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PHYSIOLOGICAL AND SPECTRAL RESPONSES OF SUGARCANE TO THE INJURY OF SUGARCANE BORERS: BASIS FOR ENHANCING PEST MONITORING

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Thesis presented to the School of Agricultural and Veterinary Sciences – São Paulo State University (Unesp), Jaboticabal Campus, as part of requirements to obtain the Doctorate degree in Agronomy (Agricultural Entomology)

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DADOS CURRICULARES DO AUTOR

JOÃO RAFAEL SILVA SOARES nasceu em 18 de dezembro de 1992, em Pompéu – MG. É filho de Francisco Lino Soares Bisneto e Sônia Martinha da Silva Soares. Formou-se Engenheiro Agrônomo pela Universidade Federal de Viçosa (UFV), campus Viçosa, título obtido em janeiro de 2018. Foi estagiário do Laboratório de Manejo Integrado de Pragas da UFV, sob a supervisão do professor Marcelo Coutinho Picanço, onde desenvolveu projetos de iniciação científica com bolsa FUNAPE-Embrapa Café durante a graduação. Ainda durante a graduação foi agraciado com uma bolsa pelo programa Ciências sem Fronteiras, fornecida pela Coordenadoria de Aperfeiçoamento Pessoal de Nível Superior (CAPES), permanecendo na Michigan State University por 1,5 anos. Foi aprovado para cursar o mestrado em Fitotecnia pela UFV – campus Viçosa, sob a orientação do Prof. Dr. Marcelo Coutinho Picanço em fevereiro de 2018. Em janeiro de 2020, foi aprovado para cursar o Doutorado em Agronomia (Entomologia Agrícola) na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista (Unesp) – câmpus Jaboticabal, sob a orientação do Prof. Dr. Odair Aparecido Fernandes, na qual teve a oportunidade de aprofundar seus conhecimentos sobre Manejo Integrado de Pragas de grandes culturas, especialmente pragas da cana-de-açúcar.

AUTHOR'S CURRICULUM DATA

JOÃO RAFAEL SILVA SOARES was born on December 18, 1992, in Pompéu, Minas Gerais state, Brazil. He is the son of Francisco Lino Soares Bisneto and Sônia Martinha da Silva Soares. In January 2018, he graduated as an agronomist at the Federal University of Viçosa (UFV), Viçosa Campus. During his undergraduate studies, he was an intern at the UFV Laboratory for Integrated Pest Management, under the supervision of Professor Dr. Marcelo Coutinho Picanço, where he developed scientific projects with FUNAPE - Embrapa Café (Embrapa coffee). He was awarded a scholarship for the Science Without Borders program, provided by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and stayed at Michigan State University for 1.5 years. The UFV-Viçosa campus approved his master's degree in Plant Science in February 2018, under the supervision of Prof. Dr. Marcelo Coutinho Picanço.In January 2020, he was approved to pursue a doctorate degree in Agronomy (Agricultural Entomology) at the School of Agricultural and Veterinary Sciences of São Paulo State University (Unesp) Jaboticabal campus, under the supervision of Prof. Dr. Odair Aparecido Fernandes, in which he had the opportunity to deepen his knowledge on integrated pest management of field crops, especially sugarcane pests.

“If you think education is expensive, try ignorance.”

Derek Bok, former president of Harvard University

To my dear and beloved family. Especially my mother, Sônia Martinha da Silva Soares, who was my greatest encourager and great example of moral and affective conduct,

I dedicate.

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RESPOSTAS FISIOLÓGICAS E ESPECTRAIS DA CANA-DE-AÇÚCAR ÀS INJÚRIAS CAUSADAS POR FITÓFAGOS BROQUEDORES: BASES PARA A MELHORIA DO MONITORAMENTO DE PRAGAS

RESUMO – O monitoramento de pragas é essencial para programas de Manejo Integrado de Pragas (MIP), pois permite a detecção precoce de infestações e auxilia na tomada de decisão para a implementação de estratégias de controle. No entanto, muitos estudos ainda negligenciam as interações entre pragas e outros fatores estressores, como os abióticos, que podem agravar os prejuízos ao desenvolvimento das plantas. Na cultura da cana-de-açúcar, pragas como a broca-da-cana e o bicudo-da-cana causam perdas significativas, especialmente em condições de estresse hídrico. Técnicas emergentes, como o sensoriamento remoto, que utiliza sensores para detectar mudanças fisiológicas nas plantas, mostram-se promissoras para o monitoramento de pragas. Este estudo explora como as infestações dessas pragas afetam as respostas fisiológicas e espectrais da cana-de-açúcar e investiga a eficácia de sensores multiespectrais para detectar alterações na reflectância espectral das plantas causados pelo bicudo-da-cana. Inicialmente, foi investigado em casa-de-vegetação, como a infestação pela broca-da-cana e diferentes regimes hídricos alteram parâmetros de trocas gasosas e a reflectância espectral da cana-de-açúcar. Posteriormente, foi realizado monitoramento mensal de áreas experimentais infestadas com o bicudo-da-cana em canaviais comerciais. A resposta espectral das plantas foi registrada por sensores multiespectrais embarcados em veículos aéreos não tripulados (VANTs) e satélites. Os resultados revelaram que tanto o déficit hídrico quanto a infestação pela broca-da-cana, isolados ou combinados, afetam significativamente parâmetros como transpiração (E), condutância estomática (gs) e fotossíntese líquida (A), dificultando a distinção entre os efeitos da broca-da-cana e os causados pelo déficit hídrico. As respostas das variedades de cana diferiram, com a variedade tolerante ao estresse hídrico apresentando menores valores para os parâmetros gasosos, sugerindo interações complexas entre a fisiologia da planta e os estressores. Além disso, a detecção do ataque da broca-da-cana via sensoriamento remoto mostrou-se desafiadora, com os algoritmos de *machine learning* (ML) apresentando dificuldades em distinguir corretamente os estresses. No monitoramento do bicudo-da-cana, foi identificada uma relação significativa entre os níveis de infestação e a reflectância espectral das plantas, variando conforme a variedade de cana e o nível de infestação. Os modelos de ML foram eficazes ao estimar a intensidade de ataque acumulado, especialmente quando os dados consideraram a fase de crescimento da cana e a fase danosa da praga, sendo os algoritmos de árvores de decisão os mais eficientes. A análise também destacou a importância de bandas espectrais específicas e índices de vegetação como o MCARI, sensíveis às alterações causadas pelo ataque do bicudo-da-cana. Surpreendentemente, os dados provenientes de satélites superaram os VANTs em termos de poder preditivo. Esses achados reforçam a necessidade de considerar a tolerância da cana a múltiplos estressores na estimativa de limiares econômicos de dano e apontam para a incorporação de ferramentas digitais como uma abordagem promissora para o monitoramento em larga escala do bicudo-da-cana.

PALAVRAS-CHAVE: Agricultura de precisão, *Diatraea saccharalis*, Ecofisiologia, Monitoramento inteligente, Tomada de decisão, Sensoriamento remoto, *Sphenophorus levis***.**

PHYSIOLOGICAL AND SPECTRAL RESPONSES OF SUGARCANE TO THE INJURY OF SUGARCANE BORERS: BASIS FOR ENHANCING PEST MONITORING

ABSTRACT – Pest monitoring is essential for Integrated Pest Management (IPM) programs, as it allows early detection of pest infestations and aids decision-making for the implementation of control strategies. However, many studies still neglect the interactions between pests and other stressors, such as abiotic ones, which can aggravate plant damage. Pests in sugarcane crops, such as the sugarcane borer and the sugarcane billbug, cause significant losses, especially under water shortage conditions. Emerging techniques, such as remote sensing, which uses sensors to detect physiological changes in plants, show promise for pest monitoring. This study explores the impact of pest infestations on the physiological and spectral responses of sugarcane, as well as the efficacy of multispectral sensors in identifying damage from the sugarcane billbug. Initially, it was investigated how sugarcane borer infestation and different water regimes alter gas exchange parameters and spectral reflectance of sugarcane under semi-field conditions. Subsequently, monthly monitoring of sugarcane billbug was carried out in experimental areas infested with this pest in commercial sugarcane fields. Multispectral sensors on board unmanned aerial vehicles (UAVs) and satellites recorded the spectral response of the plants. The results revealed that both water deficit and sugarcane borer infestation, isolated or combined, significantly affect parameters such as transpiration (E), stomatal conductance (gs), and net photosynthesis (A), making it difficult to distinguish between the effects of the sugarcane borer and those caused by water deficit. The responses of sugarcane varieties differed, with the drought-tolerant variety presenting lower values for gas exchange parameters, suggesting complex interactions between plant physiology and stressors. Furthermore, detecting sugarcane borer attacks via remote sensing proved challenging, with machine learning (ML) algorithms struggling to correctly distinguish stresses. In sugarcane billbug monitoring, a significant relationship was identified between infestation levels and plant spectral reflectance, varying according to sugarcane variety and infestation level. ML models were effective in estimating the intensity of the accumulated attack, especially when the data considered the sugarcane growth phase and the damage phase of the pest, with decision tree algorithms being the most efficient. The analysis also highlighted the importance of specific spectral bands and vegetation indices, such as MCARI, which are sensitive to changes caused by sugarcane billbug attacks. Surprisingly, satellite data outperformed UAVs in terms of predictive power. These findings reinforce the need to consider sugarcane tolerance to multiple stressors when estimating economic damage thresholds, and they point to the incorporation of digital tools as a promising approach for large-scale monitoring of the sugarcane billbug.

KEYWORDS: *Diatraea saccharalis*, Decision making, Ecophysiology, Precision agriculture, Remote sensing, Smart monitoring, *Sphenophorus levis*.

CHAPTER 1 – General considerations

Pest monitoring is one of the key pillars of the Integrated Pest Management Program (IPM). At this stage, quantitative and qualitative data are collected that support decision-making, enabling the implementation of more efficient and cost-effective management strategies and tactics (Pedigo, Hutchins and Higley, 1986; Dara, 2019). By detecting pests in the early stages of infestation, monitoring enables early interventions, preventing severe damage and consequently significant economic losses (Higley and Pedigo, 1996).

The development of precise sampling plans requires in-depth studies of how pest insects affect plants to accurately identify infestation signs, and the specific damage caused (Peterson, Higley and Pedigo, 2018). However, most studies evaluating the impact of pest-induced stress neglect the potential interactions with other stressors (Pandey *et al.*, 2017). This is often contrary to what happens in natural conditions, in which multiple stressors act simultaneously or in succession (Zandalinas *et al.*, 2024). The combination of biotic stresses (caused by living beings) and abiotic stresses (prompted by non-living environmental factors), for example, can have synergistic effects, increasing the total negative impacts on the plant (Zandalinas and Mittler, 2022). Plants subjected to a prior stressor can modulate the behavior of herbivories, such as food preferences and oviposition sites. In addition, abiotic stresses can weaken plant natural defenses, making them more susceptible to pests (Lynch, 2022; Zandalinas and Mittler, 2022).

Sugarcane (*Saccharum* spp.) is one of the most important crops for the Brazilian economy due to its diversity of applications. Brazil is the world's largest sugar producer and the second largest ethanol producer, with an estimated production of 45 million tons of sugar and 30 billion liters of ethanol on a cultivated area of 8,333.90 thousand ha (Conab, 2024). São Paulo state accounts for 49.1% of the cultivated area, with an average yield of approximately 92 tons ha^{-1} in 2023, making it the largest national grower (Conab, 2024). Although the country gains prominence as one of the largest sugarcane cultivated areas and yield, the losses caused by stress agents refrain the sector from achieving higher yield levels.

Water restriction is the most restrictive abiotic stress for sugarcane yield among the stressors (Dlamini, 2021). Under water deficit, plants retain water to balance their use, but at the cost of reducing the capture of CO_2 and, consequently, the carbon fixation in metabolic processes due to the closure of the stomates (Inman-Bamber, Lakshmanan and Park, 2012; Hussain *et al.*, 2018).

Pest infestations such as the sugarcane borer *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) and the sugarcane billbug *Sphenophorus levis* Vaurie, 1978 (Coleoptera, Curculionidae) are examples of biotic stressors and stand out as the most important insect pests in São Paulo sugarcane fields. The presence of a sugarcane borer can reduce the weight and quality of the culms, directly affecting the production of sugar and the quality of raw materials (Rossato *et al.*, 2013). Once the larvae enter the culm, they leave a hole that allows opportunistic fungi from the red rot complex, such as *Fusarium* spp. and *Colletotrichum* spp., to enter. These fungi cause the inversion of the sucrosis and reduce the industrial quality (Rossato *et al.*, 2013). In recent years, the sugarcane billbug *Sphenophorus levis* Vaurie, 1978 (Coleoptera: Curculionidae) have become one of the main pests in sugarcane (Xavier *et al.*, 2024). The larvae bore the base of the culms, causing progressive yellowing and can lead the plants to death (Dinardo-Miranda, 2005). The attack reduces the diameter, length, and number of culms, especially in dry seasons, decreasing sugarcane longevity and yield (Xavier *et al.*, 2024).

Decision-making for control of these pests is based on population surveys *in loco*. For the sugarcane borer, two sampling schemes are proposed. A direct visual inspection is carried out on two sugarcane cultivation lines with five meters each to make an average estimative of the number and development stage of the sugarcane borer larvae. However, monitoring based on delta traps containing virgin females, which release sexual pheromones to attract males, has recently emerged as an interesting option for farmers, as it makes possible to start control of eggs and larvae before boring into culms. The monitoring of the sugarcane billbug requires scouters to remove the clump from the soil with a mattock and inspect the remains of the culms, popularly known as stumps, to count injuries and any developmental stages of the insect, i.e., larvae, pupae, and adults (Dinardo-Miranda, 2005; Xavier *et al.*, 2024).

Due to the laborious nature of conventional sampling plans, walking through sugarcane fields to visually evaluate the presence of insects or their injuries is inefficient and often impractical, especially for companies (sugarcane millers and distilleries) or growers with large areas and limited labor availability. Meanwhile, in an age of intense digitization of agriculture, remote sensing can optimize pest monitoring efficiency and speed up decision-making (Nansen, 2016). Remote sensing consists of obtaining information about objects or areas at a distance without the need for direct physical contact (Jensen, 2007). Advances in sensor technology, such as radar, multispectral, and hyperspectral, offer new opportunities for application in different farming systems (Zhang *et al.*, 2019). Currently, these sensors are classified into active and passive sensors (Toth and Jóźków, 2016). Active sensors emit their own electromagnetic radiation, such as radio waves, microwaves, or light, and then measure the reflected or spread radiation by objects (Jensen, 2007). In contrast, passive sensors do not emit any signals; they only measure the radiation that is reflected, transmitted, or naturally emitted by the targets (Jensen, 2007). Both sensor types have specific applications and are chosen based on the needs of the remote sensing project (Toth and Jóźków, 2016). However, the lower cost of acquisition, data processing, and maintenance often leads to the preference for passive sensors (Zhang *et al.*, 2021).

Herbivory stress triggers a physiological defense response in plants, resulting in changes in foliar reflectance, making pest detection through remote sensing feasible (Zhang *et al.*, 2019). Biochemical changes during plant growth, such as the concentration of chlorophyll and water, affect the amount of electromagnetic radiation reflected, absorbed, or transmitted by plants (Nansen and Elliott, 2016). These changes can be observed through manual terrestrial sensors or coupled to platforms such as robots, vehicles, unmanned aerial vehicles (UAVs), airplanes, and satellites, allowing the detection of spatial and/or temporal anomalies in agricultural fields (Toth and Jóźków, 2016).

Strategies inserted in the Digital Agriculture, such as the integration of different sensors, processing systems, and data handling, assist in the manipulation of big data databases (Barbedo, 2023). Furthermore, artificial intelligence (AI) algorithms allow digital image processing and the extraction of features of interest (Barbedo, 2023; Shaikh, Rasool and Lone, 2022; Zhang *et al.*, 2019).

Machine learning (ML) is a subfield of AI in which algorithms have a high ability to recognize patterns from data (learning) and can be accurate in identifying pests or symptoms of attack in their hosts (Shinde and Shah, 2018). ML algorithms can therefore identify patterns in the raw data and extract significant information from it. Machine learning techniques have two fundamental tasks (Behmann *et al.*, 2015). Unsupervised learning begins with unlabeled data and searches for unidentified patterns that enable the creation of a new, more condensed, or more thorough representation of the information it contains (Attri, Awasthi e Sharma, 2024). The most well-known use of unsupervised learning is clustering, which is the process of organizing data instances into various clusters. Clustering methods typically create groups of samples with comparable characteristics throughout the whole dataset. Supervised learning techniques can be used if target variables with label names “y”, and the predictors are available (Bishop 2006). In this case, it is crucial to find patterns in the data that are connected to the labels (this enables the prediction of previously undiscovered new data) (Attri, Awasthi and Sharma, 2024).

The purpose of the application should guide the selection of the best machine learning data analysis technique (Shinde and Shah, 2018). As such, generic recommendations are not attainable, although some criteria can be used to find relevant algorithms. A few fundamental characteristics are crucial for choosing a method such as the quantity of features, the quantity of training samples, the nature of the label, and details regarding the data distributions (Behmann *et al.*, 2015). A supervised approach ought to be employed, presuming that the label information is supplied (Zubler and Yoon, 2020). One can only employ unsupervised approaches in the absence of labels. Regression is needed for continuous label values, while classification is needed for discrete ones (Zubler and Yoon, 2020). Artificial neural networks (ANNs), support vector machines (SVMs), random forests (RFs), and K-means clustering are a few of the most widely used machine learning techniques used for both classification or regression purposes, while K-means also can be used as clustering method (Attri, Awasthi and Sharma, 2024; Behmann *et al.*, 2015).

As in other crops, the sugarcane agroecosystem faces several stressors whose effects may be difficult to identify and quantify. Therefore, the similarity of injuries caused by biotic and abiotic agents, the genetic composition between sugarcane genotypes, and their interactions with the environment are important factors to be considered (Pascali *et al.*, 2019; Quandahor *et al.*, 2019; Savi *et al.*, 2019). This may occur due to the rupture of the vascular bundle, which aggravates the damage to plants (Haile, 2000).

This thesis investigates sugarcane's physiological and spectral responses to remote sensing when infested by the sugarcane borer and sugarcane billbug. It is assumed that attacks by *S. levis* and *D. saccharalis* may obstruct the flow of water and nutrients, creating symptoms similar to those caused by water deficit. Boring the stem or rhizome may possibly alter the sugarcane gas exchange parameters. Therefore, understanding how herbivory influences photosynthetic processes, such as water vapor transfer, respiration, and water use efficiency is fundamental. These processes determine plant growth, development, and fitness, and herbivory can have short- and long-term consequences, especially in synergy with other stressors, such as water deficit. This thesis is divided into four chapters, in which the second chapter addresses the interaction between water stress and sugarcane borer infestation and its impacts on gas exchange and the spectral response of sugarcane. The third chapter depicts a study in sugarcane fields that compares the suitability of multispectral sensors coupled to UAVs and satellites for detection and potential monitoring tools for the sugarcane billbug. The fourth chapter discusses the contributions and limitations of this study regarding current sugarcane pest monitoring tools.

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