plots (0.22, 0.17) respectively. Summer results showed that, in the control treatment, ZN 10 recorded the highest phosphorus content (0.24), while ZN 3L had the lowest (0.193). Furthermore, ZN 10 had a high

phosphorous content (0.243), while ZN 3L had the lowest (0.18). The results showed that there were no significant differences ($p>0.05$) in phosphorus content between the YSA control and infested treatments (Figure 4.10).

4.4.2.3 Potassium

Highly significant differences ($p<0.05$) in potassium levels were obtained in YSA control treatments for both winter and summer. Winter results showed that ZN 8 recorded the highest potassium content (1.49) while ZN 10 realized the least (1.21) in YSA control. Under YSA infested plots, ZN 9 recorded the highest potassium content (1.69) while ZN 10 recorded the lowest (1.19). Summer results revealed that ZN 8 sugarcane variety had the highest leaf potassium content (1.46), while ZN 10 scored the lowest (1.18) in the control treatment. In YSA infested plots, the highest potassium content (1.70) was documented in the ZN 9 sugarcane variety, whereas the lowest (1.11) was recorded in ZN 3L. Nevertheless, there were no significant differences ($p>0.05$) in potassium content among sugarcane varieties in the YSA infested plots (Figure 4.10).

4.4.2.4 Calcium

Results for the winter season showed that 96-1107 sugarcane variety recorded the highest calcium content (0.42) while ZN 9 recorded the least (0.36) in YSA control plots. In YSA infested treatments, ZN 9 recorded the highest calcium content (0.45) while ZN 3L recorded the lowest (0.37). Summer results showed that 96-1107 variety had the highest calcium content (0.42), whereas ZN 9 had the lowest (0.34) under the YSA control treatment. In YSA infested plots, ZN 9 recorded the highest calcium content (0.45) while ZN 3L recorded the lowest (0.37). This study found no significant differences ($p>0.05$) in calcium levels among sugarcane varieties for winter and summer seasons (Figure 4.10).

4.4.2.5 Magnesium

Winter season results revealed that 00-1165 sugarcane variety recorded the highest magnesium content (0.25) while ZN 9 recorded the least (0.22) in YSA control treatment. Under YSA infested treatment, 00-1165 variety recorded the highest magnesium content (0.25) while ZN 10 recorded the least (0.18). Summer results exhibited that 96-1107 variety had the highest magnesium content (0.24), whereas ZN 9 scored the lowest in the control treatment. Moreover, ZN 10 had the highest magnesium content (0.27) while 00-1165 had the lowest (0.20) in YSA

infested plot. The findings of this study revealed that there were no significant differences ($p > 0.05$) in Mg among the sugarcane varieties (Figure 4.10).

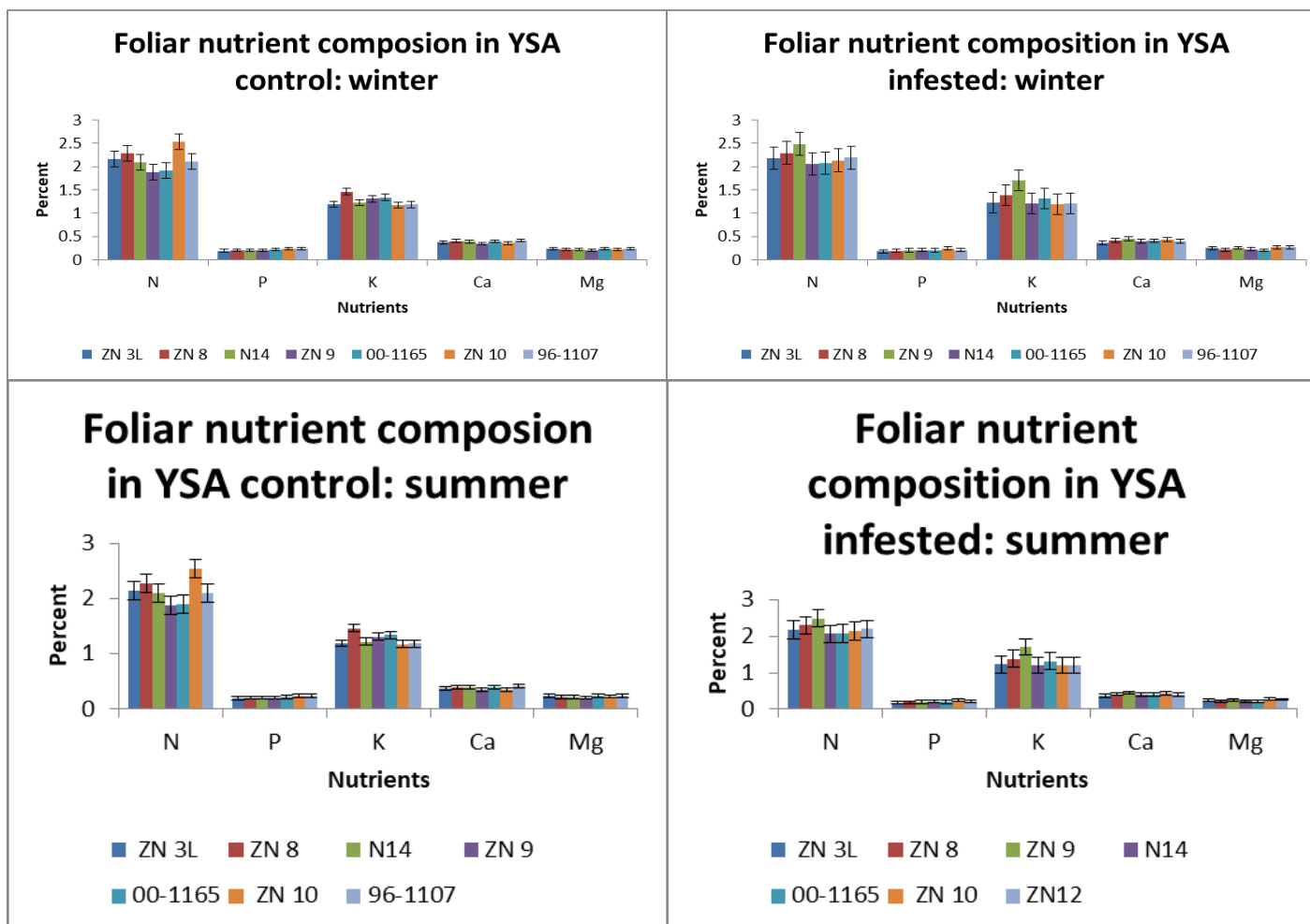


Figure 4.10: Foliar nutrient composition of sugarcane varieties in winter and summer seasons

4.4.2.6 Relationship between aphid number and percentage change in nitrogen YSA infested
Winter season results showed that the regression analysis revealed a significant ($p=0.023$) positive correlation between aphid number and percentage change in nitrogen (Figure 4.11; $Y=2.94X+149.73$, $r=0.42$). Summer results showed that there is a significant ($p=0.017$) positive correlation between aphid number and percentage change in nitrogen (Figure 4.12, $Y=6.06X+450.2$, $r=0.45$).

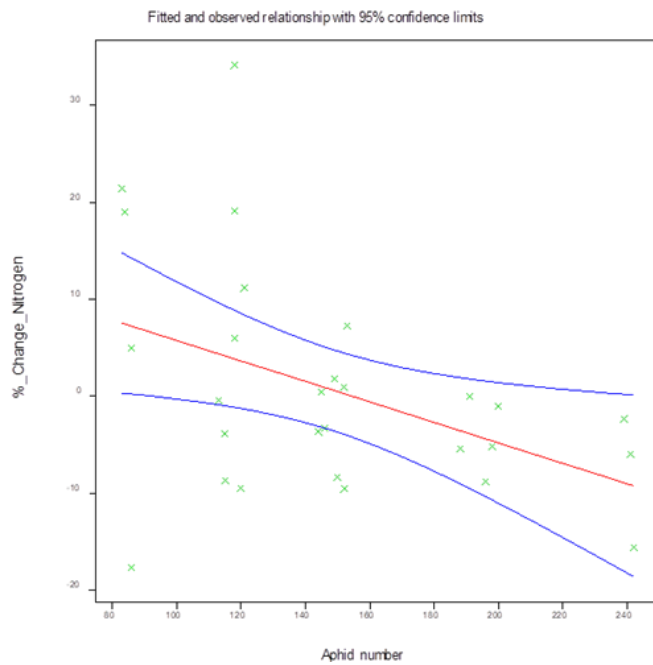


Figure 4.11: Relationship between percentage change in nitrogen and aphid number (winter)

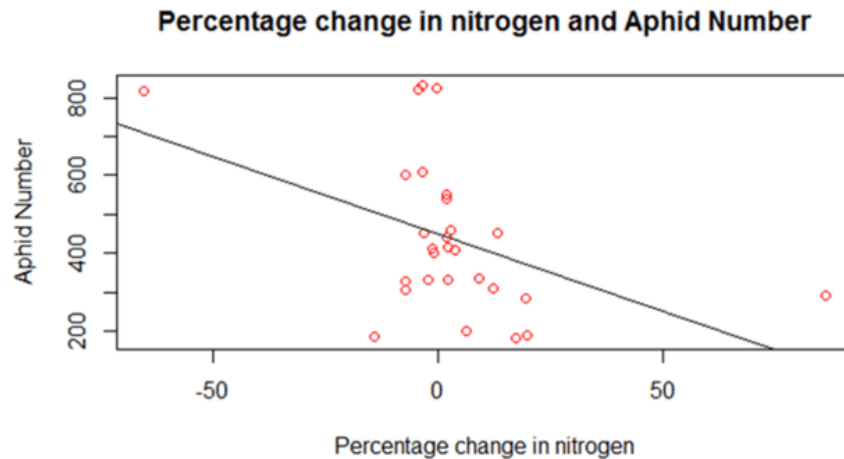


Figure 4.12: Relationship between percentage change in nitrogen and aphid number (summer)

4.4.2.7 Total leaf protein content

There were significant differences ($p < 0.05$) in leaf protein content among the sugarcane varieties in YSA control treatment for winter and summer seasons. Results of the winter season revealed that ZN 10 variety recorded the highest protein content (2.2) while N14 documented the least (1.36) in the control treatment. In YSA infested treatment, ZN 9 scored the highest leaf protein content (15.22) while 00-1165 recorded the least (12.72) although it was positive (Figure 4.13). Summer results showed that highest leaf protein content (15.86) was recorded in the ZN 10 sugarcane variety, whereas the lowest (11.73) was observed in ZN 9 in the control treatment. Under YSA infested treatment, ZN 9 recorded the highest leaf protein content (15.6) while ZN 10 recorded the lowest (13.31). There were no significant differences ($p > 0.05$) in leaf protein content or percentage change in protein among the sugarcane varieties in aphid YSA infested treatment (Figure 4.13).

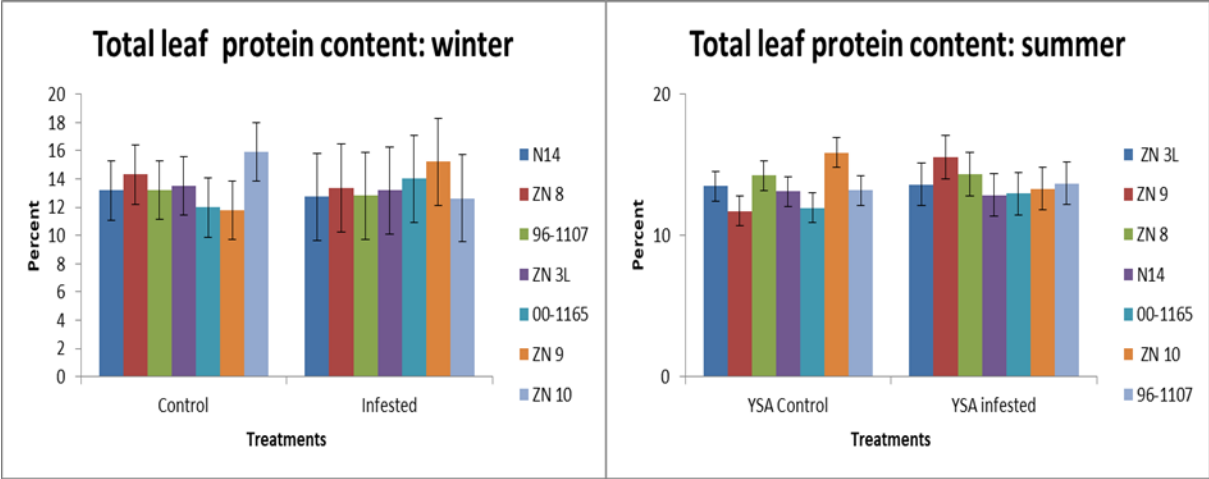


Figure 4.13: Total leaf protein content between YSA control and infested treatments

4.4.2.8 Total leaf chlorophyll content

There was highly significant interaction ($p < 0.001$) between sugarcane variety and aphid treatment (infested and control) on total leaf chlorophyll content for winter and summer seasons. Additionally, highly significant differences ($p < 0.001$) among the sugarcane accessions were recorded in leaf chlorophyll content in the control and YSA-infested plots. Winter results showed that ZN 10 variety obtained the highest (2.22) leaf chlorophyll content while N14 recorded the least (1.36) in YSA control treatment. However, YSA infested treatment revealed that ZN 9 recorded the highest (2.03) total leaf chlorophyll content while N14 recorded the least (1.20) (Figure 4.14). Summer results documented that the highest chlorophyll content (2.19) was obtained on ZN 10 while the lowest (1.34) was recorded for N14 in the control treatment. Furthermore, in aphid-infested plots, ZN 9 recorded the highest (1.92) chlorophyll content, whereas ZN 10 scored the lowest (0.99) (Figure 4.14).

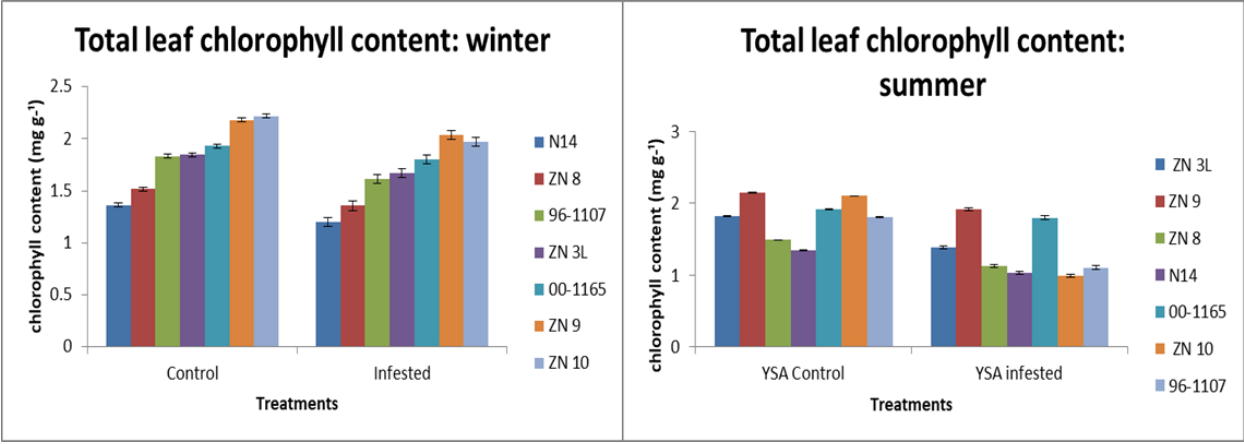


Figure 4.14: Total leaf chlorophyll content between YSA control and infested treatments

4.4.2.9 Relationship between Aphid number and percentage chlorophyll change

Results on regression results during the winter season revealed a significant ($p=0.001$) positive correlation between aphid number and percentage change in chlorophyll (Figure 4.15, $Y=-11.33X+44.5$, $r=0.58$). The regression analysis revealed a highly significant ($p<0.001$) positive correlation between aphid number and percentage chlorophyll change (Figure 4.16, $Y=5.22X+267.5$, $r=0.90$).

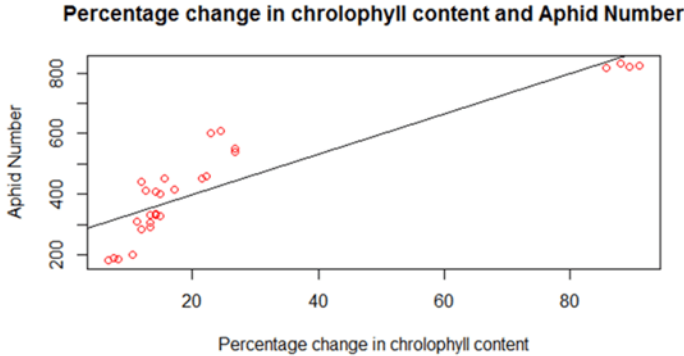


Figure 4.15: Relationship between percentage change in chlorophyll content and aphid number (winter)

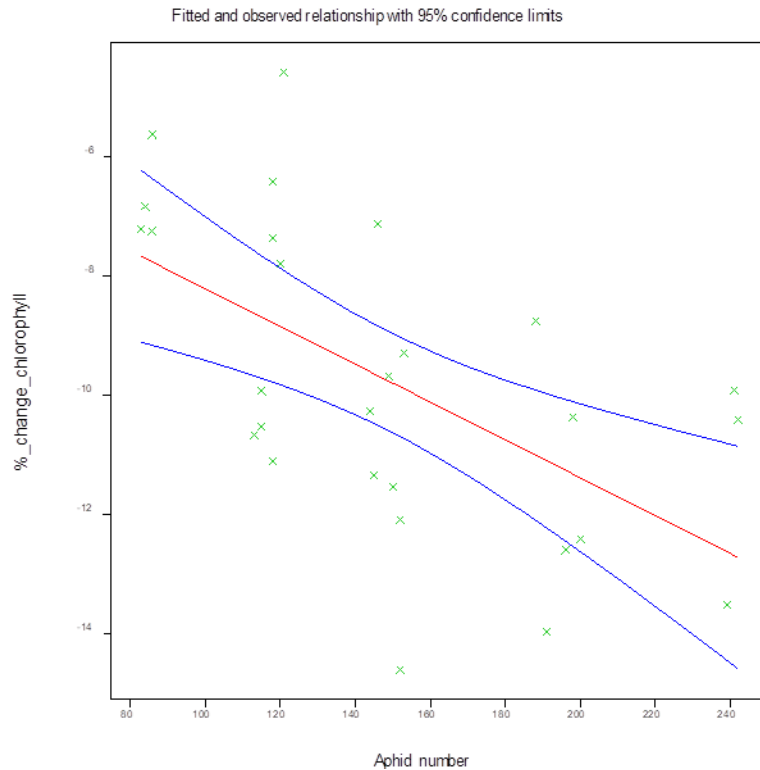


Figure 4.16: Relationship between percentage change in chlorophyll content and aphid number (summer)

4.4.2.10 Total soluble sugars

Regarding total soluble sugars, a significant interaction ($p < 0.001$) was discovered between sugarcane variety and aphid treatment on total soluble sugars. Also, highly significant differences ($p < 0.001$) among the sugarcane accessions were obtained in the total soluble sugar content in the control and YSA-infested plots. Winter results revealed that ZN 9 variety recorded the highest total soluble sugar (41.27) although it was not significantly different from ZN 8 and ZN 10. However, N14 variety recorded the least total soluble sugar (12.13) (Figure 4.17). Summer results showed that, the highest total soluble sugar content (39.27) was obtained for the ZN 9 sugarcane accession, although it was not significantly different ($p > 0.05$) from ZN 8 and ZN 10, whereas the lowest (16.78) was recorded in ZN 3L in the control treatment. Furthermore, in aphid-infested plots, the 00-1165 sugarcane accession had the highest (25.39) soluble sugar content, while N14 scored the lowest (8.13). However, the ZN 3L, ZN 8, and 96-1107 varieties were not significantly different from each other (Figure 4.17).

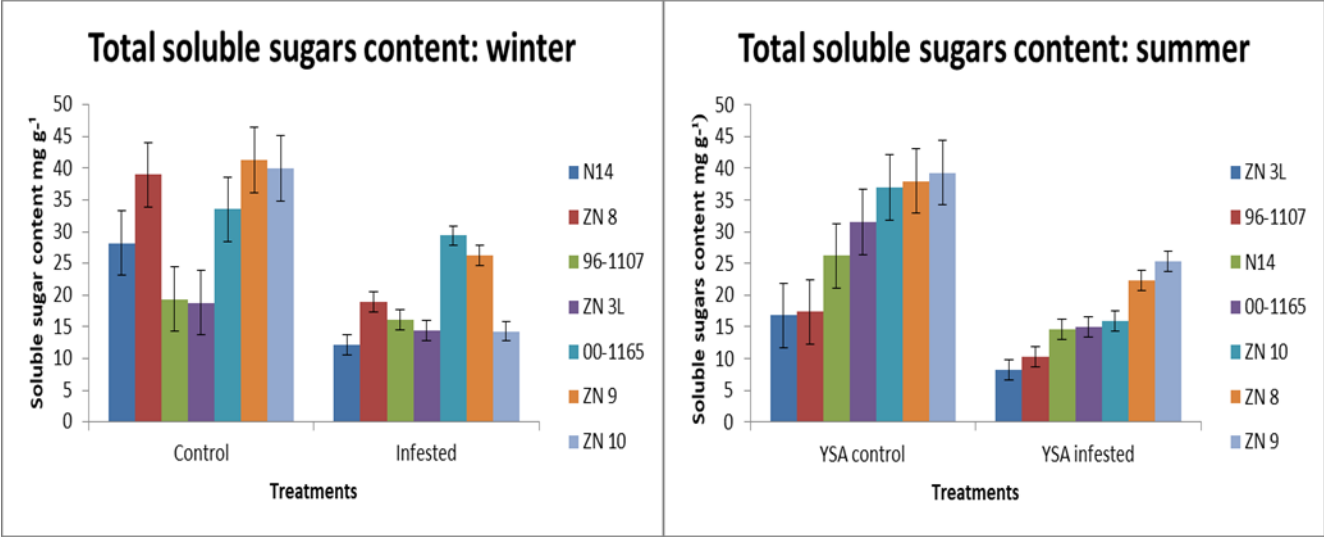


Figure 4.17: Total soluble sugar content between YSA control and infested treatments

4.4.11 Relationship between aphid number and total soluble sugar

Regression analysis showed a strong highly significant ($p < 0.001$) negative correlation between aphid number and total soluble sugars in winter (Figure 4.18, $Y = -0.07X + 30.89$, $r = 0.98$) and summer seasons (Figure 4.19, $Y = -0.023X + 26.50$, $r = 0.80$).

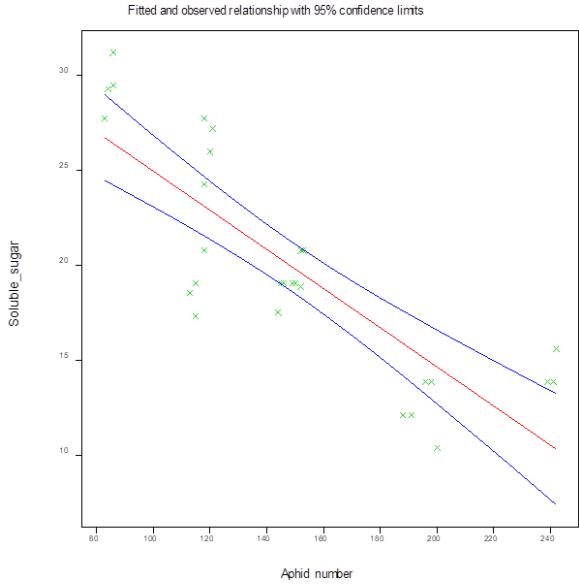


Figure 4.18: Relationship between soluble sugar and aphid number (winter) (This study)

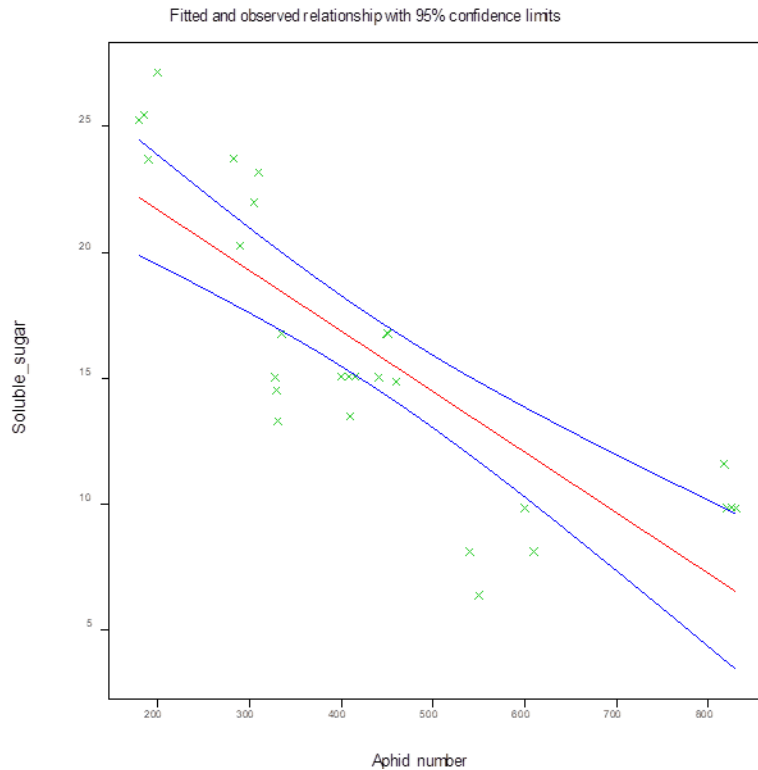


Figure 4.19: Relationship between soluble sugar and aphid number (summer) (This study)

4.5 Discussion

4.5.1 Nitrogen

The results indicated an increased production of nitrogen in tolerant sugarcane varieties (ZN 9, 00-1165, and ZN 8) in response to aphid infestation. This increased nitrogen production in sugarcane varieties translated to increased percentage change of nitrogen between YSA uninfested and infested treatments. A significant positive correlation between percentage change in nitrogen and aphid number might have been probably induced by aphid infestation. Furthermore, in the susceptible varieties (N14 and ZN 10) nitrogen was drastically reduced which might have been a contribution of nitrogen isotope and nitrogen reductase enzymes. Although the study did not measure leaf nitrogen isotope and nitrogen reductase, increased nitrogen in tolerant varieties might be a result of increased nitrogen isotopes and nitrate reductase enzymes, which offer induced compensation behavior to maintain or increase nitrogen. This theory is supported by Wilson *et al.* (2011) who emphasized that aphid-infested plants were 1.5 % to 2.0 % enriched in ^{15}N relative to uninfested control plants. Their hypothesis suggested that

the isotopic reduction of aphids compared to hosts was strongly related to host nitrogen content metabolism. Additionally, increased leaf nitrogen content in tolerant sugarcane accessions might be due to the presence of more nitrate reductase, which allows increased accumulation of ^{15}N , however, this study did not measure such results. Results by Wilson *et al.* (2011) suggested that nitrate reductase in aphid-infested plants was twice that of uninfested plants. At high and low densities, aphids significantly increased the nitrogen content in tolerant sugarcane varieties (ZN 9, 00-1165, ZN 8, and ZN 3L). YSA may serve as a new sink, boosting the photosynthetic output of the injured leaf. The results confirm those demonstrated in other systems, where the sink hypothesis is supported by the increase in nitrogen content in the leaves attacked by the apple green aphid (Syvertsen *et al.*, 2003; Urban *et al.*, 2004; Pincebourde and Ngao, 2021). Giron *et al.* (2018) pointed out that plant response to insect's manipulation of leaf metabolism is difficult to distinguish. In addition, Pincebourde and Ngao (2021) highlighted that aphids caused a rise in the nitrogen content of leaves in tolerant varieties. Resistant accessions (ZN 9, 00-1165, ZN 8, and ZN 3L) showed a percentage increase in total protein when compared to the control. This might have been enhanced by the increased nitrogen content, which compensated for the YSA-induced resistance.

These findings shed further light on the potential ways in which aphids could influence fundamental biochemical processes and potential compensatory mechanisms that tolerant plants may have developed to affect primary metabolism (Koch *et al.*, 2016). However, Waghray and Singh (1965) found that potassium and phosphorus did not affect the reproduction of *Aphis craccivora*. In contrary, low and high fertility rates have been linked to plants with high and low nitrogen content (Mahmoud's, 2005; El-Rawy *et al.*, 2007). Additionally, Slman (1997) noted that cereal aphid density responded favorably and significantly to increased nitrogen rates and their interactions with phosphorous pentoxide (P_2O_5).

4.5.2 Phosphorous

Leaf phosphorous content increased in the most susceptible accession (ZN 10), whereas it was reduced drastically in other sugarcane varieties, although it was above the critical level (0.18 %). The findings show that the presence of phosphorous (above 0.18 %) ensures resistance to YSA. This might have been as a result of biosynthesis of primary and secondary metabolites in response to YSA feeding. This is supported by Yadesa *et al.* (2019) and Wang *et al.* (2020), who

highlighted the crucial role of phosphorus (P) in the production of defense metabolites. Furthermore, Venter *et al.* (2014) highlighted that the addition of potassium phosphate induced tolerance to aphids. In addition, it is essential for energy metabolism (Battini *et al.*, 2017), thereby maintaining the physiological functions of sugarcane accessions.

4.5.3 Potassium

Leaf potassium varied among the sugarcane accessions in response to YSA feeding, although it was above the critical level (1.05 %). This may have contributed to biochemical resistance of sugarcane accessions to YSA. However, Sardans and Peñuelas (2021) pointed out that the mechanisms underlying functions of K⁺ in plant responses to pests are more complex. However, K⁺ reduction in plants has been linked to increased concentrations of carbohydrates and amino acids and decreased cell membrane resilience increasing the risk of damage from herbivores (Nissen, 2000). Higher concentrations of jasmonic acid (JA) have been linked to K⁺ deficits, making crops prone to pest attacks. Potassium regulates the opening and closing of stomata resulting in defense mechanisms and the activity of high-affinity selective K⁺ transporters in sugarcane varieties. This is confirmed in a review by Amtmann *et al.* (2008), in which fine roots were enhanced by potassium.

4.5.4 Magnesium

Feeding by YSA increased leaf magnesium in six sugarcane accessions (ZN 8, ZN 9, ZN 10, N14, and 96-1107). This might have been due to increased Mg²⁺-ATPase expression, which helps to preserve membrane integrity in response to YSA feeding and increase chlorophyll formation. However, reduced magnesium in the 00-1165 tolerant sugarcane variety might be as a result of phloem-impaired loading in the phloem inhibiting sucrose transport from the source to sink tissues (Chaudhry *et al.*, 2021). This is confirmed by the reduced soluble sugar loss on 00-1165 sugarcane variety hence supporting the hypothesis. Axelsen and Palmgren (1998) also confirmed the presence of Mg²⁺-ATPase associated with Mg²⁺ pump. Magnesium is required for photosynthesis as well as other enzymatic processes that stimulate protein synthesis (Bose *et al.*, 2011). By controlling the movement of magnesium ions into and out of cells (Liu *et al.* 2002). Mg²⁺-ATPase helps to preserve membrane integrity in sugarcane accessions.

4.5.4 Calcium

The study revealed increased calcium content in all tested sugarcane accessions. This might have been a physiological defense response to YSA feeding. Results are supported by Puri (2023) who

suggested that calcium is involved in plant defense. Furthermore, results concur with those by Yadav *et al.* (2022) who observed that calmodulin-like (CMLs) was expressed differently in soybeans after *Spodoptera litura* infestation. These results suggest that CMLs actively offer protection from larval attack a similar way in this study against YSA in sugarcane. Plants' intracellular free Ca^{2+} content fluctuates, which has an impact on a variety of physiological processes, including cell division and stress tolerance (Hepler, 2005). Calcium has an impact on a variety of physiological processes such as cell division and stress tolerance (Hepler, 2005). As soon as the plant senses an external stimulus, such as an insect or pathogen attack, calcium signaling begins instantly (Mostafa *et al.*, 2022). Furthermore, Will and Van Bel (2008) highlighted that callose generated at the feeding site by Ca^{2+} also contribute to sieve element blockage which reduces sugar and amino acids losses. The results confirm reports by Qudeimat and Faltusz (2008) that plant stress is managed in part by fluctuations in the calcium pump. Additionally, Hepler (2005) concluded that the Ca^{2+} -ATPase activity levels of the Arabidopsis were higher than those of the controls (Hepler, 2005). A similar trend was observed in this study which might have resulted in increased calcium leaf content, contributing to chlorophyll integrity and photosynthesis.

4.5.5 Total protein content (TPC)

TPC results showed that tolerant accessions (ZN 9, 00-1165, ZN 8, and ZN 3L) in the aphid-infested treatment increased when compared to the control. Although this study did not measure, pathogenesis-related (PR) proteins, this might have been induced by PR proteins, which cause a localized build-up of proteins in YSA-infested tolerant sugarcane accessions. The increase in protein content in leaves afflicted with aphids suggests that plants probably react enzymatically to aphid feeding through the expression of defense genes. The results of this study coincide with some studies that have shown that infections by pathogens or herbivores induce the production of certain proteins known as "pathogenesis-related" (PR) proteins (Hildebrand *et al.*, 1989, Hammerschmidt and Nicholson, 1999, Ni *et al.*, 2001). Additionally, results concur with those of Ni *et al.* (2001), who found that feeding by aphids caused a significant increase in total protein content when compared to the control in cereal leaves. However, susceptible sugarcane varieties (96-1107, N14, and ZN 10) did not exhibit such a mechanism of increased total protein content. In contrast, Prochaska (2015) documented that there were no significant differences in total protein content between YSA infested and YSA free control treatments in switchgrass.

Previous studies (Bi and Felton, 1995; Wilhelmina *et al.*, 2000; Torres *et al.*, 2002, Syvertsen *et al.*, 2003; Apel and Hirt 2004; Heng-Moss *et al.*, 2004; Urban *et al.*, 2004; Ralph *et al.*, 2006; Thompson and Goggin, 2006; Couldridge *et al.*, 2007; Franzen *et al.*, 2007; Browse and Howe, 2008; Gutsche *et al.*, 2009a; Liu *et al.*, 2010; Pierson *et al.*, 2010a; Wilson *et al.*, 2011; Suzuki and Mittler, 2012; Ramm *et al.*, 2013; Prochaska *et al.*, 2015; Pincebourde and Ngao, 2021) suggest that when confronted with phloem-feeding insects, plants have the ability to express many defense-related genes in both control and infested treatments. Results confirm the findings of Grover *et al.* (2022a) and Puri *et al.* (2023) who observed that sugarcane aphid (SCA) feeding down regulates and increases PR proteins in sorghum. Furthermore, other researchers (Li *et al.*, 2006; Zarate *et al.*, 2007) have confirmed the stimulated production of PR proteins by SA. Furthermore, a receptor protein of SA involved in defense was reported to be increased in sorghum in the presence of SCA (Kiani and Szczepaniec, 2018, Grover *et al.*, 2022b). In support of this study, Mohase and Van Der Westhuizen (2002) suggested that the Russian wheat aphid (*D. noxia*), induced wheat to produce SA. This might have been a similar case with YSA in our study although SA and JA were not measured. Moreover, Sandström *et al.* (2000) and Zeier (2013) reported that caterpillar feeding interfered with the amino acid content as they were raised. Apart from SA, JA has also been shown to offer defense against insect sap sucking pests (Zhu-Salzman *et al.*, 2004; Xu *et al.*, 2021). A study by Tetreault *et al.* (2019) showed that in the expression of genes encoding JA signaling transcription factors was higher in SCA-resistant sorghum genotype.

4.5.6 Total soluble sugars (TSS)

YSA significantly reduced TSS content among the sugarcane accessions. Tolerant varieties (00-1165 and ZN 9) were not significantly affected when compared to ZN 8, ZN 3L, N14, and 96-1107 sugarcane accessions. The study reported a high decrease of TSS among the sugarcane varieties in response to YSA infestation. This might be as a result of a higher preference of YSA feeding on sugars a major component present in phloem vessels. This richness of the phloem is supported by Dinant *et al.* (2010), who found significant amounts of carbohydrates and amino acids. Moreover, Hijaz *et al.* (2016) and Hijaz and Killiny (2014) noted that phloem sap is also rich in sugars and amino acids that are consumed in large volumes. In addition, the main sugar in phloem sap is sucrose (Hijaz and Killiny, 2014). Aphids and other phloem sap-feeding insects only eat phloem sap because of its richness in nutrients and free from toxins (Ae, 2006). This is

also explained by a strong positive correlation between TSS and aphid number revealed by this study. In support of our findings, Cabrera *et al.* (1994) concluded that sugar concentrations dropped five times in the presence of aphids. Reports in barley highlighted that aphid infestation resulted in more than 50 % decrease in the amount of soluble carbohydrates (Cabrera *et al.*, 1995). Similar findings regarding the reduction in total soluble sugars in infested shoots were also reported by Lokeshwari *et al.* (2014). In our study, YSA may serve as a new sink, boosting the photosynthetic output thereby draining more soluble sugars (Syvertsen *et al.*, 2003; Urban *et al.*, 2004; Pincebourde and Ngao, 2021). Insects have the ability to break down insoluble reserves and remove them continuously, resulting in a strong and continuous flow of host assimilation (Miles, 1989; Khattab, 2005). These results concur to those of Morkunas *et al.* (2016), who confirmed lower levels of sugar and fructose in aphid infested treatments.

4.5.7 Total chlorophyll leaf content

Tolerant sugarcane accessions (00-1165, ZN 9, ZN 8, and ZN 3L) exhibited a small decrease in chlorophyll loss compared to susceptible sugarcane accessions (96-1107, N14, and ZN 10) in response to YSA feeding. Maintenance of leaf chlorophyll content is associated with increased nitrogen content in response to YSA feeding as exhibited on ZN 9 and 00-1165 sugarcane accessions. However, the decreased chlorophyll content might have been caused by highly bioactive effector chemicals present in aphid saliva which resulted in yellowing and reddening of leaves in susceptible sugarcane varieties. Aphid saliva is a rich source of hydrolytic enzymes, poison and effector proteins that allow plants to respond by producing reactive oxygen species (ROS) (Shankar and Yinghua, 2021) which cause bleaching in chloroplasts and death of cells. However resistant genotypes are able to produce antioxidants which safeguard against ROS. In support of this, Shankar and Yinghua (2021) suggested that antioxidant gene expression was found to be responsible for the tolerance of the varieties to hydrogen peroxide in sorghum genotypes in response to SCA damage. Furthermore, these results support the findings of Haile *et al.* (1999) and Goławska *et al.* (2010), who observed that there might be a connection between increased production of defensive secondary metabolites such as saponins and decreased levels of chlorophyll in susceptible sugarcane varieties, although this study did not measure saponin content. Comparable findings were reported by Janave (1997), who hypothesized the oxidative bleaching pathway involved in chlorophyll degradation. Ni *et al.* (2002) provided evidence of a notable decrease in chlorophyll in susceptible wheat varieties. The results of this study will help

clarify how nutrition and primary metabolites contribute to sugarcane accessions' resistance to YSA.

4.6 Conclusion

The degree of injury is influenced by sugarcane accessions' biochemical resistance to YSA. Biochemical parameters (nitrogen, phosphorus, potassium, calcium, magnesium, total protein, total soluble sugars, and total chlorophyll content) of the tested sugarcane accessions varied in response to YSA feeding. The findings showed, that in susceptible sugarcane accessions (96-1107, N14, and ZN 10), YSA decreased the biochemical parameters. Nevertheless, in response to YSA damage, resistant sugarcane accessions (00-1165, ZN 9, and ZN 8) partially decreased or completely retained the measured biochemical parameters.

References

- Ae, D. 2006. Phloem-sap feeding by animals: problems and solutions. *Journal of Experimental Botany*. 57:747–754. doi: 10.1093/jxb/erj067.
- Akbar, W. 2009. Categorization and Identification of Mechanisms of Sugarcane Resistance to the Sugarcane Aphid (Hemiptera: Aphididae). *LSU Doctoral Dissertations*. 2785. <https://digitalcommons.lsu.edu/gradschooldissertations/2785>
- Amtmann, A., Troufflard, S., and Armengaud, P. 2008. The effect of potassium nutrition on pest and disease resistance in plants. *Physiol. Plant*. 133: 682–691.
- Apel, K., and Hirt, H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*. 55: 373–399.
- Axelsen, K. B., and Palmgren, M. G. 1998. Evolution of substrate specificities in the P-type ATPase superfamily. *J Mol Evol*. 46:84–101.
- Bak, A., Martinière, A., Blanc, S., and Drucker, M. 2013. Early interactions during the encounter of plants, aphids and arboviruses. *Plant Signaling and Behavior*. 8: e24225.
- Balman, P., and Smith D. C. 1993. Grain protein response of spring barley to high-rate and post-anthesis application of nitrogen fertilizer. *Agronomy Journal*. 85: 1109–1113.
- Battini, F., Gronlund, M., Agnolucci, M., Giovannetti, M., and Jakobsen, I. 2017. Facilitation of phosphorus uptake in maize plants by mycorrhizosphere bacteria. *Sci. Rep*. 7:4686.

- Bi, J. L., and Felton, G. W. 1995. Foliar oxidative stress and insect herbivory: Primary compounds, secondary metabolites, and reactive oxygen species are components of induced resistance. *J Chem Ecol.* 21: 1511–1530.
- Bidart-Bouzat, M. G., and Kliebenstein, D. 2011. An ecological genomic approach challenging the paradigm of differential plant responses to specialist versus generalist insect herbivores. *Oecologia.* 167: 677–689. doi: 10.1007/s00442-011-2015-z
- Bose, J., Babourina, O., and Rengel, Z. 2011. Role of magnesium in alleviation of aluminum toxicity in plants. *J Exp Bot.* 62:2251–2264.
- Browse, J., and Howe, G. A. 2008. New weapons and rapid response against insect attacks. *Plant Physiol.* 146: 832–838.
- Cabrera, H. M., Argandona, V. H., and Corcuera, L. J. 1994. Metabolic changes in barley seedlings at different aphid infestation levels. *Phytochemistry.* 35(2): 317-319.
- Cabrera, H. M., Argandoña, V. H., Zúñiga, G. E., and Corcuera, L. J. 1995. Effect of infestation by aphids on the water status of barley and insect development. *Phytochemistry.* 40(4): 1083-1088.
- Carena, M. J., and Glogoza, P. 2004. Resistance of maize to the corn leaf aphids: a review. *Maydica.* 49(4): 241-254.
- Chaudhry, A. H., Nayab, S., Hussain, S. B., Ali, M., and Pan, Z. 2021. Current understandings on magnesium deficiency and future outlooks for sustainable agriculture. *International journal of molecular sciences.* 22(4): 1819.
- Couldridge, C., Newbury, H. J., Ford-Lloyd, B., Bale, J., and Pritchard, J. 2007. Exploring plant responses to aphid feeding using a full Arabidopsis microarray revealed a small number of genes with significantly altered expression levels. *Bulletin of Entomological Research.* 97: 523–532.
- De Vos, M., and Jander, G. 2009. *Myzus persicae* (green peach aphid) salivary components induce defence responses in Arabidopsis thaliana. *Plant, Cell and Environment.* 321: 548–1560.

- De Vos, M., Van Oosten, V. R., Van Poecke, R. M. P., Van Pelt, J. A., Pozo, M. J., Mueller, M. J., *et al.* 2005. Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attacks. *Mol. Plant Microbe Interact.* 18: 923–937. doi: 10.1094/MPMI-18-0923
- Dinant, S., Bonnemain, J. L., Girousse, C., and Kehr, J. 2010. Phloem sap intricacy and interplay with aphid feeding. *Comptes Rendus Biologies.* 333:504–515. doi: 10.1016/j.crv.2010.03.008.
- Dogimont, C., Bendahmane, A., and Chovelon, V. 2010. Host plant resistance to aphids in cultivated crops: genetic and molecular bases and interactions with aphid populations. *Comp. Rendus Biol.* 333(6-7): 566-573.
- Douglas, A. E., Price, D. R. G., Minto, L. B., Jones, E., Pescod, K. V., Francois, C. L. M. J., Pritchard, J., and Boonham, N. 2006. Sweet problems: insect traits defining the limits to dietary sugar utilisation by the pea aphid, *Acyrtosiphon pisum*. *Journal of Experimental Biology.* 209: 1395–1403.
- Du, B., Zhang, W., and Liu, B. 2009. Identification and characterization of bph14, a gene that conferring resistance to brown plant hoppers in rice. *Proc. Nat. Acad. Sci.* 106(52): 22163-22168.
- Du, L., Ge, F., Zhu, S., and Parajulee, M. N. 2004. Effect of Cotton Cultivar on Development and Reproduction of *Aphis gossypii* (Homoptera: Aphididae) and Its Predator *Propylaea japonica* (Coleoptera: Coccinellidae). *J. Econ. Entomol.* 97: 1278–1283.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chem.* 28: 350-356.
- El-Rawy, A., Khaled, M. A., and Osman, S. M. 2007. Impact of NPK fertilizers, wheat varieties, and their interactions on the infestation with cereal aphids and leafminers, yield, and its components. *Journal of Plant Protection and Pathology.* 32(4): 3113-3122.
- Foyer, C. H., Rasool, B., Davey, J. W., and Hancock, R. D. 2016. Cross-tolerance to biotic and abiotic stresses in plants: a focus on resistance to aphid infestation. *Journal of experimental botany.* 67(7): 2025-2037.

- Franzen, L. D., Gutsche, A. R., Heng-Moss, T. M., Higley, L. G., Sarath, G., and Burd, J. D. 2007. Physiological and biochemical responses of resistant and susceptible wheat to injury by Russian wheat aphids. *Journal of Economic Entomology*. 100: 1692–1703.
- Giron, D., Dubreuil, G., Bennett, A., Dedeine, F., Dicke, M., Dyer, L. A., *et al.* 2018. Promises and challenges in insect-plant interactions. *Entomol. Exp. Appl.* 166: 319–343. doi: 10.1111/eea.12679
- Goławska, S., Krzyżanowski, R., and Łukasik, I. 2010. Relationship between aphid infestation and chlorophyll content in Fabaceae species. *Acta Biologica Cracoviensia Series Botanica*. 52: 76–80.
- Grover, S., Cardona, J. B., Zogli, P., Alvarez, S., Naldrett, M. J., Sattler, S.E., *et al.* 2022a. Reprogramming of the sorghum proteome in response to sugarcane aphid infestation. *Plant Sci*. 320:111289. doi: 10.1016/J.PLANTSCI.2022.111289
- Grover, S., Puri, H., Xin, Z., Sattler, S., and Louis, J. 2022b. Dichotomous role of jasmonic acid in modulating sorghum defense against aphids. *Mol. Plant Microbe Interact.* 35: 755–767. doi: 10.1094/MPMI-01-22-0005-R
- Gutsche, A., Heng-Moss, T., Sarath, G., Twigg, P., Xia, Y., Lu, G., and Mornhinweg, D. 2009a. Gene expression profiling of tolerant barley in response to *Diuraphis noxia* (Hemiptera: Aphididae) feeding. *Bulletin of Entomological Research*. 99: 163–173.
- Haile, F. J., Higley, L. G., Ni, X., and Quisenberry, S. S. 1999. Physiological and growth tolerance in wheat to Russian wheat aphid (Homoptera: Aphididae) injury. *Environ. Entomol.* 28: 787–794.
- Hammerschmidt, R., and Nicholson, R. L. 1999. A survey of plant defense responses to pathogens. Induced plant defenses against pathogens and herbivores. *Biochemistry, Ecology and Agriculture*. 55-71.
- Heng-Moss, T., Sarath, G., Baxendale, F., Novak, D., Bose, S., Ni, X., and Quisenberry, S. 2004. Characterization of oxidative enzyme changes in buffalo grass challenged with *Blissus occiduus*. *Journal of Economic Entomology*. 97: 1086–1095.

Hepler, P. K. 2005. Calcium: A central regulator of plant growth and development. *Plant Cell*. 17:2142–2155.

Hijaz, F., and Killiny, N. 2014. Collection and chemical composition of phloem sap from *Citrus sinensis* L. osbeck (sweet orange). *PLoS One*. e101830. doi: 10.1371/journal.pone.0101830.

Hijaz, F., Manthey, J., Dv, D. M., and Killiny, N. 2016. Nucleotides, micro- and macro-nutrients, limonoids, flavonoids, and hydroxycinnamates composition in the phloem sap of sweet orange. *Plant Signaling and Behavior*. 11: e1183084. doi: 10.1080/15592324.2016.1183084.

Hildebrand, D. F., Rodriguez, J. G., Legg, C. S., Brown, G. C., and Bookjans, G. 1989. The effects of wounding and mite infestation on soybean leaf lipoxygenase levels. *Z. Naturforsch.* 44c: 655- 659.

Howe, G. A., and Schaller, A. 2008. Direct defenses in plants and their induction by wounding and insect herbivores. In Schaller, A., *Induced plant resistance to herbivory*, Eds. Springer Science and Business Media, B. V.

Janave, M. T. 1997. Enzymatic degradation of chlorophyll in Cavendish vananas: In vitro evidence for two independent degradative pathways. *Plant Physiology and Biochemistry*. 35: 837-846.

Jaouannet, M., Rodriguez, P. A., Thorpe, P., Lenoir, C. J. G., MacLeod, R., Escudero-Martinez, C., and Bos. J. I. B. 2014. Plant immunity in plant-aphid interactions. *Front. Plant Sci*. 5: 1–10.

Khattab, H. 2007. The defense mechanism of cabbage plant against phloem-sucking aphid (*Brevicoryne brassicae* L.). *Australian Journal of Basic and Applied Sciences*. 1(1): 56-62.

Khattab, H. I. Kh. 2005. Responses of Eucalyptus trees to insect feeding (gall forming psyllid). *International J. Agr. and Biol*. 7: 979-984.

Kiani, M., and Szczepaniec, A. 2018. Effects of sugarcane aphid herbivory on transcriptional responses of resistant and susceptible sorghum. *BMC Genomics*. 19: 1–18. doi: 10.1186/S12864-018-5095-X

Koch, K. G., Chapman, K., Louis, J., Heng-Moss, T., Sarath, G. 2016. Plant tolerance: A unique approach to control hemipteran pests. *Frontiers in plant science*. 7:1363.

- Kumar, D., Ahlawat, D. S., Kumar, L., Kalkal, D., and Saini, V. K. 2019. Biochemical resistance in sugarcane, tentative conclusions and new research directions. *Journal of Entomology and Zoology Studies*. 7(4): 602-608.
- Kumar, D., Ahlawat, D. S., Yadav, S. S., Kalkal, D., Saini, V. K., Singh, D., et al. 2018. Phenetic resistance in sugarcane, tentative conclusions and new research directions. *Journal of Entomology and Zoology Studies*. E-ISSN: 2320-7078 P-ISSN: 2349-6800. 6(4):999-1003.
- Li, Q., Xie, Q. G., Smith-Becker, J., Navarre, D. A., and Kaloshian, I. 2006. *Mi-1*-mediated aphid resistance involves salicylic acid and mitogen-activated protein kinase signaling cascades. *Mol. Plant Microbe Interact.* 19: 655–664. doi: 10.1094/MPMI-19-0655
- Liu, G. J., Martina, D. K., Gardner, R. C., and Ryan, P. R. 2002. Large Mg²⁺-dependent currents are associated with the increased expression of *ALR1* in *Saccharomyces cerevisiae*. *FEMS Microbiol Lett.* 213:231–237.
- Liu, X., Williams, C. E., Nemacheck, J. A., Wang, H., Subramanyam, S., Zheng, C., and Chen, M. S. 2010. Reactive oxygen species are involved in plant defense against gall midges. *Plant Physiol.* 152: 985–999.
- Lokeshwari, D., Verghese, A., Shivashankar, S., Venugopalan, R., Kumar, N. K., and Manjunatha, H. 2014. Effect of *Aphis odinae* (Hemiptera: Aphididae) infestation on sugars and amino acid content in mango. *African Entomology*. 22(4): 823-827.
- Mahmoud, W. A. 2005. Ecological studies on cereal aphids and their control in the Sohag Governorate. Ph.D. Thesis, Fac. of Agric., Assiut Univ., 122 pp.
- Marimuthu, M., and Smith, C. M. 2012. Barley tolerance of the Russian wheat aphid (Hemiptera: Aphididae) biotype 2 herbivory involves the expression of defense responses and developmental genes. *Plant Signal. Behav.* 7:382–391.
- Martinez De Ilarduya, O., Xie, Q. G., and Kaloshian, I. 2003. Aphid-induced defense responses in *Mi-1*-mediated compatible and incompatible tomato interactions. *Mol. Plant Microbe Interact.* 16: 699–708. doi: 10.1094/MPMI.2003.16.8.699

- Martinez De Ilarduya, O., Xie, Q. G., and Kaloshian, I. 2003. Aphid-induced defense responses in *Mi-1*-mediated compatible and incompatible tomato interactions. *Mol. Plant Microbe Interact.* 16: 699–708. doi: 10.1094/MPMI.2003.16.8.699
- Miles, P. W. 1989. The Responses of Plant to the feeding of Aphidoidea: Principles. In World Crop Pests. (ed.) Minks, A.K. and Harrewijn P. Elsevier-New york (2c) 1-63.
- Miles, P. W. 1999. Aphid saliva. *Biological Reviews of the Cambridge Philosophical Society.* 74: 41–85.
- Mithöfer, A., and Boland, W. 2012. Plant defence against herbivores: chemical aspects. *Annu. Rev. Plant Biol.* 63: 431–450.
- Mohase, L., and Van Der Westhuizen, A. J. 2002. Salicylic acid is involved in the resistance responses in the Russian wheat aphid-wheat interaction. *J. Plant Physiol.* 159: 585–590. doi: 10.1078/0176-1617-0633
- Morkunas, I., Woźniak, A., Formela, M., Mai, V. C., Marczak, Ł., Narożna, D., and Grimm, B. 2016. Pea aphid infestation induces changes in flavonoids, antioxidative defence, soluble sugars and sugar transporter expression in leaves of pea seedlings. *Protoplasma.* 253: 1063-1079.
- Mostafa, S., Wang, Y., Zeng, W., and Jin, B. 2022. Plant responses to herbivory, wounding, and infection. *International journal of molecular sciences.* 23(13): 7031.
- Nalam, V., Louis, J., and Shah, J. 2019. Plant defense against aphids and pest extraordinaire. *Plant Sci.* 279: 96–107. doi: 10.1016/j.plantsci.2018.04.027
- Ni, X., Quisenberry, S. S., Heng-Moss, T., Markwell, J., Sarath, G., Klucas, R., and Baxendale, F. 2001. Oxidative responses of resistant and susceptible cereal leaves to symptomatic and non-symptomatic cereal aphid (Hemiptera: Aphididae) feeding. *Journal of Economic Entomology.* 94(3): 743-751.
- Nissen, P. 2000. The structural basis of ribosome activity in peptide bond synthesis. *Science.* 289: 920–930.

- Nkongolo, K. K., Quick, J. S., Limin, A. E., and Fowler, D. B. 1991. Sources and inheritance of resistance to Russian wheat aphids in *Triticum* species Amphiploids and *Triticum tauschii*. *Can. J. Plant Sci.* 71(3): 703-708.
- Pierson, L. M., Heng-Moss, T. M., Hunt, T. E., and Reese, J. 2010a. Physiological responses of resistant and susceptible reproductive stage soybean to soybean aphid (*Aphis glycines* Matsumura) feeding. *Arthropod-Plant Interactions.* 5: 49–58.
- Pincebourde, S., and Ngao, J. 2021. The Impact of Phloem Feeding Insects on Leaf Ecophysiology Varies with Leaf Age. *Front. Plant Sci.* 12:625689. doi: 10.3389/fpls.2021.625689
- Prochaska, T. J., Donze-Reiner, T., Marchi-Werle, L., Palmer, N. A., Hunt, T. E., Sarath, G., and Heng-Moss, T. 2015. Transcriptional responses of tolerant and susceptible soybean to soybean aphid (*Aphis glycines* Matsumura) herbivory. *Arthropod-Plant Interactions.* 1–13.
- Prochaska, T. J. 2015. Biochemical, physiological, and anatomical insights into aphid-bioenergy switchgrass interactions. University of Nebraska–Lincoln.
- Puri, H. 2023. Sorghum-Sugarcane Aphid Interactions: Multi-Omic Approaches to Elucidate Plant Defense Against Sap-Feeding Insects (Doctoral dissertation, The University of Nebraska-Lincoln).
- Puri, H., Ikuze, E., Ayala, J., Rodriguez, I., Kariyat, R., Louis, J., and Grover, S. 2023. Greenbug feeding-induced resistance to sugarcane aphids in sorghum. *Frontiers in Ecology and Evolution.* 11: 85.
- Qudeimat, E., Faltusz, A. M. C., Wheeler, G., Lang, D., Holtorf, H., Brownlee, C., Reski, R., and Frank, W. 2008. A P_{1B}-type Ca²⁺-ATPase is essential for stress adaptation in *Physcomitrella patens*. *PNAS.* 105:19555–19560.
- Ralph, S. G., Yueh, H., Friedmann, M., Aeschliman, D., Zeznik, J. A., Nelson, C. C., Butterfield, Y. S. N., Kirkpatrick, R., Liu, J., Jones, S. J. M., Marra, M. A., Douglas, C. J., Ritland, K., and Bohlmann, J. 2006. Conifer defense against insects: microarray gene expression profiling of Sitka spruce (*Picea sitchensis*) induced by mechanical wounding or feeding by spruce budworms

(*Choristoneura occidentalis*) or white pine weevils (*Pissodes strobi*) revealed large-scale changes in the host transcriptome. *Plant, Cell and Environment*. 29: 1545–1570.

Ramm, C. M. 2014. Buffalo grass defense response to *Blissus occiduus* feeding, chinch bug salivary gland morphology, and the role of saliva in mediating plant-insect interactions. ETD collection at the University of Nebraska, Lincoln. 1–118.

Rivas-Ubach, A., Sardans, J., Pérez-Trujillo, M.V., Estiarte, M., and Peñuelas, P. 2014. Drought enhances folivory by shifting foliar metabolomes in *Quercus ilex* trees. *New Phytol.* 202: 874–885.

Robert-Seilaniantz, A., Grant, M., and Jones, J. D. G. 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annu. Rev. Phytopathol.* 49: 317–343. doi: 10.1146/ANNUREV-PHYTO-073009-114447

Saeedi Z., and Ziaee M. 2020. Biochemical responses of two sugarcane varieties to whitefly (*Neomaskellia andropogonis*) infestation and its control by a new butenolide insecticide, flupyradifurone. *Agriculture and Forestry*. 66 (1): 69-81.

Sandström, J., Telang, A., and Moran, N. A. 2000. Nutritional enhancement of host plants by aphids: A comparison of three aphid species on grass. *J. Insect Physiol.* 46: 33–40. doi: 10.1016/S0022-1910(99)00098-0

Sardans, J., and Peñuelas, J. 2021. Potassium control of plant functions: Ecological and agricultural implications. *Plants*. 10(2): 419.

Shah, J. 2003. The salicylic acid loops in plant defense. *Curr. Opin. Plant Biol.* 6: 365–371. doi: 10.1016/S1369-5266(03)00058-X

Shankar, P., and Yinghua, H. 2021. Elevated production of reactive oxygen species is related to host plant resistance to sugarcane aphid in sorghum. *Plant Signaling and Behavior*. 16 (2): 1849523, DOI: 10.1080/15592324.2020.1849523

Slman, F. A. A. 1997. Studies on certain factors affecting the distribution of cereal aphids in wheat fields in Upper Egypt. Ph.D. Thesis, Fac. of Agriculture, El-Minia University, 178, pp.

- Sun, Y. C., Pan, L. L., Ying, F. Z., Li, P., Wang, X. W., and Liu, S. S. 2017. Jasmonic acid-related resistance in tomatoes mediates interactions between whiteflies and whitefly transmitted viruses. *Sci. Rep.* 7:566. doi: 10.1038/s41598-017-00692-w
- Suzuki, N., and Mittler, R. 2012. Reactive oxygen species-dependent wound responses in animals and plants. *Free Radical Biology and Medicine.* 53: 2269–2276.
- Syvertsen, J. P., Goñi, C., and Otero, A. 2003. Fruit load and canopy shading affect leaf characteristics and net gas exchange of ‘Spring’ navel orange trees. *Tree Physiol.* 23: 899–906. doi: 10.1093/treephys/23.13.899
- Tao, Y., Zhao, X., Wang, X., Hathorn, A., Hunt, C., Cruickshank, A. W., Oosterom, E. J., Godwin, I. D., Mace, E. S., and Jordan, D. R. 2020. Large-scale GWAS in sorghum revealed common genetic control of grain size among cereals. *Plant Biotech. J.* 18(4): 1093-1105.
- Tetreault, H. M., Grover, S., Scully, E. D., Gries, T., Palmer, N. A., Sarath, G., et al., 2019. Global responses of resistant and susceptible sorghum (*Sorghum bicolor*) to sugarcane aphids (*Melanaphis sacchari*). *Front. Plant Sci.* 10:145. doi: 10.3389/FPLS.2019.00145
- Thompson, G. A., and Goggin, F. L. 2006. Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. *J. Exp. Bot.* 57: 755–766.
- Tjallingii, W. F. 2006. Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany.* 57: 739–745.
- Torres, M. A., Dangl, J. L., and Jones, J. D. G. 2002. Arabidopsis gp91phox homologues, AtrbohD and AtrbohF, are required for the accumulation of reactive oxygen intermediates in the plant defense response. *PNAS.* 99: 517–522.
- Trumble, J. T., Kolodny-Hirsch, D. M., and Ting, I. P. 1993. Plant compensation for herbivory by arthropods. *Annu. Rev. Entomol.* 38: 93–119. doi: 10.1146/annurev.en.38.010193.000521
- Urban, L., Lechaudel, M., Lu, P. 2004. Effect of fruit load and girdling on leaf photosynthesis in *Mangifera indica* L. *J. Exp. Bot.* 55: 2075–2085. doi:10.1093/jxb/erh220
- Ussatto, S. I., and Roberto, I. C. 2004. Alternatives for detoxification of diluted-acid lignocellulosic hydrolysates for use in fermentative processes: a review. *Bioresour. Technol.* 93 (1): 1 – 10. doi : 10.1016/j.biortech.2003.10.005.

Venter, E., Mansoor, C. V., Sibisi, P., and Botha, A. M. 2014. Potassium phosphate induces tolerance against the Russian wheat aphid (*Diuraphis noxia*, Homoptera: Aphididae) in wheat. *Crop Protection*. 61: 43-50.

Waghray, R. W., and S. R. 1965. Effect of N, P and K on the fecundity of *Aphis craccivora* in groundnut plants. *Indian J. Entomol.* 27: 331-334.

Wang, C., Tian, B., Yu, Z., and Ding, J., 2020. Effect of different combinations of phosphorus and nitrogen fertilization on arbuscular mycorrhizal fungi and aphids in wheat. *Insects*. 11(6): 365.

Wang, L., and Wu, J. 2013. The essential role of jasmonic acid in plant–herbivore interactions using the wild tobacco *Nicotiana attenuata* as a model. *J. Genet. Genomics*. 40: 597–606. doi: 10.1016/J.JGG.2013.10.001

Wilhelmina, T. G., LeVesque, C. S., Perring, T. M., and Walling, L. L. 2000. Local and systemic changes in squash gene expression in response to silverleaf whitefly. *Plant Cell*. 12: 1409–1423.

Will, T., and van Bel, A. J. 2008. Induction as well as suppression: How aphid saliva may exert opposite effects on plant defense. *Plant Signaling and Behavior*. 3(6): 427-430.

Will, T., Kornemann, S. R., Furch, A. C. U., Tjallingii, W. F., and van Bel, A. J. E. 2009. Aphid watery saliva counteracts sieve-tube occlusion: a universal phenomenon. *Journal of Experimental Biology*. 212: 3305–3312.

Wilson, A. C., Sternberg, L. D. S., and Hurley, K. B., 2011. Aphids alter host-plant nitrogen isotope fractionation. *Proceedings of the National Academy of Sciences*. 108(25): 10220-10224.

Xanying, Li., and Shiqiang, Liu. 2004. Observations on morphology and tissue structure of ant-resistant plants of sorghum. *Biotechnology*. 14(1): 40-42.

Xu, J., Wang, X., Zu, H., Zeng, X., Baldwin, I. T., Lou, Y., et al., 2021. Molecular dissection of rice phytohormone signaling is involved in resistance to piercing-sucking herbivores. *New Phytol*. 230, 1639–1652. doi: 10.1111/NPH.17251

Yadav, M., Pandey, J., Chakraborty, A., Hassan, M. I., Kundu, J. K., Roy, A., and Singh, A. 2022. A comprehensive analysis of calmodulin-like proteins of glycine max indicates their role

in calcium signaling and plant defense against insect attack. *Frontiers in plant science*. 13: 817950.

Yadesa, W., Tadesse, A., Kibret, K., and Dechassa, N. E. 2019. Effect of liming and applied phosphorus on growth and P uptake of maize (*Zea mays* subsp.) plant grown in acid soils of West Wollega, Ethiopia. *J. Plant Nutr.* 42: 477–490.

Zarate, S.I., Kempema, L. A., and Walling, L. L. 2007. Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiol.* 143: 866–875. doi: 10.1104/PP.106.090035

Zeier, J. 2013. New insights into the regulation of plant immunity through amino acid metabolic pathways. *Plant Cell Environ.* 36: 2085–2103. doi: 10.1111/pce.12122

Zhang, F., Ke, F., Wang, J., Zhang, K., Duan, Y., Wu, H., Zhu, K. 2022. Transcriptomic characterization and analysis of key biological pathways associated with aphid resistance in sorghum (*Sorghum bicolor* L. Moench). *Pak. J. Bot.* 54(5): 1749-1759.

Zhang, P. J., Di Li, W., Huang, F., Zhang, J. M., Xu, F. C., and Lu, Y. B. 2013. Feeding by whiteflies suppresses downstream jasmonic acid signaling by eliciting salicylic acid signaling. *J. Chem. Ecol.* 39: 612–619. doi: 10.1007/S10886-013-0283-2

Zhu-Salzman, K., Salzman, R. A., Ahn, J. E. and Koiwa, H. 2004. Transcriptional regulation of sorghum defense determinants against phloem-feeding aphids. *Plant Physiol.* 134: 420–431. doi: 10.1104/PP.103.028324

CHAPTER FIVE

Physiological response as a tolerance mechanism to Yellow Sugarcane Aphid (YSA) (*S. flava*) herbivory on selected commercial sugarcane varieties (*S. officinarum*)

Abstract

Physiological tolerance in response to Yellow Sugarcane Aphid (YSA) feeding remains an unexplored area in the sugar industry of Zimbabwe and elsewhere. Two treatments of aphid infestation (un-infested (control) and infested) were applied to seven sugarcane genotypes (00-1165, ZN 3L, ZN 8, ZN 9, 96-1107, N14 and ZN 10). The treatments were laid out in a 7×2 factorial arrangement in a complete randomized block design (CRBD), replicated four times and carried out over summer and winter seasons. Highly significant differences ($p < 0.001$) were observed amongst the sugarcane varieties in percentage chlorophyll loss in control (sprayed) and infested (unsprayed) plots. Great increase in percentage chlorophyll loss (21.4%) was observed on ZN 10. Regression analysis displayed a highly significant ($p < 0.001$) strong positive correlation ($r = 0.85$) between chlorophyll loss and aphid number. Summer results showed highly significant differences ($p < 0.001$) in gas exchange responses in control and infested plots. Nevertheless, in YSA infested plots, 00-1165 recorded the highest compensatory photosynthetic rate (32.52), transpiration rate (4.32), and stomata conductance (218.2) when compared to the least obtained from ZN 10 and N14 at day 28. Significant positive correlations between; chlorophyll loss and photosynthesis ($r = 0.44$; $p = 0.019$) and between photosynthesis and aphid number were noticed ($r = 0.57$; $p = 0.002$). Ranking of sugarcane varieties was done according to YSA susceptibility; less susceptible (00-1165), moderate susceptible (ZN 3L, ZN 8 and ZN 9) and highly susceptible (96-1107, N14 and ZN 10). YSA reduced the chlorophyll content and gas exchange responses in susceptible varieties while tolerant varieties were able to maintain or compensate. Sugarcane growers should use YSA tolerant varieties as they exhibit physiological compensatory and maintaining behavior.

Key words: sugarcane, physiological, photosynthesis, transpiration, stomata conductance

5.1 Introduction

The global economy depends heavily on sugarcane (*Saccharum officinarum*) (Clowes and Breakwell, 1998; Esterhuzein, 2012; Shabani *et al.*, 2020). Sugarcane has become a subject of damage due to the persistent incursion of Yellow Sugarcane Aphids (YSA) (*Sipha flava*) in sugarcane fields. Feeding by YSA in sugarcane has been reported to have negative effects on stalk number and stalk height (Hall, 2001), biomass yield (Hall, 2001, Madiope *et al.*, 2021), and yield (Miskimen, 1970; Wilson, 2019).

YSA are relatively small, less than 2 mm in diameter (Wilson, 2019). Winged (alate) forms are distinguished from wingless (apterous) forms by their bright yellow abdomen. Varied climatic conditions allow the YSA to undergo two methods of reproduction; non-mating (parthenogenetically) in warmer climates (Halbert *et al.*, 2013), this results in proliferation and mating, where live individuals are produced when females mate with males in cold winters as low temperatures induce vivipary (Nuessly, 2005; Way *et al.*, 2014). The contribution of climate to YSA damage in winter and summer will be considered because heavy infestation and persistent feeding on young plant result in leaves turning from yellow to red followed by leaf senescence and stem death (Nuessly and Hentz, 2002; Way *et al.*, 2015, Wilson, 2019). Breen and Teetes (1986) as cited in Dumont *et al.* (2023) highlighted that red to purple discoloration and premature leaf necrosis are a result of injection of saliva by YSA resulting in leaf chlorosis. Moreover, Akbar *et al.* (2010) reported that leaf discoloration is associated with a chlorophyll content which cause decreased photosynthetic rate (White, 1990). Toxins injected in aphid saliva contain toxic oxygen which may cause biochemical changes within the crop resulting in bleaching of leaves and increased metabolism (Macedo *et al.*, 2003). The saliva contains a variety of effector proteins, hydrolytic enzymes, and toxic chemicals that cause plants to perceive the invasion of aphids thereby increasing the buildup of reactive oxygen species (ROS) (Shankar and Yinghua, 2021).

Photosynthesis stops when chlorophyll leaf pigments, mainly chlorophyll *a* and *b*, are reduced (Green and Durnford, 1996; Jensen, 2000; Craigie, 2022). Gonzales *et al.* (2002) reported ultrastructural damage to chloroplasts in leaves of Johnson grass (*Sorghum halepense* L.) under YSA infestation. Although nitrogen is required for chlorophyll formation, high rates of fertility of aphids were linked to plants with high nitrogen contents (Mahmoud, 2005; El-Rawy *et al.*,

2007). This clearly shows that nitrogen encourages the leaf to become soft and allows easier probing of the aphid stylet into phloem sieves. Slman (1997) suggested that the density of cereal aphids responded significantly to increased nitrogen rates and their interactions with phosphorous pentoxide (P₂ O₅).

YSA is being managed by chemical, biological, and cultural techniques. In Zimbabwe, Actara 25 Wettable Granules (WG) (25 %) thiamethoxam and Alice are registered for use. Way *et al.* (2015) observed the existence of biological agents such as earwigs, hoverflies, ladybird species, spiders and ants as natural enemies. Several authors (Nuessly, 2005, Nuessly *et al.*, 2010; Gallun *et al.*, 1966; Roberts *et al.*, 1979; Roberts and Foster, 1983; Sosa, 1990; White, 1990; ZSAES, unpublished) suggested that resistant sugarcane cultivars can be used for YSA cultural management. Aphid resistance may develop as a result of the ongoing usage of insecticides belonging to the neonicotinoid, pyrethroid, and organophosphate classes. Therefore, studies that examine physiological tolerance as a host plant defense mechanism against YSA are necessary. In light of this, they are required for integration into existing Integrated Pest Management (IPM) strategies. This strategy is environmentally friendly and is compatible with current management strategies. Physiological tolerance can be defined as the ability of plants to withstand insect injury through physiological and biochemical compensation (Painter, 1951; Koch *et al.*, 2016; Peterson *et al.*, 2017). Tolerant plants can maintain or increase physiological mechanisms (chlorophyll content and gas exchange) in response to insect herbivory as reported by various authors (Akbar, 2009; Koch *et al.*, 2015; Mbulwe, 2017; Paudyal, 2019; Paudyal *et al.*, 2020).

Peterson and Higley (2001) and Gordy *et al.* (2019) indicated that understanding the physiological changes resulting from aphid feeding is an important step in developing accurate economic damage levels for different crop cultivars. Much focus of physiological tolerance studies has been directed on sugarcane aphid (SCA) (*Melanaphis sacchari*) (Singh *et al.*, 2004; Akbar, 2009; Armstrong *et al.*, 2015, 2017; Bowling *et al.*, 2016a, b; Mbulwe *et al.*, 2016; Mbulwe, 2017; Paudyal, 2019; Paudyal *et al.*, 2020) in sorghum. Existing conclusions were made that the aforementioned aphid induced tolerance compensatory mechanisms such as increased photosynthesis, transpiration, and stomata conductance in tolerant varieties while in susceptible genotypes, gaseous exchange processes were drastically reduced by sugarcane aphid. This mechanism if captured and utilized enhances sugarcane productivity. However, limited

studies have reported on how sugarcane crop can exhibit tolerance to YSA feeding. Thus, this study will evaluate how sugarcane plants may physiologically tolerate YSA infestation. This will be an interesting and worthy research avenue in sugarcane in the presence of YSA feeding as suggested by Macedo *et al.* (2003), that phloem feeders such as aphids can dramatically decrease host plant photosynthesis, while sap feeders such as scale insects can cause an increase in leaf assimilation rate (Retuerto *et al.*, 2004). An increase in photosynthesis after insect herbivory is interpreted as a plant strategy to compensate for the effect of the herbivore (Trumble *et al.*, 1993). This hypothesis has driven the need for such a study to conclusively document the existence of such a mechanism among sugarcane varieties in response to YSA feeding.

Studies have indicated that aphids have the potential to alter host chlorophyll content and gas exchange responses (Warrington *et al.*, 1989; Welter, 1989; Macedo *et al.*, 2003a; Peterson *et al.*, 2004; Aldea *et al.*, 2005; Delaney and Higley, 2006; Shannag, 2007; Paudyal, 2019). Numerous authors (Capinera, 1981; Ryan *et al.*, 1987; Meyer and Whitlow; 1992; Larson, 1998; Haile *et al.*, 1999; Macedo *et al.*, 2003a, b; Diaz-Montano *et al.*, 2007; Pierson *et al.*, 2011; Paudyal, 2019) have emphasized that this change ultimately affects productivity. Overreliance on symptoms caused by YSA feeding has been the major talk by farmers hence side-lining the science behind contributing to the symptoms.

Welter (1989) emphasized that increased rates of transpiration caused by foliage eating pests interferes with leaf integrity. This proves a compensatory behavior that gives plants a competitive advantage to carry out their physiological processes without being disadvantaged by insect pest. On the other hand, Hoad *et al.* (1998) noted that uncontrolled water loss is a result of cut edges on leaves. Thus, the primary goal of this work is to explore these hypothesized physiological modifications in order to produce concrete sugarcane literature. Furthermore, this study seeks to understand how YSA injury affects these primary metabolic parameters so as to develop general models of plant response as suggested by Peterson (2001). According to some authors (Peterson and Higley, 1993; Macedo *et al.*, 2003; Shannag, 2007), gas exchange is an important physiological function that occurs during a plant's existence.

Other studies (Miller *et al.*, 1994; Deol *et al.*, 2001; Diaz-Montano *et al.*, 2007; Limaje *et al.*, 2017) have reported the effects of aphid feeding on the physiological response of plants. They reported reduced chlorophyll content a major driver for photosynthesis to occur. Therefore, physiological tolerance is important for sugarcane physiological integrity to compensate for YSA injury. In addition to this, Haile *et al.* (1999) and Frazen *et al.* (2007) described that aphids have the potential to directly reduce photosynthesis by causing stomata closure hence affecting nutrient flow and CO₂ assimilation. In contrast, Nagaraj *et al.* (2002) suggested that net reduction in the rate of photosynthesis is not always as a result of reduced chlorophyll. Moreover, Retuerto *et al.* (2004) and Gutsche *et al.* (2009) reported plant compensatory behavior in response to insect infestation. However, among other phloem feeders, aphids have the capacity to significantly and actively alter leaf metabolism through effectors (Giron *et al.*, 2018), which can undermine the plant's mitigation approach.

The physiological mechanisms underlying indirect damage to leaf arthropods through photosynthesis, transpiration, and stomata conductance are not well understood (Hunter, 2001; Peterson *et al.*, 2004). There is a vacuum on physiological tolerance of existing sugarcane varieties as more studies were directed towards other aphid species and SCA in sorghum (Singh *et al.*, 2004; Akbar, 2009; Armstrong *et al.*, 2015, 2017; Bowling *et al.*, 2016a, b; Koch *et al.*, 2015; Mbulwe *et al.*, 2016; Mbulwe, 2017; Paudyal, 2019; Paudyal *et al.*, 2020) in other cereal crops. The above-mentioned studies conclude by indicating that, aphids reduced chlorophyll content and gaseous exchange process in susceptible varieties. However, tolerant varieties were able to compensate and maintain chlorophyll content and gas exchange processes in response to insect feeding.

Entomologists, plant physiologists, and plant breeders have long been intrigued by the interactions between plants but the mechanisms behind plant responses have not received much research attention (Macedo *et al.*, 2003). Moreover, a large body of research has concentrated on how plants react to insect herbivory, but it has not thoroughly examined the biochemical and physiological responses of the insect (Peterson and Higley, 2001). Visual damage assessments through the use of traditional phenomic screening can introduce bias and limit precision. Therefore, there is need for integrating the use of phenotypic approaches that are not labor intensive such as using SPAD and CIRAS-4 portable photosynthetic system devices to determine

tolerance. The use of plant metabolite assays, insect population assays, hand-held spectrophotometry (SPAD meter) to measure chlorophyll content in leaves and the electrical penetration graph (EPG) technique to measure feeding behavior are some of the methods that have received a lot of support (McLean and Kinsey, 1964; Tjallingii, 1988; Deol *et al.*, 1997; Girma *et al.*, 1998; Walker, 2000; Chan *et al.*, 2010; Chen *et al.*, 2012; Ménard *et al.*, 2013, Koch *et al.*, 2016).

To comprehend piercing-sucking insects like YSA, a thorough approach is necessary. Such studies are a prerequisite in yield prediction due to YSA damage at early stages of growth (≤ 3 months) (Wilson, 2019). To date, hardly any study has reported an association between YSA herbivory, chlorophyll content loss, and gas exchange response in response to YSA incursion. This knowledge of how YSA affects sugarcane physiology, particularly in resistant and susceptible lines, may help explain the physiological mechanisms underlying tolerance. The purpose of these experiments is to gain insight into the physiological responses of selected resistant and susceptible sugarcane genotypes to YSA. Results will help to model and predict yield losses at early stages of growth. The specific objective is to assess physiological tolerance of sugarcane varieties in response to YSA infestation.

5.2 Materials and methods

5.2.1 Selection of varietal entries

A total of seven sugarcane accessions were selected and grown according to the Zimbabwe Sugar Production Manual (Clowes and Breakwell, 1998). Selection of experimental material (varieties) was done by considering moderate level of resistance, susceptibility, previous cane yield and Estimated Recoverable Crystal (ERC) (%) based on recommendations from the Plant Breeding and Entomology Departments. Two preleased accessions namely; 00-1165 (low susceptible) and 96-1107 (highly susceptible) were selected. Moderate resistant accessions based on visual assessments; ZN 8, ZN 9 and ZN 3L, hence ZN 8 and ZN 9 have the ability to recover from damage inflicted by YSA. Lastly, ZN 10 and N14 were chosen despite being susceptible because of their high biomass and high ERC %, hence they are leading in terms of area under production in both out-grower farmers and estates. Plant cane and ratoon cane were assessed for chlorophyll content, physiological tolerance and aphid number. Cut back of sugarcane was done

on the 22th of September 2023 in order to mark the start of the new season in summer. Figure 5.1 shows field planting, established and cut back sugarcane crop.



Figure 5.1: Planting, established and cut back sugarcane crop. Source (This study)

5.2.2 Experimental design

A 7×2 factorial design in a complete randomized block design (CRBD) was used, replicated four times. Genotype was the first factor under seven levels (00-1165, 96-1107, ZN 10, ZN 8, ZN 9, ZN 3L and N14) and Aphid treatment was the second factor under two levels (un-infested (control) and infested).

5.2.3 Data collection

Data was collected on aphid number, chlorophyll content and gas exchange responses (photosynthesis, transpiration and stomata conductance).

5.2.3.1 Aphid number

Aphid assessments were done weekly on five selected primary tillers spaced 1 meter from each other on a row length of 15 meters. Tillers were randomly marked with a string on control (un-infested/sprayed) and aphid infested plots. These tillers were used for taking measurements throughout the experiment period. After natural YSA infestation, aphids were physically counted per plant/tiller on all leaves and summed so as to determine aphid number for winter and summer seasons.

5.5.2 Measurement of photosynthetic parameters

Chlorophyll content and gas exchange responses were determined. Taking of readings was done after aphid infestation on marked tillers for both control and YSA infested plots as discussed in detail.

5.5.2.1 Chlorophyll concentration

A chlorophyll meter (Model SPAD-502, Minolta Camera Co., Osaka, Japan) was used to measure the chlorophyll content in the sugarcane leaves (Figure 5.2). Handheld spectrophotometry (SPAD meter) is a portable device that absorbs light at wavelengths between 430 and 750 nm when passed over a leaf and estimates the chlorophyll content (Wood *et al.* 1992). Three readings from each leaf were taken from YSA un-infested (control) and infested (unsprayed) plots. These readings were averaged and a SPAD chlorophyll index (% chlorophyll loss) was calculated using the mean SPAD based on the formula:

$$\% \text{ chlorophyll loss} = \frac{(C-I)}{C},$$

The formula was modified from the one proposed by Deol *et al.* (1997); Akbar (2010) and Paudyal (2019) and Paudyal *et al.* (2019) where C is the SPAD measurement from the YSA un-infested and I is the SPAD measurement from YSA infested sugarcane leaves. Golawska *et al.* (2010) indicated that SPAD measurements are reliable, quick and non-destructive for studying chlorophyll build up rates in different parts of the same leaf and also in determining insect-plant interactions.



Figure 5.2: Chlorophyll content determination with a SPAD meter on sugarcane crop.
Source (This study)

5.5.2.2 Gas exchange

Gas exchange responses were measured from YSA infested and un-infested (control) sugarcane leaves on the seven genotypes by using a portable photosynthetic system (CIRAS-3 DC CO₂/H₂O Gas Analyzer) after every two weeks (Figure 5.3). The CIRAS photosystem was equipped with a LED chamber (1.75 cm²) so as to cover the leaf lamina excluding the midrib. The photosynthetic parameters were measured 60 days after natural infestation in both experimental plots. The collected data also included net photosynthesis rate (A , $\mu\text{molm}^{-2} \text{s}^{-1}$), transpiration rate (E , $\text{mmolm}^{-2} \text{s}^{-1}$), and stomatal conductance (g_s , $\text{mmolm}^{-2} \text{s}^{-1}$). The A equals the rate of photosynthetic (CO₂ fixation minus the rate of CO₂ loss) during respiration. Stomatal conductance is the rate at which CO₂ enters or water vapor escapes through the stomata, and internal CO₂ (C_i , Pascals) is the concentration of carbon dioxide inside the leaf (Meyer and Whitlow 1992; Paudyal, 2019). Desiccants (soda lime, molecular sieve and drierite) were changed when 50 % of the colour changes. Every time, when going into the field to take measurements, the CO₂ canister was changed. Measurements were taken on the uppermost fully

expanded total visible dew lap (TVD) leaf of aphid-infested and control plants between 11:00 and 14:00 h (CST) on days with full sunlight at a light intensity of $1200 \text{ umol photons m}^{-2} \text{ s}^{-1}$ and a reference CO_2 of $\pm 400 \text{ ppm}$ generated from a 12g CO_2 cylinder connected to the meter. The TVD leaf was selected because it is the photosynthetic active leaf which utilises all the nutrients in the plant. The machine was switched on and allowed to warm up to a temperature of $55 \text{ }^\circ\text{C}$ before taking measurements. Prior to taking measurements, aphids were removed from the leaf by a fine brush so that they don't interfere with results. Sugarcane leaves of control (un-infested) plants were also brushed to avoid undesirable effect resulting from the use of brush on the leaf tissues of aphid-infested plants. Measurements were taken in winter and summer of 2023/2024 season in both plant and first ratoon sugarcane (≤ 3 months).



Figure 5.3: Gas exchange measurements by using a CIRAS-3 photosystem on sugarcane crop. Source (This study)

5.3 Data analysis

For each measurement, analysis of variance using GenStat 18th version was used to examine the differences in chlorophyll content, net photosynthesis rate and stomata conductance. Fisher's Protected Least Significance was used to separate means at 5 % significance level. Regression analysis was used to determine. The regression analysis was performed in order to integrate variable contribution to physiological tolerance among the sugarcane varieties.

5.4 Results

5.4.1 Aphid number per plant days after YSA natural infestation in winter and summer seasons

There were highly significant differences ($p < 0.001$) between number of aphids per plant in control and infested treatments. In winter, ZN 10 scored the highest aphid number (104) while 00-1165 recorded the least (31) in the control treatment (Figure 5.4). ZN 10 scored the highest aphid number (295) while 00-1165 scored the least. However, ZN 3L and ZN 9 were not significantly different from each other so as ZN 8 and 96-1107. In summer, in the control treatment, ZN 10 sugarcane variety recorded the highest (219) aphid number when compared to the least (74) realised by 00-1165 (Figure 5.4). In aphid infested plots, ZN 10 sugarcane variety scored the highest (962) aphid number while 00-1165 (237) recorded the least. Table 5.1 shows the temperature regimes and dates at which aphid numbers were recorded.

Table 5.1: Temperature regimes and dates at which aphid numbers were recorded

Temp (°C)	April		May		June		October		November		December	
	Date	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
1	30.6	16.7	29.1	19.0	26.6	12.7	36.2	20.5	19.2	13.5	31.2	18.2
2	30.9	17.0	30.9	15.0	26.6	10	22.1	16.5	24.6	14.5	32.9	17
3	32.2	17.5	31.2	13.7	26.1	13	28.5	16.5	28.1	15.7	33.7	18
4	32.9	16.0	30.3	17.0	27.1	11.2	33.5	12	30.2	16.5	35.1	18.9
5	27.2	20.7	31.5	12.3	27.9	10	34.3	13.2	32.4	16.7	37.1	19.5
6	28.9	16.0	31.4	13.3	24.1	10	36.4	16	35.8	18.6	33.7	22
7	31.4	14.5	31.9	12.0	25.4	10.5	37.9	20.5	36.9	18.7	33.4	23
8	35.9	15.5	28.6	16.0	28.1	10	36.5	20.7	38.1	19.1	36.4	20.5
9	34.7	15.9	26.5	15.9	30.1	9.5	31.1	21	31.3	19.5	40.5	22
10	34.2	18.6	27.7	15.5	18.4	14.9	34.5	19.5	30.7	20.1	37.4	24.9
11	27.9	20.8	29.7	14.0	18.6	11	36.1	20.1	29.6	19.3	30.5	21.2
12	28.6	19.4	32.2	13.5	19.6	10.7	38.2	19.2	37.2	19.7	36.7	22.4
13	31.7	14.1	31.7	14.0	22	9.4	40.4	21.5	31.5	17	28.2	22
14	34.5	12.8	30.5	14.7	22.1	12.5	24.5	20.5	23	20.1	32	21.9
15	23.2	18.3	29.6	13.4	26	11	23.2	18.8	29.8	17.6	35.9	22
16	23.9	18.5	23.5	17.2	22.5	10	20.9	18.5	32	16.8	35.5	24
17	28.0	13.4	29.5	15.9	26.5	8.9	21.3	18.8	34.2	15.9	29.1	21.4
18	28.9	13.0	31.5	17.8	26.7	7.9	26.5	18.6	37.3	15.8	33.7	22.7
19	29.0	14.5	33.2	16.8	28.4	7	30	16	38	19	34.4	24
20	29.4	14.3	24.9	18.2	23.7	9.9	31.3	19	39.6	21	32.7	22.5
21	34.4	13.3	31.0	18.3	15.7	15	35.9	16.3	39.6	23	34.9	20
22	31.2	16.9	29.9	13.8	18.6	14.1	39.9	20	40	25.5	33.4	21.5
23	36.5	14.5	25.5	18.2	26.4	9	23.9	18.9	39.8	22.5	35.4	22.6
24	30.1	19.5	29.4	17.0	30.4	8.7	36	19	38.9	20.2	36.3	23.5
25	32.6	15.7	30.0	14.4	22	12.3	26.8	22	40.9	21.5	37.3	23.2
26	30.9	17.5	32.4	13.0	23.5	14	31.4	21.5	38.5	23.8	29.6	23.2
27	32.0	16.0	27.6	15.9	27.3	9.2	35.5	18	38.4	23.7	20.7	28.9
28	33.3	15.0	27.7	12.0	29.6	10	36.6	19	39.3	22.1	22.2	23.4
29	32.3	16.0	29.1	11.5	32.9	10	31.2	21	41.3	19	23.4	19
30	32.0	13.5	29.1	12.6	29.6	12.8	16.1	13.8	23.2	17.5	25	20
31			31.6	14.8	29.8	8.8	15.4	13			30.5	20
Total	929.3	485.4	918.7	466.7	782.3	334	952.1	569.9	1019.4	573.9	1008.8	673.4
Average	31.0	23.6	29.6	15.1	25.2	10.8	30.7	18.4	34	19.1	32.5	21.7

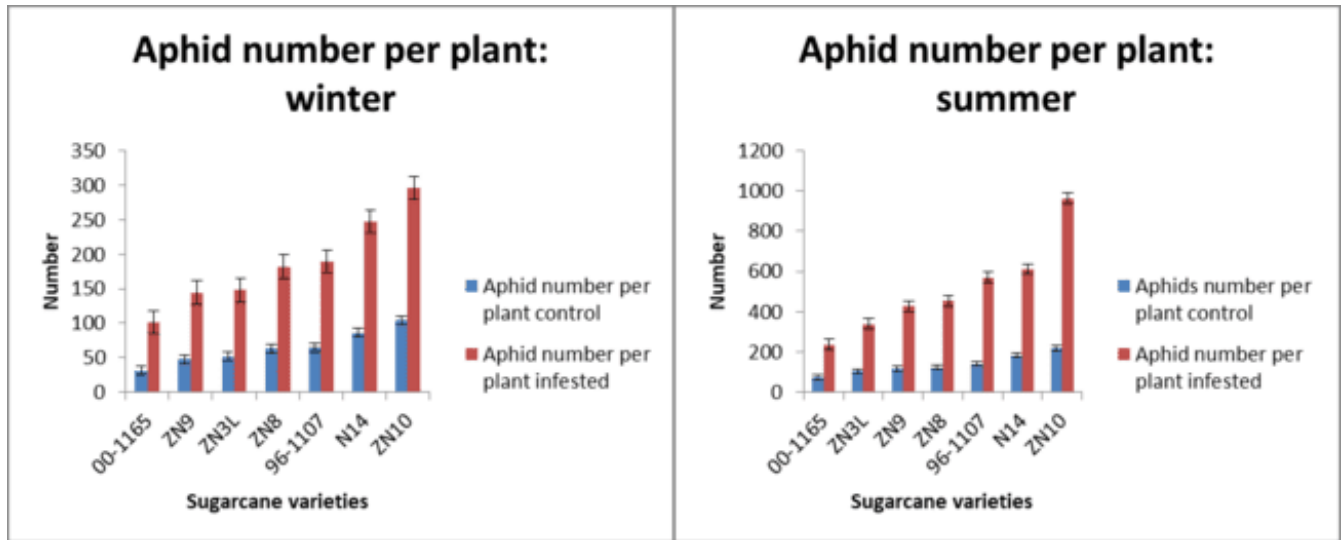


Figure 5.4: Number of aphids per plant 40 days after natural YSA infestation

5.4.2 Effects of YSA on Chlorophyll content and percentage chlorophyll loss in selected sugarcane varieties

Highly significant differences ($p < 0.001$) among the sugarcane varieties on chlorophyll content and percentage chlorophyll loss were recorded. In winter, in all the days of SPAD reading, ZN 3L scored the highest SPAD units (48.31) whilst N14 scored the least (43.34) at day 70 the control treatment (un-infested) and infested treatments (44.32, 38.85) respectively (Figure 5.5). Summer results showed that ZN 3L scored the highest chlorophyll content (48.34) while ZN 9 scored the least (43.37) at day 42 of SPAD reading. Furthermore, on aphid infested plots, ZN 3L recorded the highest (46.82) while ZN 10 scored the least (39.45) (Figure 5.6). In terms of chlorophyll loss, all the varieties scored $< 10\%$ chlorophyll loss (Figure 5.7). Furthermore, 14 days after the initial SPAD reading, ZN 10 scored the highest chlorophyll loss (7.3 %) which was not significantly different ($p > 0.05$) from 96-1107 and N14 sugarcane varieties. However, 00-1165 scored the least chlorophyll loss (4.3 %) which was not significantly different ($p > 0.05$) from ZN 3L. The same trend of results was realised in summer except that there was a great increase in percentage chlorophyll loss margin whereby ZN 10 recorded 21.4 % chlorophyll loss zero days of initial SPAD reading.

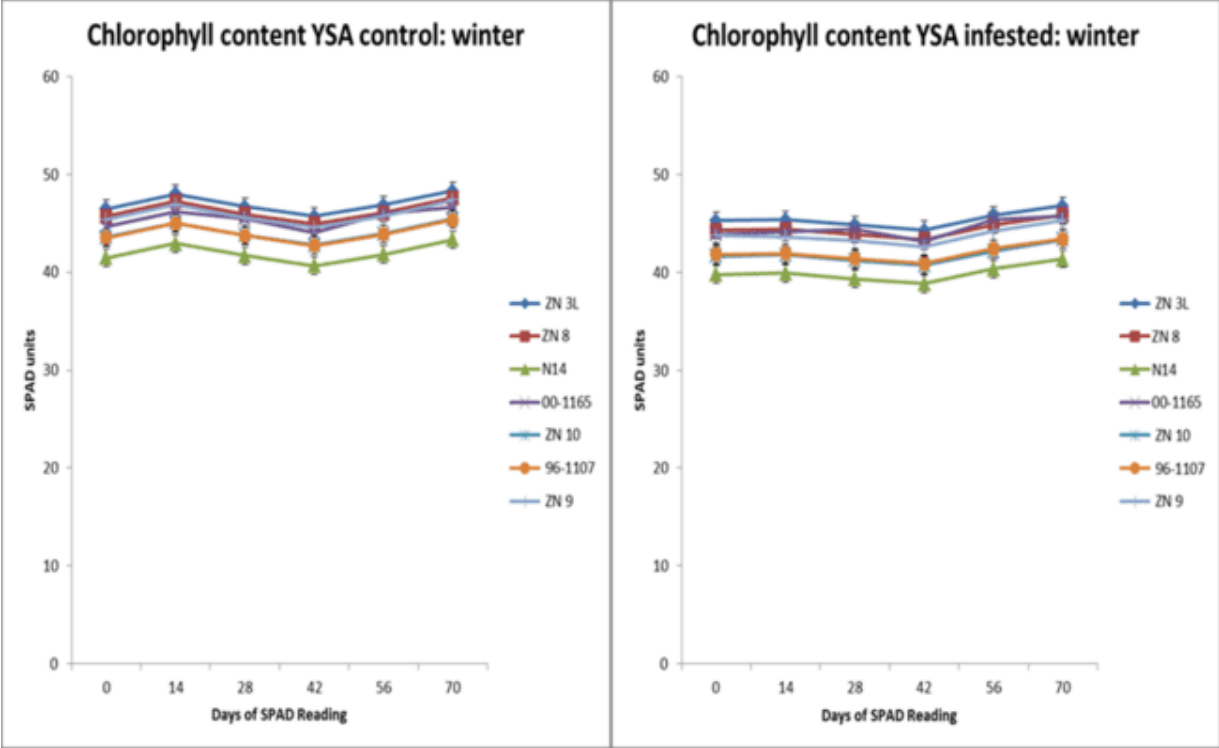


Figure 5.5: Chlorophyll content of control and YSA infested sugarcane varieties in winter

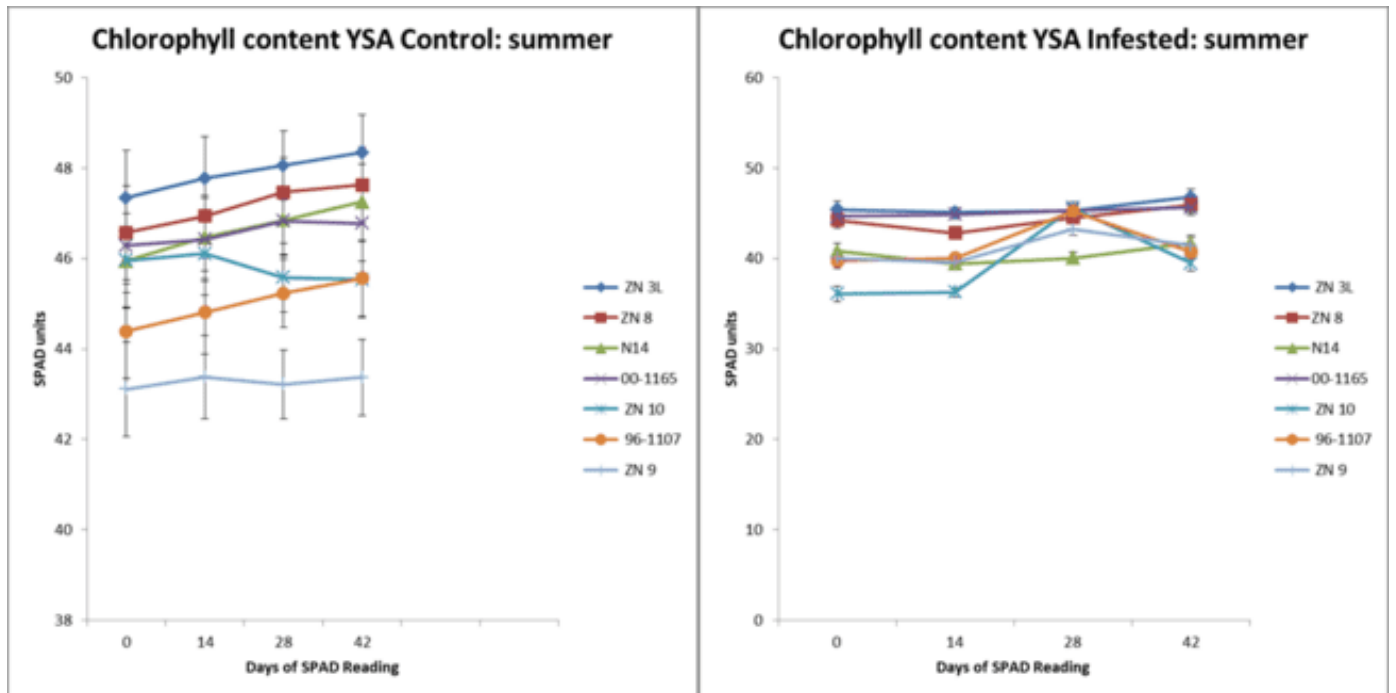


Figure 5.6: Chlorophyll content of control and YSA infested sugarcane varieties in summer

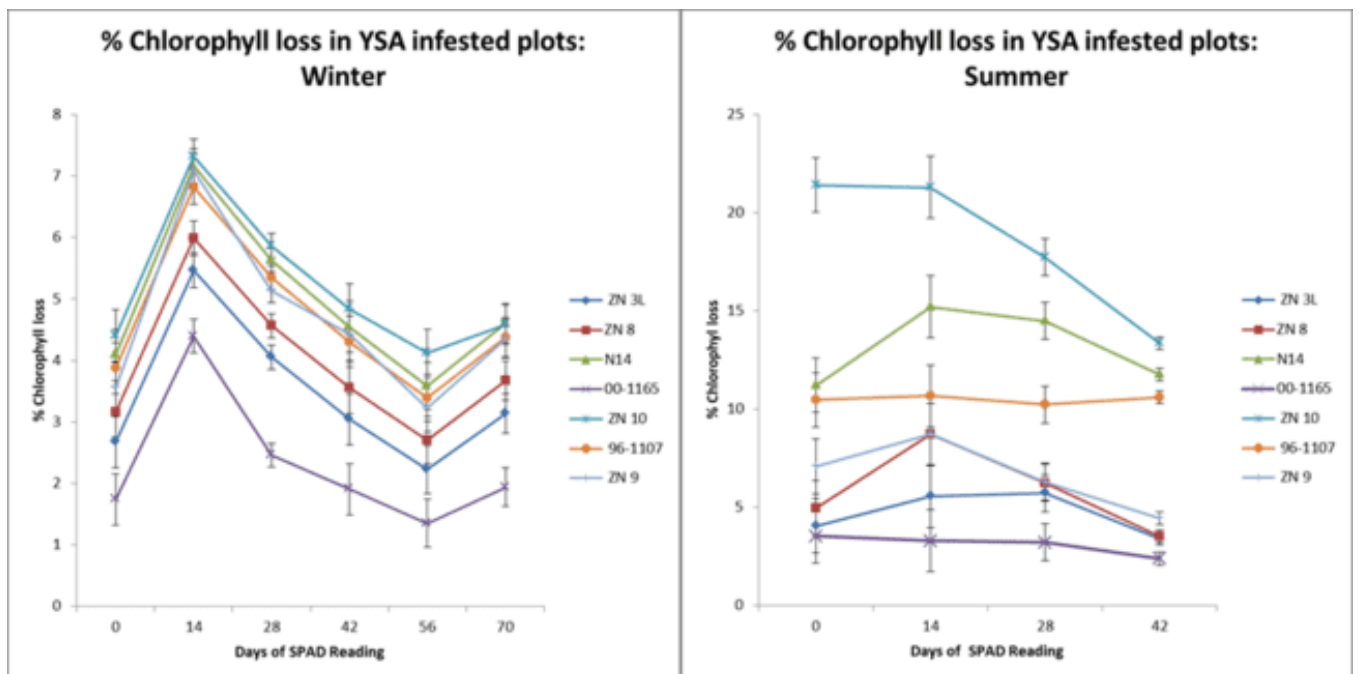


Figure 5.7: Percentage chlorophyll loss in YSA infested sugarcane varieties

5.4.3 Relationship between SPAD values and YSA aphid number on infested sugarcane varieties

Regression analysis showed highly significant ($p < 0.001$) negative correlation between SPAD values and Aphid number (Figure 5.8, $Y = -39.5X + 2209$, $p < 0.001$, $r = 0.69$).

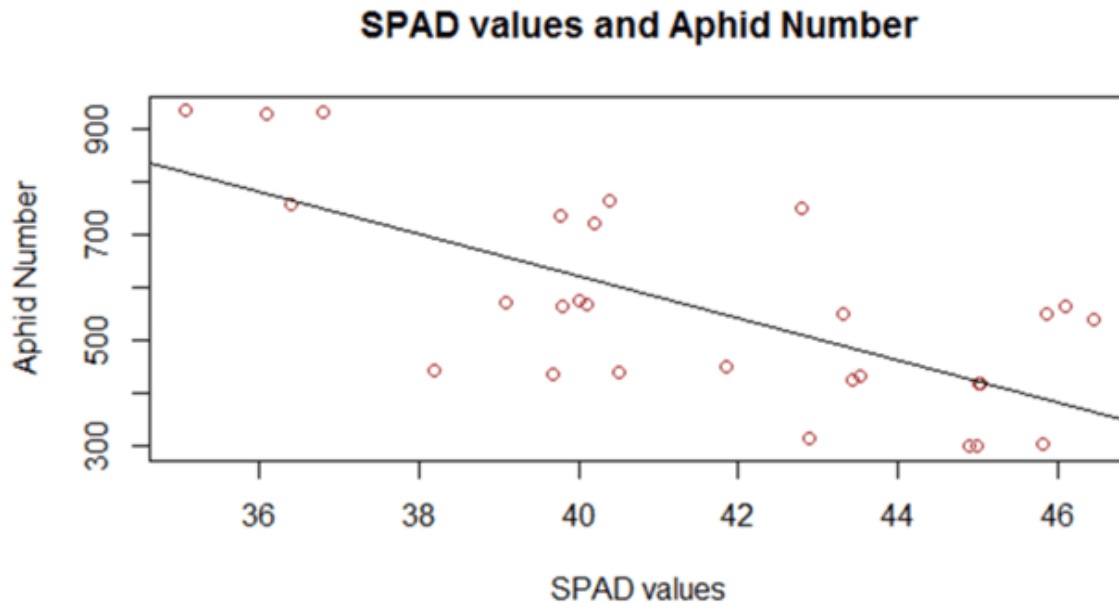


Figure 5.8: Relationship between SPAD values and YSA aphid number on infested sugarcane varieties

5.4.4 Relationship between chlorophyll loss and YSA aphid number on infested sugarcane varieties

Findings of the regression analysis showed a highly significant ($p < 0.001$) strong positive correlation between chlorophyll loss and aphid number after natural infestation. (Figure 5.9, $Y = 0.028X - 6.58$, $p < 0.001$, $r = 0.85$).

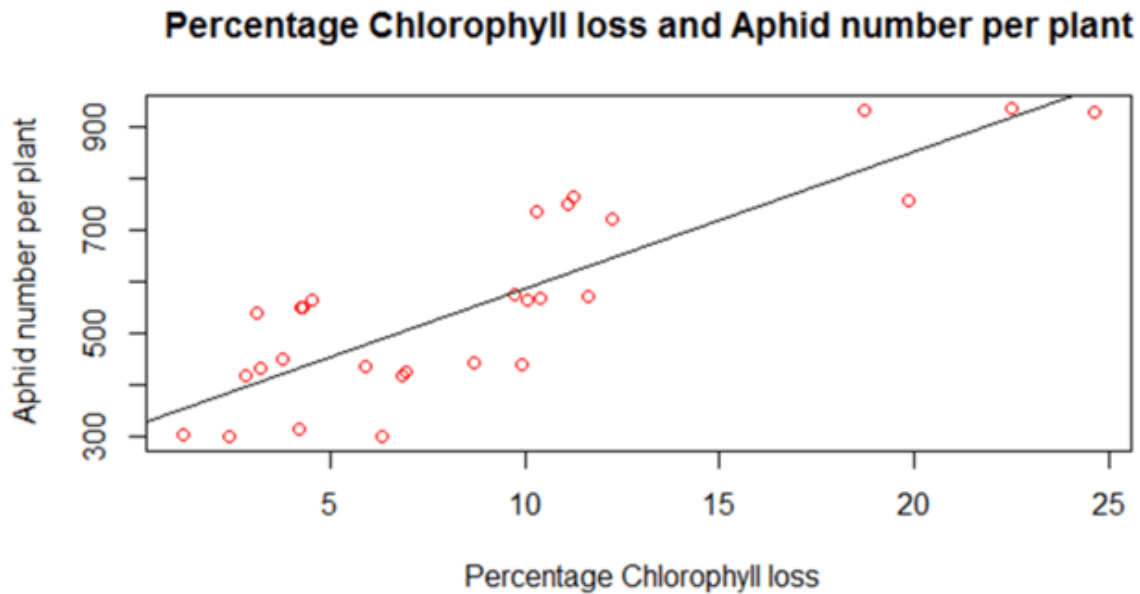


Figure 5.9: Relationship between chlorophyll loss and YSA aphid number on infested sugarcane varieties

5.4.5 Effect of YSA on gas exchange responses of selected commercial sugarcane varieties

5.4.5.1 Photosynthesis

YSA had a significant effect ($p < 0.05$) on photosynthetic rate amongst the sugarcane varieties. In winter, 70 days after aphid infestation, ZN 3L scored the highest photosynthesis (31.81) as compared to N14 which scored the least (18.43) in the control treatment (Figure 5.10). In the aphid infested treatment, 00-1165 sugarcane variety scored the highest photosynthesis as from day zero up to day 48 while N14 scored the lowest (15.92) (Figure 5.10). Summer results displayed highly significant differences ($p < 0.001$) on photosynthetic rate. The highest photosynthesis was obtained at day 28 from ZN 3L which recorded (30.75) when compared to the least (18.96) realised on N14 in the control treatment which was not significantly different ($p > 0.05$) from 96-1107 (Figure 5.11). However, in YSA infested plots, 00-1165 recorded the highest compensatory photosynthetic rate (32.52) when compared to the least (12.34) obtained from ZN 10 at day 28 (Figure 5.11).

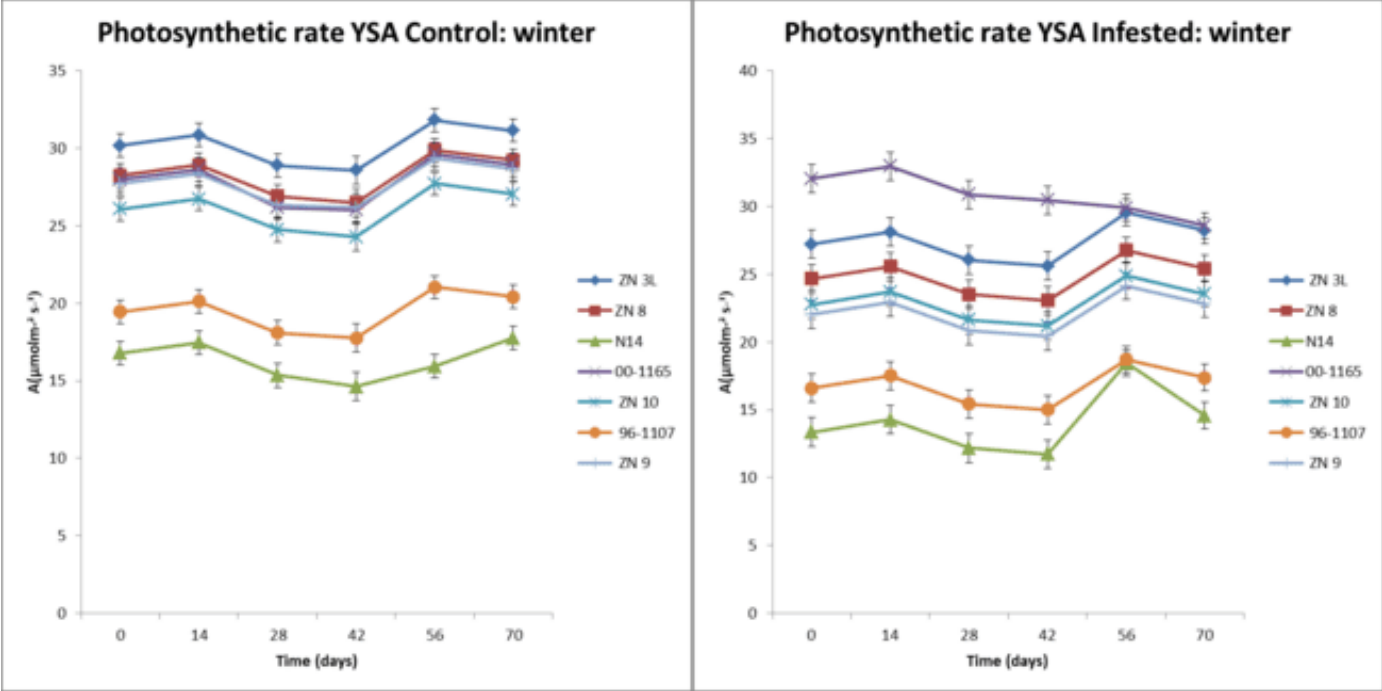


Figure 5.10: Photosynthetic rates (A) of control and YSA infested sugarcane varieties in winter

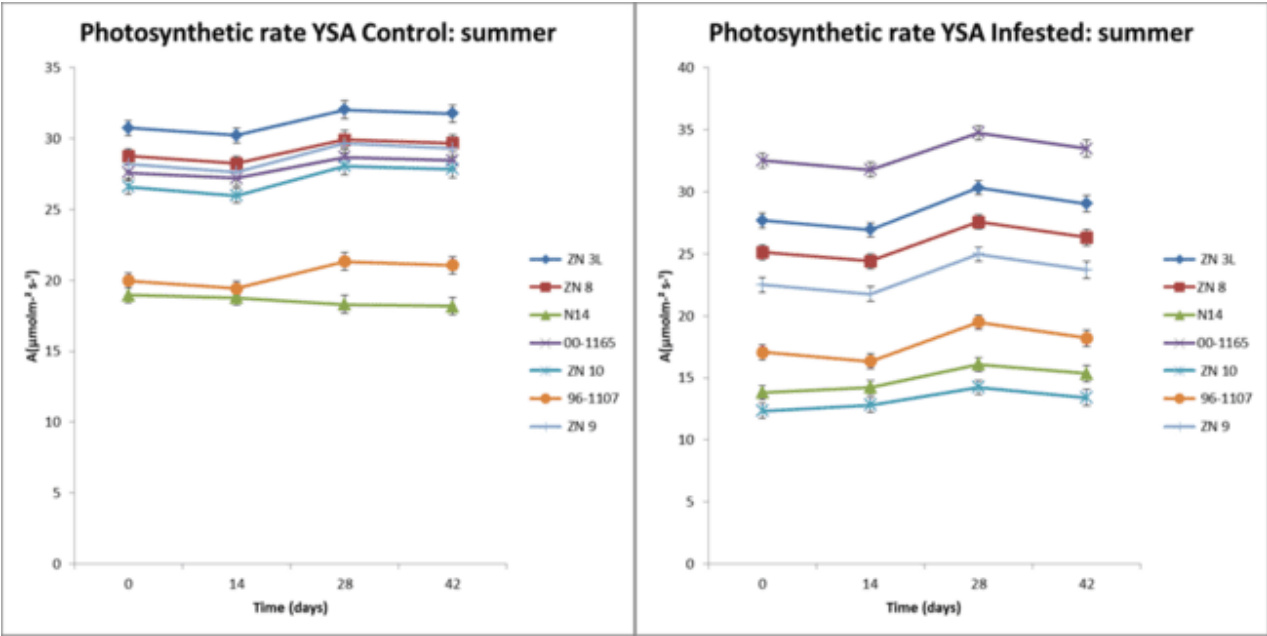


Figure 5.11: Photosynthetic rates (A) of control and YSA infested sugarcane varieties in summer

5.4.5.2 Relationship between photosynthesis and chlorophyll loss on infested YSA sugarcane varieties

Regression analysis revealed highly significant ($p=0.019$) negative correlation between chlorophyll loss and photosynthesis (Figure 5.12, $Y = -0.40X + 27.03$, $p= 0.019$, $r= 0.44$).

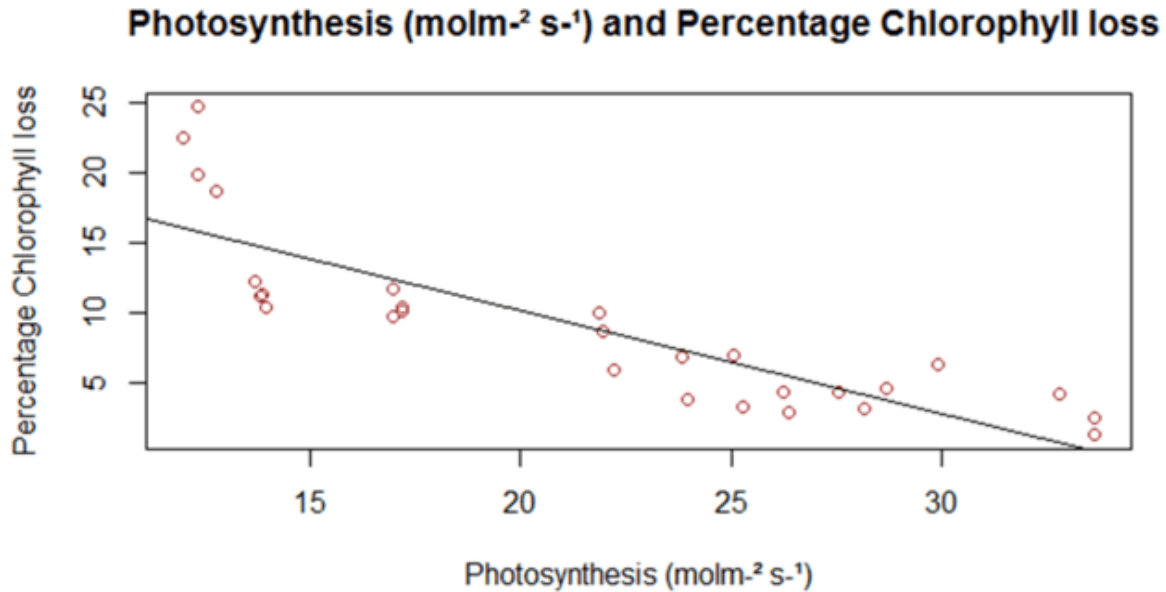


Figure 5.12: Relationship between photosynthesis and chlorophyll loss on infested YSA sugarcane varieties

5.4.5.3 Relationship between photosynthesis and aphid number on infested YSA sugarcane varieties

Regression analysis exhibited highly significant ($p=0.002$) negative correlation between photosynthesis and aphid number. (Figure 5.13, $Y = -0.01X + 33.25$, $p=0.002$, $r=0.57$).

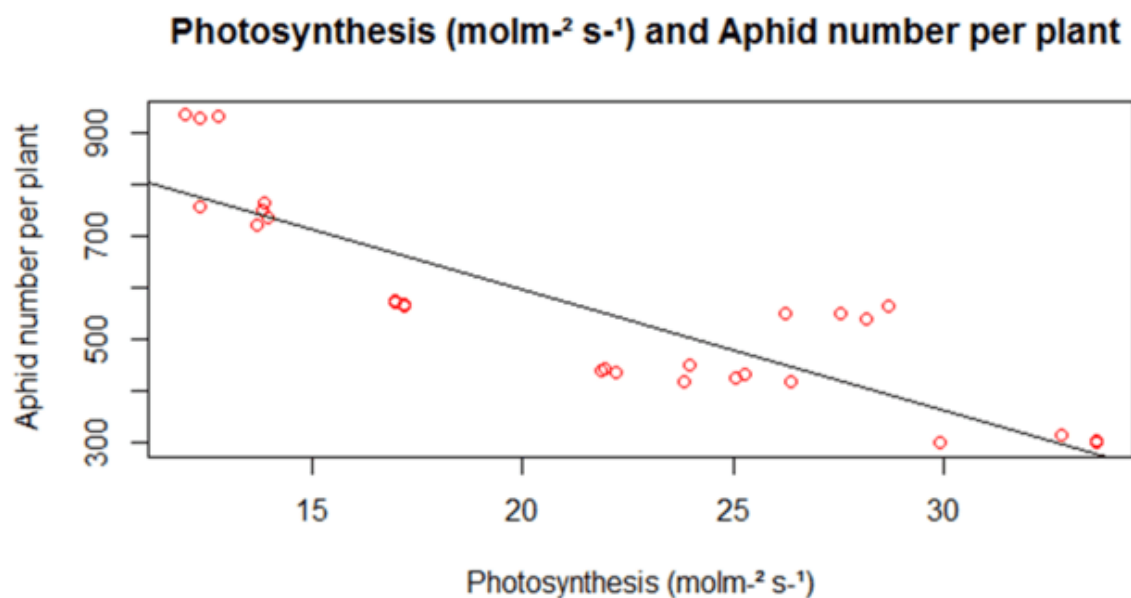


Figure 5.13: Relationship between photosynthesis and aphid number on infested YSA sugarcane varieties

5.4.5.4 Relationship between photosynthesis and SPAD values on infested YSA sugarcane varieties

Regression analysis showed highly significant ($p=0.004$) positive correlation between photosynthesis and SPAD values (Figure 5.14, $Y = 0.96X - 16.6$, $p=0.004$, $r=0.52$).

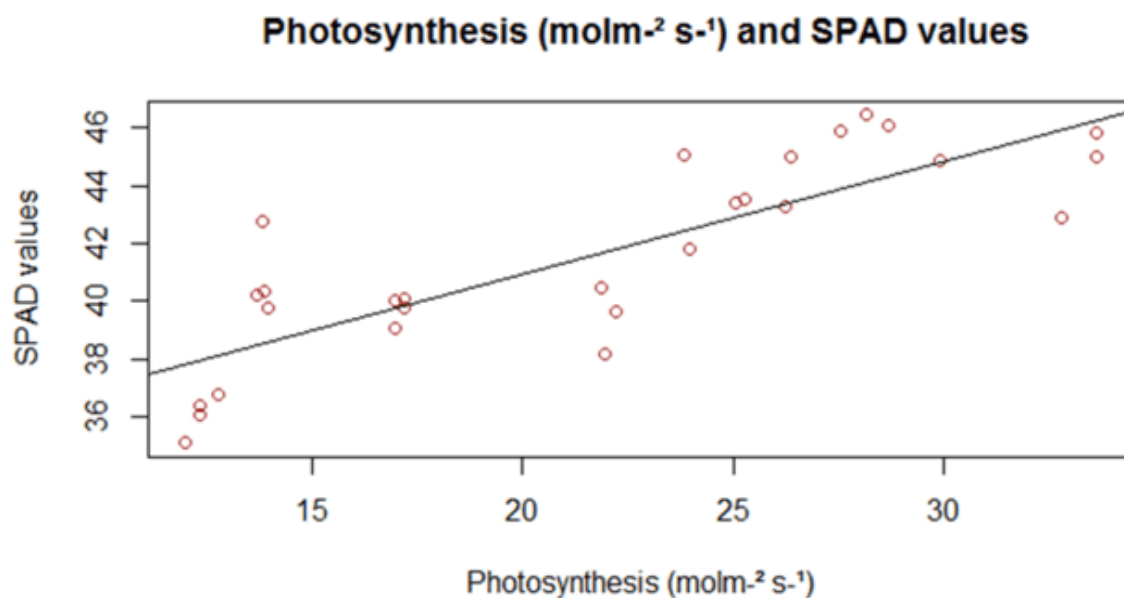


Figure 5.14: Relationship between photosynthesis and SPAD values on infested YSA sugarcane varieties

5.4.5.5 Transpiration

YSA had a significant effect ($p < 0.05$) on transpiration rate among the sugarcane varieties. In winter, ZN 3L scored the highest transpiration rate (3.5) on day 28 which was significantly different ($p < 0.05$) from the other control treatments. The lowest transpiration rate (2.40) was recorded on 96-1107 which was not significantly different ($p > 0.05$) from N14 and ZN 10 sugarcane varieties (Figure 5.15). On aphid infested plots, 00-1165 sugarcane variety scored the highest transpiration rate (2.96) while N14 recorded the lowest (1.32) (Figure 5.15). As from day 48 up to day 70, ZN 3L sugarcane variety resumed its high transpiration rate whilst N14 continued to record the least when compared to other sugarcane varieties as YSA are less active. During summer, the highest transpiration rate in the control treatment, was recorded on ZN 3L (4.78) while N14 recorded the least (3.60) which was not significantly different from 96-1107 and ZN 10 (Figure 5.16). However, on YSA infested plots, 00-1165 scored the highest transpiration rate (4.32) when compared to N14 which recorded the lowest (2.12) (Figure 5.16).

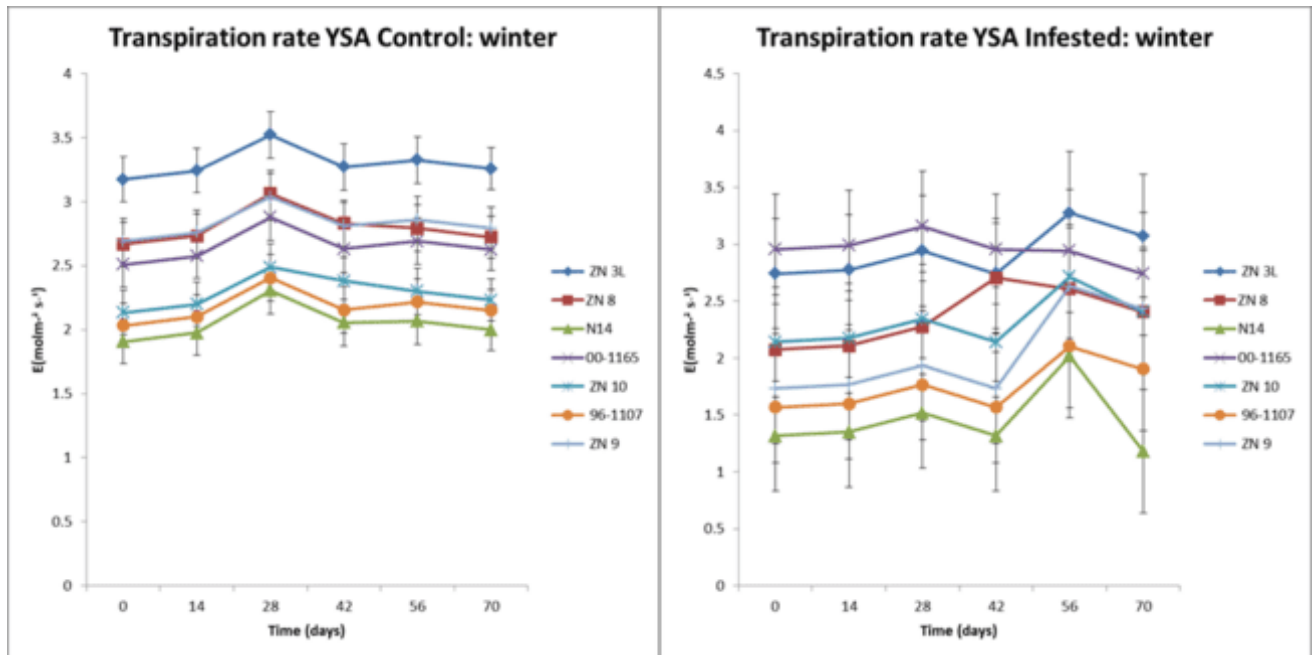


Figure 5.15: Transpiration rates (E) of control and YSA infested sugarcane varieties in winter

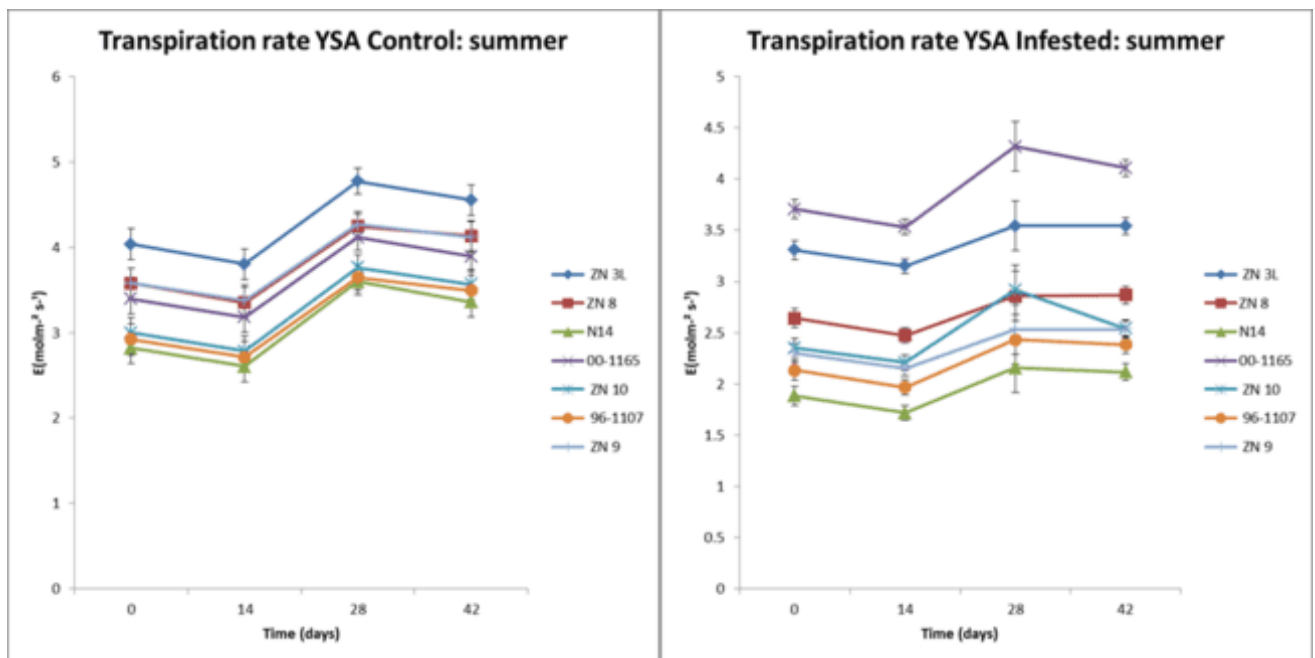


Figure 5.16: Transpiration rates (E) of control and YSA infested sugarcane varieties in summer

5.4.5.6 Stomata conductance

YSA had a significant effect ($p < 0.05$) on stomata conductance among the sugarcane varieties. In winter, there were highly significant differences ($p < 0.001$) among the sugarcane varieties in control plots; 00-1165 scored the highest stomata conductance (308.8) compared to N14 which recorded (165.7) at day 14 (Figure 5.17). In YSA infested plots, at day 56, 00-1165 variety scored the highest stomata conductance (237.5) in comparison to the lowest (123.6) reported on sugarcane variety 96-1107, which was not significantly different ($p > 0.05$) from N14 and ZN 8 (Figure 5.17). The summer results displayed highly significant differences ($p < 0.001$) on stomata conductance. In aphid control (un-infested) plots, at day zero, 00-1165 variety scored the highest (286.9) while N14 recorded the least (149) (Figure 5.18). In aphid infested plots, at day 28, 00-1165 sugarcane variety recorded the highest (218.2) stomata conductance when compared to the least (152.9) obtained in N14 (Figure 5.18). However, 96-1107 and ZN 9 sugarcane varieties were not significantly different ($p > 0.05$) from each other. The results highlight that; tolerant sugarcane varieties have the ability to increase or maintain gas exchange in response to YSA infestation.

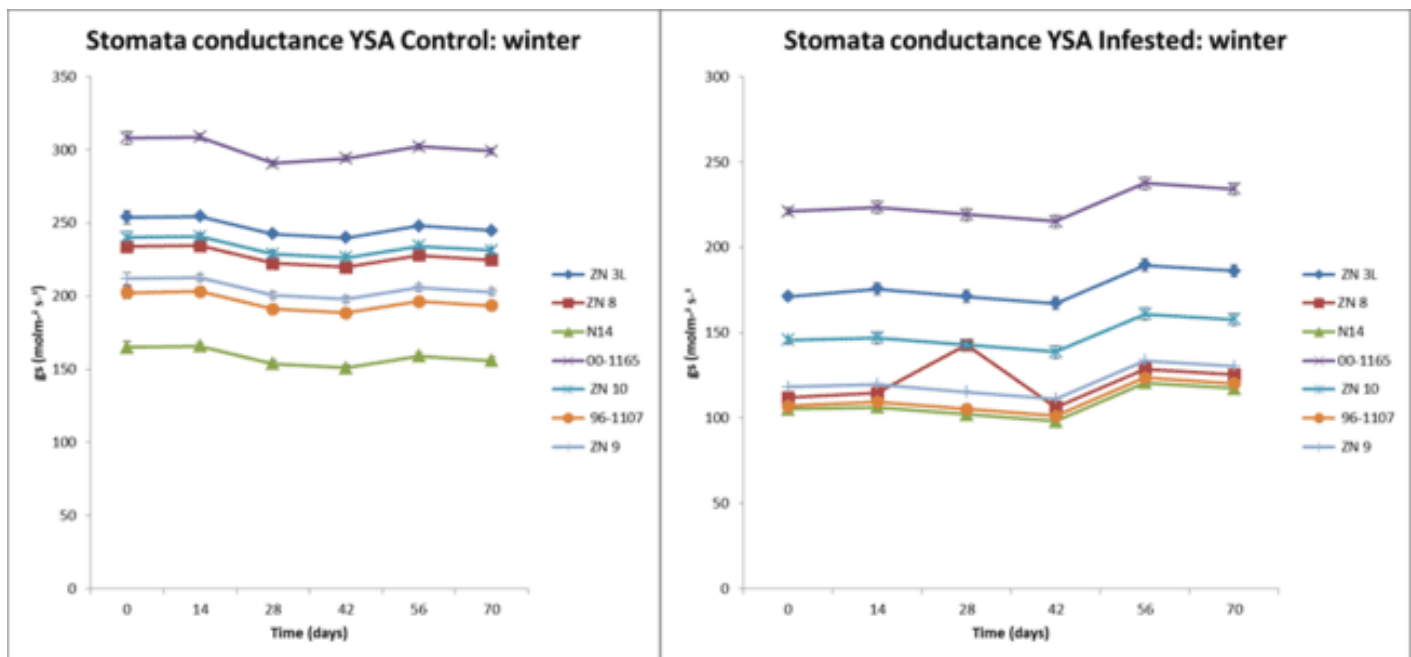


Figure 5.17: Stomata conductance (gs) of control and YSA infested sugarcane varieties in winter

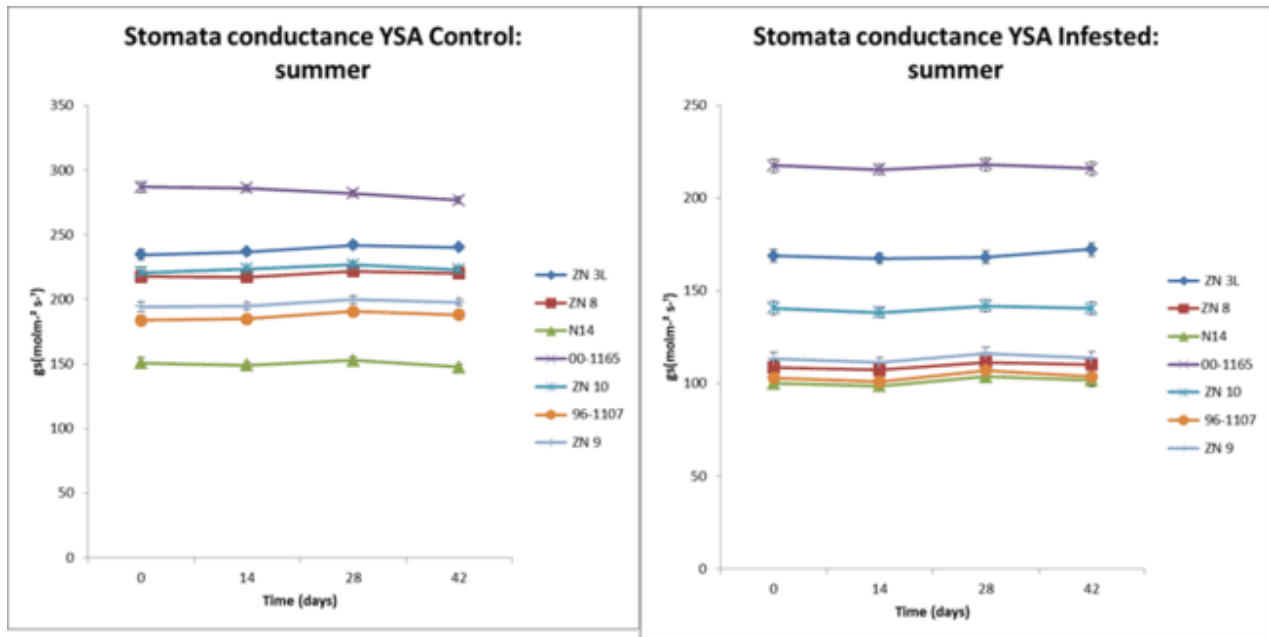


Figure 5.18: Stomata conductance (gs) of control and YSA infested sugarcane varieties in summer

5.5 Discussion

5.5.1 Aphid number

The study's findings demonstrated that the Lowveld's summer and winter seasons have an impact on the YSA number. This could be the problem of temperature-dependent polymorphism in reproduction (ovivipary and parthenogenesis). Compared to asexual reproduction, which results in greater fertility, the sexual method diminishes fecundity. Some researchers also reported similar results, indicating that YSA can reproduce in two ways depending on climate: mating (which produces live individuals and permits females to mate with males in cold winters when low temperatures induce vivipary (Halbert *et al.*, 2013) and non-mating (parthenogenetically) in warmer climates (Nuessly, 2005; Way *et al.*, 2015).

The greatest abiotic factor influencing insect life history parameters is temperature (Harrison *et al.*, 1985; Aalbersberg *et al.*, 1987; Bleicher and Parra, 1990; Davis *et al.*, 2006; Keena, 2006; Ozder and Saglam, 2013; Souza and Davis, 2018). According to Souza and Davis (2018), aphids exhibit a significant degree of phenotypic plasticity in response to prevailing environmental

circumstances. Body temperature of insect is dependent on the surrounding air temperature (Briere *et al.*, 1999; Rosenzweig *et al.*, 2001; Bale *et al.*, 2002; Menendez, 2007). According to Sharpe and DeMichele's (1977), temperature thresholds regulate the growth and development of certain insect species.

Temperature variations have an impact on metabolic and neurological and endocrine processes (Neven, 1998, 2000). Reports indicate that at lower and higher temperatures, insect growth is lengthened and shortened respectively (Angilletta *et al.*, 2004; Pigliucci, 2005; Sibly *et al.*, 2007). The physiology and palatability of the host plant are also altered changes in temperature (Acreman and Dixon, 1989). In addition, temperature regimes affect the polymorphism, feeding behaviour and migration of aphids (Dixon, 1977, 1987; Leather and Dixon, 1982; Liu, 1994; Collins and Leather, 2001; Müller *et al.*, 2001; Langer *et al.*, 2004; Kuo *et al.*, 2006; Harrington *et al.*, 2007; Hassal *et al.*, 2007; Colinet and Hance, 2009; Oliveira *et al.*, 2009b; Zheng *et al.*, 2015; Auad *et al.*, 2009; Auad *et al.*, 2012; Auad *et al.*, 2015; Schlemmer, 2018; Souza, 2018). Hinson (2017) investigated how temperature affects the SCA life cycle. Furthermore, Oliveira *et al.* (2009a) concluded that the optimal temperature range for YSA development and reproduction range from 20 °C-24 °C. Research conducted by Flynn *et al.* (2006) and Auad *et al.* (2012) highlighted that rising temperatures and CO₂ levels typically have an indirect impact on insectivores through changes in host plant physiology and phytochemistry. Furthermore, Souza *et al.* (2018) and (2019) noted that aphids' development rate slows down as a result of high temperature physiological stress.

5.5.2 Chlorophyll content

YSA infestation can damage the chlorophyll content of sugarcane leaves (>10% of loss), however resistant plants (00-1165, ZN 9, ZN 8, and ZN 3L) had less chlorophyll loss (< 10% of loss) for both winter and summer seasons. This might be a contribution of genes that code for more production of nitrogen required for chlorophyll formation. Similar results were reported by Wilson *et al.* (2011) who reported increased nitrogen in aphid-tolerant-infested plants due to increased nitrogen reductase. Susceptible genotypes (96-1107, N14, and ZN 10) might not have the same gene expression as intolerant varieties resulting in an increased percentage of chlorophyll loss. Reports of an increase in nitrogen content in the leaves attacked by the apple green aphid supports the sink theory (Syvertsen *et al.*, 2003; Urban *et al.*, 2004; Pincebourde and

Ngao, 2021). Furthermore, similar range of results was reported in sorghum against SCA by Paudyal (2019) on tolerant and susceptible tested varieties. As revealed by this study, the measurement of chlorophyll content in sorghum has been used as an indication of tolerance for SCA and YSA (Deol *et al.* 1997; Diaz-Montano *et al.* 2007b; Akbar, 2009; Paudyal, 2009). Results of reduced chlorophyll content in susceptible sugarcane genotypes (96-1107, N14, and ZN 10) might have been caused by degradation of chlorophyll or increased production of secondary metabolites. Results corroborate findings by Haile *et al.* (1999) and Goławska *et al.* (2010) who reported reduced chlorophyll content due to secondary metabolite production defence. Natural degradation of chlorophyll might have occurred in susceptible varieties although it was not determined. However, Janave (1997) suggested that oxidative bleaching pathway is a pathway of natural degradation of chlorophyll. Furthermore, Gonzales *et al.* (2002) found ultrastructural damage to chloroplasts in leaves of Johnson grass (*Sorghum halepense*) infested with YSA. Increased chlorophyllase activity has been reported in plants that have been colonized by aphids (Ni *et al.*, 2002; Ciepiela *et al.*, 2005; Sytykiewicz 2007), which hydrolyze free chlorophyll substrates into their corresponding chlorophyllide forms (Sytykiewicz *et al.*, 2013). Ni *et al.* (2002) showed that feeding by greenbug caused significant loss of chlorophyll in wheat. Regression analysis showed a positive strong correlation between chlorophyll loss and aphid number confirming studies by Golawska *et al.* (2010).

Plants infested with aphids may suffer significant harm as exhibited by ZN 10 from highly bioactive effector chemicals found in their salivary gland secretions resulting in yellowing and reddening of leaves. White (1990) confirmed that YSA feeding results in leaf discoloration with possible photosynthetic decline in susceptible varieties. Also, reports by Akbar (2009) state that chlorophyll loss from YSA was above 20 % but less than 50 %. However, chlorophyll loss from this study falls short in susceptible sugarcane varieties when compared to the above-mentioned range. This trend of results is supported by a number of authors (Cooper *et al.*, 2010; Cooper *et al.*, 2011; Nicholson *et al.*, 2012; Rao *et al.*, 2013; Stytykiewicz *et al.*, 2013). In support of these findings, Lage *et al.* (2003) pointed out that a variety that maintains relatively high chlorophyll content despite infestation is considered a good indicator of plant tolerance to herbivores as proven by 00-1165. However, ZN 9, ZN 8, and ZN 3L exhibited moderate physiological tolerance although they were not significantly different from each other. Chlorophyll ranges in this study confirm possible yield forecast that feeding by YSA at early stages of growth can

drastically lower output from 6 % to 19 % (Reagan, 1994; Hentz and Nuessly, 2004; Nuessly, 2005; Nuessly *et al.*, 2010; Wilson, 2019). According to this research, resistant sugarcane genotypes (00-1165, ZN 9, ZN 8, and ZN 3L) may have high levels of hydrogen peroxide (H₂O₂) buildup and robust antioxidant gene overexpression which may have aided in the development of host plant tolerance. Similar confirmation of such results was reported in resistant sorghum genotypes in response to SCA damage (Shankar and Yinghua, 2021).

5.5.3 Gas exchange responses

Gas exchange responses of commercial sugarcane varieties contribute significantly to physiological tolerance against YSA. In aphid-infested plots, 00-1165 was more tolerant when compared to other varieties as it recorded high photosynthetic rate, transpiration rate, and stomata conductance. A moderate level of physiological tolerance was exhibited in ZN 9, ZN 8, and ZN 3L sugarcane accessions. The reduction of conductance in ZN 10, N14, and 96-1107 showed they are not tolerant, this further suggests that stomatal interference contributes to decreased photosynthetic rates in susceptible varieties (Meyer and Whitlow, 1992).

In this study, infestation of YSA reduced the photosynthetic rate. This might be a reduction in chlorophyll content which is a pre-requisite for photosynthesis to occur. Phloem cells can be injected with saliva when there is an aphid infestation. Saliva is known to contain a range of effector proteins, hydrolytic enzymes, and poisonous compounds that trigger plant perception of aphid invasion and may exacerbate the accumulation of reactive oxygen species (ROS), a precursor to oxidative stress (Shankar and Yinghua, 2021). Oxidative stress, defined as high levels of reactive oxygen species (ROS) production within a cell, results in oxidative damage to membranes (lipid peroxidation), pigments, proteins, and nucleic acids, ultimately leading to cell death (Mittler, 2002; Gechev, 2006). The resistant sugarcane genotype (00-1165) might have evolved in an antioxidant mechanism to counteract the harmful effects of ROS and shield the plant from oxidative damage by eliminating excess ROS from the cell as highlighted in a study by Shankar and Yinghua (2021). The antioxidant properties have been mentioned by several authors (Mittler *et al.*, 2004; Apel and Hirt, 2004; Pekker *et al.*, 2002; Foyer and Noctor, 2005; Gelhaye *et al.*, 2005; Noctor and Foyer, 1998; Pei *et al.*, 2000; Dat *et al.*, 2000; Grant and Loake, 2000; Shankar and Huang, 2021). ROS are recognized for signaling, cellular growth control and stomatal closure (Apel and Hirt, 2004; Foyer and Noctor, 2005; Pei *et al.*, 2000; Dat *et al.*, 2000;

Grant and Loake, 2000; Shankar and Huang, 2021), programmed cell death (Gechev *et al.*, 2006), and response to biotic and abiotic stresses (Gechev *et al.*, 2006; Suzuki *et al.*, 2012). Moreover, increased ROS accumulation can also trigger host plant defense response mediated by systemic acquired immune responses SARS (Wu *et al.*, 1997; Cao *et al.*, 1998; Zhang *et al.*, 1999; Asada *et al.*, 2006). In support of this study, plants that experience ROS suppression become more susceptible to aphid infestation, while ROS buildup may also lead to aphid resistance (Shoala *et al.*, 2018). Macedo *et al.* (2003) revealed two potential mechanisms underlying the plant responses by considering transient analysis of chlorophyll fluorescence: (i) end-product inhibition induced by source-sink manipulation) or (ii) photo-inhibition on the light reactions due to ROS. This might be the case associated with susceptibility in tested sugarcane varieties (96-1107, N14, and ZN 10) although this study did not consider further investigation of the aforementioned mechanisms.

Results indicating lower stomata conductance and transpiration in susceptible sugarcane accessions (96-1107, N14, and ZN 10) may be as a result of reduced stomata aperture size, stimulated by abscisic acid signaling pathway in response to YSA's feeding. Similar findings are confirmed by Sun *et al.* (2015), who suggested that aphid infestation interferes with ABA signaling pathway. This, in turn, reduces leaf transpiration, increases xylem feeding time and decreases hemolymph osmolarity. Moreover, feeding by YSA might have caused decreased stomata conductance by upregulating ABA carbonic anhydrase in stressed YSA-infested susceptible sugarcane varieties. The results are in agreement with those of Guo *et al.* (2016), who found that, probing by aphids increased the expression of carbonic anhydrase, an ABA enzyme.

The stress from YSA might have increased the expression of mitogen-activated protein kinase (MPK) in susceptible sugarcane accessions thereby increasing the amount of ABA produced, although this study did not determine MPK and ABA. The expression of mitogen-activated protein kinases-4 (MPK4) might have increase plant susceptibility to YSA activating anion in the guard cells to close the stomata. The findings are corroborated by Jakobson *et al.* (2016), who observed that MPK4 cause stomatal closure. Guo *et al.* (2017) also concluded that MPK4 suppresses the jasmonic acid (JA) signaling pathway, resulting in reduced transpiration and stomata conductance, a similar trend observed in susceptible sugarcane varieties (96-1107, N14, and ZN 10).

Furthermore, the regression analysis indicated a positive correlation ($r=0.57$) between photosynthesis and aphid number. This shows that aphid density influences the photosynthesis outcome as they inflict stress and injury to susceptible sugarcane genotypes. Similar results of an inverse relationship between the number of Russian wheat aphids and photosynthesis in wheat (Haile *et al.*, 1999). This study reveals that aphids interfere negatively with physiological processes in plants in susceptible sugarcane varieties. Similar discoveries were reported by Nagaraj *et al.* (2002) that photosynthesis was highly significantly positively correlated with chlorophyll content on sorghum damaged by greenbug. Furthermore, Nagaraj *et al.* (2002) observed that with a small drop in chlorophyll content, a drastic decrease in photosynthesis was noticed which falls within the correlation of 0.44 obtained in this study. In line with this discovery, a significant decline in chlorophyll content was reported by Burd and Eliot (1996) in aphid infested wheat and barley leaves. The same decrease was also noticed in 96-1107, N14, and ZN 10 sugarcane varieties indicating that they cannot cope up with high aphid infestations. Sugarcane accessions (ZN 9, ZN 8, and ZN 3L) were able to retain chlorophyll and maintain photosynthesis. In addition to this, Macedo *et al.* (2003a) reported similar results under continuous light in wheat. Furthermore, Frazen *et al.* (2007) showed that tolerant wheat had similar rates of photosynthesis compared to the control treatments. This similar trend was observed on the tolerant accession (00-1165) and moderate tolerant accessions (ZN 9, ZN 8, and ZN 3L). These accessions can be used in environments where YSA is a menace because they were able to express physiological tolerance. Contrary to this research, Gomez *et al.* (2006) found no change in the rate of photosynthesis in cotton in the presence of cotton aphids.

Reduction in gas exchange responses has been documented in infested hosts inflicted by sucking insect pests in rice (Watanabe and Kitagawa, 2000) and in cotton (Lin *et al.*, 1999). However, on the contrary, Macedo *et al.* (2009) reported that greenbug does not affect transpiration rate in wheat. Furthermore, Hawkins *et al.* (2006) found out that aphid-infested plants had more net CO₂ exchange rates than their respective controls. Moreover, Heng-Moss *et al.* (2003) speculated that feeding mainly on phloem tissue by the aphids results in pH change in the stroma. Additionally, Burd and Elliott (1996) showed that aphid feeding has the ability to reduce protein synthesis causing photo-inhibition. Also, Haile *et al.* (1999) and Heng-Moss *et al.* (2003) reported reduced photosynthesis in aphid infested crops. Their study speculated that this might have resulted from increased biochemical resistance in response to insect herbivory hence a

possible contribution to this study. The decline in chlorophyll concentration as exhibited by yellowish and reddish discoloration in this study might also be due to the increased production of defensive compounds. These results suggest that the capacity to maintain or raise rubisco and ribulose biphosphate (RuBP) activity may be linked to compensatory mechanisms present in tolerant sugarcane accessions. Reductions and compensatory mechanisms specifically in crops that demonstrated tolerance to specific aphid species has been documented (Haile *et al.*, 1999; Haile and Higley, 2003; Macedo *et al.*, 2003a, b; Frazen *et al.*, 2007; Gutsche *et al.*, 2009a; Pierson *et al.*, 2010a; Pierson *et al.*, 2011; Prochaska, 2015). The plant can partially compensate for insect pest herbivory by increasing the photosynthetic rate per unit area in the surviving leaf tissues as a result of the loss of photosynthetic tissue caused by defoliating insects (Welter, 1989).

Zangerl *et al.* (2002) suggested that herbivory can cause a decrease in the rate of assimilation in the remaining tissues. Furthermore, plants infested by mesophyll feeders such as stink bugs (Velikova *et al.*, 2010) and spider mites (Welter, 1989; Haile and Higley, 2003) recorded significant reductions in photosynthesis. In order to improve herbivore performance, it has been demonstrated that effectors of spider mites and aphids reduce plant defense signals and responses (Atamian *et al.*, 2013; Naessens *et al.*, 2015, Schimmel *et al.*, 2017). The effects of insect feeding on leaf stomata conductivity and transpiration rate also vary widely among the sugarcane genotypes as demonstrated by our study. Similar results were confirmed by Shannag (2007) who indicated that aphid inject saliva with metabolically active substances into the phloem, resulting in increased transpiration and stomatal conductance rates. This study revealed that there are significant genotype-specific differences in the impacts of YSA feeding on the conductivity of leaf stomata and the rate of transpiration. Shannag (2007) confirm similar findings, highlighting the fact that aphids inject saliva containing substances that are metabolically active into the phloem, thereby increasing the rates of transpiration and stomatal conductance.

Yellowing and reddening of leaves inflicted by YSA in our study reduced leaf surface area for physiological processes. In support of this observation, Aldea *et al.* (2005) confirm that insect damage increased water loss around damaged tissue in soybean. Additionally, Tang *et al.* (2006) discovered that, photosynthesis was limited by water stress brought on by both increased water loss near the wounded tissue in *Arabidopsis thaliana* and decreased stomatal conductivity in

tissues farther away from the injury induced by the *Lepidoptera Trichoplusia ni*. Pincebourde and Ngao (2021) concluded that photosynthesis decreases when assimilation and transpiration rates are affected concurrently. However, although full compensation is rare (Peterson, 2000), mitigation is possible which may contribute to plant tolerance to herbivores (Pincebourde *et al.*, 2006). It is still difficult to explain the observed variability in sugarcane leaf gas exchange responses to YSA damage, as Pincebourde and Ngao (2021) pointed out.

The findings of this research demonstrate that YSA sugarcane-resistant plants appear to be able to compensate for injury caused by YSA feeding, through increased chlorophyll content, maintaining or increasing; photosynthetic integrity, transpiration, and stomata conductance. These findings support previous research on some aphid species (Retuerto *et al.*, 2004; Heng-Moss *et al.*, 2006; Frazen *et al.*, 2007; Gutsche *et al.*, 2009; Akbar *et al.*, 2009; Paudyal, 2019; Paudyal *et al.*, 2019). However, other variables like; leaf age, nutritional composition, and micro-environment parameters, may also influence photosynthesis (Nagaraj *et al.*, 2002). Knowledge of the physiological alterations occurring in sugarcane leaves infested by YSA may provide information on resistance and defense responses. This will be leveraged to develop new YSA-resistant sugarcane accessions.

5.6 Conclusion

Sugarcane accessions displayed significant differences in chlorophyll content and gas exchange responses (photosynthesis, transpiration, and stomata conductance). Furthermore, 00-1165 exhibited high physiological tolerance while moderate tolerance was exhibited on ZN 9, ZN 8, and ZN 3L. These accessions were able to compensate for/maintain chlorophyll content and gas exchange in response to YSA feeding. However, 96-1107, N14, and ZN 10 were the most susceptible to physiological damage posed by YSA.

5.7 Future studies

To gain a better understanding of the molecular mechanisms of ROS-mediated aphid resistance in sugarcane, additional experiments involving additional enzymes and remaining members of the enzyme families are required. This study has been restricted to supporting discussions on specific genes associated with the ROS scavenging pathways that may be involved in sugarcane resistance to YSA. To close the information gap regarding the genetic and metabolic basis of

aphid resistance in sugarcane, detailed elucidation of H₂ O₂ interactions with other plant defense pathways, such as plant hormones and transcription factors, is also necessary.

References

- Aalbersberg, Y. K., Du Toit, F., Van Der Westhuizen, M. C., and Hewitt, P. H. 1987. Development rate, fecundity and lifespan of apterae of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko)(Hemiptera: Aphididae), under controlled conditions. *Bulletin of Entomological Research*. 77(4): 629-635.
- Acreman, S. J., and Dixon, A. F. G. 1989. The effects of temperature and host quality on the rate of increase of the grain aphid (*Sitobion avenae*) on wheat. *Annals of Applied Biology*. 115: 3–9.
- Akbar, W. 2009. Categorization and Identification of Mechanisms of Sugarcane Resistance to the Sugarcane Aphid (Hemiptera: Aphididae). *LSU Doctoral Dissertations*. 2785. <https://digitalcommons.lsu.edu/gradschooldissertations/2785>
- Akbar, W., Showler, A. T. Reagan, T. E., and White. W. H. 2010. Categorizing sugarcane cultivar resistance to the sugarcane aphid and yellow sugarcane aphid (Hemiptera: Aphididae). *J. Econ. Entomol.* 103: 1431–1437.
- Aldea, M., Hamilton, J. G., Resti J. P., Zangerl, A. R., Berenbaum, M. R., and DeLucia, H. 2005. Indirect effects of insect herbivory on leaf gas exchange in soybean. *Plant, Cell and Environment*. 28: 402–411.
- Angilletta, M. J., Steury, T. D., and Sears, M. W. 2004. Temperature, growth rate, and body size in ectotherms: wetting pieces of a life-history puzzle. *Integrative and Comparative Biology*. 44: 498–509.
- Apel, K., and Hirt, H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol.* 55:373-399. doi:10.1146/annurev.arplant.55.031903.141701.
- Armstrong, J. S., Rooney, W. L., Gary Peterson, C. Villeneuve, R. T. Brewer, M. J., and Sekula-Ortiz. D. 2015. Sugarcane Aphid (Hemiptera: Aphididae): Host range and sorghum resistance including Cross-Resistance from greenbug sources. *J. Econ. Entomol.* 108: 576–582.

- Armstrong, J. S., Rooney, W. L., Mbulwe, L., Sekula-Ortiz, D., and Villanueva, R. T. 2017. Host plant resistance to sugarcane aphid *Melanaphis sacchari*, (Hemiptera: Aphididae) in forage and grain sorghums. *J. Econ Entomol.* 110: 259–265.
- Asada, K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiol.* 141:391-396. doi:10.1104/pp.106.082040.
- Asin, L., and Pons, X. 2001. Effect of high temperature on the growth and reproduction of corn aphids (Homoptera: Aphididae) and implications for their population dynamics on the northeastern Iberian Peninsula. *Environ. Entomol.* 30: 1127- 1134.
- Auad, A. M., Alves, S. O., Carvalho, C. A., Silva, D. M., Resende, T. T., and Veríssimo, B. A. 2009. The impact of temperature on biological aspects and life table of *Rhopalosiphum padi* (Hemiptera: Aphididae) fed with signal grass. *Florida Entomol.* 92: 569-577.
- Auad, A. M., Fonseca, M. G., Resende, T. T., and Maddalena, Í. S. 2012. Effect of climate change on longevity and reproduction of *Sipha flava* (Hemiptera: Aphididae). *Florida Entomologist.* 95(2): 433-444.
- Auad, A. M., Silva, S. E. B., Santos, J. C., and Vieira, T. M. 2015. Impact of fluctuating and constant temperatures on key life history parameters of *Sipha flava* (Hemiptera: Aphididae). *Florida Entomologist.* 98 (2): 424-429.
- Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D., and Whittaker, J. B. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology.* 8: 1-16.
- Bleicher, E., and Parra, J. R. P. 1990. Species of *Trichogramma* parasitoid of *Alabama argillacea*. iii. Determination of thermal requirement of three strains. *Brazilian Agricultural Research.* 25: 215–219.
- 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. 2016a. Sugarcane aphid (Hemiptera: Aphididae):

A new pest on sorghum in Northern America. *Journal of Integrated Pest Management*. 7(1): 1–13.

Bowling, R., Brewer, M. J., Knutson, A., Way, M., Biles, S., and Sekula-Ortiz, D. 2016b. Scouting sugarcane aphids in South, Central, and West Texas. Texas A & M Agrilife Extension. NTO-043, College Station, TX, 2p. (<http://ccag.tamu.edu/sorghum-insect-pests/>).

Breen J. P., and Teetes, G. L. 1986. Relationships of the yellow sugarcane aphid (Homoptera: Aphididae) density to sorghum damage. *Journal of Economic Entomology*. 79: 1106-1110.

Briere, J. F., Pracros, P., Le Roux, A. Y and Pierre, S. 1999. A novel rate model of temperature dependent development for arthropods. *Environmental Entomology*. 28: 22-29.

Burd, J. D., and Elliott, N. C. 1996. Changes in chlorophyll *a* fluorescence induction kinetics in cereals infested with Russian wheat aphid (Homoptera: Aphididae). *Journal of Economic Entomology*. 89: 1332–1337.

Cao, H., Li, X., and Dong, X. 1998. Generation of broad-spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance. *Proc Natl Acad Sci USA*. 6531-6536. doi:10.1073/pnas.95.11.6531.

Capinera, J. L. 1981. Some effects of infestation by bean aphid, *Aphis fabae* Scopoli, on carbohydrate and protein levels in sugar beet plants, and procedures for estimating economic injury levels. *Zeitschrift für angewandte Entomologie*. 92: 374–384.

Chan, E. K., Rowe, H. C., and Kliebenstein, D. J. 2010. Understanding the evolution of defense metabolites in *Arabidopsis thaliana* using genome-wide association mapping. *Genetics*. 185: 991–1007. doi: 10.1534/genetics.109.108522

Chen, X., Vosman, B., Visser, R. G. F., van der Vlugt, R. A. A., and Broekgaarden, C. 2012. High throughput phenotyping for aphid resistance in large plant collections. *Plant Methods*. 8:33. doi: 10.1186/1746-4811-8-33

Ciepiela, A. P., Sytykiewicz, H., and Czerniewicz, P. 2005. Changes in the activity of chlorophyllase and Mgdechelatase in bird cherry leaves induced by *Rhopalosiphum padi* L. feeding. *Aphids and Other Hemipterous Insects*. 11: 25-31.

- Clowes, M. J., and Breakwell, W. L. 1998. Zimbabwe Sugarcane Production Manual. Zimbabwe Sugar Association, Chiredzi, Zimbabwe.
- Colinet, H., and Hance, T. 2009. Male reproductive potential of *Aphidius colemani* (Hymenoptera: Aphidiinae) exposed to constant or fluctuating thermal regimens. *Environmental Entomology*. 1: 242–249.
- Collins, C. M., and Leather, S. R. 2001. Effect of temperature on fecundity and development of the giant willow aphid, *Tuberolachnus salignus* (Sternorrhyncha: Aphididae). *European Journal of Entomology*. 98: 177–182.
- Cooper, W. R., Dillwith, J. W., and Puterka, G. J. 2010. Salivary proteins of Russian wheat aphid (Hemiptera: Aphididae). *Environmental Entomology*. 39: 223-231.
- Cooper, W. R., Dillwith, J.W., and Puterka, G.J. 2011. Comparisons of salivary proteins from five aphid (Hemiptera: Aphididae) species. *Environmental Entomology*. 40: 151-156.
- Craigie, G. 2022. Detecting Sugarcane Aphid (*Melanaphis sacchari*) infestation in Grain Sorghum (*Sorghum bicolor*) using leaf spectral response (Doctoral dissertation).
- Dat, J., Vandenabeele, S., Vranova, E. V. M. M., Van Montagu, M., Inze, D., and Van Breusegem F. 2005. Dual action of the active oxygen species during plant stress responses. *Cell Mol Life Sci*. 57:779-795.doi:10.1007/s000180050041.
- Davis, J. A., Radcliffe, E. B., and Ragsdale, D. W. 2006. Effects of high and fluctuating temperatures on *Myzus persicae* (Hemiptera: Aphididae). *Environmental Entomology*. 35: 1461–1468.
- Delaney, K. J and., Higley, L. G. 2006. An insect countermeasure impacts plant physiology: midrib vein cutting, defoliation and leaf photosynthesis. *Plant, Cell and Environment*. 29: 1245–1258.
- Deol, G. S., Reese, J. C., and Gill, B. S. 1997. A rapid, nondestructive technique for assessing chlorophyll loss from greenbug (Homoptera: Aphididae) feeding damage on sorghum leaves. *J. Kans. Entomol. Soc.* 70: 305–312.

- Deol, G. S., Reese, J. C., Gill, B. S., Wilde, G. E., and Campbell, L. R. 2001. Comparative chlorophyll losses in susceptible wheat leaves fed by Russian wheat aphids or greenbugs (Homoptera: Aphididae). *J. Kans. Entomol. Soc.* 74: 192–198.
- Diaz-Montano, J., Reese, J. C., Schapaugh, W. T and Campbell, L. R. 2007. Chlorophyll loss caused by soybean aphid (Hemiptera: Aphididae) feeding on Soybean. *J. Econ. Entomol.* 100: 1657–1662.
- Dixon, A. F. G. 2000. *Aphid Ecology: An Optimization Approach*, 2nd edition. Springer Science & Business Media, New York, USA.
- Dixon, A. F. G. 1972. Crowding and nutrition in the induction of macropterous alatae in *Drepanosiphum dixonii*. *Journal of Insect Physiology.* 18: 459–464.
- Dixon, A. F. G., and Glen, D.M. 1971. Morph determination in the bird cherry-oat aphid, *Rhopalosiphum padi* L. *Annals of Applied Biology.* 68: 11–21.
- Dumont, T., Marodon, J., Nibouche, S., Hoarau, J. Y., and Barau, L. 2023. Genetic variability of sugarcane varieties for resistance to *Sipha flava*. In *Proceedings of the International Society of Sugar Cane Technologists.* 31: 628-636.
- El-Rawy, A., Khaled, M. A., and Osman, S. M. 2007. Impact of NPK fertilizers, wheat varieties and their interactions on the infestation with cereal aphids and leafminer, yield and its components. *Journal of Plant Protection and Pathology.* 32(4): 3113-3122.
- Esterhuzein, D. 2012. The report focuses on the production and usage of sugar in Zimbabwe. USDA Foreign Agricultural Service, United States of America.
- Flynn, D. F. B., Sudderth, E. A., and Bazzaz, F. A. 2006. Effects of aphid herbivory on biomass and leaf-level physiology of *Solanum dulcamara* under elevated temperature and CO₂. *Environ. Expt. Bot.* 56: 10-18.
- Foyer, C. H., and Noctor, G. 2005. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell.* 17:1866-1875. doi:10.1105/tpc.105.033589.

- Franzen, L. D., Gutsche, A. R. T., Heng-Moss, M., Higley, L. G., Sarath, G., and Burd, J. D. 2007. Physiological and biochemical responses of resistant and susceptible wheat to injury by Russian wheat aphid. *Journal of Economic Entomology*. 100: 1692–1703.
- Gallun, R. L., Ruppel, R., and Everson, E. H. 1966. Resistance of small grains to the cereal leaf beetle. *J. Econ. Entomol.* 59: 827-829.
- Gechev, T. S., Van Breusegem, F., Stone, J. M., Denev, I., and Laloi, C. 2006. Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. *Bioessays*. 28:1091-1101.
- Gelhaye, E., Rouhier, N., Navrot, N., and Jacquot, J. P. 2005. The plant thioredoxin system. *Cell Mol Life Sci.* 62:24-35. doi:10.1007/s00018-004- 4296-4.
- Girma, M., Kofoed, K. D., and Reese, J. C. 1998. Sorghum germplasm tolerant to greenbug (Homoptera: Aphididae) feeding damage as measured by reduced chlorophyll loss. *J. Kans. Entomol. Soc.* 71: 108–115.
- Giron, D., Dubreuil, G., Bennett, A., Dedeine, F., Dicke, M., Dyer, L. A., et al., 2018. Promises and challenges in insect-plant interactions. *Entomol. Exp. Appl.* 166: 319–343. doi: 10.1111/eea.12679
- Goławska, S., Krzyżanowski, R., and Łukasik, I. 2010. Relationship between aphid infestation and chlorophyll content in Fabaceae species. *Acta Biologica Cracoviensia Series Botanica.* 52: 76-80.
- Gomez, S. K., Oosterhuis, D. M., Hendrix, D. L., Johnson, D. R., and Steinkraus, D. C. 2006. Diurnal pattern of aphid feeding and its effect on cotton leaf physiology. *Environ. Exper. Bot.* 55: 77–86.
- Gonzales, W. L., Ramirez, C. C., Olea, N., and Niemeyer, H. M. 2002. Host plant changes produced by the aphid *Sipha flava*: consequences for aphid feeding behaviour and growth. *Entomologia Experimentalis et Applicata.* 103: 107-113.
- Gordy, J. W., Brewer, M. J., Bowling, R. D., Buntin, G. D., Seiter, N. J., Kerns, D. L., Reay-Jones, F. P. F., and Way. M. O. 2019. Development of economic thresholds for sugarcane aphid

(Hemiptera: Aphididae) in susceptible grain sorghum hybrids. *J. Econ. Entomol.* toz028, <https://doi.org/10.1093/jee/toz028>

Grant, J. J., and Loake, G. J. Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. *Plant Physiol.* 124:21-30. doi:10.1104/pp.124.1.21.

Green, B. R., and D. G. 1996. The Chlorophyll-Carotenoid Proteins of Oxygenic Photosynthesis. *Annual Reviews of Plant Physiology and Plant Molecular Biology.* 47(1):685-714. *Annual Reviews*, <https://doi.org/10.1146/annurev.arplant.47.1.685>.

Guo, H., Peng, X., Gu, L., Wu, J., Ge, F., and Sun, Y. 2017. Up-regulation of MPK4 increases the feeding efficiency of the green peach aphid under elevated CO₂ in *Nicotiana attenuata*. *Journal of Experimental Botany.* 68(21-22): 5923-5935.

Guo, H., Sun, Y., Peng, X., Wang, Q., Harris, M., and Ge, F. 2016. Up-regulation of abscisic acid signaling pathway facilitates aphid xylem absorption and osmoregulation under drought stress. *Journal of experimental botany.* 67(3): 681-693.

Gutsche, A. R., Heng-Moss, T. M., Higley, L. G., Sarath, G., and Mornhinweg, D. W. 2009. Physiological responses of resistant and susceptible barley, *Hordeum vulgare* to the Russian wheat aphid, *Diuraphis noxia* (Mordvilko). *Arthropod-Plant Interactions.* 3: 233-240.

Haile, F. J., and Higley, L. G. 2003. Changes in soybean gas-exchange after moisture stress and spider mite injury. *Environ. Entomol.* 32: 433-440. doi: 10.1603/0046-225X-32.3.433

Haile, F. J., and Higley, L. G. 2003. Changes in soybean gas-exchange after moisture stress and spider mite injury. *Environ Entomol.* 32: 433-440.

Haile, F. J., Higley, L. G., Ni, X., and Quisenberry, S. S. 1999. Physiological and growth tolerance in wheat to Russian wheat aphid (Homoptera: Aphididae) injury. *Environ. Entomol.* 28: 787-794.

Halbert, S. E., Miller, G. L., and AMES, L. M. 2013. *The genus Sipha Passerini (Hemiptera: Aphididae) in North America.*- Ins. Mundi, Paper 831.

- Hall, D. G. 2001. Notes on the yellow sugarcane aphid *Sipha flava* (Homoptera: Aphididea) and the lady beetle *Diomus terminates* (Coleoptera: Coccinellidae) in Florida. *Journal of the American Society of Sugar Cane Technologists*. 21: 21-29.
- Harrington, R., Clark, S. J., Welham, S. J., Verrier, P. J., Denholm, C. H., Hulle, M., Maurice, D., Rounsevell, M. D., and Cocu, N. 2007. European Union Examine Consortium. Environmental change and the phenology of European aphids. *Global Change Biology*. 13: 1550-1564.
- Harrison, W. W., King, E. G., and Ouzts, J. D. 1985. Development of *Trichogramma exiguum* and *T. pretiosum* at five temperature regimes. *Environmental Entomology*. 14: 118–121.
- Hassall, C., Thompson, D. J., French, G. C., and Harvey, I. F. 2007. Historical changes in the phenology of British Odonata are related to climate. *Global Change Biology*. 13: 933-941.
- Heng-moss, T. M., Ni, X., Macedo, T., Markwell, J. P., Baxendale, F. P., Quisenberry, S. S., and Tolmay, V. 2003. Comparison of chlorophyll and carotenoid concentrations among Russian wheat aphid (Homoptera: Aphididae)-infested wheat isolines. *Plant Resistance*. 96: 475–481.
- Hentz, M., and Nuessly, G. 2004. Development, longevity, and fecundity of *Sipha flava* (Homoptera: Aphididae) feeding on *Sorghum bicolor*. *Environmental entomology*. 33(3): 546-553.
- Hinson, P. O. 2017. Effect of temperature on the development of sugarcane aphid, *Melanaphis sacchari*, on sorghum. A Thesis Submitted in Partial Fulfilment of the Requirement for the Degree Masters of Science in West Texas A & M University Canyon, Texas, USA.
- Hoad, S. P., Marzoli A., Grace A., and Jeffree C. E. 1998. Response of leaf surfaces and gas exchange to wind stress and acid mist in birch (*Betula pubescens*). *Trees – Structure and Function*. 13: 1–12.
- Hunter, M. D. 2001. Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Agricultural and Forest Entomology*. 3: 153–159.
- Janave, M. T. 1997. Enzymic degradation of chlorophyll in Cavendish bananas: in vitro evidence for two independent degradative pathways. 837-846

- Jakobson, L., Vaahtera, L., Töldsepp, K., et al., 2016. Natural variation in *Arabidopsis* Cvi-0 accession reveals an important role of MPK12 in guard cell CO₂ signaling. *PLoS Biology*.14, e2000322.
- Jensen, J. 2000. Remote Sensing of the Environment: An Earth Resource Perspective. Low Price Edition, Pearson Education, 2000.
- Keena, M. A. 2006. Effects of temperature on *Anoplophora glabripennis* (Coleoptera: Cerambycidae) adult survival, reproduction, and egg hatch. *Environmental Entomology*. 35: 912–921.
- Koch, K. G., Chapman, K., Louis, J., Heng-Moss, T., and Sarath, G. 2016. Plant tolerance: a unique approach to control hemipteran pests. *Frontiers in Plant Science*. 7: 1363.
- Kuo, M. H., Chiu, M. C., and Perng, J. J. 2006. Temperature effects on life history traits of the corn leaf aphid, *Rhopalosiphum madis* (Homoptera: Aphididae) on corn in Taiwan. *Appl. Entomol. Zool*. 41: 171-177.
- Lage, J., Skovmand, B., and Andersen, S. B. 2003. Characterization of greenbug (Homoptera: Aphididae) resistance in synthetic hexaploid wheats. *J. Econ. Entomol*. 96(6): 1922–1928.
- Langer, A., Boivin, G., and Hance, T. 2004. Oviposition, flight and walking capacity at low temperatures of four aphid parasitoid species (Hymenoptera: Aphidiinae). *European Journal of Entomology*. 101: 473–480.
- Larson, K. C. 1998. The impact of two gall-forming arthropods on the photosynthetic rates on their hosts. *Oecologia*. 115: 161–166.
- Leather, S. R., and Dixon, A. F. G. 1982. Secondary host preferences and reproductive activity of the bird cherry oat aphid, *Rhopalosiphum padi*. *Annals of Applied Biology*. 101: 219–228.
- Lees, A. D. 1963. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid *Megoura viciae* Buckton – III. Further properties of the maternal switching mechanism in apterous aphids. *Journal of Insect Physiology*. 9: 153–164.

- Lei, J., and Zhu-Salzman, K. 2015. Enhanced aphid detoxification when confronted by a host with elevated ROS production. *Plant Signal Behav.* 10: e1010936.
- Limaje, A., Armstrong, S., Hoback, W., Zarrabi, A., Chad, H., Paudyal, S., and Burke, J. 2017. Antibiosis and Tolerance Discovered in USDA-ARS Sorghums Resistant to the Sugarcane Aphid *Melanaphis sacchari* (Hemiptera: Aphididae). *J. Entomol. Sci.* 53(2): 230–241.
- Lin, T. B., Schwartz, A., and Saranga, Y. 1999. Non stomatal factors limit cotton photosynthesis under Silverleaf whitefly stress. *Physiol. Plant.* 107: 303-311.
- Liu, S. S. 1994. Production of alatae in response to low temperature in aphids: a trait of seasonal adaptation, pp. 245–261 *In* Danks HV [ed.], *Insect Life-cycle Polymorphism*. Kluwer, Dordrecht, Netherlands.
- Macedo, T. B. 2003. Physiological responses of plants to piercing-sucking arthropods. The University of Nebraska-Lincoln.
- Macedo, T. B., Bastos, C. S., Highley, L. G., Ostlie, K. R., and Madhavan. S. 2003a. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. *J. Econ. Entomol.* 96: 188–193.
- Macedo, T. B., Highley, L. G., Ni, X., and Quisenberry. S. S. 2003b. Light activation of Russian wheat-elicited physiological responses in susceptible wheat. *J. Econ. Entomol.* 96(1): 194–201.
- Macedo, T. B., Peterson, R. K. D., Weaver, D. K., and Xinzhi, N. I. 2009. Impact of *Diuraphis noxia* and *Rhopalosiphum padi* (Hemiptera: Aphididae) on primary physiology of four near isogenic Wheat lines. *J. Econ. Ent.* 102: 412-421.
- Madiope, K. W., Keeping, M. G., and Fourie, D. V. 2021. Quantifying yield loss to yellow sugarcane aphid in potted sugarcane. Short non-refereed paper. Conference Paper in South African Journal of Education, August 2021.
- Mahmoud, W. A. 2005. Ecological studies on cereal aphids and their control in Sohag Governorate. Ph.D. Thesis, Fac. of Agric., Assiut Univ., 122 pp.
- Mbulwe, L. 2017. Evaluating Host-Plant Resistance against Sugarcane Aphid (*Melanaphis sacchari* (Zehntner)) in Sorghum (*Sorghum bicolor* (L.) Moench) (Doctoral dissertation).

- Mbulwe, L., Peterson, G. C., Armstrong, J. S., and Rooney, W. L. 2016. Registration of sorghum germplasm Tx3408 and Tx3409 with tolerance to sugarcane aphid [*Melanaphis sacchari* (Zehntner)]. *J. Plant Regist.* 10: 51–56.
- McLean, D. L., and Kinsey, M. G. 1964. A technique for electronically recording aphid feeding and salivation. *Nature.* 202: 1358–1359. doi: 10.1038/2021358a0
- Ménard, G., Biais, B., Prodhomme, D., Ballias, P., Petit, J., Just, D., et al., 2013. High-throughput biochemical phenotyping for plants. *Adv. Bot. Res.* 67: 407–439. doi: 10.1016/b978-0-12-397922-3.00009-5
- Menendez, R. 2007. How are insects responding to global-warming. *Tijdschrift voor Entomologie.* 150: 355-365.
- Meyer, G. A., and Whitlow, T. H. 1992. Effects of leaf and sap feeding insects on photosynthetic rate of goldenrod. *Oecologia.* 92: 480–489.
- Miller, H., Porter, D. R., Burd, J. D., Mornhinweg, D. W and Burton. R. L. 1994. Physiological effects of Russian wheat aphid (Homoptera: Aphididae) on resistant and susceptible barley. *J. Econ. Entomol.* 87: 493–499.
- Miskimen, G. W. 1970. Population dynamics of the yellow sugarcane aphid, *Sipha flava*, in Puerto Rico, as affected by heavy rains. *Annals of the Entomological Society of America.* 63: 642–645.
- Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7:405-410. doi:10.1016/S1360-1385(02) 02312-9.
- Mittler, R., Vanderauwera, S., Gollery, M., and Van Breusegem, F. 2004. Reactive oxygen gene network of plants. *Trends Plant Sci.* 9:490-498. doi:10.1016/j.tplants.2004.08.009.
- Moloi, M., van der Westhuizen, A. J., and van der Westhuizen, A. 2006. The reactive oxygen species are involved in resistance responses of wheat to the Russian wheat aphid. *J. Plant. Physiol.* 163:1118-1125. doi:10.1016/j.jplph.2005.07.014.

- Müller, C. B., Williams, I. S., and Hardie, J. 2001. The role of nutrition, crowding and interspecific interactions in the development of winged aphids. *Ecological Entomology*. 26: 330–340.
- Naessens, E., Dubreuil, G., Giordanengo, P., Baron, O. L., and Minet-Kebdani, N. 2015. A secreted MIF cytokine enables aphid feeding and represses plant immune responses. *Curr. Biol* 25: 1898–1903. doi: 10.1016/j.cub.2015.05.047
- Nagaraj, N., Reese, J. C., Kirkham, M. B., Kofoid, K., Campbell, L. R., and Loughin. T. M. 2002. Relationship between chlorophyll loss and photosynthetic rate in greenbug (Homoptera: Aphididae) damaged sorghum. *Journal of Kansas Entomological Society*. 75:101–109.
- Neven, L. G. 1998. Respiratory response of fifth instar codling moth to rapidly changing temperatures. *Journal of Economic Entomolog.* 91: 302–308.
- Neven, L. G. 2000. Physiological responses of insects to heat. *Postharvest Biology and Technology*. 31: 103–111.
- Ni, X., Quisenberry, S. S., Heng-moss, T., Markwell, J., Higley, L., Baxendale, F., Sarath, G., and Klucas, R. 2002. Dynamic change in photosynthetic pigments and chlorophyll degradation elicited by cereal aphid feeding. *Entomologia Experimentalis et Applicata*. 105: 43-53.
- Nicholson, S. J., Hartson, S. D., and Puterka, G. J. 2012. Proteomic analysis of secreted saliva from Russian Wheat Aphid (*Diuraphis noxia* Kurd.) biotypes that differ in virulence to wheat. *Journal of Proteomics*. 75: 2252-2268.
- Noctor, G., and Foyer, C. H. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annu Rev Plant Biol*. 49:249-279. doi:10.1146/annurev.arplant.49.1.249.
- Nuessly, G. S. 2005. Featured Creatures: Yellow Sugarcane Aphid; publication #EENY-354; University of Florida: Gainesville, FL, USA; Available online: <http://entnemdept.ufl.edu/creatures/field/bugs/yellowsugarcaneaphid.htm> (accessed on 16 January 2022).
- Nuessly, G. S., and Hentz, M. G. 2002a. Feeding effects of yellow sugarcane aphid on sugarcane. *Journal of the American Society of Sugar Cane Technology*. 22: 126-127.

- Nuessly, G. S., Hentz, M. G., and Gilbert, R. A. 2010. Susceptibility of stage IV canal point (CP) sugarcane clones to yellow sugarcane aphid (*Sipha flava* (Forbes)) feeding damage. *J Am Soc Sug Cane Technol.* 30: 104-114.
- Oliveira, S. A., Auad, A. M., Souza, B., Souza, L. S., Amaral, R. L., and Silva, D. M. 2009a. Tabela de esperança de vida e de fertilidade de *Sipha flava* (Forbes) (Hemiptera, Aphididae) alimentado com capim-elefante em diferentes temperaturas. *Rev. Brasileira Entomol.* 53: 614-619.
- Oliveira, S. A., Souza, B., Auad, A. M., Silva, D. M., Souza, L. S., and Carvalho, C. A. 2009b. Desenvolvimento e reprodução de *Sipha flava* (Forbes) (Hemiptera: Aphididae) em diferentes temperaturas. *Neotrop. Entomol.* 38: 311-316.
- Özder, N., and Sağlam, Ö. 2013. The effects of temperature for development time, fecundity and reproduction on some ornamental aphid species. *J Cent Eur Agric.* 14: 149-157.
- Painter, R. H. 1951. Insect resistance in crop plants. The McMillan Co. New York, pp. 520.
- Paudyal, S. 2019. Host plant resistance to the sugarcane aphid (Hemiptera: Aphididae). A Dissertation Submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfillment of the requirements for the Degree of Doctor of philosophy.
- Paudyal, S., Armstrong, J. S., Giles, K. L., Payton, M. E., Opit, G. P., Limaje, A. 2019. Categories of resistance to sugarcane aphid (Hemiptera: Aphididae) among sorghum genotypes. *Journal of Economic Entomology.* 112(4): 1932-1940.
- Paudyal, S., Armstrong, J. S., Giles, K. L., Hoback, W., Aiken, R., and Payton, M. E. 2020. Differential responses of sorghum genotypes to sugarcane aphid feeding. *Planta.* 252: 1-9.
- Pei, Z. M., Murata, Y., Benning, G., Thomine, S., Klusener, B., Allen, G. J., Grill, E., and Schroeder, J. I. 2000. Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature.* 406:731-734. doi:10.1038/35021067.
- Pekker, I., Tel-Or, E., and Mittler, R. 2002. Reactive oxygen intermediates and glutathione regulate the expression of cytosolic ascorbate peroxidase during iron-mediated oxidative stress in bean. *Plant Mol Biol.* 49:429-438. doi:10.1023/A:1015554616358.

- Peterson, R. K. D. 2000. "Photosynthesis, yield loss, and injury guilds," in *Biotic Stress and Yield Loss*, eds R.K.D. Peterson and L. G. Higley (Boca Raton: CRC Press). doi: 10.1201/9781420040753
- Peterson, R. K. D. 2001. Photosynthesis, yield loss, and injury guilds. In *Biotic Stress and Yield Loss*, pp. 83–98. Eds R.K.D. Peterson and L.G. Higley. Boca Raton, FL: CRC Press.
- Peterson, R. K. D., and Higley, L. G. 1993. Arthropod injury and plant gas exchange: current understandings and approaches for synthesis. *Trends in Agricultural Sciences: Entomology*. 1: 93–100.
- Peterson, R. K. D., Shannon, C. L., and Lensen, A. W. 2004. Photosynthetic responses of Legume species to leaf-mass consumption injury. *Environmental Entomology*. 33: 450–456.
- Peterson, R. K. D., and L. G. Higley. 2001. Illuminating the black box: the relationship between injury and yield, pp. 1–12. In R.K.D. Peterson and L. G. Higley [eds], *Biotic stress and yield loss*. CRC, Boca Raton, FL.
- Peterson, R. K., Varella, A. C., Higley, L. G. 2017. Tolerance: the forgotten child of plant resistance. *PeerJ*. 5: e3934.
- Pierson, L. M., Heng-Moss, T. M., Hunt, T. E., and Reese, J. 2011. Physiological responses of resistant and susceptible reproductive stage soybean to soybean aphid (*Aphis glycines* Matsumura) feeding. *Arthropod-Plant Interaction*. 5: 49–58.
- Pierson, L. M., Heng-Moss, T. M., Hunt, T. E., and Reese, J. 2010a. Physiological responses of resistant and susceptible reproductive stage soybean to soybean aphid (*Aphis glycines* Matsumura) feeding. *Arthropod-Plant Interactions*. 5: 49–58.
- Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology and Evolution*. 20: 481–486.
- Pincebourde, S., and Ngao, J. 2021. The Impact of Phloem Feeding Insects on Leaf Ecophysiology Varies with Leaf Age. *Front. Plant Sci.* 12:625689. doi: 10.3389/fpls.2021.625689

Pincebourde, S., Frak, E., Sinoquet, H., Regnard, J. L., and Casas, J. 2006. Herbivory mitigation through increased water-use efficiency in a leaf-mining moth-apple tree relationship. *Plant Cell Environ.* 29: 2238–2247. doi: 10.1111/j.1365-3040.2006.01598.x

Prochaska, T. J. 2015. *Biochemical, physiological, and anatomical insights into aphid-bioenergy switchgrass interactions*. The University of Nebraska-Lincoln.

Rao, S. A., Carolan, J. C., and Wilkinson, T. L. 2013. Proteomic profiling of cereal aphid saliva reveals both ubiquitous and adaptive secreted proteins. *PLoS One.* 8: e57413. DOI: 10.1371/journal.pone.0057413.

Reagan, T. E. 1994. Pest Status, Biology and control measures of sugar cane sap suckers in the North American sugar growing regions. pp 123-124 In: AJM Carnegie and DE Conlong (Eds), Biology, Pest Status and Control Measure Relationships of Sugar Cane Insect Pests. *Proceedings of Second Sugar Cane Entomology Workshop*. Mount Edgecombe, KwaZulu-Natal, South Africa, held 30 May to 3 June 1994.

Retuerto, R., Fernandez-lema, B. Rodriguez-Roiloa., and Obeso. J. R. 2004. Increased photosynthetic performance in holly trees infested by scale insects. *Functional Ecology.* 18: 664-669. DOI: 10.1111/j.0269-8463.2004. 00889.x

Roberts, J. J., and Foster, J. E. 1983. Effect of leaf pubescence in wheat on the bird cherry oat aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 76: 1320-1322.

Roberts, J. J., Gallun, R. L., Patterson, F. L., and Foster. J. E. 1979. Effects of wheat leaf pubescence on the Hessian fly. *J. Econ. Entomol.* 72: 211-214.

Rosenzweig, C., Iglesias, A., Yang, X. B., Epstein, P. R., and Chivian, E. 2001. Climate change and extreme weather events; Implications for food production, plant diseases and pests. *Global Change and Human Health.* 2: 90-104.

Ryan, J. D., Johnson, R. C., Eikenbary, R. D., and Dorschner, K. W. 1987. Drought/greenbug interactions: photosynthesis of greenbug resistant and susceptible wheat. *Crop Sci.* 27(2): 283–288.

- Schimmel, B. C. J., Ataide, L. M. S., Chafi, R., Villarroel, C. A., and Alba, J. M. 2017. Overcompensation of herbivore reproduction through hypersuppression of plant defenses in response to competition. *New Phytol.* 214: 1688–1701. doi: 10.1111/nph.14543
- Schlemmer, M. 2018. Effect of temperature on development and reproduction of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Dissertation submitted in fulfilment of the requirements for the Masters degree in Environmental Science at the North- West University.
- Shabani, A. A., Venkataramana, P., and Mbega, E. R. 2020. Infestation Status and Farmers' Knowledge on Yellow Sugarcane Aphid (*Sipha flava*) in Tanzania. *International Journal of Biosciences.* 17 (6): 227-240.
- Shankar, P., and Yinghua, H. 2021. Elevated production of reactive oxygen species is related to host plant resistance to sugarcane aphid in sorghum. *Plant Signaling and Behavior.* 16:2: 1849523. doi: 10.1080/15592324.2020.1849523
- Shannag, H. K. 2007. Effect of black bean aphid, *Aphis fabae*, on transpiration, stomatal conductance and crude protein content of faba bean. *Ann Appl Biol.* 151: 183–188.
- Sharpe, P. J. H., and De Michele, D. W. 1977. Reaction kinetics of poikilotherm development. *Journal of Theoretical Biology.* 64: 649-670.
- Shoala, T., Edwards, M. G., Knight, M. R., and Gatehouse, A. M. R. 2018. OXII kinase plays a key role in resistance of arabidopsis towards aphids (*Myzus persicae*). *Transgenic Res.* 27:355-366. doi:10.1007/s11248-018-0078-x.
- Sibly, R. M., Barker, D., Hone, J., and Pagel, M. 2007. On the stability of populations of mammals, birds, fish and insects. *Ecology Letters.* 10: 970–976.
- Singh, B. U., Padmaja, P., and Seetharma. N. 2004. Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehnter) (Homoptera: Aphididae), in sorghum: a review. *Crop Prot.* 23 (9): 739–755.
- Slman, F. A. A. 1997. Studies on certain factors affecting the distribution of cereal aphids in wheat fields in Upper Egypt. Ph.D. Thesis, Fac. of Agric., El-Minia Univ., 178 pp.

Sosa, O. Jr. 1990. Pubescence in sugarcane as an obstacle to yellow sugarcane aphid establishment. *Sugar Y Azuca*, 85, 30.

Souza, M. A. 2018. Effects of temperature on sugarcane aphid, *Melanaphis sacchari*, life history on three different host plants. Thesis submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfilment of the requirements for the Degree of Master of science December, 2018.

Souza, M. A., Armstrong, J. S, Hoback, W. W., Mulder, P. G., Paudyal, S., et al., 2019. Temperature Dependent Development of Sugarcane Aphids *Melanaphis Sacchari*, (Hemiptera: Aphididae) on Three Different Host Plants with Estimates of the Lower and Upper Threshold for Fecundity. *Curr Trends Entomol Zool Stds* 2: 1011. DOI:10.29011/CTEZS-1011.001011

Sun, Y., Guo, H., Yuan, L., Wei, J., Zhang, W., and Ge, F. 2015. Plant stomatal closure improves aphid feeding under elevated CO₂. *Global Change Biology*. 21(7): 2739-2748.

Suzuki, N., Koussevitzky, S., Mittler, R., and Miller, G. 2012. ROS and redox signalling in the response of plants to abiotic stress. *Plant Cell Environ*. 35:259-270. doi:10.1111/j.1365-3040.2011.02336.x.

Sytykiewicz, H. 2007. Biochemiczne i anatomiczne aspekty żerowania mszycy czeremchowo-zbożowej (*Rhopalosiphum padi* L.) na żywicielu pierwotnym. PhD dissertation, Akademia Podlaska, Wydział Rolniczy, Siedlce.

Sytykiewicz, H., Sprawka, I., Czerniewicz, P., Sempruch, C., Leszczyński, B and Sikora, M. 2013. Biochemical characterization of chlorophyllase from leaves of selected Prunus species - A comparative study. *Acta Biochimica Polonica*. 60: 457-465.

Syvertsen, J. P., Goñi, C., and Otero, A. 2003. Fruit load and canopy shading affect leaf characteristics and net gas exchange of ‘Spring’ navel orange trees. *Tree Physiol*. 23: 899–906. doi: 10.1093/treephys/23.13.899

Tang, J. Y., Zielinski, R. E., Zangerl, A. R., Crofts, A. R., Berenbaum, M. R., and DeLucia, E. H. 2006. The differential effects of herbivory by first and fourth instars of *Trichoplusia ni* (Lepidoptera: Noctuidae) on photosynthesis in *Arabidopsis thaliana*. *J. Exp. Bot*. 57: 527–536. doi: 10.1093/jxb/erj032

- Tjallingii, W. F. 1988. "Electrical recording of stylet penetration activities," in *Aphids, Their Biology, Natural Enemies and Control*, eds A. K. Minks and P. Harrewijn (Amsterdam: Elsevier). 95–108.
- Trumble, J. T., Kolodny-Hirsch, D. M., and Ting, I. P. 1993. Plant compensation for arthropod herbivory. *Annu. Rev. Entomo*, 38: 93–119. doi: 10.1146/annurev.en.38.010193.000521
- Urban, L., Lechaudel, M., and Lu, P. 2004. Effect of fruit load and girdling on leaf photosynthesis in *Mangifera indica* L. *J. Exp. Bot.* 55, 2075–2085. doi:10.1093/jxb/erh220
- Velikova, V., Salerno, G., Frati, F., Peri, E., Conti, E., Colazza, S., et al., 2010. Influence of feeding and oviposition by phytophagous pentatomids on photosynthesis of herbaceous plants. *J. Chem. Ecol.* 36: 629–641. doi: 10.1007/s10886-010-9801-7
- Waghray, R. W., and S. R. 1965. Effect of N, P and K on the fecundity of *Aphis craccivora* for groundnut plants. *Indian J. Entomol.* 27: 331-334.
- Walker, G. P. 2000. "A beginner's guide to electronic monitoring of homopteran probing behavior," in *Principles and Applications of Electronic Monitoring and Other Techniques in the Study of Homopteran Feeding Behavior*, eds G. P. Walker and E. A. Backus (Lanham, MD: Thomas Say Publications in Entomology, *Entomological Society of America*. 14–40.
- Warrington, S., Cottam, D. A., and Whittaker, J. B. 1989. Effects of insect damage on photosynthesis, transpiration and SO₂ uptake by sycamore. *Oecologia*. 80: 136–139.
- Watanabe, T., and Kitagawa, H. 2000. Photosynthesis and translocation of assimilates in rice plants following phloem feeding by the plant hopper *Nilaparvata lugens* (Homoptera: Delphacidae). *J. Econ. Ent.* 93: 1192- 1197.
- Way, M. J., Conlong D. E., Martin, L. A., McFarlane, S. A., Stranack, R., Keeping M. G., and Rutherford, R. S. 2015. First record of yellow sugarcane aphid, *Sipha flava* (Homoptera: Aphididae), in the South African sugarcane industry. *Proceeding of the South African Sugar Technology Association*. 87: 53–57.
- Welter, S. C. 1989. "Arthropod impact on plant gas exchange," in *Insect-Plant Interactions*, ed. E. A. Bernays (Boca Raton, FL, USA: CRC Press). 1: 135–150. doi: 10.1201/9780429290916-5

- White, W. H. 1990. Yellow Sugarcane Aphid (Homoptera: Aphididae) resistance mechanisms in selected sugarcane cultivars. *J Econ Ent.* 83(5): 2111-2114.
- Wilson, A. C., Sternberg, L. D. S and Hurley, K. B. 2011. Aphids alter host-plant nitrogen isotope fractionation. *Proceedings of the National Academy of Sciences.* 108(25): 10220-10224.
- Wilson, B. E. 2019. Hemipteran Pests of Sugarcane in North America. *Insects.* 10 (107): 1-15. doi:10.3390/insects10040107
- Wood, C. W., Tracy, P. W. Reeves, D. W., and Edmisten, K. L 1992. Determination of cotton nitrogen status with a handheld chlorophyll meter, *J. Plant Nutr.* 15(9): 1435–1448, DOI: 10.1080/01904169209364409
- Wu, G., Shortt, B., Lawrence, E., Leon, J., Fitzsimmons, K., Levine, E., Raskin, I., and Shah, D. M. 1997. Activation of host defense mechanisms by elevated production of H₂O₂ in transgenic plants. *Plant Physiol.* 115:427-435. doi:10.1104/pp.115.2.427.
- Zangerl, A. R., Hamilton, J. G., Miller, T. J., Crofts, A. R., Oxborough, K., Berenbaum, M. R., et al., 2002. Impact of folivory on photosynthesis is greater than the sum of its holes. *Proc. Nat. Acad. Sci U.S.A.* 99: 1088–1091. doi: 10.1073/pnas.022647099
- Zhang, Y., Fan, W., Kinkema, M., Li, X., and Dong, X. 1999. Interaction of NPR1 with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the PR-1 gene. *Proc Natl Acad Sci USA.* 96:6523-6528. doi:10.1073/pnas.96.11.6523.
- Zheng, X., Huang, Q., Cao, W., Lu, W., Wang, G., Yu, S., Yang, Z., and Wang, X. 2015. Modelling climate change impacts on overwintering of *Spodoptera exigua* Hubner in regions of China. *Chilean Journal of Agricultural Research.* 75(3): 328-333.

CHAPTER SIX

Phytochemical profiling of metabolites in commercial sugarcane (*S. officinarum*) varieties that confer resistance to *S. flava* feeding by using Gas Chromatography Mass Spectrometry (GC-MS) Metabolomics Approach

Abstract

Secondary metabolites serve a variety of ecological purposes, including defense against biotic and abiotic stressors. The aim of this study was to quantify the total phenol and flavonoid contents in sugarcane leaves that mediate resistance to yellow sugarcane aphids (YSA) (*Sipha flava*). A 7×2 factorial experiment was conducted in a complete randomized block design (CRBD). Seven sugarcane varieties namely 00-1165, 96-1107, ZN 8, ZN 9, ZN 10, ZN 3L, and N14 under two aphid treatments [uninfested (control) and infested] were used. 00-1165 showed medium resistance, as shown by its aphid quantity ratio (AQR), which fell between 0.30-0.60. Moreover, ZN 10 is regarded a highly sensitive variety because its AQR was more than 1.50. Highly significant ($p < 0.001$) differences were recorded in both uninfested and infested treatments on total phenol and flavonoid content. In the YSA infested plots, 96-1107 recorded the highest phenol content of 50.31 $\mu\text{g/g}$, while ZN 3L had the lowest (25.92 $\mu\text{g/g}$). Furthermore, N14 recorded the highest flavonoid content of 6.47 $\mu\text{g/g}$, whereas ZN 3L produced the lowest (1.60 $\mu\text{g/g}$) in YSA infested plots. Notably, there was a significant positive correlation ($p = 0.002$, $r = 0.58$) between the percentage change in phenol concentration and the number of aphids. Moreover, the regression results showed a significant positive correlation ($p < 0.001$, $r = 0.70$) between the percentage change in flavonoid concentration and the number of aphids. Feeding by YSA increased phenol and flavonoid induced resistance. There is a need to incorporate sugarcane varieties with high phenol and flavonoid content into the existing YSA management strategies.

Keywords: sugarcane, resistance, flavonoids, phenols, *Sipha flava*

6.1 Introduction

Sugarcane (*Saccharum officinarum* L.) has become a subject of damage due to the persistent infestation of Yellow Sugarcane Aphids (YSA) (*Sipha flava*) (Sakadzo *et al.*, 2024). In November 2006, the pest was first reported in Morocco in North Africa (Adbelmajid, 2008). In May 2013, the insect pest made its way to southern Africa, specifically South Africa (Conlong and Way, 2014). It then proceeded to other sugarcane-producing regions, including Zimbabwe, Swaziland, Malawi, and Zambia (Way *et al.*, 2014; Conlong and Way, 2014); Kenya in 2016 (Mutonyi and Babikha, 2019); and Tanzania in 2019 (January *et al.*, 2020). It is currently regarded as a significant sugarcane pest, along with the well-known Black Maize Beetle and Sugarcane Stalk Borer (*Eldana saccharina*) (ZSAES, unpublished).

YSA has emerged as a polyphagous insect pest, and its host range includes plants in the genera *Digitaria*, *Panicum*, *Paspalum*, and *Pennisetum*; cultivated cereal crops such as rice (*Oryza sativa*), wheat (*Triticum aestivum*), and sorghum (*Sorghum bicolor*); and non-crop members of the same genus (Blackman and Eastop, 1984). The host range of Zimbabwe consists of guinea grass, maize, sorghum, and sugarcane (ZSAES, unpublished). Further investigation is necessary, as there is a chance that the pest's host range will expand as it becomes more entrenched.

Cultural, biological, and chemical techniques are available as control strategies for YSA. The main approach in Zimbabwe's sugar business is the use of synthetic chemicals. As of right now, Allice (Acetamiprid) and Actara have been approved for use. It is essential to create an environmentally benign strategy, such as the adoption of host plant resistance (HPRs), given the economic significance of YSA and the environmental concerns associated with employing chemicals to manage aphids. The only management strategy that has been tried thus far to control the YSA has not worked independently. To control YSA, it is crucial to incorporate the development of sugarcane varieties with high levels of secondary metabolites (phytochemicals) as a sustainable option to curb the rapid increase in YSA populations in the sugar industry.

Phytochemicals are secondary metabolites found in plant species and are chemical constituents of plants (Mercy *et al.*, 2017). Although these substances do not hinder plant growth, they make tissues less appetizing to herbivorous insects (Howe and Jander, 2008; Ahman *et al.*, 2019). According to Risebrow and Dixon (1987) and Pickett *et al.* (1992), aphid behavior and performance are affected by primary nutrients, as well as secondary chemicals. The effects of

allelochemicals on the function of other organisms can be either beneficial or detrimental (Rice, 1984; Pejman *et al.*, 2011; Thi *et al.*, 2015; Rawat *et al.*, 2017). This is because of their many benefits such as biodegradation, affordability, safety for the environment, and ease of use, these organic molecules are a good replacement for synthetic chemicals (Farooq *et al.*, 2011a, Sharma and Satsangi, 2013). These substances may be utilized instead of traditional insecticides if they are hazardous to insects (Akbar *et al.*, 2009). Synthetic insecticides are used frequently, such that insects have become resistant to them (Mulungu *et al.*, 2007; Khater *et al.*, 2012; Belmain *et al.*, 2013; Parwada *et al.*, 2018) hence, there is a need for natural substances that have been found to be effective against insect pests (Farooq *et al.*, 2011; Ajayi *et al.*, 2018). The concept of allelopathy has been successfully used to manage insect pests, according to research that have been published (Hongo and Karel, 1986; Saljoqi *et al.*, 2006; Farooq *et al.*, 2011; Zia *et al.*, 2011; Ajayi *et al.*, 2018).

Phenol is the most prevalent compound in the Poaceae family, and several herbivorous insects have been shown to be harmful when exposed to it (Kessler and Baldwin, 2002; Sharma *et al.*, 2009; Usha Rani and Jyothsna, 2010; War *et al.*, 2011b; Ahman *et al.*, 2019). Some studies (Leszczynski *et al.*, 1995; Kessler and Baldwin, 2002) have demonstrated that aphid life-table parameters are affected by plants with high phenol concentrations. Additionally, phenols have been shown to have anti-feedant qualities against the cereal aphid (Urbanska *et al.*, 2002).

Numerous secondary metabolites, such as phenols, sterols, terpenoids, lignins, and policosanols (Singh *et al.*, 2015) are found in sugarcane, particularly in its juice (Feng *et al.*, 2014; Ali *et al.*, 2019). Godshall and Legendre (1988) documented that sugarcane and its by-products contain phenols. Several authors (Mollyneux *et al.*, 2007; Tinky *et al.*, 2020, Kerdchan *et al.*, 2020) postulated that secondary metabolites promote development and activation of defensive mechanisms to safeguard plants. Moreover, it has been observed that they also give color, taste, and smell, resulting in pest deterrents. In support of this, War *et al.* (2012) indicated that these metabolites are constitutively generated by plants or induced in response to an insect attack. This may result in antixenosis (non-preference behavior), which discourages insects from feeding, ovipositing, and hiding on plants (Kogan and Ortman, 1978; Smith and Clement, 2012; Padmaja, 2016; Puri *et al.*, 2023). Moreover, plants may exhibit antibiosis, which has a deleterious effect

on the biology of the insect when it feeds on the plant (Painter, 1951; Padmaja, 2016; Puri *et al.*, 2023).

Singh *et al.* (2015) reported the presence of phenolic compounds such as flavonoids in sugarcane leaves. Furthermore, Colombo *et al.* (2006) suggested that sugar cane juice contain flavonoids and those produced via the phenylpropanoid pathway, are among the major secondary metabolites (Falcone Ferreyra *et al.*, 2012; Mierziak *et al.*, 2014; Singh *et al.*, 2021) involved in sugarcane defence. A well-known flavonoid called pisatin found in pea (Morkunas *et al.*, 2016) offers protection against the pea aphid. Similarly, resistance against *R. maidis* in sorghum was recently shown to be conferred by the flavonoid 3-deoxyanthocyanidin (Kariyat *et al.*, 2019).

The idea that insect damage could alter the phenolic chemicals in sugarcane was first proposed by Akbar *et al.* (2009). The total phenol content significantly increased when the root-sucking froghopper attacked sugarcane leaves (Silva *et al.*, 2005). Furthermore, feeding by the white pit was found to significantly alter the amount and type of phenols in 15 sugarcane clones. The phenol and flavonoid response of sugarcane genotypes to YSA herbivory has not been studied to date. Understanding the secondary metabolite response of YSA feeding on sugarcane would provide basic insights into the defensive mechanisms and resistance responses. According to Paudyal (2019), host plant resistance can assist raise economic thresholds (ETs), eliminating the need for insecticide use. This makes it an efficient and least disruptive form of integrated pest management. The scientific method of examination, investigation, extraction, and testing by identifying many classes of metabolites present is known as phytochemical profiling (Tinky *et al.*, 2020). Flavonoids in sugarcane leaves, juice, and bagasse were characterized by using a variety of chromatographic techniques (Colombo *et al.*, 2006; Colombo *et al.*, 2008). Therefore, the purpose of this study is to characterize the secondary metabolites in sugarcane (*S. officinarum*) leaves that confer resistance against *S. flava* through Gas Chromatography Mass Spectrometry (GCMS) profiling.

6.2 Materials and methods

The study was conducted in the 2023/24 season at the ZSAES, which is owned by Tongaat Hullets, in the southeast Lowveld of Masvingo Province, in Chiredzi district. It is found in agro-ecological region V, which is characterized by very low and erratic rainfall of less than 500 mm

per annum. The site is situated on a 99 km peg along the Ngundu-Tanganda road. It is located 430 m above sea level at latitudes of 200 01' S and longitude 280 38' E (Sakadzo *et al.*, 2024).

6.2.1 Agronomic practices

Seven different sugarcane accessions (ZN 10, N14, ZN 3L, ZN 8, 96-1107, ZN 9, and 00-1165) were chosen and planted in accordance with the Zimbabwe Sugar Production Manual (Clowes and Breakwell, 1998).

6.2.2 Preparation of plant extract

For this investigation, the leaves of seven sugarcane cultivars were used: ZN 10, N14, ZN 3L, ZN 8, 96-1107, ZN 9, and 00-1165. Three months of sugarcane cultivation, as recommended by Rao *et al.* (2021), was used following a natural aphid infestation. The leaves of YSA-infested and uninfested sugarcane were defoliated from the plant (Figure 6.1). They were cleaned under running tap water to remove dust, dried in an oven at 60 °C for 18 h, crushed into small pieces, and stored at room temperature in a sealed bag. The crude leaf extract was extracted using methanol. Figure 6.2 shows the contribution of leaf position on quantity of metabolites.



Figure 6.1: YSA un-infested and infested leaves as exhibited by damage symptoms on sugarcane crop. Source (This study).

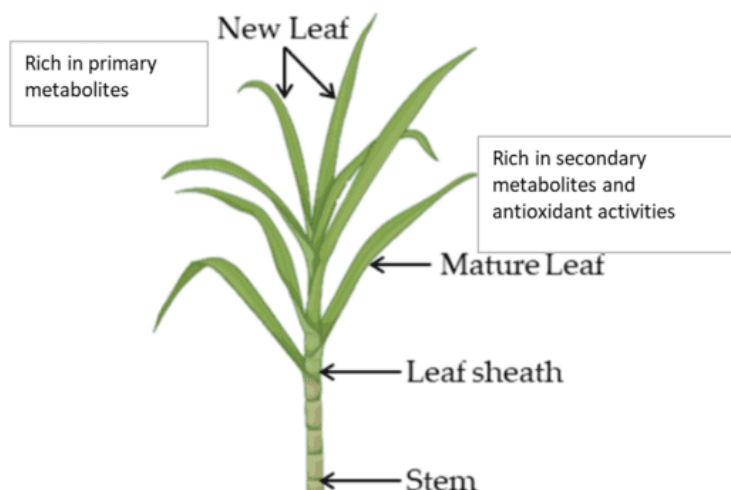


Figure 6.2: Contribution of leaf position on quantity of metabolites (Rao et al., 2021).

6.2.3 Sample Preparation

A 1 g of sugarcane leaf sample was weighed into a clean 100 ml Low Actinic Volumetric flask. Fifty millilitres of gradient-grade methanol was added and extracted with ultrasonic maceration for 1 h. Top up to volume with methanol. The mixture was then centrifuged at 1000 rpm for 15 min. The supernatant was collected and the residue was discarded. Concentration portion of the sample was placed in speed vacuum. The sample was reconstituted with gradient-grade chloroform in a 5 ml low actinic volumetric flask. The sample was passed through a solid-phase extraction vacuum station and a reverse-phase octadecyl (C18, 6 mL, 500 mg) column connected to a 3kDa Amicon filtering device.

6.2.4 Standard Concentration

The standard concentration was prepared by combining standard stock (100 ng/ml) diluted in chloroform to 10, 20, 40 and 50 ng/ml.

6.2.5 Sample analysis

Gas chromatography mass spectrometry with electron ionization (Shimadzu Nexis GC2030, GCMS TQ8040NX triple quadrupole mass spectrometer, HS-20NX (Figure 6.3) mode was used to examine the phenolic and flavonoid composition of both infested and un-infested sugarcane leaves. Seven sugarcane varieties were subjected to four replications, resulting in 56 samples under the two aphid treatments (infested and uninfested (control)). The procedure of Rajendran *et al.* (2017) was followed to soak about 1 gram of leaf extract in a 1:2 ratio of hexane. The leaf extract was incubated at room temperature overnight and then filtered through blotting paper by using a sample concentrator and nitrogen gas flushing. To remove sediments and any remaining

moisture content in the filtrate, the filtrate concentrate was filtered once more using Whatman No. 41 filter paper combined with sodium sulfate (Rajendran *et al.*, 2017). This process guarantees reproducible sample injection and an accurate derivatization time. Following the extraction and filtration process, a split-mode injection port received 1 µl of the sample injected at a ratio of 1:10. Helium Carrier Gas Control at 32.0 cm/sec FR; 1.0 ml/min was used.

The column of 30 m long, 0.25 mm internal diameter, and 0.25 µm thick fused in silica capillary column of BR-5MS (5 % Diphenyl/95 % Dimethyl poly siloxane) was used. The temperature of the oven was raised from 40 °C for two minutes to 160 °C at a rate of 20 °C/min without holding, then increased once more to 280 °C at a rate of 5 °C/min without holding, and finally to 300 °C at a rate of 12 °C/min with an 8-minute hold) (Rajendran *et al.*, 2017).

A 275 °C injector temperature of 275 °C and a GC operating duration of 41 min were observed. The purpose of this final increase was to thoroughly elute the components of the sample from the column and to remove any remaining residue. With an ionization energy of 70 eV, the mass spectrometer was operated in positive electron ionization (EI) mode. A 0-3.0 minute solvent delay was observed. Fragments from m/z 50 to 500 kDa were programmed with a scan interval of 0.5 seconds. The temperature of the filament source used was 250 °C, and the transfer line temperature was set to 280 °C. The individual peak areas were compared with the total peak ion areas to determine the proportion of each component. Analytical work done at Tinnac Scientific Laboratory is thoroughly documented, providing full details of all analyses, including acceptance criteria and actual results, analytical methods and run conditions, chromatograms, and spectral data (if applicable), and analytical method validation and verification data are also available upon request (**Certificate of Analysis No: TSL8330ZW**). Customer COAs and reports can be produced according to customer specifications for custom projects. Figure 6.4 shows a summary of the sample analysis flowchart.



Figure 6.3: Shimadzu Nexis GC2030, GCMS TQ8040NX Triple Quadrupole Mass Spectrometer, HS-20NX mode. Source (This study)

6.2.6. Summaried analysis flow chart of phenol and flavonoid identification

Summarised flow chart for identification of phenols and flavonoids is an important step for quick reference, time saving, simplification of complex procedures, enhanced accuracy, method development, and standardization (Figure 6.4).

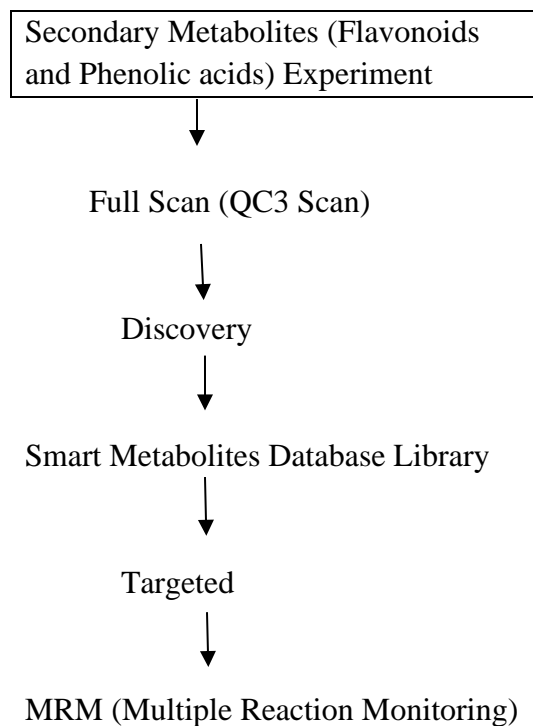


Figure 6.4: Analysis flow chart phenols and flavonoids

6.3 Phytochemical Screening

The phytochemicals screened in this study included flavonoids, tannins, phenols, terpenoids, saponins, coumarins, and anthraquinones.

6.3.1 Testing for flavonoids

The sodium hydroxide (NaOH) test was used to test for flavonoids; 1 ml of the stock solution was placed in a test tube, and a few drops of 1 M NaOH solution were added. The presence of flavonoids was indicated by the formation of an intense yellow color that disappeared after adding a few drops of 1M hydrochloric acid (Hossain *et al.*, 2013).

6.3.2 Testing for tannins

A ferric chloride test was performed. A few drops of 5 % ferric chloride were added to a test tube containing 1 ml of the stock solution. Wait *et al.* (2011) inferred the presence of tannins when a greenish, blue-black, or blue-green color is obtained.

6.3.3 Testing for saponins

This was performed by adding 2 ml of distilled water to 2 ml of the extract solution and shaking the mixture in a test tube for 10s. Foam development indicates the presence of saponins (Kumar Bargah, 2015).

6.3.4 Testing for Coumarins

One milliliter of 10 % NaOH (sodium hydroxide) was added to 1 ml of the extract in a test tube. Yellow indicates the presence of coumarins (Vimalkumar *et al.*, 2014).

6.3.5 Testing for terpenoids

This was performed by mixing 2 ml of chloroform and a few drops of concentrated sulfuric acid, which was later added to the extract (0.5 ml of the extract). Raphael (2012) emphasized that a red-brown color indicates the presence of terpenoids.

6.3.6 Testing for phenols

A combination of 2 ml distilled water and a few drops of 10 % ferric chloride were added to 1 ml of the extract. The presence of blue or green indicates the presence of phenols are present (Gowri and Vasantha, 2010).

6.3.7 Testing for anthraquinones

A few drops of 10 % ammonia solution were added to 1 ml of the plant extract, formation of a pink color indicated the presence of anthraquinones (Geetha and Geetha, 2014).

6.4 Data collection

6.4.1 Aphid number

Aphids were physically counted on all leaves (Figure 6.5) of five marked tillers to correlate aphid numbers with phenol and flavonoid contents.



Figure 6.5: Aphids on sugarcane leaf

6.4.2 Aphid quantity ratio (AQR)

The Chinese Agricultural Standard was used to determine the AQR (Chen *et al.*, 2007; Xu *et al.*, 2021). At the sampling stages, the number of aphids on the five plants in each treatment was counted. The following formula was used to determine AQR: $AQR =$

$$\frac{\text{Average number of aphids on a variety}}{\text{Average number of aphids on all varieties}}$$

Based on AQR, the following scale was used to evaluate aphid resistance (Xu *et al.*, 2021) (Table 6.1).

Table 6.1: Aphid resistance scale based on AQR

Level Description	AQR
1 High resistance	$0.01 < AQR \leq 0.30$
2 Medium resistance	$0.30 < AQR \leq 0.60$
3 Low resistance	$0.60 < AQR \leq 0.90$
4 Low sensitivity	$0.90 < AQR \leq 1.20$
5 Medium sensitivity	$1.20 < AQR \leq 1.50$
6 High sensitivity	$AQR > 1.50$

6.4.3 Qualitative data on secondary metabolites

Qualitative data were obtained for phenols (P), flavonoids (F), terpenoids (T), saponins (S), coumarins (C), tannins (T), and anthraquinones (A).

6.4.4 Quantitative data on phenols and flavonoids

Total phenolic content (TPC) and total flavonoid content (TFC) were determined from 56 samples by GC-MS by adding the quantified phenols and flavonoids. Furthermore, the relationship between Aphid Quantity Ratio (AQR) and accumulation of total phenol content and flavonoid content in the seven sugarcane varieties was also determined.

6.5 Data analysis

Two way and one way analysis of variance was used to test the effects of variety and aphid treatment and their interaction (variety \times aphid treatment). Genstat version 18th edition was used to analyze data on aphid number, total phenol content (TPC), and total flavonoid content (TFC), and mean separation was done by using Fishers Least Significance at 5 % Significance Level. Regression analysis was used to determine the correlation between the percentage change in total phenol and flavonoid contents and aphid number.

6.6 Results

The profiling of secondary metabolites revealed that the leaves of both infested and uninfested sugarcane leaves contained the following: phenols (P), flavonoids (F), terpenoids (T), saponins (S), coumarins (C), tannins (T), and anthraquinones (A) (Table 6.2). The identified and quantified phenols and flavonoids are listed in Table 6.3.

Table 6.2: Secondary metabolites screening of seven sugarcane varieties

Phytochemical screening							
Sugarcane variety	Phenols	Flavonoids	Terpenoids	Saponins	Coumarins	Tannins	Antraquinones
ZN 10	+	+	+	+	+	+	+
ZN 9	+	+	+	+	+	+	+
ZN 8	+	+	+	+	+	+	+
ZN 3L	+	+	+	+	+	+	+
00-1165	+	+	+	+	+	+	+
96-1107	+	+	+	+	+	+	+
N14	+	+	+	+	+	+	+

+ indicates the presence of the tested: Phenols (P), Flavonoids (F), Terpenoids (T), Saponins (S), Coumarins (C), Tannins (T), Anthraquinones (A).

Table 6.3: Identified common phenols and flavonoids in sugarcane leaves amongst the seven sugarcane varieties in YSA uninfested and infested plots

Identified Compound Name	Match Score	Name
(1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid	99.1	Chlorogenic Acid
(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid	99.36	Caffeic Acid
(2R,3S,4S,5S)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3,4,5-trihydroxyhexanedioic acid	99.22	2-O-caffeoylglucarate
1-(4-Hydroxy-3,5-dimethoxyphenyl)ethanone	99.8	Acetosyringone
Feruloyl quinic acid: 1,3,5-trihydroxy-4-[(E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]oxycyclohexane-1-carboxylic acid	99.4	Feruloylquinic acid isomer 1
Unknown	99.18	Feruloylquinic acid isomer 2
Unknown	98.27	Feruloylquinic acid isomer 3
Unknown	98.24	Feruloylquinic acid isomer 4
3,5-Dimethoxy-4-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxybenzoic acid	99.24	Glucosyringic acid
2-(4-Hydroxy-3-methoxyphenyl)acetic acid	98.21	Homovanillic acid
(1R,3S,4S,5S)-1,3,4-trihydroxy-5-[(E)-3-(4-hydroxyphenyl)prop-2-enoyl]oxycyclohexane-1-carboxylic acid	98.36	1 p-Coumaroylquinic acid:
3,4-Dihydroxybenzoic acid	99.15	p-Coumaroylquinic acid
4-Hydroxy-3-methoxybenzoic acid	97.92	Protocatechuic acid
4-Hydroxy-3,5-dimethoxybenzoic acid	98.96	Vanillic acid
(E)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enal	99.54	Syringic acid
(2E)-3-(2-hydroxyphenyl)prop-2-enoic acid	99.84	Sinapaldehyde
(2E)-3-(3-hydroxyphenyl)prop-2-enoic acid	97.77	o-Coumaric acid
(2R,3S)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	99.14	m-Coumaric acid
5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chromen-4-one	98.84	Catechin
5,7-Dihydroxy-2-(4-hydroxyphenyl)chromen-4-one	99.3	Diosmetin
3-[[6-[2-(3,4-Dihydroxyphenyl)-5,7-dihydroxychromenyl]-3-yl]oxy-3,4,5-trihydroxyoxan-2-(3,4-Dihydroxyphenyl)-5,7-dihydroxychromen-4-one	99.09	Apigenin
5,7-Dihydroxy-3-(4-hydroxyphenyl)chromen-4-one	98.02	Cyanidin-3-malonyl-glucoside
[(2R,3S,4S,5R,6S)-6-[2-(3-Ethenyl-5-methoxy-4-methylphenyl)-5,7-dihydroxychromenyl]-3-yl]oxy-3,4,5-trihydroxyoxan	98.54	Luteolin
5,7-Dihydroxy-2-(4-hydroxyphenyl)-6-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-	99.63	Genistein
	99.25	Malvidin-3-caffeoyl-glucoside
	99.74	Isovitexin (apigenin-6-C-glucoside)

yl]chromen-4-one		
2-(3,4-Dihydroxyphenyl)-5-hydroxy-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	99.28	Luteolin-7-O-glucoside
3-[[6-[2-(3,4-Dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-3-oxopropanoic acid	99.08	Cyanidin-3-malonyl-glucoside
5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methylloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	99.73	Diosmin (diosmetin-7-Orhamonglucoside)
[(2R,3S,4S,5R,6S)-6-[2-(3-Ethenyl-5-methoxy-4-methylphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate	98.48	Malvidin-3-caffeoyl-glucoside
2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-6-[4-hydroxy-6-methyl-5-oxo-3-(3,4,5-trihydroxy-6-methylloxan-2-yl)oxyoxan-2-yl]chromen-4-one	99.03	Maysin (luteolin-6-C-diglycoside)
(2S,4S,5S)-2-[7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-[(2S,5S)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromenylium-5-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	99.5	Peonidin-3,5-diglucoside
6-[(2S,3R,4S,5S,6R)-4,5-Dihydroxy-6-(hydroxymethyl)-3-[(3R,4R,5R,6S)-3,4,5-trihydroxy-6-methylloxan-2-yl]oxyoxan-2-yl]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one	96.15	Isoorientin 2''-C-rhamnoside
5-Hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-7-[(3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	95.11	Tricin-7-O-glucoside
3-O-(6''-succinyl)-rhamnoside	97.54	Petunidin
Vitexin (apigenin-8-C-glucoside) 5,7-Dihydroxy-2-(4-hydroxyphenyl)-8-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one	99.95	Vitexin (apigenin-8-C-glucoside)

Source: This study

6.7 Aphid number

There was a highly significant interaction ($p < 0.001$) between sugarcane variety and aphid treatment (infested and uninfested) on aphid number. Aphid number showed highly significant differences ($p < 0.001$) among the sugarcane varieties in both the YSA uninfested (control) and infested treatments. The ZN 10 sugarcane variety recorded the highest YSA number (220) while the 00-1165 recorded the lowest (75). In aphid infested plots, ZN 10 recorded the highest YSA number (888) while 00-1165 recorded the lowest (309) (Figure 6).

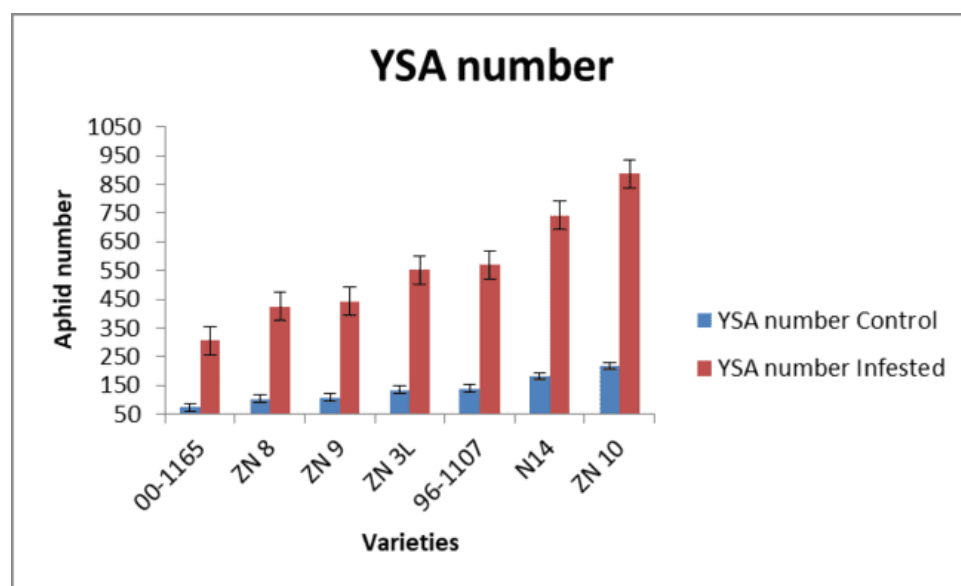


Figure 6.6: Aphid number in control and infested plots

6.8 Evaluation of YSA resistance

The AQR of cultivar 00-1165 was in the range of 0.30-0.60 indicating that it was a medium resistant cultivar to YSA stress, while ZN 8 and ZN 9 were low resistant. Furthermore, ZN 3 L and 96-1107 showed low sensitivity to YSA. Moreover, N14 was moderately sensitive. Lastly, cultivar ZN 10 represents a highly susceptible cultivar because its AQR was more than 1.50 (Table 6.4).

Table 6.4: The AQR and aphid resistance levels of seven sugarcane varieties

Varieties	AQR	Aphid resistance
00-1165	0.55	Medium resistance
ZN 8	0.76	Low resistance
ZN 9	0.79	Low resistance
ZN 3L	0.98	Low sensitivity
96-1107	1.02	Low sensitivity
N14	1.32	Medium sensitivity
ZN 10	1.58	High sensitivity

6.9 Effects of sugarcane variety on total phenol content (TPC)

A highly significant interaction ($p < 0.001$) between sugarcane variety and aphid treatment on total phenol content was recorded. Highly significant differences ($p < 0.001$) in TPC among the sugarcane varieties were observed. The results showed that N14 had the highest phenol content of 35.79 $\mu\text{g/g}$ while ZN 8 had the lowest phenol content of 20.71 $\mu\text{g/g}$ in the YSA uninfested (control) treatment. In the YSA infested plots, 96-1107 sugarcane variety had the highest phenol content of 50.31 $\mu\text{g/g}$, while ZN 3L had the lowest phenol content of 25.92 $\mu\text{g/g}$ (Figure 6.7).

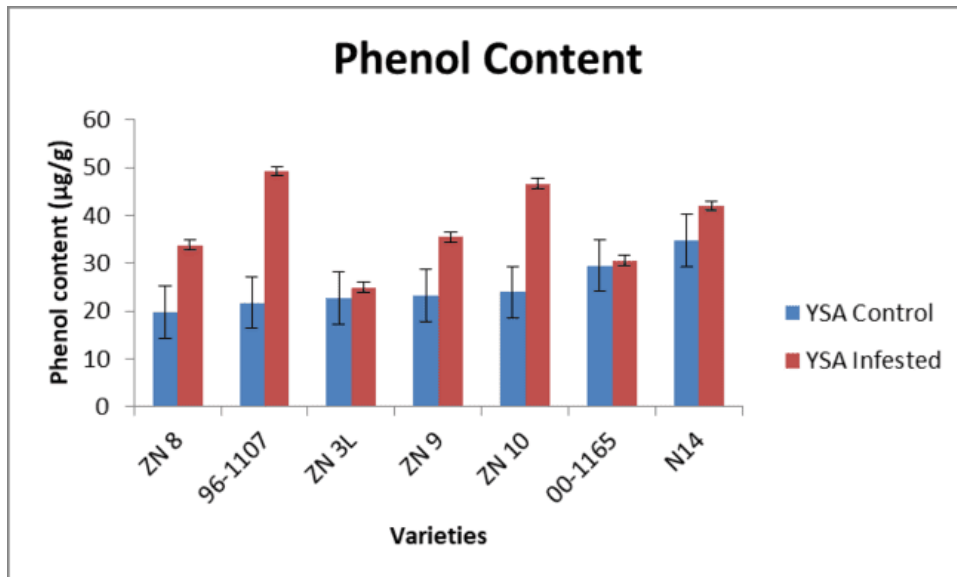


Figure 6.7: Phenol content of different sugarcane varieties

6.10 Effects of sugarcane variety on total flavonoid content (TFC)

There was highly significant interaction ($p < 0.001$) between sugarcane variety and aphid treatment on TFC. Furthermore, highly significant ($p < 0.001$) differences in total flavonoid content were recorded among the sugarcane varieties. 00-1165 sugarcane variety recorded the highest flavonoid content of $2.99 \mu\text{g/g}$ whereas ZN 9 had the lowest flavonoid content of $1.5 \mu\text{g/g}$ in the YSA uninfested (control) treatment. In the YSA infested plots, the N14 sugarcane variety had the highest total flavonoid content of $6.47 \mu\text{g/g}$, whereas ZN 3L had the lowest flavonoid content of $1.60 \mu\text{g/g}$ (Figure 6.8).

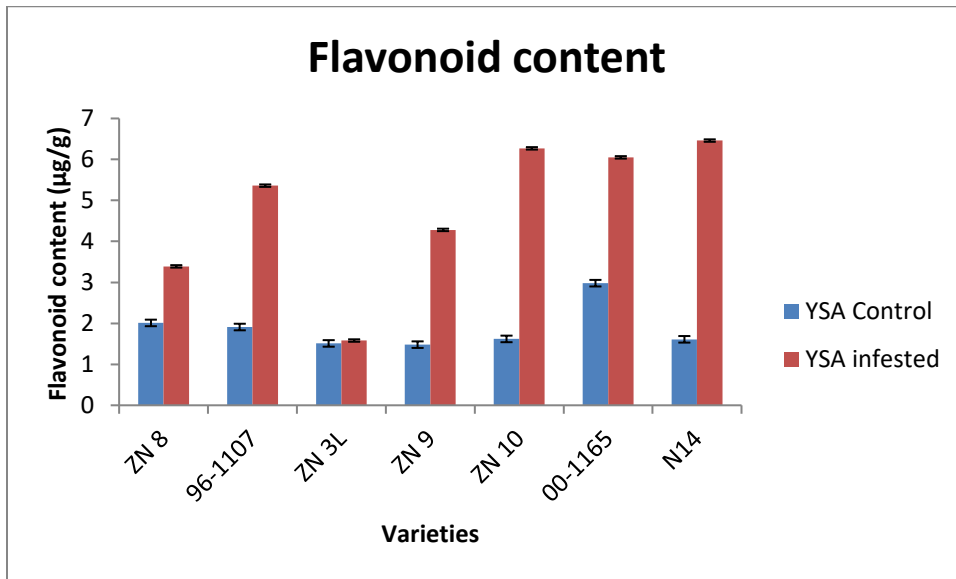


Figure 6.8: Flavonoid content of different sugarcane varieties

6.11 Relationship between percentage change in phenol content and aphid number

The regression analysis showed a highly significant ($p = 0.002$) positive correlation between percent change in phenol content and YSA number (Figure 6.9, $Y = 0.15X - 23.6$, $p = 0.002$, $r = 0.58$). An increase in YSA number or feeding stimulates the plant to produce more phenols as a defence strategy thereby causing a positive correlation between the two variables.

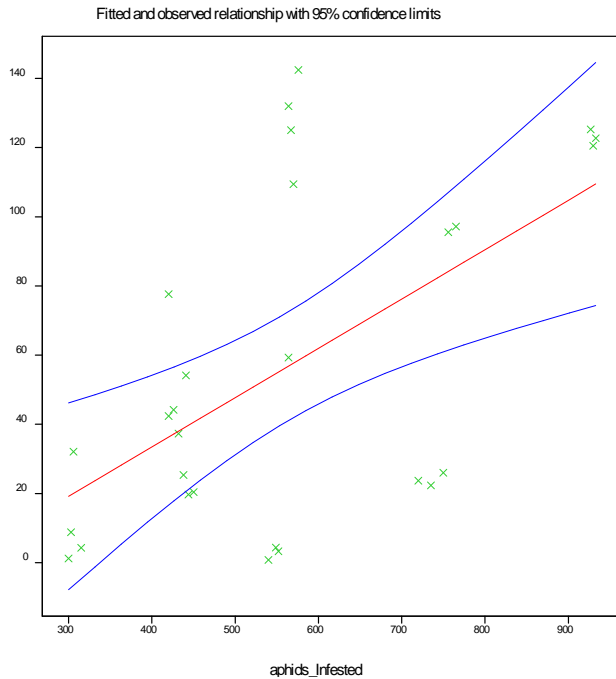


Figure 6.9: Relationship between percentage change in phenol content and aphid number

6.12 Relationship between percent change in flavonoid content and aphid number

Regression analysis showed a highly significant ($p < 0.001$) positive correlation between percent change in flavonoid content and YSA aphid number (Figure 6.10, $Y = 1.17X - 50.2$, $p < 0.001$, $r = 0.70$). A positive percentage change in flavonoid is a result of a rise in the YSA number, which raises the flavonoid content and serves as a defense mechanism against YSA aphid infestation leading to a positive correlation between these two variables.

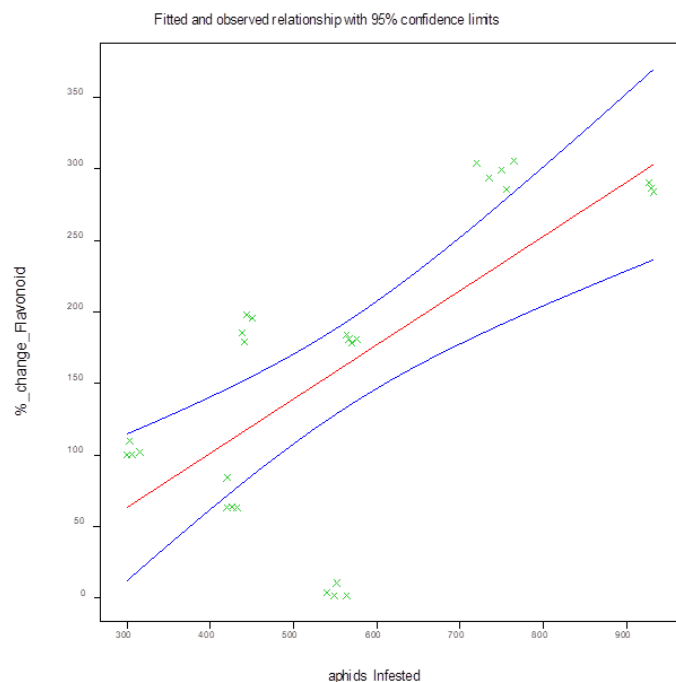


Figure 6.10: Relationship between percent change in flavonoid content and aphid number

6.13 Discussion

The YSA number of the sugarcane varieties affected the AQR resistant evaluation. According to the AQR resistance evaluations, 00-1165 sugarcane variety is a medium resistant cultivar to YSA stress, while ZN 8 and ZN 9 are low resistant. Furthermore, ZN 3L and 96-1107 showed low sensitivity. In addition, N14 is moderately sensitive, while ZN 10 variety is regarded as highly sensitive. This trend of aphid number and AQR might have been influenced by secondary metabolites and defensive enzymes although defensive enzymes were not measured. Similar trend of results on AQR resistant evaluations were reported by Xu *et al.* (2021) in wheat under cereal aphid stress. The study also focused on the identification and quantification of flavonoids and phenols, as well as secondary metabolite profiling. The results of this study showed that sugarcane contains a myriad of secondary metabolites (Tables 6.2 and 6.3). Similar results on sugarcane secondary metabolites have been reported to be present in sugarcane generally by a number of authors (Colombo *et al.*, 2005, 2006, 2008; Duarte-Almeida; Feng *et al.*, 2014; Singh *et al.*, 2014; Pinheiro *et al.*, 2017; Rajendran *et al.*, 2017; Koch *et al.*, 2018; Ali *et al.*, 2019; Salgado, 2020; Kerdchan *et al.*, 2020; Ni *et al.*, 2021; Srihanam *et al.*, 2021; Rao *et al.*, 2022; Shafiqa-atikah *et al.*, 2022. Moreover, some authors (Feng *et al.*, 2014; Kraphankhieo and Srihanam, 2016; Naowaset and Srihanam, 2017) have suggested that the type and location of

sugarcane planting affects the phytochemical content of the crop. The accumulation of phenols and flavonoids is part of the defense response of sugarcane to YSA feeding, with the aim of repelling or toxifying aphids. YSA feeding might have induced the expression of phenol and flavonoid biosynthetic genes, leading to increased production. This study demonstrated that susceptible sugarcane varieties (96-1107, N14, and ZN 10) were able to increase total phenolics and flavonoids in YSA-infested plots. Previous studies by Malekshah *et al.* (2022) documented the impact of the physicochemical characteristics of sugarcane varieties on population dynamics of stem borer (*Sesamia nonagrioides*). A decrease in the body size of aphids was reported in wheat cultivars high in hydroxamic acid (Fuentes-Contreras and Niemeyer, 1998). In addition, plants generate several secondary metabolites known as phytoanticipins and or phytoalexins (War *et al.*, 2012). These secondary compounds hinder insects' ability to feed continuously on plants (Nalam *et al.*, 2019).

In the present study, different sugarcane varieties exhibited varying levels of phenolic and flavonoid accumulation in response to YSA infestation. This significant increase between varieties was reported by Zhu *et al.* (2011). The N14 sugarcane variety seemed to be able to accumulate sufficient phenol and flavonoid content in response to YSA attack. Similar results were reported by Green *et al.* (2003) on pigeonpea (*Cajanus cajan*) varieties' susceptibility to podworms (*Helicoverpa armigera*). Furthermore, Zhang *et al.* (2022) reported statistically strong associations between percent change in flavonoid content and aphid number, although a strong positive correlation was reported on both phenols and flavonoids. Moreover, Haviola *et al.* (2007) found positive correlations similar to this study. Additionally, Zhang *et al.* (2022) conjectured those correlations between total phenol compounds and insect performance ranged from negative to zero.

Under various environmental stressors, common wheat shows enhanced phenylpropanoid metabolism and elevated levels of phenolic metabolites (Sakihama and Yamasaki, 2002). Similar results were reported by Chen *et al.* (2003), who indicated that wheat resistance to aphids improved when the total phenol content increased. The total phenol levels in the seven sugarcane cultivars were substantially higher than those in the control plants. The results of Chen *et al.* (2003), Sakihama and Yamasaki (2002), Ramos *et al.* (2017), and Xu *et al.* (2021) were in good agreement with these findings.

Our results confirm the findings of Akbar (2009), who reported an increased phenolic content in response to aphid feeding in sugarcane. These results concur with the findings of Silva *et al.* (2005), who indicated increased phenol in response to root-sucking froghopper (*Mahanarva fimbriolata* Stal.). In support of this, insect herbivore has been reported to frequently cause changes in phenol and flavonoid levels in plants (Treutter, 2007; Zhang *et al.*, 2017; Wang *et al.*, 2019). Higher phenol concentrations in plants have been shown to deter pests (Zhang *et al.*, 2022). In addition, it was discovered that phenol content increased in 15 sugarcane clones that attacked by the white pit (*Antitrogus parvulus*) (Silva *et al.*, 2005). Of the seven sugarcane accessions, 96-1107 and N14 displayed high total phenolic and flavonoid content, respectively. Furthermore, the susceptible varieties (96-1107, N14, and ZN 10) exhibited yellowish and purplish leaf color. These findings corroborate those of Haile *et al.* (1999) and Goławska *et al.* (2010), who observed that lower levels of chlorophyll in susceptible varieties may be linked to higher synthesis of defensive secondary metabolites. During feeding, aphids secrete phytotoxins that disrupt physiology and activate defense mechanisms (Botha *et al.*, 2006; Smith *et al.*, 2010). The leaf chloroplasts of aphid-infested plants can be broken down by enzymes in aphid saliva, resulting in longitudinal streaks that are white, yellow, purple, or reddish-purple on the leaves (Fouché *et al.*, 1984; Pike and Allison, 1991; Ma *et al.*, 1998), although enzymes were not measured in this study.

The ability of sugarcane varieties to withstand the damaging effects of reactive oxygen species (ROS) caused by aphid infestation depends on their antioxidant capacity. Moreover, plants have evolved antioxidant systems to counteract the harmful effects of ROS. This mechanism eliminates excess ROS from the cell and shields the plant from oxidative damage (Shankar and Yinghua, 2021). Smith and clement (2012) discovered that shikimate kinase was elevated in wheat infested by Russian wheat aphid. Therefore, the study findings suggest that the YSA infestation in our study could have triggered the shikimate route, which could subsequently trigger the creation of secondary metabolites via the plant defense mechanism pathway, thereby increasing the resistance of infested sugarcane types to aphids. However, the ROS and the shikimate route were not investigated by this study.

The increased phenolic and flavonoid contents in sugarcane leaves may correlate with enhanced antioxidant activity, helping to counteract the oxidative stress caused by YSA infestation. Elevated accumulation of ROS can also result in a defense response in the host plant, mediated by SAR-induced systemic acquired immunity (Wu *et al.*, 1997; Cao *et al.*, 1998; Zhang *et al.*, 1999; Asada *et al.*, 2006). Plants that experience ROS suppression are more vulnerable to aphid attacks, whereas ROS accumulation may result in aphid resistance, supporting the findings of this study (Shoala *et al.*, 2018). This may be a similar case in YSA-resistant (00-1165) and moderately resistant (ZN 8, ZN 9, and ZN 3L) sugarcane varieties. According to Shankar and Yinghua (2021), saliva is known to contain a variety of toxic chemicals that cause plants to perceive invasion by aphids. This may worsen the build-up of ROS, a precursor to oxidative stress. This condition ultimately results in cell death (Mittler, 2002; Gechev 2006; Shankar and Yinghua, 2021). The resistant sugarcane genotype (00-1165) may have evolved as an antioxidant mechanism to combat the negative effects of ROS and to protect the plant from oxidative damage by removing excess ROS from the cell, which triggers the production of secondary metabolites. Moreover, resistant sugarcane varieties might have a regulatory antioxidant gene that regulates the toxic effects of the absence of ROS from susceptible sugarcane varieties.

The action of ROS scavenging enzymes neutralizes excessively generated ROS molecules to prevent oxidative damage that otherwise could result in needless cell death and maintain ROS homeostasis in plants (Ye *et al.*, 2021). The resistant variety (00-1165) in this study might have had a higher concentration of the aforementioned detoxifying enzymes that regulated the exhibition of aphid-infested symptoms compared to susceptible varieties. These enzymes are referred to as detoxifying enzymes (Das and Roychoudhury, 2014; Puri, 2023).

In addition to being potent antioxidants, flavonoids play a variety of functions in plants, including defense against pathogens, resistance against insect pests and squelching of free radicals (Solovchenko, 2010; Pourcel *et al.*, 2007). Studies on a wide range of plant species have found that plants exhibit high levels of flavonoid content (Pieta, 2000; Rao *et al.*, 2019, 2020). A high concentration of antioxidant and flavonoids is generally linked with tolerance to biotic and abiotic stresses (Rao *et al.*, 2020, 2021). Therefore, herbivores are less drawn to plant species with greater secondary metabolite levels (Rao *et al.*, 2018; Rao *et al.*, 2021).

The study's findings showed that sugarcane leaves contain a wide variety of secondary metabolites, including anthraquinones, phenols, flavonoids, terpenoids, saponins, coumarins, and tannins (Singh *et al.*, 2015; Sanarat *et al.*, 2021). This confirms findings by Puri *et al.* (2023), who postulated that many secondary metabolites are produced for defense against invaders. War *et al.* (2012) stressed that these metabolites are constitutively or induced by pest attack. Phenols, flavonoids, and tannins exhibit antixenosis (Kogan and Ortman, 1978; Smith and Clement, 2012; War *et al.*, 2012; Padmaja, 2016, Puri *et al.*, 2023) and antibiosis properties (Painter, 1951; Padmaja, 2016). This study highlights the importance of phenols and flavonoids in sugarcane defense against YSA, and suggests potential targets for breeding resistant varieties.

6.10 Conclusion

ZN 10 sugarcane variety recorded the highest aphid number, while 00-1165 had the lowest. Furthermore, variety 00-1165 exhibited a medium resistance to YSA stress, as indicated by its AQR, whereas ZN 8 and ZN 9 demonstrated low resistance. Moreover, ZN 3L and 96-1107 showed low sensitivity. The N14 variety is moderately sensitive while ZN 10 is considered a highly sensitive variety. Phenols and flavonoids confer sugarcane resistance to YSA feeding. The tested sugarcane accessions showed increased phenolic and flavonoid content in response to YSA feeding. Different sugarcane varieties exhibit varying levels of phenolic and flavonoid accumulation in response to YSA exposure. In the YSA infested plots, 96-1107 sugarcane variety recorded the highest phenol content, while ZN 3L recorded the lowest flavonoid content. Finally, the N14 sugarcane variety had the highest flavonoid content, whereas ZN 3L scored the lowest in the infested treatments.

References

- Abdelmajid, N. 2008. Première apparition de *Sipha flava* (Homoptera, Aphididae) sur la canne à sucre au Maroc.- Bull. OEPP/EPPO Bull. 38: 220-222.
- Åhman, I., Kim, S. Y., and Zhu L. H 2019. Plant Genes Benefitting Aphids—Potential for Exploitation in Resistance Breeding. *Front. Plant Sci.* 10:1452. doi: 10.3389/fpls.2019.01452
- Ajayi, O. A., Akinola, M. O., and Awodoyin, R. O. 2018. Applications of Allelopathy in Crop Protection. *International Journal of Agriculture and Economic Development.* 6(1): 9-23
- Akar, Z., and Burnaz, N. A. 2019. A new colorimetric method for CUPRAC assay with using of TLC plate. *LWT*, 112, 108212.8. Geetha, T.S and Geetha, N. 2014. Phytochemical screening,

quantitative analysis of primary and secondary metabolites of *Cymbopogon citratus* (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu. *International Journal of Pharmtech Research*. 6(2): 521-529.

Ali, S. E., El Gedaily, R. A., Mocan, A., Farag, M. A., and El-Seedi. H. R. 2019. Profiling Metabolites and Biological Activities of Sugarcane (*Saccharum officinarum* Linn.) Juice and Its Product Molasses via a Multiplex Metabolomics Approach. *Molecules*. 24: 934.

Asada, K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiol*. 141:391-396. doi:10.1104/pp.106.082040.

Belmain, S. R., Haggard, J., Holt, J., and Stevenson, P. C. 2013. Managing legume pests in sub Saharan Africa: *Challenges and prospects for improving food security and nutrition through agro-ecological intensification*. Chatham Maritime (United Kingdom): Natural Resources Institute, University of Greenwich. 34p.

Blackman, R. L., and Eastop, V.F. 1984. *Aphids on the World's Crops—An Identification Guide*; John Wiley and Sons: New York, NY, USA.

Botha, A. M., Lacock, L., van Niekerk, C., Matsioloko, M. T, du Preez, F. B, Loots, S., Venter, E., Kunert, K. J., and Cullis, C. A. 2006. Is photosynthetic transcriptional regulation in *Triticum aestivum* L. cv. 'TugelaDN' a contributing factor for tolerance to *Diuraphis noxia*? *Plant Cell Reports*. 25: 41–54.

Cao, H., Li, X., and Dong, X. 1998. Generation of broad-spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance. *Proc Natl Acad Sci USA*. 6531-6536. doi:10.1073/pnas.95.11.6531.

Chen, J. L., Ni, H. X., Sun, J. R., Cheng, D. F. 2003. Effects of major secondary chemicals of wheat plant on enzyme activity in *Sitobion avenae*. *Acta Entomol Sin*. 46:144–149.

Chen, W. Q., Liu, T. G., Chen, J. L., Cheng, D. F., and Chao, Y. Z. 2007. Agricultural standards of the People's Republic of China—rule for aphid resistance evaluation in wheat. (NY/T1443.7—2007), p 1–3.

Clowes, M. J and Breakwell, W. L. 1998. *Zimbabwe Sugarcane Production Manual*. Zimbabwe Sugar Association, Chiredzi, Zimbabwe.

- Colombo, R., Lancas, F. M., and Yariwake, J. H. 2006. Determination of flavonoids in cultivated sugarcane leaves, bagasse, juice and in transgenic sugarcane by liquid chromatography-UV detection. *J. Chromatogr. A.* 1103:118–124.
- Colombo, R., Yariwake, J. H., and McCullagh, M. 2008. Study of C- and O-glycosylflavones in sugarcane extracts using liquid chromatography: Exact mass measurement mass spectrometry. *J. Braz. Chem. Soc.* 19: 483–490.
- Colombo, R., Yariwake, J. H., Queiroz, E. F., Ndjoko, K., and Hostettmann, K. 2005. On-line identification of sugarcane (*Saccharum officinarum* L.) methoxyflavones by liquid chromatography-UV detection using post-column derivatization and liquid chromatography-mass spectrometry. *J. Chromatogr. A.* 1082: 51–59.
- Colombo, R., Yariwake, J. H., Queiroz, E. F., Ndjoko, K., and Hostettmann, K. 2005. On-line identification of sugarcane (*Saccharum officinarum* L.) methoxyflavones by liquid chromatography-UV detection using post-column derivatization and liquid chromatography-mass spectrometry. *J. Chromatogr. A.* 1082: 51–59.
- Conlong, D., and Way, M. 2014. New Pest Alert, Yellow Sugarcane Aphid. *Links.* 23(12): e.13.
- Das, K., and Roychoudhury, A. 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science.* 2: 53.
- Duarte-Almeida, J. M., Negri, G., Salatino, A., de Carvalho, J. E., and Lajolo, F. M. 2007. Antiproliferative and antioxidant activities of a triclin acylated glycoside from sugarcane (*Saccharum officinarum* L.) juice. *Phytochemistry.* 68(8): 1165-1171. doi:10.1016/j.phytochem.2007.01.015
- Falcone Ferreyra, M. L., Rius, S. P., and Casati, P. 2012. Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Front. Plant Sci.* 3:222. doi: 10.3389/FPLS.2012.00222
- Farooq, M., Jabran, K., Cheema, Z. A., Wahid, A., and Siddique, K. H. M. 2011. The role of allelopathy in agricultural pest management. *Pest Management Science.* 67:493-506

- Feng, S., Luo, Z., Zhang, Y., Zhong, Z., and Lu, B. 2014. Phytochemical contents and antioxidant capacities of different parts of two sugarcane (*Saccharum officinarum* L.) cultivars. *Food Chem.* 151: 452–458.
- Fouché, A., Verhoeven, R. L., Hewitt, P. H., Walters, M. C, Kriel, C. F., and De Jager, J. 1984. Russian aphid (*Diuraphis noxia*) feeding damage on wheat, related cereals and *Bromus* grass species. In: Walters M C, ed., *Progress in Russian Wheat Aphid (D. Noxia) Research in the Republic of South Africa*. Technical Communication from the Department of Agriculture, Republic of South Africa. pp. 22–33.
- Fuentes-Contreras, E., and Niemeyer, H. M. 1998. Dimboa glucoside, a wheat chemical defense, affects host acceptance and suitability of *Sitobion avenae* to the cereal aphid parasitoid *Aphidius rhopalosiphii*. *J. Chem. Ecol.* 24: 371-381.
- Gechev, T. S., Van Breusegem, F., Stone, J. M., Denev, I., and Laloi, C. 2006. Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. *Bioessays.* 28:1091-1101.
- Geetha, T. S., and Geetha, N. 2014. Phytochemical screening, quantitative analysis of primary and secondary metabolites of *Cymbopogon citratus* (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu. *International Journal of Pharmtech Research.* 6(2): 521-529.
- Godshall, M. A., and Legendre. B. L. 1988. Phenolic content of maturing sugar cane. *Internat. Sugar J.* 90: 16-19.
- Goławska, S., Krzyżanowski, R., and Łukasik I. 2010. Relationship between aphid infestation and chlorophyll content in Fabaceae species. *Acta Biologica Cracoviensia Series Botanica.* 52: 76-80.
- Gowri, S. S., and Vasantha, K. 2010. Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.)(Myrtaceae) leaves extracts. *Int J Pharm Tech Res.* 2(2): 1569-1573.
- Green, P. W., Stevenson, P. C., Simmonds, M. S., and Sharma, H. C. 2003. Phenolic compounds on the pod-surface of pigeonpea, *Cajanus cajan*, mediate feeding behavior of *Helicoverpa armigera* larvae. *J. Chem. Ecol.* 29: 811–821.

- Haile, F. J., Higley, L. G., Ni, X., and Quisenberry, S. S. 1999. Physiological and growth tolerance in wheat to Russian wheat aphid (Homoptera: Aphididae) injury. *Environmental Entomology*. 28(5): 787-794.
- Haviola, S., Kapari, L., Ossipov, V., Rantala, M. J., Ruuhola, T., and Haukioja, E. 2007. Foliar phenolics are differently associated with *Epirrita autumnata* growth and immunocompetence. *J. Chem. Ecol.* 33: 1013–1023.
- Hongo, H., and Karel, A. K. 1986. Effects of plant extracts on insect pest of common beans. *Journal of Applied Entomology*. 102:164-169.
- Hossain, M. A., AL-Raqmi, K. A. S., Al-Mijizy, Z. H., Weli, A. M., and Al-Riyami, Q. 2013. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. *Asian Pacific journal of tropical biomedicine*. 3(9): 705-710.
- Howe, G. A., and Jander G. 2008. Plant immunity to insect herbivores. *Annu Rev Plant Biol.* 59:41-66; PMID:18031220; <http://dx.doi.org/10.1146/annurev.arplant.59.032607.092825>.
- January, B., Yusufu, A., and Urassa, F. 2020. First report of yellow sugarcane aphid *Sipha flava* Forbes (Homoptera: Aphididae) in Tanzania. *Journal of Plant Protection Research*. 60(3).
- Kariyat, R. R., Gaffoor, I., Sattar, S., Dixon, C. W., Frock, N., Moen, J., et al. 2019. Sorghum 3-deoxyanthocyanidin flavonoids confer resistance against corn leaf aphid. *J. Chem. Ecol.* 45: 502–514. doi: 10.1007/S10886-019-01062-8
- Kerdchan, K., Kotsaeng, N., and Srihanam, P. 2020. Oxidative Compounds Screening in the Extracts of Sugarcane (*Saccharum officinarum* L.) Planted in Maha Sarakham Province, Thailand. *Asian Journal of Plant Science*. 19(4): 390-397.
- Kessler, A., and Baldwin, I. T. 2002. Plant response to insect herbivory: the emerging molecular analysis. *Annu. Rev. Plant Biol.* 53: 299-328.
- Khater, H. F. 2012. Prospects of botanical biopesticides in insect pest management. *Journal of Applied Pharmaceutical Science*. 2: 244– 259.

Koch, K. G., Donze-Reiner, T., Baird, L. M., Louis, J., Amundsen, K., Sarath, G., Bradshaw, J. D., and Heng-Moss, T. 2018. Evaluation of greenbug and yellow sugarcane aphid feeding behavior on resistant and susceptible switchgrass cultivars. *BioEnergy Research*. 11: 480–490.

Kogan, M., and Ortman, E. F. 1978. Antixenosis—a new term proposed to define painter’s “nonpreference” modality of resistance. *Bull. Entomol. Soc. Am.* 24, 175–176. doi: 10.1093/BESA/24.2.175

Kraphankhieo, W., and Srihanam, P. 2016. Investigation of phytochemical and antioxidant activity of different parts of sugarcane planted in Buriram province. In *Proceedings of the 12th Mahasarakham University Research Conference* (pp. 728-743).

Kumar Bargah, R. 2015. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn. *Journal of Pharmacognosy and Phytochemistry*. 4(1).

Leszczynski B., Tjallingii, W. F., Dixon, A. F. G., and Swiderski, R. 1995. Effect of methoxyphenols on grain aphid feeding behaviour. *Entomol. Exp. Appl.* 76: 157-162.

Ma, R. Z., Reese, J. C., Black, IV W. C., and Bramel-Cox, P. 1998. Chlorophyll loss in a greenbug-susceptible sorghum due to pectinases and pectin fragments. *Journal of the Kansas Entomological Society*. 71: 51–60.

Mercy, A. G., Light, W. F., and Gospel, S.A. 2017. Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta region of Nigeria, *Journal of food and Nutrition Sciences*. 5(5): 198-205 <https://doi.org/10.11648/j.jfns.20170505.16>.

Mierziak, J., Kostyn, K., and Kulma, A. 2014. Flavonoids as important molecules of plant interactions with the environment. *Molecules* 19, 16240–16265. doi: 10.3390/MOLECULES191016240

Mittler, R., Vanderauwera, S., Gollery, M., and Van Breusegem, F. 2004. Reactive oxygen gene network of plants. *Trends Plant Sci.* 9:490-498. doi:10.1016/j.tplants.2004.08.009.

Morkunas, I., Woźniak, A., Formela, M., Mai, V. C., Marczak, Ł., Narożna, D., and Grimm, B. 2016. Pea aphid infestation induces changes in flavonoids, antioxidative defence, soluble sugars and sugar transporter expression in leaves of pea seedlings. *Protoplasma*. 253: 1063-1079.

Molyneux, R. J., Lee, S. T., Gardener, L. E., and Panter K. E. 2007. Phytochemicals: The good, the bad, and the ugly? *Phytochem.* 68 (22-24): 2973-2985. <https://doi.org/10.1016/j.phytochem.2007.09.004>.

Mortazavi Malekshah, S. A., Naseri, B., Ranjbar Aghdam, H., Razmjou, J., Fathi, S. A. A., Ebadollahi, A., and Changbunjong, T. 2022. Physicochemical Properties of Sugarcane Cultivars Affected Life History and Population Growth Parameters of *Sesamia nonagrioides* (Lefebvre)(Lepidoptera: Noctuidae). *Insects.* 13(10): 901.

Mulungu L. S., Lupenza, G., Reuben, S. O. W. M and Misangu, R. N. 2007. Evaluation of Botanical products as stored Grain Protectant against Maize weevil, *Sitophilus zeamais* (L.) on maize. *Journal of Entomology.* 4: 258-202.

Mutonyi, J., and Babikha, S.J . 2019. The occurrence of sugarcane yellow aphid (*Sipha flava* (Homoptera: Aphididae) in Kakamega North Sub-County, Kenya. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS).* 12 (6): 34-38.

Nalam, V., Louis, J., and Shah, J. 2019. Plant defense against aphids, the pest extraordinaire. *Plant Sci.* 279:96–107.

Naowaset, D., and Srihanam, P. 2017. Phytochemical contents and antioxidant activity of partially purified sugarcane extract by silica gel column. In *Proceedings of the 13th Mahasarakham University Research Conference* (pp. 444-453).

Padmaja, P. G. 2016. “Insect pest resistance in sorghum” in *Biotic stress resistance in millets* (UK: Elsevier Academic Press). 105–145.

Painter, R. 1951. *Insect resistance in crop plants*. Macmillan, New York, 72, 481.

Parwada, C., Chikuvire, T.J, Kamota, A., Mandumbu, R., and Mutsengi, K. 2018. Use of Botanical Pesticides in Controlling *Sitophilus Zeamais* (Maize Weevil) on Stored *Zea Mays* (Maize) Grain. *Mod Concep Dev Agrono.* 1(4). MCDA.000517.2018.DOI: 10.31031/MCDA.2018.01.000517.

Paudyal, S. 2019. Host plant resistance to the sugarcane aphid (Hemiptera: Aphididae). A Dissertation Submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfillment of the requirements for the Degree of Doctor of philosophy.

- Pejman, N., Karimmojeni, H., Moghanibashi, M., and Hosseini, N. 2011. Allelopathic potential of sunflower on weed management in safflower and wheat. *Australian Journal of Crop Science*. 5(11):1434-1440.
- Pickett, J. A., Wadhams, L. J., Woodcock, C. M., and Hardi, J. 1992. The chemical ecology of aphids. *Annu. Rev. Entomol.* 37: 67-90.
- Pietta, P. G. 2000. Flavonoids as antioxidants. *J. Nat. Prod.* 63: 1035–1042.
- Pike, K. S., and Allison, D. 1991. *Russian Wheat Aphid-Biology, Damage and Management*. Washington, Oregon, Idaho Pacific Northwest Extension Publication. PNW371. Pullman, WA.
- Pinheiro, F. G. C., Soares, A. K. L., Santaella, S. T., Alexandre e Silva, L. M., Canuto, K. M., Cáceres, C. A., Rosa, M. D. F., Feitosa, J. P. D. A., and Leitão, R. C. 2017. Optimization of the acetosolv extraction of lignin from sugarcane bagasse for phenolic resin production. *Ind. Crops Prod.* 96: 80–90.
- Pourcel, L., Routaboul, J. M., Cheynier, V., Lepiniec, L., and Debeaujon, I. 2007. Flavonoid oxidation in plants: from biochemical properties to physiological functions. *Trends Plant Sci.* 12: 29–36.
- Puri, H. 2023. *Sorghum-Sugarcane Aphid Interactions: Multi-Omic Approaches to Elucidate Plant Defense Against Sap-Feeding Insects* (Doctoral dissertation, The University of Nebraska-Lincoln).
- Puri, H., Ikuze, E., Ayala, J., Rodriguez, I., Kariyat, R., Louis, J., and Grover, S. 2023. Greenbug feeding-induced resistance to sugarcane aphids in sorghum. *Front. Ecol. Evol.* 11:1105725. doi: 10.3389/fevo.2023.1105725
- Rajendran, P., Bharathidasan, R., and Sureka, I. 2017. Phytochemical Screening GC-MS and FT-IR Analysis of Sugarcane Juice. *Int J Pharma Res Health Sci.* 5 (6): 1962-67 doi:10.21276/ijprhs.2017.06.12
- Rajendran, P., Bharathidasan, R., and Sureshkumar, K. 2017. GC-MS Analysis of Phyto-components in raw and treated Sugarcane Juice. *Int.J.Curr.Microbiol.App.Sci.* 6(7): 51-61.

- Ramos, O. F., Smith, C. M., Fritz, A. K., and Madl, R. L. 2017. Bird-cherry oat aphid (*Rhopalosiphum padi*) feeding stress induces enhanced levels of phenolics in mature wheat grains. *Crop Sci.* 57:2073–2079.
- Rao, M. J., Ahmed, U., Ahmed, M. H., Duan, M., Wang, J., Wang, Y., and Wang, L. 2021. Comparison and quantification of metabolites and their antioxidant activities in young and mature leaves of sugarcane. *ACS Food Science & Technology.* 1(3): 362-373.
- Rao, M. J., Ding, F., Wang, N., Deng, X., and Xu, Q. 2018. Metabolic mechanisms of host species against *Citrus Huanglongbing* (Greening Disease). *Crit. Rev. Plant Sci.* 37: 496–511.
- Rao, M. J., Duan, M., Yang, M., Li, M., and Wang, L. 2022. Sugarcane rind secondary metabolites and their antioxidant activities in eleven cultivated sugarcane varieties. *Sugar Tech.* 24(5): 1570-1582.
- Rao, M. J., Xu, Y., Huang, Y., Tang, X., Deng, X., Xu, Q. 2019. Ectopic expression of citrus UDP-GLUCOSYL TRANSFERASE gene enhances anthocyanin and proanthocyanidins contents and confers high light tolerance in Arabidopsis. *BMC Plant Biol.* 19: 603
- Rao, M. J., Xu, Y., Tang, X., Huang, Y., Liu, J., Deng, X., Xu, Q. 2020. CsCYT75B1, a citrus CYTOCHROME P450 gene, is involved in accumulation of antioxidant flavonoids and induces drought tolerance in transgenic Arabidopsis. *Antioxidants.* 9: 161.
- Raphael, E. 2012. Phytochemical constituents of some leaves extract of Aloe vera and *Azadirachta indica* plant species. *Global Advanced Research Journal of Environmental Science and Toxicology.* 1(2): 014-017.
- Rawat, L. S., Maikhuri, R. K., Bahuguma, Y. M., Jha, N. K., and Phondani, P. C. 2017. Sunflower allelopathy for weed control in Agricultural systems. *Crop Science Biotechnology.* (1): 45-60.
- Rice, E.L. 1984. *Allelopathy*. Academic Press, Orlando, F.L. 422pp.
- Risebrow, A., and Dixon, A. F. G. 1987. Nutritional ecology of phloem-feeding insects, pp. 421-448. In F. Slansky and J. G. Rodriguez [eds.], *Nutritional ecology of insects, mites, spiders, and related invertebrates*. Wiley-Interscience Publication.

- Sakadzo, N., Mubvuma, M., Mukanga, C., Mabveni, A. R. S., and Musundire, R. 2024. Physiological response as a tolerance mechanism to yellow sugarcane aphid (YSA) (*S. Flava* Forbes) herbivory on selected commercial sugarcane varieties (*S. Officinarum*). *Int J Trop Insect Sci.* 44(3):1-16 <https://doi.org/10.1007/s42690-024-01293-7>
- Sakihama, Y., and Yamasaki, H. 2002. Lipid peroxidation induced by phenolics in conjunction with aluminum ions. *Biol Plant.* 45:249–254.
- Salgado, L. D. 2020. Characterization of Resistance to Two Crambid Stem Borers Among Louisiana Sugarcane Cultivars. Louisiana State University and Agricultural and Mechanical College
- Saljoqi, A. U. R., Afridi, M. K., Khan, S. A., and Rehman, S. 2006. Effect of six plant extracts on rice weevil *Sitophilus oryzae* in stored wheat grains. *Journal of Agric. Biological Science.* 1:1-5.
- Sanarat, P., Motham, P., Thonpho, A., and Srihanam, P. 2021. Phytochemicals and Antioxidant Activity in Sugarcane (*Saccharum officinarum* L.) Bagasse Extracts. *Suan Sunandha Science and Technology Journal.* 08(02): 26-35.
- Shafiq-atikah, M. K., Mahyudin, N. A., Abas, F., Nur-syifa', J., and Nor-khaizura, M. A. R. (2022). The proximate composition and metabolite profiling of sugarcane (*Saccharum officinarum*) molasses. *Malays Appl. Biol.* 51(2): 63-68. <https://doi.org/10.55230/mabjournal>
- Shankar, P., and Yinghua H. 2021. Elevated production of reactive oxygen species is related to host plant resistance to sugarcane aphid in sorghum. *Plant Signaling and Behavior.* 16:2, 1849523, DOI: 10.1080/15592324.2020.1849523
- Sharma, A. R., and Satsangi, S. 2013. Potential allelopathic Influence of Sunflower (*Helianthus annuus* L.) on Germination and Growth behaviour of two weeds in- Vitro Conditions. *international Journal of Biotechnology and Bioengineering Research.* 4 (5): 421-426.
- Sharma, H. C., Sujana, G., and Rao, D. M. 2009. Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeonpea. *Arthropod-Plant Interact.* 3:151-61; [http:// dx.doi.org/10.1007/s11829-009-9068-5](http://dx.doi.org/10.1007/s11829-009-9068-5).

- Shoala, T., Edwards, M. G., Knight, M. R., and Gatehouse, A. M. 2018. OXI1 kinase plays a key role in resistance of Arabidopsis towards aphids (*Myzus persicae*). *Transgenic research*. 27:355-366.
- Silva, R. J. N. da., Guimaraes, E. R. Garcia, J. F. Botelho, P. S. M., Ferro, M. I. T., Mutton, M. A., and Mutton, M. J. R. 2005. Infestation of froghopper nymphs changes the amounts of total phenolics in sugarcane. *Sci. Agric. (Piracicaba, Braz.)*. 62: 543-546.
- Singh, A., Lal, U. R., Mukhtar, H. M., Singh, P. S., Shah, G., and Dhawan, R. K. 2015. Phytochemical profile of sugarcane and its potential health aspects. *Pharmacogn. Rev*, 9: 45–54.
- Singh, A., Lal, U. R., Mukhtar, H. M., Singh, P. S., Shah, G., and Dhawan, R. K. 2014. Phytochemical profile of sugarcane and its potential health aspects. *Pharmacognosy Reviews*, 9(17), 45-54.
- Singh, S., Kaur, I., and Kariyat, R. 2021. The multifunctional roles of polyphenols in plant-herbivore interactions. *Int. J. Mol. Sci.* 22:1442. doi: 10.3390/IJMS22031442
- Smith, C. M., Liu, X., Wang, L. J., Liu, X., Chen, M. S, Starkey, S., and Bai, J. 2010. Aphid feeding activates expression of a transcriptome of oxylipin-based defense signals in wheat involved in resistance to herbivory. *Journal of Chemical Ecology*. 36: 260–216.
- Smith, C., and Clement, S. L. 2012. Molecular bases of plant resistance to arthropods. *Annu. Rev. Entomol.* 57: 309–328. doi: 10.1146/annurev-ento-120710-100642
- Solovchenko, A. 2010. Optical Screening-Based Mechanisms. In *Photoprotection in Plants*; Springer-Verlag: Berlin. Vol. 14.
- Srihanam, P., Thonpho, A., Sanarat, P., and Motham, P. (2021). Phytochemicals and antioxidant activity in sugarcane (*Saccharum officinarum* L.) bagasse extracts. *Suan Sunandha Science and Technology Journal*. 8(2): 26-35.
- Thi, H. L., Hyuk, P., and Ji, P. Y. 2015. Allelopathy in *Sorghum bicolor* (L.) Moench: a review on environmentally friendly solution for weed control. *Res. Crops*. 16(4): 657–662.
- Tinky, S., Binjita, P., Bishnu, K. S., Gayatri M. K, Rojeena, T., Nabin, Karki. 2020. Phytochemical screening of medicinal plants and study of the effect of phytoconstituents in seed

germination. *Tribhuvan University Journal*. 35(2): 1-11. doi:
<https://doi.org/10.3126/tuj.v35i2.36183>

Treutter, D. Significance of flavonoids in plant resistance: *A review*. *Environ. Chem. Lett.* 4: 147–157.

Urbanska, A., Leszczynski, B., Tjallingii, W. F., and Matok, H. 2002. Probing behaviour and enzymatic defense of the grain aphid against cereal phenolics. *Electronic Journal of Polish Agricultural Universities*. 5(2): 02.

Usha Rani, P., and Jyothsna Y. 2010. Biochemical and enzymatic changes in rice as a mechanism of defense. *Acta Physiol Plant*. 32:695-701; <http://dx.doi.org/10.1007/s11738-009-0449-2>.

Vimalkumar, C. S., Hosagaudar, V. B., Suja, S. R., Vilash, V., Krishnakumar, N. M., and Latha, P.G. 2014. Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of *Olea dioica* Roxb infected with the rust fungus *Zaghouania oleae* (EJ Butler) Cummins and non-infected plants. *Journal of Pharmacognosy and phytochemistry*. 3(4).

Wang, Z., Nur, F. A., Ma, J., Wang, J., and Cao, C. 2019. Effects of poplar secondary metabolites on performance and detoxification enzyme activity of *Lymantria dispar*. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 225: 108587.

Wani, J. A., Achur, R. N., and Nema, R.K. 2011. Phytochemical screening and aphrodisiac activity of *Asparagus racemosus*. *International Journal of Pharmaceutical sciences and Drug Research*. 3(2): 112-115.

War, A. R, Paulraj, M. G., War, M. Y., and Ignacimuthu, S. 2011b. Herbivore- and elicitor-induced resistance in groundnut to Asian armyworm, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Plant Signal Behav.* 6:1769-77; PMID:22042128; <http://dx.doi.org/10.4161/psb.6.11.17323>.

Way, M. J., Conlong, D. E., Martin, L. A., Mcfarlane, S. A., Stranack, R., Keeping, M. G., and Rutherford, R. S. 2014. First record of yellow sugarcane aphid, *Sipha flava* (Homoptera: Aphididae), in the South African Sugarcane Industry. *Proc S Afr Sug Technol.* 87: 53 – 57.

Wu, G., Shortt, B., Lawrence, E., Leon, J., Fitzsimmons, K., Levine, E., Raskin, I., and Shah, D. M. 1997. Activation of host defense mechanisms by elevated production of H₂ O₂ in transgenic plants. *Plant Physiol.* 115:427-435. doi:10.1104/pp.115.2.427.

- Xu, Yi., Guo, H., Geng, G., Zhang, Q., and Zhang, S. 2021. Changes in defense-related enzymes and phenolics in resistant and susceptible common wheat cultivars under aphid stress. *Acta Physiologiae Plantarum*. 43:36.
- Ye, C., Zheng, S., Jiang, D., Lu, J., Huang, Z., Liu, Z., and Li, J. 2021. Initiation and execution of programmed cell death and regulation of reactive oxygen species in plants. *International Journal of Molecular Sciences*. 22(23):12942.
- Zhang, K. X., Li, H. Y., Quandahor, P., Gou, Y. P., Li, C. C., Zhang, Q. Y., Haq, I. U., Ma, Y., Liu, C. Z. 2022. Responses of Six Wheat Cultivars (*Triticum aestivum*) to Wheat Aphid (*Sitobion avenae*) Infestation. *Insects*. 13: 508. <https://doi.org/10.3390/insects13060508>
- Zhang, X., Sun, X., Zhao, H.P., Xue, M., and Wang, D. 2017. Phenolic compounds induced by *Bemisia tabaci* and *Trialeurodes vaporariorum* in *Nicotiana tabacum* L. and their relationship with the salicylic acid signaling pathway. *Arthropod Plant Interact*. 11: 659–667.
- Zhang, Y., Fan, W., Kinkema, M., Li, X., and Dong, X. 1999. Interaction of NPR1 with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the PR-1 gene. *Proc Natl Acad Sci USA*. 96:6523-6528. doi:10.1073/pnas.96.11.6523.
- Zhu, Y. F., Chen, M., and Liu, C. Z. 2011. Variations of several substances in the seedlings of five oat cultivars infested by aphids. *Plant. Prot*. 37: 55–58.
- Zia, A., Aslam, M., Naz, F., and Ilyas, M. 2011. Bio-efficacy of some plant extracts against chickpea beetle, *Callosobrucus chinensis* L. attacking chickpea. *Pakistan Journal of Zoology*. 43:733-737.

CHAPTER SEVEN

Reconnoitering commercial sugarcane (*S. officinarum*) germplasm's plant resistance index (PRI) categories in response to the incursion of YSA (*S. flava*) in the sugar industry of Zimbabwe

Abstract

The study aimed at examining tolerance, antixenosis, and antibiosis expression in seven sugarcane (*Saccharum officinarum*) (L.) germplasm for Yellow Sugarcane Aphid (YSA) (*Sipha flava*) resistance. A 7×2 factorial design, laid out in a complete randomized block design and replicated four times was used. Variety was the first factor, with seven treatment levels (96-1107, 00-1165, ZN 10, ZN 8, ZN 9, ZN 3L, and N14). Aphid infestation was a second factor, with two treatments (un-infested (control) and infested). Data was collected on tolerance parameters (chlorophyll loss, net photosynthesis, transpiration and stomatal conductance), antibiosis parameters (total protein content, total soluble sugars, nitrogen, phosphorus, potassium, calcium, and magnesium) and on antixenosis parameters (trichomes and aphid numbers). GenStat 18th edition was used for statistical analysis and Fisher's Least Significance Difference ($p < 0.05$) was used for mean treatment separation. Different sugarcane varieties varied significantly ($p < 0.05$) on resistance mechanisms to YSA. Three levels of PRI (tolerance, antibiosis, and antixenosis) was expressed in 00-1165 sugarcane accession, while varieties; ZN 8, ZN 9 and ZN 3L showed tolerance and antixenosis. Interaction effects ($p < 0.001$) between treatments were observed on gas exchange, total soluble sugars, chlorophyll loss, phenol content, and flavonoid content. Variety 00-1165 did not show any differences in chlorophyll loss between the aphids infested and uninfested treatments. Similarly, variety ZN 3L did not show significant variation in flavonoid concentration between the aphids infested and uninfested treatments. Indeed, the study clearly shows that sugarcane varieties have resistance mechanisms to YSA.

Key words: *Sipha flava*, sugarcane, antixenosis, tolerance, antibiosis

7.1 Introduction

The yellow sugar aphid (YSA) (*Sipha flava*) was first detected in sorghum (*Sorghum bicolor* L.) in the United States (Hall, 2001). Its continental range has been progressively expanding and is currently being reported in seven African countries (Morocco, South Africa, Zimbabwe, Swaziland, Malawi, Zambia, Tanzania, and Kenya). Since the arrival of the Yellow Sugarcane Aphid (*Sipha flava*) in 2014 (Zimbabwe Sugar Association Experiment Station (ZSAES) unpublished), sugarcane production in Zimbabwe has been facing a serious economic threat. Yield estimates losses in Zimbabwe in terms of percentage total cane per hectare (TCH) due to *S. flava* in the following seasons were as follows: 2019 (7.2 %), 2021 (5.1 %), 2022 (7.6 %), and 2023 (5.4 %) (ZSAES, unpublished).

Sipha flava is capable of sucking sap from leaves, as it has a tendency to colonize the underside of lower leaves, and the colonies move upward, eventually making their way to the newly formed leaves similar to the sugarcane aphid (SCA) feeding mechanism reported by some authors (Singh *et al.*, 2004; Akbar, 2009; Bayoumy *et al.*, 2016; Paudyal, 2019; Paudyal *et al.*, 2019). Colonies increase rapidly in warm dry weather owing to their sexual polymorphism (Nuessly, 2005; Way *et al.*, 2014; Halbert *et al.*, 2013) causing plant injury from significant loss of plant assimilates, and heavily infested leaves turn yellowish and reddish. This reddish leaf discoloration arises from the injection of a toxin, which leads to chlorosis and necrosis (Breen and Teetes, 1986; Webster, 1990; Akbar, 2009). Another concern associated with direct YSA feeding is the possibility of spreading sugarcane mosaic potyvirus (Hall and Bennett, 1994; Blackman and Eastop, 2000).

The use of resistant sugarcane germplasm as a defense against *S. flava* is anticipated to form the foundation for future sustainable management initiatives in the sugarcane industry. Resistance refers to a plant's heritable characteristics that influence the extent of damage an insect produces. According to Mbulwe (2017), this mechanism can either be constitutive or initiated by eating insects. Painter (1951), Horber (1980), and Smith (2005) documented three mechanisms of plant resistance: tolerance within a host plant, antixenosis (non-preference), and antibiosis. Balikai (2001) emphasized that insects depend on overlapping mechanisms in addition to morphological and metabolic aspects. Stout (2013) divided these into two categories: resistance (antixenosis)

and tolerance. While antixenosis is an insect non-preference response of aphids toward particular genotypes that are affected by physical barriers, antibiosis is a physiological response (Painter, 1951; Mbulwe, 2017). Kogan (1994) also identified other antixenosis components, such as stimulating, suppressive, and discouraging actions of host plants. Antixenosis is a key category of crop aphid resistance since it influences the early stage of plant infestation, according to a number of studies (Gallun *et al.*, 1966; Webster *et al.*, 1987; Dixon *et al.*, 1990; Webster *et al.*, 1994; Flinn *et al.*, 2001, Andarge and Westhuizen, 2004; Mbulwe, 2017; Paudyal, 2019; Paudyal *et al.*, 2019).

Furthermore, Painter (1951) defined antibiosis, another type of plant resistance, as a detrimental impact on insects caused by feeding on resistant plants. Dixon (1998) reported adverse effects on arthropod insects, such as a reduced capacity for reproduction, shorter lifespans, higher mortality, and reduced growth. According to Smith (2005), reduced fertility is a consequence of antibiosis. Additionally, Painter (1951) discovered tolerance to be the third mechanism of plant resistance. This mechanism is the ability of a plant to grow and spread even in the face of an infestation of pests that are almost equal in number to those that cause harm to a susceptible host.

Chemical, biological, and cultural control is complemented by host plant resistance, which increases the efficacy of these control methods (Peters and Starks, 1990; Knutson *et al.*, 2016; Mbulwe, 2017). According to van Emden (2007), plant resistance is an effective management tool to protect crop plants from insects. Girma *et al.* (1998) advanced the knowledge of the relationship between chlorophyll loss and the degree of tolerance of sorghum to aphids. Several studies have measured chlorophyll loss to determine the relationship between aphids and plant tolerance (Flinn *et al.*, 2001; Lage *et al.*, 2003; Akbar, 2009; Paudyal, 2019; Paudyal *et al.*, 2019; Sakadzo *et al.*, 2024).

The aim of this study was to use objective screening techniques that incorporate the three mechanisms of plant resistance to identify the sources of *S. flava* resistance in sugarcane. The discovery of resistant sugarcane genotypes in this study will facilitate the creation of economically viable sugarcane varieties for the sugar industry in Zimbabwe. This study used the methods adopted from previous studies (Inayatullah *et al.*, 1990; Paudyal, 2019; Paudyal *et al.*, 2019) which they employed for plant resistance evaluation in sorghum. The influence of each

resistance category was combined to generate the plant resistance index (PRI). Several authors (Webster *et al.*, 1987; Inayatullah *et al.*, 1990; Webster and Porter, 2000; Lage *et al.*, 2003; Akbar, 2010; Razmjou *et al.*, 2012; Paudyal, 2019; Paudyal *et al.*, 2019) defined Plant Resistance Index (PRI) as a measure of the total resistance expression that combines three resistance categories: antibiosis, antixenosis, and tolerance.

7.2 Materials and methods

7.2.1 Experimental procedure

Seven sugarcane varieties were selected and grown according to the Zimbabwe Sugar Production Manual (Clowes and Breakwell, 1998) (Table 7.1). Conceptual methodology on three major mechanisms of host plant insect defense, modified from Manikandan (2019), was used for resistance evaluations (Figure 7.1).

Table 7.1: Sugarcane genotypes evaluated for resistance against YSA

Entry	Genotype	Maturity	Cane t/ha	ERC %	ERC t/ha	Smut	Lodging	Stalks/ha	YSA/visual symptoms
1	ZN 8	Early	158	12.9	19.7	resistant	susceptible	124000	Less susceptible
		Mid	156	15.2	23.3	resistant	susceptible	124000	
		Late	116	15.6	18.2	resistant	susceptible	124000	
2	ZN 9	Early	178	11.9	21.1	susceptible	susceptible	118000	Less susceptible
		Mid	160	15.4	24.6	susceptible	susceptible	118000	
		Late	113	15.6	17.7	susceptible	susceptible	118000	
3	ZN 10	Early	173	12.7	22.1	resistant	susceptible	144000	Susceptible
		Mid	165	14.7	24.2	resistant	susceptible	144000	
		Late	143	15.1	21.4	resistant	susceptible	144000	
4	ZN 3L	Early	135	14.9	19.5	resistant	tolerant	121000	Less susceptible
		Mid	132	15.6	20.5	resistant	tolerant	121000	
		Late	100	15.2	15.2	resistant	tolerant	121000	
5	N 14	Early	165	12.3	20.1	resistant	tolerant	118000	Susceptible
		Mid	156	14.1	21.8	resistant	tolerant	118000	
		Late				moderate			
6	00-1165	Early	131.2	14.4	18.9	resistant	susceptible	135800	Moderate resistant
		Mid							
		Late							
7	96-1107	Early	152.5	14	21.4	moderate	tolerant	120000	Susceptible
		Mid							
		Late							

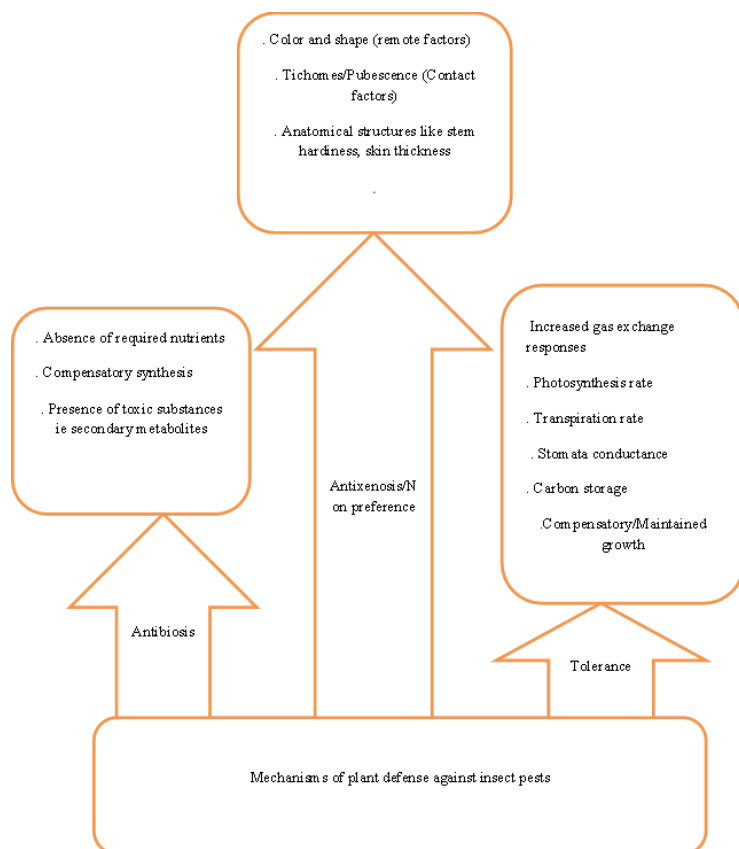


Figure 7.1: Conceptual methodology on three major mechanisms of host plant insect defense. Modified from Manikandan (2019)

7.2.2 Host plant resistance evaluations

7.2.2.1 Antixenosis test

Seven sugarcane genotypes with characteristics stated in Table 1. Planting date of plant cane was on the 24th of February 2023. Two preleased varieties namely; 00-1165 (low susceptibility to YSA) and 96-1107 (highly susceptible to YSA), were also considered for evaluations. The seven sugarcane varieties were examined for trichomes per mm² on the abaxial surfaces. Trichome density was determined using a light microscope, according to the protocols of Batyrshina *et al.* (2020b) and Singh *et al.* (2021). Aphids were physically counted once at each sampled leaf position (leaf-1, leaf-2, and leaf-3) when the sugarcane crop reached the three-leaf stage (one to two weeks after germination and natural aphid infestation). The number of trichomes was determined on an area covering 1 cm² on the middle sections of leaf-1, leaf-2, and leaf-3, from untreated and aphid-infested plants. A light microscope with a 10 × objective and 10 × ocular lenses was used following the protocol of Moghadam *et al.* (2013).

7.2.2.3 Data analysis

Comparison of number of aphids in the antixenosis test was conducted following square root transformation to correct for heterogeneous variances and the lack of normality in the aphid numbers. GenStat 18th version was used under a two-way analysis of variance (ANOVA) to test the effects of leaf position, accession, treatment, and their interaction (leaf position × accession × treatment). Fisher's Least Significance Difference (LSD) test ($p < 0.05$) was used in both two way and one-way ANOVA to test for differences between the accessions.

7.2.2.4 Tolerance test

A free-choice test was conducted in order to determine the relative level of tolerance among the sugarcane germplasm entries according to the protocol by Sakadzo *et al.* (2024). The crop that was cultivated on February 24, 2023, provided the initial experimental data, which was collected every two weeks. On September 22, 2023, the cutback crop provided the data for the second experiment. All genotypes were compared with known moderate resistant (00-1165) and the most susceptible genotype ZN 10. Aphid damage rating through consideration of chlorophyll loss, gas exchange responses (photosynthesis, transpiration and stomata conductance) was used to assess the level of tolerance in each of the sugarcane entries 30 days after YSA infestation. Chlorophyll loss was determined with an aid of a handheld spectrophotometry device (SPAD) meter (Sakadzo *et al.*, 2024). Three readings from each leaf were taken for YSA un-infested (control) and infested (unsprayed) plots. Alice acetamiprid pesticide was sprayed after every two weeks in aphid sprayed plots (300g/250 liters water). Measurements were for the first and second experiment in both plant and first ratoon sugarcane (≤ 3 months). These readings were averaged and a SPAD chlorophyll index (% chlorophyll loss) was calculated using the mean SPAD based on the formula: $(C-I)/C$ modified from the one proposed by Deol *et al.* (1997); Akbar (2010); Paudyal (2019); Paudyal *et al.* (2019), and Sakadzo *et al.* (2024) where C is the SPAD measurement from the YSA un-infested and I is the SPAD measurement from YSA infested sugarcane leaves. Gas exchange responses were measured from YSA infested and un-infested (control) sugarcane. Total Visible Dewlap (TVD) leaf for all seven genotypes using a portable photosynthetic system (CIRAS-3 DC CO₂/H₂O Gas Analyzer) after every two weeks (Sakadzo *et al.*, 2024). The TVD leaf was selected because it is the photosynthetic active leaf which utilises all the nutrients in the plant. Collected data included net photosynthesis rate (A , $\mu\text{molm}^{-2} \text{s}^{-1}$), transpiration rate (E , $\text{mmolm}^{-2} \text{s}^{-1}$), and stomatal conductance (g_s , $\text{mmolm}^{-2} \text{s}^{-1}$) (Sakadzo *et al.*, 2024).

7.4.2.5 Data analysis

Two way and oneway analysis of variance was used. GenStat 18th version was used for analysis to examine differences in chlorophyll content, net photosynthesis rate, stomatal conductance, and aphid number. Parameters were analyzed using 7 (sugarcane genotypes) × 2 (YSA infested and control (sprayed) replicates four times. Means were separated using Fisher's Protected Least Significance at 5 %.

7.4.2.6 Antibiosis (No-choice experiments) test

No-choice experiment 1. On February 24, 2023, a field experiment was conducted at the Zimbabwe Sugar Association Experiment Station (ZSAES). On September 22, 2023, to commemorate the start of the second season, sugarcane was cut back to analyze the effect of the plant's biochemical traits, providing some resistance against YSA infestation of the sugarcane crop 90 days after the natural YSA infestation. For each biochemical trait, four replicates, including infested and control (uninfested) leaves, of seven sugarcane cultivars were collected. The leaves were placed in plastic bags and taken to the laboratory for oven drying at 70°C for 24 hours. The samples that had been dried in the oven were cooled, pulverized in a Willey grinding mill using a 1 mm screen, and then moved to a bow mill for additional grinding. The study employed a complete randomized block design (CRBD) with four replications using a 7 × 2 factorial design. The first factor under seven levels (00-1165, 96-1107, ZN 10, ZN 8, ZN 9, ZN 3L, and N14) was genotype, whereas the second factor under two levels (un-infested (control) and infested) was aphid treatment. Data on aphid number, nitrogen (N), phosphorous (P), potassium (P), calcium (Ca), magnesium (Mg), total protein content (TPC), total chlorophyll content (TCC), and total soluble sugars (TSS) were collected. Weekly aphid assessments were conducted on five randomly chosen primary tillers from both aphid-infested and control (un-infested/sprayed) plots. Aphids were physically counted on all leaves of each tiller 90 days after natural YSA infestation during foliar sample collection to correlate aphid numbers with soluble sugar, percentage nitrogen change, and total chlorophyll content. *No-choice experiment 2.* For this investigation quantification of phenols and flavonoids was done from the leaves used in determining biochemical parameters by using Gas Chromatography Mass Spectrometry (GCMS). Three-month-old leaves of seven sugarcane cultivars; ZN 10, N14, ZN 3L, ZN 8, 96-1107, ZN 9, and 00-1165 were used following the protocol by Rao (2021). Following a natural

aphid infestation, the YSAinfested and uninfested sugarcane leaves were defoliated from the plant, cleaned under running tap water to remove dust, dried in an oven at 60°C for 18 hrs, crushed into small pieces, and stored at room temperature in a sealed bag. The leafy crude extracts were extracted using methanol. After weighing and adding one gram of crushed leaves (1g), the volumetric flask was completely filled with methanol (25 mL) and shaken for 48 hours. For every sample, three duplicate extractions were performed. The solvents were thereafter extracted from the extracts using a rotary evaporator. After taking the extract powder out of the round-bottom bottle, we weighed it. Last, the exact dry weight of the crude extracts were measured before adding methanol (1:10 w/v) to dissolve the obtained crude extract and store it in a freezer at -20°C.

7.4.2.7 Data analysis

The GenStat 18th edition was utilized for analysis to examine variations in aphid number, total protein content, total chlorophyll content, total soluble carbohydrates, and nitrogen, phosphorus, potassium, calcium, and magnesium for each measurement. A 7 (sugarcane genotypes) × 2 (YSA-infested and control (sprayed) replicated four times) was used to analyze the parameters. Fisher's Protected Least Significance test was used to separate the means at 5%.

7.4.2.8 Plant Resistance Index (PRI)

PRI was determined by using statistically analyzed data from the three resistance mechanisms. PRI was used as adopted from several authors (Webster *et al.*, 1987; Inayatullah *et al.*, 1990; Webster and Porter, 2000; Lage *et al.*, 2003; Razmjou *et al.*, 2012; Akbar *et al.*, 2010; Paudyal, 2019; Paudyal *et al.*, 2019) have defined plant resistance index (PRI) as a measure of total resistance expression that takes into account three resistance categories: antibiosis, antixenosis, and tolerance. To normalize the data, it was divided by the resistance category from seven sugarcane genotypes (selected for the antibiosis assay; no-choice experiment 1 and 2), tolerance (X=damage ratings (chlorophyll loss and gas exchange responses)), antixenosis, (Y= aphid number rating from trichome density No-choice experiment 1), by the number of aphids per plant (Y), antibiosis (Z = biochemical response No choice experiment 1 + phytochemical resistance from No choice experiment 2). By doing so, this has allowed us to normalize our data by every category's highest mean. Utilizing the modified formula $PRI = 1/XYZ$ from Razmjou *et al.* (2012); Akbar *et al.* (2010), Paudyal (2019), and Paudyal *et al.* (2019), it was determined by using normalized values for X (tolerance index), Y (antixenosis index), and Z (antibiosis index).

7.5 Results

7.5.1 Tolerance

Table 7.3 shows gas exchange responses to YSA feeding. There were highly significant interactions ($p < 0.001$) between sugarcane genotype and aphid infestation. The nature of the interaction was in such a way that genotype 00-1165 did not show significant differences on chlorophyll loss between YSA-infested and uninfested treatments, while these genotypes (96-1107, N14, ZN 3L, ZN 8, and ZN 10) showed significant variation ($p < 0.001$) in chlorophyll content between YSA-infested and uninfested plots in the free-choice tolerance assay. Chlorophyll loss is an indicator of aphid induced stress showing the extent of damage or tolerance of the genotypes. The seven sugarcane genotypes' varying levels of gene expression and susceptibility to YSA-induced response account for this tolerant variance. In the 2nd experiment, ZN 10, had the highest chlorophyll index rating (21.35 %) in November (Table 7.2) when compared to the 1st experiment (5.86 %) in April (Table 7.2). YSA populations are most active during warm conditions due to their reproductive polymorphism as highlighted by a high chlorophyll index. High chlorophyll index rating signifies that the genotype is more susceptible to YSA damage. The damage ratings of the susceptible check (ZN 10) and 00-1165 differed significantly ($p < 0.001$), indicating that ZN 10 was more susceptible while 00-1165 was tolerant. With chlorophyll loss of ≤ 4 , the genotype 00-1165 demonstrated a high expression of resistance and was much lower than the susceptible checks; ZN 10, 96-1107, and N14, which had damage ratings of > 10 . Differences in the SPAD values (YSA control (uninfested) minus infested, expressed as a percentage chlorophyll loss) led to the allocation of ranges based on the results was used to define the resistance classification. For ZN 8, ZN 9, and ZN 3L, moderately resistant genotypes (> 5 to < 10) were found. The genotype is said to be resistant when it maintains a low percentage of chlorophyll loss even in the presence of YSA infestation. It was determined that the sugarcane genotypes; 96-1107, N14, and ZN 10 were sensitive to YSA infestation as indicated by the chlorophyll loss (> 10 %).

Genotype-to-genotype differences in chlorophyll loss from *S. flava* damage were significant, ranging from 1.64 % (00-1165) to 21.35% (ZN 10) (Table 7.2). This protects the ongoing use and cultivation of genotypes that are more sensitive than those that are now included into the cropping system. In the no choice evaluation, the genotypes; 00-1165, ZN 8, ZN 9, and ZN 3L

had chlorophyll losses of less than 10 % indicating tolerance and moderate tolerance. A strong correlation ($r=0.91$) (free-choice assay), $p<0.001$) was found in the regression analysis between aphid number and chlorophyll loss, as the aphid number increases more chlorophyll is lost indicating damage and genetic differences in susceptibility or tolerance. Additionally, there was a positive correlation ($r=0.52$) between photosynthesis and chlorophyll content (SPAD values) (free choice assay; $p=0.004$). This implies that photosynthesis increases with chlorophyll content on different sugarcane genotypes. Results indicated that there were highly significant differences ($p<0.001$) on number of aphids per plant in control and infested treatments on gas exchange responses (photosynthesis, transpiration and stomata conductance). This indicates that sugarcane genotypes exhibit different physiological mechanisms of resistance to YSA. YSA infested plots, 00-1165 recorded the highest compensatory photosynthetic rate (34.12) when compared to the least (13.82) obtained from ZN 10 (Table 7.3). The genotype's ability to sustain photosynthesis in the face of YSA infestation is explained by compensation. Highly significant ($p=0.001$) positive correlation ($r= 0.58$) between photosynthesis and aphid number was observed. In YSA infested plots, 00-1165 scored the highest transpiration rate (4.16) while N14 recorded the lowest (2.86) in the month of December (Table 3). This demonstrates that a genotype can boost transpiration rate to make up for feeding harm caused by YSA. Finally, in aphid infested plots, 00-1165 sugarcane genotype recorded the highest (217) stomata conductance whereas N14 recorded the least (101) in December (Table 7.3). This variation explains that increased stomata conductance influence diffusion of gases and water vapour movement on two critical processes (transpiration and photosynthesis) in the life stage of sugarcane.

Table 7.2: Chlorophyll loss for the sugarcane genotypes infested with YSA in a no-choice (tolerance) assay

Variety	1st experiment			2nd experiment	
	April	May	June	Nov	Dec
00-1165	3.07a	2.18a	1.64a	3.42a	2.80a
ZN 3L	4.07b	3.55b	2.68b	4.80a	4.43b
ZN 8	4.58bc	4.07c	3.19b	6.46b	4.89bc
ZN 9	5.32cd	4.78d	3.84cd	7.89b	5.37c
96-1107	5.35cd	4.82d	4.82d	10.57c	10.42d
N14	5.63d	5.09d	4.10d	13.22d	13.12e
ZN 10	5.86d	5.35d	4.35d	21.35e	15.55f
Pvalue	<0.001	<0.001	<0.001	<0.001	<0.001
Lsd	0.919	0.798	0.683	1.995	0.873
Cv%	12.8	12.6	13.6	13.8	7.3

***Means followed by the same letter are not significantly different at 5 % significance level**

Table 7.3: Gaseous exchange responses for the sugarcane genotypes infested with Yellow Sugarcane Aphid in a no-choice (antibiosis) assay

Variety	Photosynthesis rate					Transpiration rate					Stomata conductance				
	Apr	May	Jun	Nov	Dec	Apr	May	Jun	Nov	Dec	Apr	May	Jun	Nov	Dec
N14	13.81a	11.96a	15.25a	14.02b	15.71b	1.33a	1.42a	1.65a	1.80a	2.86a	135.7a	100.1a	118.5a	99.5a	101.7a
96-1107	17.06b	15.23b	18.04b	16.71c	18.84c	1.58a	1.67a	1.82ab	2.05b	3.02ab	156b	103.2ab	125.8b	102.2ab	104.4ab
ZN 9	22.48c	20.64c	23.46c	22.12cd	24.33d	1.75ab	1.83ab	2.16bc	2.23c	3.40c	165.8c	113.3c	135.8c	112.3c	11c4.2
ZN 10	23.23cd	21.40cd	24.22cd	12.58a	13.82a	2.24b	2.18b	2.41c	2.56d	3.56c	265.8g	217.2f	134.1c	139.6d	140.7d
ZN 8	25.12d	23.28d	26.10d	24.77e	26.94e	2.09b	2.24b	2.32c	2.28c	3.15b	174.2d	108.3bc	163.2d	108.1bc	110bc
ZN 3L	27.65e	25.82e	28.88e	27.30f	29.67f	2.76c	2.84c	2.99d	3.23e	4.16d	214.8f	169.2e	190e	168.2e	169.7e
00-1165	32.50f	30.67f	29.23e	32.15g	34.12g	2.98c	3.06c	2.93d	3.62f	4.11d	193.6e	140.7d	163.2d	216.5f	217f
Pvalue	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Lsd	2.091	2.092	1.947	1.168	1.21	0.4835	0.4844	0.4854	0.1232	0.1969	33.93	6.947	6.0247	6.841	6.71
Cv%	6.1	6.6	5.6	3.7	3.5	15.5	15	14.1	3.3	3.8	1.4	0.4	2.6	3.4	3.3

***Means followed by the same letter are not significantly different at 5 % significance level**

7.5.2 Antixenosis

There was no significant interaction ($p>0.05$) between leaf position and genotype on aphid number. This indicates that sugarcane genotype might be the primary factor controlling aphid resistance rather than leaf position, and it shows that genotype resistance is consistent across different leaf positions. Significant differences ($p<0.001$) were observed in trichome density among the studied genotypes. Trichomes are a crucial component of plant morphology that discourages herbivores; as a result, their density may have created discomfort and changed the feeding preferences of YSA on sugarcane genotypes. ZN 10 had the lowest trichome density across all leaf positions, whereas the 00-1165 sugarcane cultivar had the highest (Table 7.4). Furthermore, ZN 10 had the highest (41) average number of *S. flava* across all the three leaf positions, whereas 00-1165 had the lowest (11) (Table 7.4). Dense trichomes in 00-1165 might have reduced aphid infestation when compared to ZN 10 which had low trichome density. Nonetheless, among the seven genotypes examined, significant variations in the number of aphids were noted (Table 7.5). Based on the high *S. flava*, the sugarcane variety that seemed to be most favored was ZN 10 (susceptible check), where more than 35 aphids were recorded for each leaf position at three leaf stage due to reduced trichome density. Throughout the entire winter and summer seasons, there were fewer aphids per plant in the moderately resistant genotypes ZN 8, ZN 9, and ZN 3L. Other sugarcane genotypes that had significant lower number of aphids than the susceptible check (ZN 10) was N14 and 96-1107.

Table 7.4: Trichome density on different leaf positions in different sugarcane varieties infested with Yellow Sugarcane Aphid

Sugarcane variety	Leaf position one trichome density	Leaf position two trichome density	Leaf position three trichome density
00-1165	93.07g	111.15f	124.90g
ZN 8	78.85f	94.50e	110.88f
ZN 9	70.57e	85.33d	99.28e
ZN 3L	62.92d	76.41c	89.46d
96-1107	51.12c	66.12b	80.20c
N14	46.32b	65.68b	76.40b
ZN 10	20.97a	35.85a	50.98a
Lsd	0.98	0.882	0.7647
Pvalue	<0.001	<0.001	<0.001
Cv%	1.1	0.1	0.6

***Means followed by the same letter are not significantly different at 5 % significance level**

Table 7.5: Aphid number on different leaf positions in different sugarcane varieties infested with Yellow Sugarcane Aphid

Sugarcane variety	Leaf position one aphid number	Leaf position two aphid number	Leaf position three aphid number
00-1165	16.5a	12.50a	6.5a
ZN 8	22.25b	17b	8.5b
ZN 9	25.50c	21c	12.75c
ZN 3L	28.75d	25d	15d
96-1107	33.50e	27e	18e
N14	37f	32f	33.5f
ZN 10	46.50g	39.75g	37.5g
Lsd	1.728	0.5844	0.995
Pvalue	<0.001	<0.001	<0.001
Cv%	3.9	1.6	3.6

***Means followed by the same letter are not significantly different at 5 % significance level**

7.5.3 Antibiosis

7.5.3.1 No-choice Experiment 1

In response to YSA feeding, there were no significant differences ($p>0.05$) in the foliar nutrient (N, P, K, Ca, and Mg composition among the sugarcane genotypes; however, ZN 9, 00-1165, and ZN 8 showed an insignificant rise in nitrogen percentage change ((unsprayed minus sprayed)/ (unsprayed)*100)) in response to YSA infestation. This shows the ability of the genotypes to compensate for chlorophyll loss by producing more nitrogen required for chlorophyll synthesis. The above-mentioned genotypes have high levels of chlorophyll, suggesting a favorable connection between nitrogen and chlorophyll. However, an increase in calcium was recorded on all the genotypes highlighting the role of calcium in plant defense in response to insect infestation. The genotype and biochemical parameters (total chlorophyll content, and soluble sugar content) showed highly significant interaction ($p<0.001$). This demonstrates how gene expression affects biochemical resistance in the sugarcane response to YSA infestation. Differences among genotypes in the no-choice assay were significant ($p<0.001$) on aphid number, total chlorophyll content and soluble sugar content (Table 6). This variation clarifies how biochemical losses brought on by YSA feeding are regulated by genotype resistance ability. The total number of aphids at 90 days post-infestation varied from 300 to 870. In aphid-infested plots, ZN 9 recorded the highest (1.92) chlorophyll content, whereas ZN 10 scored the lowest (0.99). Regression analysis revealed a highly significant ($p<0.001$) positive correlation ($r=0.90$) between aphid number and percentage chlorophyll change. This suggests

that there is a direct and proportional relationship between the variables; in genotypes that are vulnerable, a higher number of aphids feeding on sugarcane results in a bigger percentage change in chlorophyll. Moreover, in aphid-infested plots, 00-1165 sugarcane variety had the highest (25.39) soluble sugar content, while N14 had the lowest (8.13). Additionally, regression analysis showed a strong, highly significant ($p < 0.001$) negative correlation ($r = -0.80$) between aphid number and total soluble sugars. As aphid number increases, soluble sugar decreases among the sugarcane genotypes because aphids feed on plant sap which is rich in soluble sugars, this indicates an inverse and a proportional relationship. In contrast to other genotypes, the resistant 00-1165 genotype exhibits a negligible drop in soluble sugar loss, indicating its ability to tolerate aphid pressure

7.5.3.2 No choice experiment 2

Highly significant interaction ($p < 0.001$) between genotype and secondary metabolites (phenol and flavonoid) content in response to YSA infestation was observed. This interaction shows that different genotypes respond differently to aphid infestation. Additionally, the interaction demonstrates that the genotype determines the effectiveness of the plant's defense against aphids and that secondary metabolites are involved in this defense. In aphid infested plots, ZN 10 recorded the highest (886) aphid number while 00-1165 scored the lowest (306) as significant differences were noted among the sugarcane varieties. This indicates that aphid infestations are more common in genotypes that are more susceptible. In YSA infested plots, 96-1107 sugarcane variety scored the highest (49.20) phenol content while ZN 3L scored the lowest (24.91) (Table 7.7). Elevated phenol concentration suggests a good defense response triggered to reduce aphid pressure. Similarly, the level of flavonoid content was affected by levels of aphid infestation. In YSA infested plots, N14 sugarcane variety scored the highest (6.46) flavonoid content while ZN 3L scored the lowest (1.58) (Table 7.7). However, ZN 3L genotype did not show significant different flavonoid concentration between uninfested (1.51) and infested (1.58) treatments while all other genotypes (ZN 10, ZN 8, ZN 9, N14, 96-1107, and 00-1165) showed significant effect between uninfested and infested plants. This tendency of rising flavonoid content is in line with the previously mentioned higher phenol content as rise is genotype dependent and influenced by aphid infestation. The regression analysis showed highly significant ($p = 0.002$) positive correlation ($r = 0.56$) between percentage change in phenol content and aphid number. This illustrates the direct and proportionate variation, that is, as one variable rises, the other tends to

rise as well. Additionally, findings of the study showed highly significant ($p < 0.002$) positive correlation ($r = 0.69$) between percentage change in flavonoid content and aphid number. This demonstrates that the relationship is strong and indicates a high degree of association in conferring resistance to YSA feeding. Although agronomic practices were carried out in accordance with recommendations, the experiment was conducted in a field rather than a laboratory controlled environment, which may have contributed to the modest results. Replication of treatments was done to cater for other factors that were not taken into account which could also be at play. Table 7.8 show normalized indices of seven sugarcane genotypes' components of resistance against *S. flava* and their PRI.

Table 7.6: Biochemical tolerance in different sugarcane varieties infested with YSA

Variety	N	P	K	Ca	Mg	TPC	TCC	TSS	YSA number Infested
00-1165	2.07	0.2	1.32	0.41	0.2	12.95	1.80e	25.39e	424.5b
96-1107	2.19	0.21	1.21	0.4	0.27	13.69	1.57c	15.87c	569.2c
N14	2.06	0.21	1.2	0.4	0.23	12.86	1a	8.13a	551.2c
ZN 10	2.13	0.24	1.2	0.43	0.27	13.31	0.99a	10.29b	443.2b
ZN 3L	2.18	0.18	1.11	0.37	0.25	13.61	1.62d	14.66c	886e
ZN 8	2.29	0.19	1.38	0.41	0.22	14.31	1.30b	14.92c	306a
ZN 9	2.49	0.22	1.7	0.45	0.26	15.55	1.99f	22.28d	742.5d
Pvalue	0.571	0.695	0.179	0.517	0.257	0.571	<0.001	<0.001	< 0.001
Lsd	0.489	0.076	0.45	0.084	0.066	3.058	0.049	1.59	49.1
Cv%	15	24.7	23.2	13.9	18.4	15	2.3	6.7	5.9

***Means followed by the same letter are not significantly different at 5% significance level**

Table 7.7: Quantified phenol, flavonoid and aphid number

Variety	YSA Infested phenol	YSA infested flavonoid	YSA number Infested
ZN 8	33.76c	3.39b	424.5b
96-1107	49.20g	5.36d	569.2c
ZN 3L	24.91a	1.58a	551.2c
ZN 9	35.46d	4.28c	443.2b
ZN 10	46.56f	6.27f	886e
00-1165	30.55b	6.05e	306a
N14	41.96e	6.46c	742.5d
Pvalue	< 0.001	< 0.001	< 0.001
Lsd	1.04	0.03	49.1
Cv	1.9	0.5	5.9

***Means followed by the same letter are not significantly different at 5% significance level**

Table 7.8: Normalized indices of seven sugarcane genotypes' components of resistance against *S. flava* and their PRI

Sugarcane variety	Tolerance (X)	Antixenosis (Y)	Antibiosis (Z)	PRI
00-1165	0.29	0.27	0.34	37.56
ZN 3L	0.48	0.39	0.62	8.61
ZN 8	0.45	0.48	0.48	9.6
ZN 9	0.5	0.56	0.5	7.1
96-1107	0.64	0.64	0.64	3.8
N14	0.79	0.83	0.83	1.8
ZN 10 (S)	1	1	1	1

PRI is equal to $(1/XYZ)$ divided by the highest mean rating in each category, with X representing the damage rating of sugarcane genotypes in the free-choice assay (Tables 7.2 and 7.3), Y representing the mean number of YSA per plant on different leaf positions (Tables 7.4 and 7.5), and Z representing the damage rating of sugarcane genotypes in the no-choice assay (Tables 7.6 and 7.7).

7.5.4 Plant Resistance Index (PRI)

00-1165 sugarcane genotype scored the highest PRI value of 37.6 while ZN scored the lowest (1), this was obtained from the normalized indices across all the three resistance mechanisms (Table 8). A high PRI shows that a genotype has a broad spectrum multi-layered defense system which makes it less susceptible to YSA damage. Moreover, genotypes such as ZN 8, ZN 3L and ZN 9 had relatively > 5 PRI compared with susceptible check (ZN 10), although they indicated antixenosis and tolerance. Researchers and farmers can create efficient plans for managing YSA and producing sugarcane that is more resistant to this threat by comprehending the PRI in connection to the three mechanisms.

7.6 Discussion

Findings of this study showed that PRI comprise of normalized indices of three resistance mechanisms (antixenosis, antibiosis, and tolerance) of the seven sugarcane genotypes' (00-1165, ZN 8, ZN 9, ZN 10, ZN 3L, N14, and 96-1107) against *S. flava*. According to this study, a high PRI across all resistant mechanisms may be a sign of a genotype with strong resistance, low susceptibility, and useful parents for breeding programs, all of which would increase resistance in offspring. The findings support the study by Paudyal *et al.* (2019), which showed that tolerant plants are more likely to experience less aphid selection pressure. Data from this study indicated that YSA feeding inflicts stress resulting in trichome density variation among sugarcane genotypes resulting in antixenosis resistance. However, the number of aphids decreased in the antixenosis resistant genotype. Sugarcane resistant genotypes showed a strong non preference by YSA which is probably because of their antixenosis property. Dense trichomes might have modified the feeding behavior and preference of YSA resulting in reduced infestation. These findings corroborate those of Boughalleb and Hajlaoui (2011) who noted that an increase in stomata and trichomes was produced by stress based on the anatomical features of leaves. Additionally, Karabourniotis *et al.* (2020) stressed that trichomes are an important feature of plants that need to be taken into account. Furthermore, even though there was no interaction between genotype and leaf position, the results demonstrated that trichomes varied based on leaf position. This study showcased an increased trichome density in relation to leaf position. These findings are in line with those of Leybourne *et al.* (2019); Gyan *et al.* (2020), and Singh *et al.* (2021) who highlighted the impact that trichome density can have based on different leaf positions.

Numerous research, particularly directed to sorghum, have reported on the determination of aphid tolerance in cereal plants based on the assessment of SPAD leaf chlorophyll loss (Deol *et al.*, 1997; Flinn *et al.*, 2001; Lage *et al.*, 2003; Akbar *et al.*, 2010; Paudyal, 2019; Paudyal *et al.*, 2019). On the other hand, physiological tolerance variation was noted on seven commercial sugarcane genotypes examined in a recent study by Sakadzo *et al.* (2024). Plant tolerance is thought to be well indicated by maintaining or compensating reasonably high chlorophyll content and gas exchange responses despite infestation (Lage *et al.*, 2003; Akbar, 2010; Paudyal, 2019; Sakadzo *et al.*, 2024). Reduced yellowing and reddish leaves were observed in genotypes with lower chlorophyll loss (%) (< 5) damage ratings in this study indicating variation in physiological tolerance response to YSA.

This study further confirms previous studies conducted in grain and forage sorghum conducted by Akbar (2009); Armstrong *et al.* (2017); Mbulwe (2017); Paudyal (2019), and Paudyal *et al.* (2019) who established three mechanisms of resistance to the sugarcane aphid (SCA) (*Melanaphis sacchari*). The lack of more research on sugarcane in the presence of YSA has made these assessments necessary. The study's findings on PRI demonstrated that 00-1165 genotype has an advantage over other examined genotypes in that it could fit into the three resistance mechanisms of tolerance, antixenosis, and antibiosis. Possessing all three resistance mechanisms will potentially increase yield during YSA infestation by maintaining a lower selection pressure against YSA outbreak on the resistant genotype. Based on these findings, molecular breeding strategies in the future will rely heavily on this data to isolate the gene that has the overall advantage of reducing YSA populations when screening future sugarcane varieties for resistance. Furthermore, these traits hold significant potential as sources of resistance against *S. flava* in sugarcane breeding initiatives. Moreover, the genotypes ZN 8, ZN 9, and ZN 3L might be interesting choices for resistance management due to their modest levels, because of their high tillering prolificacy, yield and ERC %, they are quite popular among sugarcane farmers. Furthermore, they also show modest levels of antibiosis and antixenosis as compared to the susceptible check (ZN 10). Modest results might be due to the inherent characteristics of the genotypes. In support of this study, Akbar (2009); Armstrong *et al.* (2017); Limaje *et al.* (2018); Paudyal (2019), and Paudyal *et al.* (2019) showed that the resistant

sorghum genotype likewise exhibited significant level of antibiosis and tolerance. Growing a genotype of sugarcane with all three mechanisms; antixenosis, which the YSA does not prefer, antibiosis effect, which lowers the YSA population, and tolerance, which minimizes sugarcane damage will be more advantageous. However, when working with large genotype populations, it is necessary to take into account integrating the use of labor-saving phenotypic approaches, such as the use of portable photosynthetic systems (CIRAS-4) and handheld spectrometry devices (SPAD) to determine tolerance, biochemical analysis using gas chromatography mass spectrometry (GCMS) to determine antibiosis, and image processing to determine antixenosis during early stages of growth. Farmers are primarily concerned with profit making; therefore, the screening process will only focus on genotypes that will generate revenue rather than squandering time and resources on unproductive genotypes.

The PRI observed in this study could be the consequence of host unsuitability, which could explain the significant differences in the total number of aphids among the genotypes of sugarcane that were investigated. This is supported by various authors who suggested that a more accurate picture of the impacts of antibiosis is provided by the number of insects that feed on plants (Webster and Porter, 2000, Lage *et al.*, 2003, Smith, 2005; Akbar *et al.*, 2010; Paudyal, 2019; Paudyal *et al.*, 2019). In addition to this, lower relative aphid number suggests that *S. flava* limited population dynamics may lead to less plant damage as confirmed by studies in sorghum in the presence of SCA (Paudyal, 2019; Paudyal *et al.*, 2019). Sakadzo *et al.* (2024) provided evidence in support of this by demonstrating a comparable pattern in damage ratings among the seven genotypes of sugarcane in the presence of YSA infestation. The PRI provides a thorough foundation for upcoming breeding programs aimed at identifying more tolerant sugarcane genotypes based on the results of this investigation. While there are multiple functional categories of resistance that can exist, the weighted PRI index supports these findings by offering a clear explanation and standardization of the overall expected effects of plant resistance (Webster *et al.*, 1987; Inayatullah *et al.*, 1990; Webster and Porter, 2000; Lage *et al.*, 2003; Razmjou *et al.*, 2012; Mbulwe, 2017; Limage *et al.*, 2018; Paudyal, 2019; Paudyal *et al.*, 2019). It may be more advantageous to control YSA using genotypes that combine tolerance traits that limit plant damage with high PRI that impede population growth. Paudyal (2019) and Paudyal *et al.* (2019) have provided support for the idea that genotypes exhibiting several resistance

categories offer a competitive advantage hence supporting the importance of PRI. In order to protect sugarcane genotypes from YSA biotypes, ongoing screening is required in the sugar industry. This study demonstrates that a vital first step toward more sustainable integrated pest management (IPM) systems is the development of sugarcane that is resistant to YSA.

7.7 Conclusion

Despite being less than 50, the sugarcane genotype 00-1165 was able to score a PRI of 37.6, which combines all three mechanisms of resistance: antixenosis, tolerance, and antibiosis. In addition, ZN 8, ZN 9, and ZN 3L exhibited tolerance and antixenosis resistance mechanisms. The low PRI suggests that the susceptible genotypes, 96-1107, N14, and ZN 10, were unable to demonstrate any of the three mechanisms of resistance.

References

- Akbar, W. 2009. Categorization and Identification of Mechanisms of Sugarcane Resistance to the Sugarcane Aphid (Hemiptera: Aphididae). *LSU Doctoral Dissertations*. 2785. <https://digitalcommons.lsu.edu/gradschooldissertations/2785>
- Akbar, W., Showler, A. T. Reagan, T. E., and White. W. H. 2010. Categorizing sugarcane cultivar resistance to the sugarcane aphid and yellow sugarcane aphid (Hemiptera: Aphididae). *J. Econ. Entomol.* 103: 1431–1437.
- Andarge, A., and Van Der Westhuizen. M. C. 2004. Mechanisms of resistance of lentil *Lens culinaris* Medikus, genotypes to the pea aphid *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae). *Int. J. Tropical Insect Sci.* 24: 249-254.
- Balikai, R. A. 2001. Bioecology and management of the sorghum aphid, *Melanaphis sacchari*. Ph.D. thesis, University of Agricultural Sciences, Dharwad, Karnataka, India.
- Blackman, R. L., and Eastop. V. F. 2000. The aphids, pp. 297-298, pp. 339-340. In R. L. Blackman, and V. F. Eastop [eds.], *Aphids on the world's crops-an identification and information guide*, 2nd ed. John Wiley and Sons, West Sussex, England.
- Boughalleb, F., and Hajlaoui, H. 2011. Physiological and anatomical changes induced by drought in two olive cultivars (cv Zalmati and Chemlali). *Acta Physiologiae Plantarum*. 33: 53-65.

- Breen, J. P., and Teetes, G. L. 1986. Relationships of yellow sugarcane aphid (Homoptera: Aphididae) density to sorghum damage. *Journal of Economic Entomology*. 79(4): 1106-1110.
- Clowes, M. J., and Breakwell, W. L. 1998. Zimbabwe Sugarcane Production Manual. Zimbabwe Sugar Association, Chiredzi, Zimbabwe.
- Dixon, A. F. G. 1998. Aphid ecology an optimization approach. Springer Science and Business Media, London. p. 1-7.
- Dixon, A. G. O., Bramel-Cox, P. J., Reese, J. C., and Harvey, T. L. 1990. Mechanisms of resistance and their interactions in twelve sources of resistance to biotype E greenbug (Homoptera: Aphididae) in sorghum. *J. Econ. Entomol.* 83: 234-240.
- Flinn, M., Smith, C. M., Reese, J. H., and Gill. G. 2001. Categories of resistance to greenbug (Homoptera: Aphididae) biotype 1 in *Aegilops tauschii* germplasm. *J. Econ. Entomol.* 94: 558-563.
- Gallun, R. L., Ruppel, R., and Everson, E. H. 1966. Resistance of small grains to the cereal leaf beetle. *J. Econ. Entomol.* 59: 827-829.
- Goławska, S., Krzyżanowski, R., and Łukasik, I. 2010. Relationship between aphid infestation and chlorophyll content in Fabaceae species. *Acta Biologica Cracoviensia Series Botanica*. 52: 76-80.
- Gyan, N. M., Yaakov, B., Weinblum, N., Singh, A., Cna'ani, A., Ben-Zeev, S., Saranga, Y., Tzin, V. 2020. Variation Between Three Eragrostis tef Accessions in Defense Responses to *Rhopalosiphum padi* Aphid Infestation. *Front. Plant Sci* 11. 598483. doi: 10.3389/fpls.2020.598483
- Halbert, S. E., Miller, G. L., and Ames, L. M. 2013. 0326. The genus *Sipha* Passerini (Hemiptera: Aphididae) in North America. *Insecta Mundi*.
- Hall, D. G., and Bennett, F. D. 1994. Biological control and IPM of sugarcane pests in Florida, pp. 287-325. In D. Rosen, F. D. Bennett, and J. L. Capinera [eds.], Pest management in the subtropics, biological control – a Florida perspective. Intercept Ltd., Andover, UK.

Horber, E. 1980. Types and classification of resistance, pp. 15-21. *In* F. G. Maxwell and P. R. Jennings [eds.], *Breeding Plants Resistant to Insects*. Wiley, New York.

Inayatullah, C., Webster, J. A., and Fargo, W. S. 1990. Index for measuring plant resistance to insects. *Entomologists*. 109: 146–152.

Knutson, A., Bowling, R., Brewer, M. J., Bynum, E., and Porter, P. 2016. The sugarcane aphid: Management guidelines for grain and forage sorghum in Texas. Texas A & M AgriLife Extension and Research. Texas A & M University. <http://ccag.tamu.edu/sorghum-insect-pests>

Lage, J., Skovmand, B., and Andersen, S. B. 2003. Characterization of Greenbug (Homoptera: Aphididae) Resistance in synthetic hexaploid wheats. *J. Econ. Entomol.* 96: 1922–1928.

Leybourne, D. J., Valentine, T. A., Robertson, J. A. H., Pérez-Fernández, E., Main, A. M., Karley, A. J., et al., 2019. Defence gene expression and phloem quality contribute to mesophyll and phloem resistance to aphids in wild barley. *J. Exp. Bot.* 70: 4011–4026. doi: 10.1093/jxb/erz163

Limaje, A., Armstrong, S., Hoback, W., Zarrabi, A., Chad, H., Paudyal, S., and Burke, J. 2018. Antibiosis and Tolerance Discovered in USDA-ARS Sorghums Resistant to the Sugarcane Aphid *Melanaphis sacchari* (Hemiptera: Aphididae). *J. Entomol. Sci.* 53: 230–241.

Manikandan, P. 2019. Chapter-6 Host Plant Resistance: A Fundamental Approach to Pest Management. *Recent Trends in.* 85.

Nuessly, G. S. 2005. Featured Creatures: Yellow Sugarcane Aphid; publication #EENY-354; University of Florida: Gainesville, FL, USA; Available online: http://entnemdept.ufl.edu/creatures/field/bugs/yellow_sugarcane_aphid.htm (accessed on 16 January 2022).

Painter, R. H. 1951. *Insect Resistance in Crop Plants*. Macmillan & Co., New York.

Paudyal, S. 2019. Host plant resistance to the sugarcane aphid (Hemiptera: Aphididae). A Dissertation Submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfillment of the requirements for the Degree of Doctor of philosophy.

- Paudyal, S., Armstrong, J. S., Giles, K. L., Payton, M. E., Opit, G. P., Limaje, A. 2019. Categories of resistance to sugarcane aphid (Hemiptera: Aphididae) among sorghum genotypes. *Journal of Economic Entomology*. 112(4): 1932-1940.
- Peters, D. C., and Starks, K. J. 1990. Pest management systems for sorghum insects. *CRC Hand Book of Pest Management in Agriculture*. 3:549-562.
- Razmjou, J., Mohamadi, P., Golizadeh, A., Hasanpour, M., and Naseri, B. 2012. Resistance of wheat lines to *Rhopalosiphum padi* (Hemiptera: Aphididae) under laboratory conditions. *J. Econ. Entomol.* 105: 592–597. doi: 10.1603/EC11290
- Singh, A., Dilkes, B., Sela, H, Tzin, V. 2021. The Effectiveness of Physical and Chemical Defense Responses of Wild Emmer Wheat Against Aphids Depends on Leaf Position and Genotype. *Front. Plant Sci.* 12:667820. doi: 10.3389/fpls.2021.667820
- Sakadzo, N., Mubvuma, M., Mukanga, C., Mabveni, A.R.S., and Musundire, R. 2024. Physiological response as a tolerance mechanism to yellow sugarcane aphid (YSA) (*S. Flava* Forbes) herbivory on selected commercial sugarcane varieties (*S. Officinarum*). *Int J Trop Insect Sci.* <https://doi.org/10.1007/s42690-024-01293-7>
- Smith, C. M. 2005. Plant resistance to arthropods: Molecular and conventional approaches. Springer Science and Business Media, Dordrecht. p. 19-122.
- Stout, M. J., Brovont, R. A., and Duffey, S. S. 1998. Effect of nitrogen availability on expression of constitutive and inducible chemical defenses in tomato, *Lycopersicon esculentum*. *J. Chem. Ecol.* 24: 945-963.
- van Emden, H. F. 2007. Plant resistance, pp. 447–468. In H. F. van Emden and R. Harrington [eds.], *Aphids as Crop Pests*. CABI Bioscience, Oxfordshire, UK
- Way, M. J., Conlong, D. E., Martin, L. A., McFarlane, S. A., Stranack, R., Keeping, M. G., and Rutherford, R. S. 2014. First record of yellow sugarcane aphid, *Sipha flava* (Homoptera: Aphididae), in the South African sugarcane industry. *Proceeding of the South African Sugar Technology Association*. 87: 53–57.

Webster, J. A., and Porter, D. R. 2000. Reactions of four aphid species on Russian wheat aphid resistant wheat. *Southwest. Entomol.* 25: 83–90.

Webster, J. A. 1990. Yellow sugarcane aphid (Homoptera: Aphididae): detection and mechanisms of resistance among Ethiopian sorghum lines. *J. Econ. Entomol.* 83: 1053-1057.

Webster, J. A., Inayatullah, C., Hamissou, M., and Mirkes, K. A. 1994. Leaf pubescence effects in wheat on yellow sugarcane aphids and greenbugs (Homoptera: Aphididae). *J. Econ. Entomol.* 87: 231-240.

Webster, J. A., Starks, K. J., and Burton, R. L. 1987. Plant resistance studies with *Diuraphis noxia* (Homoptera: Aphididae), a new United States wheat pest. *J. Econ. Entomol.* 80: 944-949.

CHAPTER EIGHT: General discussion

A thorough review of the literature was conducted in order to determine whether there are any differences in the following areas: leaf pubescence (trichomes); biochemical properties impacted by *S. flava* in sugarcane; physiological responses of sugarcane to *S. flava* herbivory; and phytochemical metabolites mediating resistance to YSA in sugarcane varieties. The majority of the research was skewed toward crops other than *S. flava* in sugarcane, insect arthropods, and other aphid species. A promising management method for controlling *S. flava* is to combine biological, chemical, and cultural with sugarcane genotypes resistant to YSA.

The study showed how little is known currently about leaf pubescence's role in providing resistance against YSA in commercial sugarcane cultivars across the globe. Furthermore, a significant morphological characteristic in response to *S. flava* is leaf pubescence. The findings of other authors (Gallun *et al.*, 1966; Roberts *et al.*, 1979; Roberts and Foster, 1983, Sosa, 1990, 1991; White, 1989, 1990; Nuessly, 2005; Nuessly *et al.*, 2010) who decisively revealed that leaf pubescent sugarcane types give resistance to *S. flava* are in agreement with these results.

The SLR of the study found that crops react differentially to biochemical parameters in response to insect infestations. There are currently no independent published data on the effects of YSA on biochemical parameters in sugarcane except for switchgrass. The systematic evaluation of the literature also found that sucking pests have a detrimental impact on the level of phosphorus, total protein, nitrogen, chlorophyll, and soluble sugar. The results of the current study confirm those by Saeedi and Ziaee (2020), who found that whitefly (*Neomaskellia Andropogonis*) in sugarcane lowers the previously described biochemical parameters, which lends support to this.

The majority of the research surveyed in the SLR focused on cereals and other aphid species, ignoring sugarcane and the YSA. Furthermore, the SLR results emphasize that the general physiological characteristics of relevance in sugarcane include chlorophyll content and gas-exchange reactions. Several authors (Ryan *et al.*, 1987; Meyer and Whitlow, 1992; Larson, 1998; Haile *et al.*, 1999; Macedo *et al.*, 2003a; Diaz-Montano *et al.*, 2007; Pierson *et al.*, 2011; Paudyal, 2019) attest to the effects of aphids on physiological parameters because they have the

capacity to modify plant physiological processes, which in turn affects plant growth, development, and yield.

A comprehensive analysis of the literature also demonstrates that sugarcane and its byproducts contain phytochemicals. Furthermore, it has been suggested that phenols protect against herbivorous insects. Changes in total phenols in sugarcane in the presence of froghopper nymphs and white grubs were reported by Silva *et al.* (2005) and Nutt *et al.* (2004), respectively. Furthermore, Akbar *et al.* (2009) noted that insect feeding may cause alterations in the phenolic content in sugarcane. Moreover, feeding of the white pit (*Antitrogus parvulus* Britton) in sugarcane roots resulted in a considerable increase or decrease in the quantity and kind of phenols in 15 sugarcane clones evaluated (Nutt *et al.*, 2004).

This study's primary goal was to identify and classify commercial sugarcane cultivars in response to YSA herbivory in irrigated cropping systems based on three host-plant resistance mechanisms by considering morphology, physiology, and biochemistry. This study found that sugarcane leaves have structures called non-glandular trichomes that resemble epidermal hair. Furthermore, these trichomes varied in relation to leaf position across all the seven sugarcane genotypes. An increase in trichome density resulted in sugarcane resistance to YSA infestation. Finally, the level of resistance was ranked from high to low: 00-1165 > ZN 8 > ZN 9 > ZN 3L > 96-1107 > N14 > ZN 10. These outcomes support the findings by Sosa (1991), who suggested that a crucial morphological characteristic for resistance to YSA may be leaf surface pubescence. On the leaves of other cereals, including barley and wheat, similar patterns of trichome presence were noted (Leybourne *et al.*, 2019; Correa *et al.*, 2020; Batyrshina *et al.*, 2020b). Furthermore, our findings align with the research conducted by some authors (Leybourne *et al.*, 2019; Gyan *et al.*, 2020; Singh *et al.*, 2021) which highlighted the various factors that can impact trichome density.

In terms of biochemical response, YSA feeding enhanced the amount of nitrogen produced in tolerant accessions (ZN 9, 00-1165, and ZN 8). This might be compensatory response of increased nitrogen isotope and nitrogen reductase enzyme activity although this study did not measure the variables. Wilson *et al.* (2011) corroborate similar results on green peach aphid

(*Myzus persicae*) of increased nitrogen and nitrogen reductase. Additionally, Pincebourde and Ngao (2021) proposed that aphids elevated the nitrogen content of leaves in tolerant varieties, with the difference between infested and non-infested leaves increasing with age. In six sugarcane accessions (ZN 8, ZN 9, ZN 10, ZN 3L, N14, and 96-1107), feeding by YSA increased leaf magnesium. Magnesium is required for chlorophyll formation to compensate for chlorophyll bleaching caused by aphid saliva as postulated by Shankar and Yinghua (2021). This might have been as a result of increased Mg^{2+} -ATPase expression although it was not measured. Liu *et al.* (2002) have confirmed that membrane integrity is supported by increased Mg^{2+} -ATPase expression in response to aphid feeding. Conversely, the sugarcane cultivar 00-1165 showed a lower magnesium level. This trend of decreased magnesium may have been brought on by YSA's decreased phloem feeding, which raised the concentration of soluble sugar. Results concur to findings by Chaudhry *et al.* (2021), who suggested that lower phloem loading results in the prevention of sucrose from being transferred from the source to sink tissues.

The findings of the study showed that, all examined sugarcane accessions had higher calcium contents, indicating an induction of calcium defense in sugarcane varieties as a result of YSA infestation. Furthermore, although it was not established, calmodulin (CMLs) may have contributed to the above mentioned response. Findings are consistent with those of Yadav *et al.* (2022), who noted that following a *Spodoptera litura* infestation, soybeans expressed calmodulin-like (CMLs) in a different way. According to Puri (2023), calcium is a crucial secondary messenger that plays a major role in plant defense. Variations in the amount of intracellular free Ca^{2+} in plants affect several physiological functions, such as stress tolerance and cell division (Hepler, 2005).

When compared to the control, the total protein content (TPC) results indicated that the tolerant accessions (ZN 9, 00-1165, ZN 8, and ZN 3L) increased in response to YSA incursion. These results of protein content are correlated to the increased nitrogen content reported in this study. Results confirm with findings by Wilson *et al.* (2011) who indicated increased nitrogen isotope and reduced nitrogen reductase activity. Moreover, the results of the study are consistent with other investigations showing that plant tissues are driven to create particular proteins in response to pathogen or herbivore infestation, although these proteins were not analyzed. These proteins

are referred to as "pathogenesis-related" (PR) proteins (Hildebrand *et al.*, 1989; Hammerschmidt and Nicholson, 1999; Ni *et al.*, 2001).

YSA infestation lowered the amount of total soluble sugars (TSS) in the sugarcane accessions. This pattern of findings indicates that the only food source for YSA in the phloem vessels is sugar. These results confirm those of Dinant *et al.* (2010), who identified significant levels of organic and inorganic acids, vitamins, amino acids, and carbohydrates in the phloem sap, supporting the richness of the phloem. Furthermore, it was observed by Hijaz *et al.* (2016) and Hijaz and Killiny (2014) that the primary constituents of phloem sap are high-volume-consumed sugars and amino acids. In response to YSA feeding, sensitive sugarcane accessions (96-1107, N14, and ZN 10) showed a decrease in chlorophyll loss compared to tolerant sugarcane accessions (00-1165, ZN 9, ZN 8, and ZN 3L). This reduced chlorophyll content is associated with reduced nitrogen content in susceptible sugarcane varieties as supported by earlier discussions. These findings corroborate those of Shankar and Yinghua (2021) who found that aphid saliva is a rich source of hydrolytic enzymes, toxic compounds, and effector proteins that enable plants to detect aphid invasions and may even hasten the production of reactive oxygen species (ROS), which cause chloroplast bleaching and cell death.

YSA infestation reduced gas exchange responses in susceptible sugarcane accessions. When compared to other accessions, 00-1165 showed greater tolerance in aphid-infested plots because of its high photosynthetic rate, transpiration rate, and stomata conductance. The sugarcane accessions ZN 9, ZN 8, and ZN 3L showed a moderate degree of physiological tolerance. Due to their lack of tolerance as evidenced by the drop in conductance, it is possible that stomatal interference lowers photosynthetic rates in sensitive accessions (Meyer and Whitlow, 1992). Furthermore, in stressed, YSA-infested sensitive sugarcane cultivars, abscisic acid (ABA) carbonic anhydrase may have been upregulated by YSA, which could account for the decreased stomata conductance. However, this study did not measure ABA, although the outcomes of this study are consistent with those of Guo *et al.* (2016), who discovered that aphid probing boosted the expression of the carbonic anhydrase an ABA-independent enzyme. The present study's findings that YSA sugarcane-resistant plants seem to be able to make up for damage brought on by YSA feeding by having improved transpiration, stomata conductance, and photosynthetic

integrity. The present results corroborate earlier investigations conducted on different species of aphids (Heng-Moss *et al.*, 2003; Retuerto *et al.*, 2004; Frazen *et al.*, 2007; Gutsche *et al.*, 2009; Akbar *et al.*, 2009; Paudyal, 2019; Paudyal *et al.*, 2019).

YSA feeding induced production of high phenols and flavonoids in sugarcane accessions. Furthermore, this study found that sugarcane accession affected how much was produced in the form of flavonoids and phenols in response to YSA damage. The rate of increase differed considerably between cultivars as found by Zhu *et al.* (2011). N14 sugarcane accession appears to be able to acquire sufficient amounts of phenol and flavonoid content in response to YSA attack in contrast to the other accessions. In addition, variations in the amounts of phenol components and the sensitivity of cultivars of pigeon pea (*Cajanus cajan*) to pod worms (*Helicoverpa armigera*) were reported by Green *et al.* (2003). Furthermore, even though there was a significant and strong positive link between a rise in total phenol content and an increase in flavonoid content, Zhang *et al.* (2022) showed statistically robust relationships between the percent change in flavonoid content and the number of aphids.

PRI of the sugarcane varieties varied in relation to antixenosis, antibiosis and tolerance. This trend might be as a result of variation of genetic responses induced by YSA feeding. This study identified resistance categories (tolerance, antixenosis, and antibiosis) and resistant entries for each of the seven sugarcane genotypes (00-1165, ZN 8, ZN 9, ZN 10, ZN 3L, N14, and 96-1107) with regard to YSA feeding. The study's findings demonstrated that 00-1165 possessed a high level of antibiosis and tolerance according to the PRI. These results might be due to the ability of the variety to maintain and compensate for injury inflicted by YSA during feeding. These findings support those of Armstrong *et al.* (2017); Limaje *et al.* (2018) and Paudyal (2019), who demonstrated that the resistant check displayed notable levels of tolerance and antibiosis in sorghum.

References

Akbar, W. 2009. Categorization and identification of mechanisms of sugarcane resistance to the sugarcane aphid (Hemiptera: Aphididae). Louisiana State University and Agricultural and Mechanical College.

- Armstrong, J. S., Rooney, W. L., Mbulwe, L., Sekula-Ortiz, D., and Villanueva, R. T. 2017. Host plant resistance to sugarcane aphid *Melanaphis sacchari*, (Hemiptera: Aphididae) in forage and grain sorghums. *J. Econ Entomol.* 110: 259–265.
- Batyrshina, Z. S., Yaakov, B., Shavit, R., Singh, A and., Tzin, V. 2020b. Comparative transcriptomic and metabolic analysis of wild and domesticated wheat genotypes reveals differences in chemical and physical defense responses against aphids. *BMC Plant Biol.* 20:19. doi: 10.1186/s12870-019-2214-z
- Chaudhry, A. H., Nayab, S., Hussain, S. B., Ali, M., and Pan, Z. 2021. Current understandings on magnesium deficiency and future outlooks for sustainable agriculture. *International journal of molecular sciences.* 22(4): 1819.
- Correa, L. J., Maciel, O. V. B., Bucker-Neto, L., Pilati, L., Morozini, A. M., Faria, M. V., et al. 2020. A comprehensive analysis of wheat resistance to *Rhopalosiphum padi* (Hemiptera: Aphididae) in Brazilian wheat cultivars. *J. Econ. Entomol.* 113:1493–1503. doi: 10.1093/jee/toaa059
- Diaz-Montano, J., Reese, J. C., Schapaugh, W. T., and Campbell, L. R. 2007. Chlorophyll loss caused by soybean aphid (Hemiptera: Aphididae) feeding on Soybean. *J. Econ. Entomol.* 100: 1657–1662.
- Dinant, S., Bonnemain, J. L., Girusse, C., and Kehr, J. 2010. Phloem sap intricacy and interplay with aphid feeding. *Comptes Rendus Biologies.* 333:504–515. doi: 10.1016/j.crv.2010.03.008.
- Franzen, L. D., Gutsche, A. R. T., Heng-Moss, M., Higley, L. G., Sarath, G., and Burd, J. D. 2007. Physiological and biochemical responses of resistant and susceptible wheat to injury by Russian wheat aphid. *Journal of Economic Entomology.* 100: 1692–1703.
- Gallun, R. L., Ruppel, R., and Everson, E. H. 1966. Resistance of small grains to the cereal leaf beetle. *J. Econ. Entomol.* 59: 827-829.
- Green, P. W., Stevenson, P. C., Simmonds, M. S., and Sharma, H. C. 2003. Phenolic compounds on the pod-surface of pigeonpea, *Cajanus cajan*, mediate feeding behavior of *Helicoverpa armigera* larvae. *J. Chem. Ecol.* 29: 811–821.

- Guo, H., Sun, Y., Peng, X., Wang, Q., Harris, M., and Ge, F. 2016. Up-regulation of abscisic acid signaling pathway facilitates aphid xylem absorption and osmoregulation under drought stress. *Journal of experimental botany*. 67(3): 681-693.
- Gutsche, A. R., Heng-Moss, T. M., Higley, L. G., Sarath, G., and Mornhinweg, D. W. 2009. Physiological responses of resistant and susceptible barley, *Hordeum vulgare* to the Russian wheat aphid, *Diuraphis noxia* (Mordvilko). *Arthropod-Plant Interactions*. 3: 233–240.
- Gutsche, A. R., Heng-Moss, T. M., Higley, L. G., Sarath, G., and Mornhinweg, D. W. 2009. Physiological responses of resistant and susceptible barley, *Hordeum vulgare* to the Russian wheat aphid, *Diuraphis noxia* (Mordvilko). *Arthropod-Plant Interactions*. 3: 233–240.
- Gyan N. M., Yaakov, B., Weinblum, N., Singh, A., Cna'ani, A., Ben-Zeev, S., Saranga, Y and., Tzin, V. 2020. Variation Between Three Eragrostis tef Accessions in Defense Responses to *Rhopalosiphum padi* Aphid Infestation. *Front. Plant Sci.* 11:598483. doi: 10.3389/fpls.2020.598483
- Haile, F. J., Higley, L. G., Ni, X., and Quisenberry, S. S. 1999. Physiological and growth tolerance in wheat to Russian wheat aphid (Homoptera: Aphididae) injury. *Environ. Entomol.* 28: 787–794.
- Hammerschmidt, R., and Nicholson, R. L. 1999. A survey of plant defense responses to pathogens. Induced plant defenses against pathogens and herbivores. *Biochemistry, Ecology and Agriculture*. 55-71.
- Heng-Moss, T. M., Ni, X., Macedo, T., Markwell, J. P., Baxendale, F. P., Quisenberry, S. S., and Tolmay, V. 2003. Comparison of chlorophyll and carotenoid concentrations among Russian wheat aphid (Homoptera: Aphididae) infested wheat isolines. *J. Econ. Entomol.* 96: 475- 481.
- Hepler, P. K. 2005. Calcium: A central regulator of plant growth and development. *Plant Cell*. 17:2142–2155.
- Hijaz, F., and Killiny, N. 2014. Collection and chemical composition of phloem sap from *Citrus sinensis* L. osbeck (sweet orange). *PLoS One*. e101830. doi: 10.1371/journal.pone.0101830.

- Hijaz, F., Manthey, J., Dv, D. M., and Killiny, N. 2016. Nucleotides, micro- and macro-nutrients, limonoids, flavonoids, and hydroxycinnamates composition in the phloem sap of sweet orange. *Plant Signaling and Behavior*. 11:e1183084. doi: 10.1080/15592324.2016.1183084.
- Hildebrand, D. F., Rodriguez, J. G., Legg, C. S., Brown, G. C., and Bookjans, G. 1989. The effects of wounding and mite infestation on soybean leaf lipoxygenase levels. *Z. Naturforsch.* 44c: 655- 659.
- Larson, K. C. 1998. The impact of two gall-forming arthropods on the photosynthetic rates on their hosts. *Oecologia*. 115: 161–166.
- Leybourne, D. J., Valentine, T. A., Robertson, J. A. H., Pérez-Fernández, E., Main, A. M., Karley, A. J., et al. 2019. Defence gene expression and phloem quality contribute to mesophyll and phloem resistance to aphids in wild barley. *J. Exp. Bot.* 70: 4011–4026. doi: 10.1093/jxb/erz163
- Limaje, A., Armstrong, S., Hoback, W., Zarrabi, A., Chad, H., Paudyal, S., and Burke. J. 2018. Antibiosis and Tolerance Discovered in USDA-ARS Sorghums Resistant to the Sugarcane Aphid *Melanaphis sacchari* (Hemiptera: Aphididae). *J. Entomol. Sci.* 53: 230–241.
- Liu, G. J., Martina, D. K., Gardner, R. C., and Ryan, P. R. 2002. Large Mg²⁺-dependent currents are associated with the increased expression of *ALR1* in *Saccharomyces cerevisiae*. *FEMS Microbiol Lett.* 213:231–237.
- Macedo, T. B., Bastos, C. S., Highley, L. G., Ostlie, K. R., and Madhavan. S. 2003a. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. *J. Econ. Entomol.* 96: 188–193.
- Meyer, G. A., and Whitlow, T. H. 1992. Effects of leaf and sap feeding insects on photosynthetic rate of goldenrod. *Oecologia*. 92: 480–489.
- Meyer, G. A., and Whitlow, T. H. 1992. Effects of leaf and sap feeding insects on photosynthetic rate of goldenrod. *Oecologia*. 92: 480–489.
- Ni, X., Quisenberry, S. S., Heng-Moss, T., Markwell, J., Sarath, G., Klucas, R., and Baxendale, F. 2001. Oxidative responses of resistant and susceptible cereal leaves to symptomatic and non-

symptomatic cereal aphid (Hemiptera: Aphididae) feeding. *Journal of Economic Entomology*. 94(3): 743-751.

Nuessly, G. S. (2005). Featured Creatures: Yellow Sugarcane Aphid; publication #EENY-354; University of Florida: Gainesville, FL, USA; Available online: <http://entnemdept.ufl.edu/creatures/field/bugs/yellowsugarcaneaphid.htm> (accessed on 16 January 2022).

Nuessly, G. S., Hentz, M. G., and Gilbert, R. A. 2010. Susceptibility of stage IV canal point (CP) sugarcane clones to yellow sugarcane aphid (*Sipha flava* (Forbes)) feeding damage. *J Am Soc Sug Cane Technol*. 30: 104-114.

Nutt, K. A., O'Shea, M. G., and Allsopp, P. G. 2004. Feeding by sugarcane whitegrubs induces changes in the types and amounts of phenolics in the roots of sugarcane. *Env. and Exper. Botany*. 51: 155-165.

Paudyal, S. 2019. Host plant resistance to the sugarcane aphid (Hemiptera: Aphididae). A Dissertation Submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfillment of the requirements for the Degree of Doctor of philosophy.

Paudyal, S., Armstrong, J. S., Giles, K. L., Hoback, W., Aiken, R., and Payton, M. E. 2020. Differential responses of sorghum genotypes to sugarcane aphid feeding. *Planta*. 252: 1-9.

Pierson, L. M., Heng-Moss, T. M., Hunt, T. E., Reese, J. 2011. Physiological responses of resistant and susceptible reproductive stage soybean to soybean aphid (*Aphis glycines* Matsumura) feeding. *Arthropod-Plant Interaction*. 5: 49–58.

Pincebourde, S., and Ngao, J. 2021. The Impact of Phloem Feeding Insects on Leaf Ecophysiology Varies with Leaf Age. *Front. Plant Sci*. 12: 625689. doi: 10.3389/fpls.2021.625689

Puri, H. 2023. Sorghum-Sugarcane Aphid Interactions: Multi-Omic Approaches to Elucidate Plant Defense Against Sap-Feeding Insects (Doctoral dissertation, The University of Nebraska-Lincoln).

- Retuerto, R., Fernandez-lema, B. Rodriguez-Roiloa., and Obeso. J. R. 2004. Increased photosynthetic performance in holly trees infested by scale insects. *Functional Ecology*. 18: 664-669. DOI: 10.1111/j.0269-8463.2004. 00889.x
- Roberts, J. J., and Foster, J. E. 1983. Effect of leaf pubescence in wheat on the bird cherry oat aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 76: 1320-1322.
- Roberts, J. J., Gallun, R. L., Patterson, F. L., and Foster. J. E. 1979. Effects of wheat leaf pubescence on the Hessian fly. *J. Econ. Entomol.* 72: 211-214.
- Ryan, J. D., Johnson, R. C., Eikenbary, R. D and Dorschner, K. W. 1987. Drought/greenbug interactions: photosynthesis of greenbug resistant and susceptible wheat. *Crop Sci.* 27(2): 283–288.
- Saeedi, Z., and Ziaee, M. 2020. Biochemical responses of two sugarcane varieties to whitefly (*Neomaskellia andropogonis*) Infestation and its control by a new butenolide insecticide, flupyradifurone. *Agriculture and Forestry*. 66 (1): 69-81.
- Shankar, P., and Yinghua, H. 2021. Elevated production of reactive oxygen species is related to host plant resistance to sugarcane aphid in sorghum. *Plant Signaling and Behavior*. 16 (2): 1849523, DOI: 10.1080/15592324.2020.1849523
- Silva, R. J. N. da., Guimaraes, E. R. Garcia, J. F. Botelho, P. S. M., Ferro, M. I. T., Mutton, M. A., and Mutton, M. J .R. 2005. Infestation of froghopper nymphs changes the amounts of total phenolics in sugarcane. *Sci. Agric. (Piracicaba, Braz.)*. 62: 543-546.
- Singh, A., Dilkes, B., Sela, H., and Tzin, V. 2021. The Effectiveness of Physical and Chemical Defense Responses of Wild Emmer Wheat Against Aphids Depends on Leaf Position and Genotype. *Front. Plant Sci.* 12: 667820. doi: 10.3389/fpls.2021.667820
- Sosa, O. Jr. 1990. Pubescence in sugarcane as an obstacle to yellow sugarcane aphid establishment. *Sugar Y Azuca.* 85: 30.
- Sosa, O., Jr. 1991. Yellow sugarcane aphid (Homoptera: Aphididae) antixenosis in sugarcane. *Sugar Cane.* 2: 9-10.

Wilson, A. C., Sternberg, L. D. S., and Hurley, K. B., 2011. Aphids alter host-plant nitrogen isotope fractionation. *Proceedings of the National Academy of Sciences*. 108(25): 10220-10224.

Yadav, M., Pandey, J., Chakraborty, A., Hassan, M. I., Kundu, J. K., Roy, A., and Singh, A. 2022. A comprehensive analysis of calmodulin-like proteins of glycine max indicates their role in calcium signaling and plant defense against insect attack. *Frontiers in plant science*. 13: 817950.

Zhang, K. X., Li, H. Y., Quandahor, P., Gou, Y. P., Li, C. C., Zhang, Q. Y., Haq, I. U., Ma, Y., Liu, C. Z. 2022. Responses of Six Wheat Cultivars (*Triticum aestivum*) to Wheat Aphid (*Sitobion avenae*) Infestation. *Insects*. 13: 508. <https://doi.org/10.3390/insects13060508>

Zhu, Y. F., Chen, M., and Liu, C. Z. 2011. Variations of several substances in the seedlings of five oat cultivars infested by aphids. *Plant. Prot.* 37: 55–58.

CHAPTER NINE: Conclusions and Recommendations

9.1 Objective 1

Determine leaf pubescence that mediates resistance to YSA in selected sugarcane varieties.

9.1.1 Hypothesis

Leaf pubescence does not mediate resistance to YSA in selected sugarcane varieties.

9.1.2 Conclusion on objective 1

Leaf pubescence (dense trichomes) mediates resistance to YSA in selected sugarcane varieties. Trichome density increased in relation to aphid number and leaf position.

9.2 Objective 2

Evaluate biochemical resistance in commercial sugarcane varieties in retort to YSA injury.

9.2.1 Hypothesis 2

There is no biochemical resistance in sugarcane varieties in retort to YSA injury.

9.2.2 Conclusion on objective

Biochemical resistance in retort to YSA aphid injury exists among the sugarcane varieties. The biochemical parameters that were examined in vulnerable sugarcane cultivars were dramatically lowered by YSA.

9.3 Objective 3

Assess physiological response as a tolerance mechanism to YSA herbivory on selected sugarcane varieties.

9.3.1 Hypothesis 3

There is no physiological response as a tolerance mechanism to YSA feeding in selected sugarcane varieties.

9.3.2 Conclusion on objective 3

Physiological tolerance occurs among the selected sugarcane varieties in response to YSA feeding. Tolerant sugarcane varieties were able to compensate or maintain chlorophyll content and gas exchange response in the presence of YSA infestation.

9.4 Objective 4

Perform phytochemical profiling of secondary metabolites in sugarcane that confer resistance to YSA feeding.

9.4.1 Hypothesis 4

Secondary metabolites do not confer resistance to YSA feeding among the sugarcane varieties.

9.4.2 Conclusion on objective 4

Secondary metabolites confer resistance to feeding of YSA among the sugarcane varieties. This study observed that the synthesis of flavonoids and phenols was much higher in infested leaves compared to control (uninfested leaves).

9.5 Objective 5

Reconnoiter plant resistant index (PRI) of tested commercial sugarcane germplasm in response to YSA incursion.

9.5.1 Hypothesis 5

Plant resistant indexes (PRI) of tested sugarcane germplasm do not vary in response to YSA incursion.

9.5.2 Conclusion on objective 5

Plant resistant indexes (PRI) of selected sugarcane germplasm vary in response to YSA incursion. Tolerant sugarcane varieties have high PRI indexes compared as to susceptible sugarcane germplasm.

9.6 Recommendations

- There is a need for more studies in the Zimbabwean sugar industry that address the highlighted resistance mechanisms to ameliorate the effects of YSA to cover the four objectives: (i) leaf pubescence (trichomes) that mediate surface resistance to yellow sugarcane aphids (*S. flava* Forbes) in sugarcane (*Saccharum officinarum* L.) varieties; (ii) biochemical properties affected by *S. flava* in sugarcane; (iii) physiological responses of sugarcane to *S. flava* herbivory; and (iv) phytochemical profiling of metabolites that confer resistance to *S. flava* in sugarcane.

- The Zimbabwe sugar industry should incorporate host plant resistance mechanisms in Integrated Pest Management strategies for the sustainable management of YSA to curb the challenges associated with the use of chemicals, as it is currently the only alternative.
- The ZSAES should integrate leaf pubescence, physiological, biochemical and secondary metabolites options in existing integrated pest management (IPM) strategies as they confer resistance to YSA in sugarcane.
- ZSAES plant breeding department should create innovative biotechnological molecular breeding techniques that will strengthen sugarcane's tolerance and resistance to phloem-feeding insects.
- Sugarcane growers in Zimbabwe should incorporate the 00-1165 pre-released sugarcane variety in their YSA management tactics as proven by its moderate PRI.
- ZSAES plant breeder should incorporate the gene expression of physiological tolerance and high producing phenol and flavonoid gene among susceptible sugarcane accessions thereby enhancing sugarcane productivity in the presence of YSA using molecular techniques.
- There is need for continuous screening of the existing sugarcane genotypes in the sugar industry of Zimbabwe for persuasive informative results in order to construct a gene bank for resistance to YSA.
- There is a need for follow up studies by sugarcane scientists in Zimbabwe to further evaluate sugarcane varieties up to the harvesting stage to incorporate the yield and ERC % of the tested sugarcane varieties.

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Physiological response as a tolerance mechanism to yellow sugarcane aphid (YSA) (*S. Flava* Forbes) herbivory on selected commercial sugarcane varieties (*S. Officinarum*)

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Abstract

Physiological tolerance in response to Yellow Sugarcane Aphid feeding remains an unexplored area in the sugar industry of Zimbabwe and elsewhere. Two treatments of aphid infestation (un-infested (control) and infested) were applied to seven sugarcane genotypes (00-1165, ZN 3 L, ZN 8, ZN 9, 96-1107, N14 and ZN 10). The treatments were laid out in a 7 × 2 factorial arrangement in a complete randomized block design (CRBD), replicated four times and carried out over summer and winter seasons. Highly significant differences ($p < 0.001$) were observed amongst the sugarcane varieties in percentage chlorophyll loss in control (sprayed) and infested (unsprayed) plots. Great increase in percentage chlorophyll loss (21.4%) was observed on ZN 10. Regression analysis displayed a highly significant ($p < 0.001$) strong positive correlation ($r = 0.87$) between chlorophyll loss and aphid number. Summer results showed highly significant differences ($p < 0.001$) in gas exchange responses in control and infested plots. Nevertheless, in YSA infested plot, 00-1165 recorded the highest compensatory photosynthetic rate (32.52), transpiration rate (4.32), and stomata conductance (218.2) when compared to the least obtained from ZN 10 and N14 at day 28. Significant positive correlations between; chlorophyll loss and photosynthesis ($r = 0.44$; $p = 0.019$) and between photosynthesis and aphid number were noticed ($r = 0.57$; $p = 0.002$). Ranking of sugarcane varieties was done according to YSA susceptibility; less susceptible (00-1165), moderate susceptible (ZN 3 L, ZN 8 and ZN 9) and highly susceptible (96-1107, N14 and ZN 10). Sugarcane growers should use YSA tolerant varieties as they exhibit physiological compensatory and maintaining behavior.

Keywords Sugarcane · Physiological · Photosynthesis · Transpiration · Stomata conductance

Introduction

Sugarcane is a vital crop that produces sugar, ethanol, and other byproducts including molasses, filter cake, and bagasse globally (Clowes and Breakwell 1998; Esterhuysen 2012; Shabazi et al. 2020). Sugarcane has become a subject of

damage due to the persistent incursion of Yellow Sugarcane Aphids (YSA) (*Siphum flava*) in sugarcane fields. Feeding by yellow sugarcane aphids in sugarcane has been reported to have negative effects on stalk number and stalk height (Hall 2001), biomass yield (Hall 2001; Madzope et al. 2021), and yield (Mirkkinen 1970; Wilson 2019).

Breen and Teetes (1986) as cited in Dumont et al. (2023) highlighted that red to purple discoloration and premature leaf necrosis are a result of injection of saliva by YSA resulting in leaf chlorosis. Moreover, Akbar et al. (2010) reported that leaf discoloration is associated with a chlorophyll content decline associated with a drastic decrease in the photosynthetic rate (White 1990). Toxic oxygen contained in aphid saliva causes biochemical changes within the crop resulting in bleaching of leaves and increased metabolism (Macedo et al. 2003a, b, c). Plants perceive the invasion of aphids and may potentially increase the buildup of reactive

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Dr. Sakadzo, N
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Dear Dr. Sakadzo

Thank you for your interest in publishing in the Arab Journal for Plant Protection.
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List of appendices

Appendix 1: Trichome density vs Aphid number Regression analysis

Response variate: Aphid_number
Fitted terms: Constant, Leaf_position_1_3

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	40584.	40584.1	49.91	<.001
Residual	166	134991.	813.2		
Total	167	175575.	1051.3		

Estimates of parameters

Parameter	estimate	s.e.	t(166)	t pr.
Constant	82.11	5.82	14.11	<.001
Leaf_position_1_3	-19.04	2.69	-7.06	<.001

Parameter	lower95%	upper95%
Constant	70.62	93.61
Leaf_position_1_3	-24.36	-13.72

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ Leaf_position_1_3	1	40584.1	40584.1	49.91	<.001
Residual	166	134990.6	813.2		
Total	167	175574.7	1051.3		

Wald tests for dropping terms

Term	Wald statistic	d.f.	F statistic	F pr.
Leaf_position_1_3	49.91	1	49.91	<0.001

Residual d.f. 166

Appendix 2: Trichome density vs Leaf position Regression analysis

Response variate: Trichome_Density_mm2
Fitted terms: Constant, Leaf_position_1_3

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	22254.	22254.0	42.31	<.001
Residual	166	87320.	526.0		
Total	167	109574.	656.1		

Estimates of parameters

Parameter	estimate	s.e.	t(166)	t pr.
Constant	50.97	4.68	10.89	<.001
Leaf_position_1_3	14.10	2.17	6.50	<.001

Parameter	lower95%	upper95%
Constant	41.73	60.21
Leaf_position_1_3	9.817	18.37

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ Leaf_position_1_3	1	22254.0	22254.0	42.31	<.001
Residual	166	87320.0	526.0		
Total	167	109574.0	656.1		

Wald tests for dropping terms

Term	Wald statistic	d.f.	F statistic	F pr.
Leaf_position_1_3	42.31	1	42.31	<0.001

Residual d.f. 166

Appendix 3: Analysis of variance of soluble sugar

Variate: Soluble_sugar

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	66.613	22.204	3.23	
Rep.*Units* stratum					
Variety	6	1836.965	306.161	44.56	<.001
Treatment	1	2547.324	2547.324	370.78	<.001
Variety.Treatment	6	1251.753	208.626	30.37	<.001
Residual	39	267.937	6.870		
Total	55	5970.593			

Appendix 4: Analysis of variance of chlorophyll content

Variate: Chlorophyll_content_mg_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.0003482	0.0001161	0.20	
Rep.*Units* stratum					
Variety	6	4.1668929	0.6944821	1215.83	<.001
Treatment	1	1.7045161	1.7045161	2984.10	<.001
Variety.Treatment	6	1.7404214	0.2900702	507.83	<.001
Residual	39	0.0222768	0.0005712		
Total	55	7.6344554			

Appendix 5: Soluble sugar vs Aphid number Regression analysis

Response variate: Soluble_sugar
Fitted terms: Constant, Aphid_number

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	608.3	608.31	48.79	<.001
Residual	26	324.2	12.47		
Total	27	932.5	34.54		

Estimates of parameters

Parameter	estimate	s.e.	t(26)	t pr.
Constant	26.50	1.65	16.03	<.001
Aphid_number	-0.02406	0.00344	-6.99	<.001

Parameter	lower95%	upper95%
Constant	23.10	29.90
Aphid_number	-0.03114	-0.01698

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ Aphid_number	1	608.31	608.31	48.79	<.001
Residual	26	324.15	12.47		
Total	27	932.46	34.54		

Wald tests for dropping terms

Term	Wald statistic	d.f.	F statistic	F pr.
Aphid_number	48.79	1	48.79	<0.001

Residual d.f. 26

Appendix 6: Analysis of variance for flavonoid content

Variate: Flavonoid_Concentration_ug_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.001471	0.000490	0.25	
Rep.*Units* stratum					
Variety	6	49.233418	8.205570	4206.40	<.001
Treatment	1	117.334350	117.334350	60148.86	<.001
Variety.Treatment	6	35.101225	5.850204	2998.98	<.001
Residual	39	0.076079	0.001951		
Total	55	201.746543			

Appendix 7: Analysis of variance for phenol concentration

Variate: Phenol_Concentration_ug_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	10.514	3.505	0.52	
Rep.*Units* stratum					
Variety	6	1319.529	219.922	32.43	<.001
Treatment	1	2156.610	2156.610	318.04	<.001
Variety.Treatment	6	1194.930	199.155	29.37	<.001
Residual	39	264.453	6.781		
Total	55	4946.037			

Appendix 8: % change flavonoid vs Aphids Regression analysis

Response variate: %_change_Flavonoid
 Fitted terms: Constant, aphids_Infested

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	139331.	139331.	23.69	<.001
Residual	26	152908.	5881.		
Total	27	292239.	10824.		

Estimates of parameters

Parameter	estimate	s.e.	t(26)	t pr.
Constant	-50.2	46.0	-1.09	0.285
aphids_Infested	0.3788	0.0778	4.87	<.001
Parameter	lower95%	upper95%		
Constant	-144.7	44.26		
aphids_Infested	0.2189	0.5388		

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ aphids_Infested	1	139331.	139331.	23.69	<.001
Residual	26	152908.	5881.		
Total	27	292239.	10824.		

Wald tests for dropping terms

Term	Wald statistic	d.f.	F statistic	F pr.
aphids_Infested	23.69	1	23.69	<0.001

Residual d.f. 26

Appendix 9: % change phenol vs Aphids Regression analysis

Response variate: %_change phenol
Fitted terms: Constant, aphids_Infested

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	19742.	19742.	12.08	0.002
Residual	26	42500.	1635.		
Total	27	62242.	2305.		

Estimates of parameters

Parameter	estimate	s.e.	t(26)	t pr.
Constant	-23.6	24.2	-0.97	0.339
aphids_Infested	0.1426	0.0410	3.48	0.002

Parameter	lower95%	upper95%
Constant	-73.40	26.22
aphids_Infested	0.05826	0.2269

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ aphids_Infested	1	19742.	19742.	12.08	0.002
Residual	26	42500.	1635.		
Total	27	62242.	2305.		

Wald tests for dropping terms

Term	Wald statistic	d.f.	F statistic	F pr.
aphids_Infested	12.08	1	12.08	0.002

Residual d.f. 26

Appendix 10: Soluble sugar UVV spectral data

Lab no	Plot	Rep	Variety	Gross Abs	Blank	Net Abs	Gradient	intercept	y-c	Blank	Conc
				0.023	0.023	0.001	0.006	-0.009	0.032	5.569583	0.00
ZF3032	1	1	N14	0.070	0.023	0.047	0.006	-0.009	0.079	5.569583	8.13
ZF3034	2	1	ZN 10	0.080	0.023	0.057	0.006	-0.009	0.089	5.569583	9.86
ZF3036	3	1	96-1107	0.109	0.023	0.086	0.006	-0.009	0.118	5.569583	14.88
ZF3038	4	1	00-1165	0.169	0.023	0.146	0.006	-0.009	0.178	5.569583	25.26
ZF3040	5	1	ZN 9	0.157	0.023	0.134	0.006	-0.009	0.166	5.569583	23.18
ZF3042	6	1	ZN 3L	0.101	0.023	0.078	0.006	-0.009	0.110	5.569583	13.50
ZF3044	7	1	ZN 8	0.107	0.023	0.084	0.006	-0.009	0.116	5.569583	14.53
ZF3046	8	2	N14	0.080	0.023	0.057	0.006	-0.009	0.089	5.569583	9.86
ZF3048	9	2	96-1107	0.120	0.023	0.097	0.006	-0.009	0.129	5.569583	16.78
ZF3050	10	2	ZN 10	0.090	0.023	0.067	0.006	-0.009	0.099	5.569583	11.59
ZF3052	11	2	ZN 9	0.150	0.023	0.127	0.006	-0.009	0.159	5.569583	21.97
ZF3054	12	2	ZN 3L	0.110	0.061	0.049	0.006	-0.009	0.119	5.569583	15.05
ZF3056	13	2	ZN 8	0.120	0.023	0.097	0.006	-0.009	0.129	5.569583	16.78
ZF3058	14	2	00-1165	0.180	0.023	0.157	0.006	-0.009	0.189	5.569583	27.16
ZF3060	15	3	ZN 3L	0.110	0.023	0.087	0.006	-0.009	0.119	5.569583	15.05
ZF3062	16	3	96-1107	0.120	0.023	0.097	0.006	-0.009	0.129	5.569583	16.78
ZF3064	17	3	ZN 10	0.080	0.023	0.057	0.006	-0.009	0.089	5.569583	9.86
ZF3066	18	3	N14	0.060	0.023	0.037	0.006	-0.009	0.069	5.569583	6.40
ZF3068	19	3	ZN 8	0.100	0.023	0.077	0.006	-0.009	0.109	5.569583	13.32
ZF3070	20	3	00-1165	0.170	0.023	0.147	0.006	-0.009	0.179	5.569583	25.43
ZF3072	21	3	ZN 9	0.140	0.023	0.117	0.006	-0.009	0.149	5.569583	20.24
ZF3074	22	4	ZN 10	0.080	0.023	0.057	0.006	-0.009	0.089	5.569583	9.86
ZF3076	23	4	ZN 3L	0.11	0.023	0.087	0.006	-0.009	0.119	5.569583	15.05
ZF3078	24	4	96-1107	0.11	0.023	0.087	0.006	-0.009	0.119	5.569583	15.05
ZF3080	25	4	ZN 9	0.16	0.023	0.137	0.006	-0.009	0.169	5.569583	23.70
ZF3082	26	4	ZN 8	0.11	0.023	0.087	0.006	-0.009	0.119	5.569583	15.05
ZF3084	27	4	00-1165	0.16	0.023	0.137	0.006	-0.009	0.169	5.569583	23.70
ZF3086	28	4	N14	0.07	0.023	0.047	0.006	-0.009	0.079	5.569583	8.13

Appendix 11: Soluble sugar UVV spectral data

Lab no	Plot	Rep	Variety	Gross Abs	Blank	Net Abs	Gradient	Intercept	y-c	Blank	Conc
				0.023	0.023	0.001	0.006	-0.009	0.032	5.569583	0.00
ZF3032	1	1	N14	0.17	0.023	0.144	0.006	-0.009	0.176	5.569583	24.91
ZF3034	2	1	ZN 10	0.17	0.023	0.143	0.006	-0.009	0.175	5.569583	24.74
ZF3036	3	1	96-1107	0.11	0.023	0.087	0.006	-0.009	0.119	5.569583	15.05
ZF3038	4	1	00-1165	0.19	0.023	0.167	0.006	-0.009	0.199	5.569583	28.89
ZF3040	5	1	ZN 9	0.24	0.023	0.217	0.006	-0.009	0.249	5.569583	37.54
ZF3042	6	1	ZN 3L	0.11	0.023	0.087	0.006	-0.009	0.119	5.569583	15.05
ZF3044	7	1	ZN 8	0.25	0.023	0.227	0.006	-0.009	0.259	5.569583	39.27
ZF3046	8	2	N14	0.18	0.023	0.157	0.006	-0.009	0.189	5.569583	27.16
ZF3048	9	2	96-1107	0.16	0.023	0.137	0.006	-0.009	0.169	5.569583	23.70
ZF3050	10	2	ZN 10	0.26	0.023	0.237	0.006	-0.009	0.269	5.569583	41.00
ZF3052	11	2	ZN 9	0.25	0.023	0.227	0.006	-0.009	0.259	5.569583	39.27
ZF3054	12	2	ZN 3L	0.12	0.061	0.059	0.006	-0.009	0.129	5.569583	16.78
ZF3056	13	2	ZN 8	0.24	0.023	0.217	0.006	-0.009	0.249	5.569583	37.54
ZF3058	14	2	00-1165	0.20	0.023	0.177	0.006	-0.009	0.209	5.569583	30.62
ZF3060	15	3	ZN 3L	0.12	0.023	0.097	0.006	-0.009	0.129	5.569583	16.78
ZF3062	16	3	96-1107	0.10	0.023	0.080	0.006	-0.009	0.112	5.569583	13.84
ZF3064	17	3	ZN 10	0.25	0.023	0.227	0.006	-0.009	0.259	5.569583	39.27
ZF3066	18	3	N14	0.18	0.023	0.157	0.006	-0.009	0.189	5.569583	27.16
ZF3068	19	3	ZN 8	0.23	0.023	0.207	0.006	-0.009	0.239	5.569583	35.81
ZF3070	20	3	00-1165	0.21	0.023	0.187	0.006	-0.009	0.219	5.569583	32.35
ZF3072	21	3	ZN 9	0.26	0.023	0.237	0.006	-0.009	0.269	5.569583	41.00
ZF3074	22	4	ZN 10	0.27	0.023	0.247	0.006	-0.009	0.279	5.569583	42.73
ZF3076	23	4	ZN 3L	0.13	0.023	0.107	0.006	-0.009	0.139	5.569583	18.51
ZF3078	24	4	96-1107	0.12	0.023	0.097	0.006	-0.009	0.129	5.569583	16.78
ZF3080	25	4	ZN 9	0.25	0.023	0.227	0.006	-0.009	0.259	5.569583	39.27
ZF3082	26	4	ZN 8	0.25	0.023	0.227	0.006	-0.009	0.259	5.569583	39.27
ZF3084	27	4	00-1165	0.22	0.023	0.197	0.006	-0.009	0.229	5.569583	34.08
ZF3086	28	4	N14	0.17	0.023	0.147	0.006	-0.009	0.179	5.569583	25.43

Appendix 12: SPAD values vs Aphid number Regression analysis

Response variate: SPAD_values
 Fitted terms: Constant, Aphid_number_per_plant

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	144.2	144.188	24.33	<.001
Residual	26	154.1	5.927		
Total	27	298.3	11.048		

Estimates of parameters

Parameter	estimate	s.e.	t(26)	t pr.
Constant	48.40	1.46	33.17	<.001
Aphid_number_per_plant	-0.01219	0.00247	-4.93	<.001

Parameter	lower95%	upper95%
Constant	45.40	51.40
Aphid_number_per_plant	-0.01727	-0.007108

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ Aphid_number_per_plant	1	144.188	144.188	24.33	<.001
Residual	26	154.104	5.927		
Total	27	298.292	11.048		

Wald tests for dropping terms

Term	Wald statistic	d.f.	F statistic	F pr.
Aphid_number_per_plant	24.33	1	24.33	<0.001

Residual d.f. 26

Appendix 13: Photosynthesis vs % chlorophyll loss Regression analysis

Response variate: Photosynthesis
 Fitted terms: Constant, %_chlorophyl_loss

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	192.6	192.62	6.30	0.019
Residual	26	794.4	30.55		
Total	27	987.0	36.56		

Estimates of parameters

Parameter	estimate	s.e.	t(26)	t pr.
Constant	27.03	1.87	14.46	<.001
%_chlorophyl_loss	-0.434	0.173	-2.51	0.019

Parameter	lower95%	upper95%
Constant	23.18	30.87
%_chlorophyl_loss	-0.7902	-0.07879

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ %_chlorophyl_loss	1	192.62	192.62	6.30	0.019
Residual	26	794.40	30.55		
Total	27	987.01	36.56		

Wald tests for dropping terms

Term	Wald statistic	d.f.	F statistic	F pr.
%_chlorophyl_loss	6.304	1	6.30	0.019

Residual d.f. 26

Appendix 14: Photosynthesis vs Aphid number Regression analysis

Response variate: Photosynthesis
 Fitted terms: Constant, Aphid_number_per_plant

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	316.4	316.36	12.26	0.002
Residual	26	670.7	25.79		
Total	27	987.0	36.56		

Percentage variance accounted for 29.4

Estimates of parameters

Parameter	estimate	s.e.	t(26)	t pr.
Constant	33.25	3.04	10.92	<.001
Aphid_number_per_plant	-0.01805	0.00515	-3.50	0.002

Parameter	lower95%	upper95%
Constant	26.99	39.51
Aphid_number_per_plant	-0.02865	-0.007456

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ Aphid_number_per_plant	1	316.36	316.36	12.26	0.002
Residual	26	670.66	25.79		
Total	27	987.01	36.56		

Wald tests for dropping terms

Term	Wald statistic	d.f.	F statistic	F pr.
Aphid_number_per_plant	12.26	1	12.26	0.002

Residual d.f. 26