

DAIANE DAS GRAÇAS DO CARMO

**CONTROL DECISION MAKING SYSTEMS FOR *Dalbulus maidis* IN CORN
CROPS**

Thesis submitted to the Plant Sciences
Graduate Program of the Universidade
Federal de Viçosa in partial fulfillment of
the requirements for the degree of
Doctor Scientiae.

Adviser: Marcelo Coutinho Picanço

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ABSTRACT

CARMO, Daiane das Graças do, D.Sc., Universidade Federal de Viçosa, August, 2024. **Control decision making systems for *Dalbulus maidis* in corn crops.** Adviser: Marcelo Coutinho Picanço. Co-advisers: Renato de Almeida Sarmento and Abraão Almeida Santos

Dalbulus maidis (Hemiptera: Cicadellidae) is one of the main pests in corn crops. Decision-making systems are essential parts of the integrated pest management program. Pest attack intensity prediction models enable the planned use of decision-making systems. Thus, this work aimed to determine a prediction model and a sampling plan for *D. maidis* in corn crops. The work was conducted on commercial corn crops in the Atlantic Forest and Cerrado biomes. In the first part, the artificial neural network (ANN) model was determined to predict the attack intensity of *D. maidis*. In the second part, a conventional sampling plan was determined. The ANN predictors were plant age, rainfall, average air temperature and average relative humidity. ANN had a high capacity to predict the intensity of pest attack in different years, phenological stages of plants and biomes ($r_v=0.962$). The yellow sticky trap technique was used in the sampling plan. Firstly, the correlation between the densities of *D. maidis* in the trap and in corn plants was verified. The relative variance (VR) of densities was calculated. The frequency distribution of the data was evaluated and the number of samples was established. Sampling time and cost were calculated. This technique allowed accurate ($VR<25\%$) and fast (<2 min/sample) sampling. The densities evaluated by the traps showed significant correlations with the intensity of the pest attack on the plants. The data fit the negative binomial probability distribution. The sampling plan consisted of installing a trap every two hectares. The cost and execution time of the sampling plan was up to US\$/ha 0.50 and up to 2.5 min/ha. Therefore, the determined prediction model and sampling plan are promising for incorporation into integrated *D. maidis* management programs.

Keywords: Corn leafhopper. Prediction model. Sampling plan.

RESUMO

CARMO, Daiane das Graças do, D.Sc., Universidade Federal de Viçosa, agosto de 2024. **Sistemas de tomada de decisão de controle para *Dalbulus maidis* em cultivos de milho**. Orientador: Marcelo Coutinho Picanço. Coorientadores: Renato de Almeida Sarmiento e Abraão Almeida Santos.

Dalbulus maidis (Hemiptera: Cicadellidae) é uma das principais pragas nos cultivos de milho. Os sistemas de tomada de decisão são partes essenciais para o programa de manejo integrado de pragas. Os modelos de previsão da intensidade de ataque das pragas possibilitam o uso planejado dos sistemas de tomada de decisão. Assim, esse trabalho teve como objetivos a determinação de um modelo de previsão e um plano de amostragem para *D. maidis* em cultivos de milho. O trabalho foi conduzido em lavouras comerciais de milho nos biomas Mata Atlântica e Cerrado. Na primeira parte foi determinado o modelo de rede neural artificial (RNA) para prever a intensidade de ataque de *D. maidis*. Já na segunda parte foi determinado um plano de amostragem convencional. Os preditores da RNA foram a idade das plantas, precipitação pluviométrica, temperatura média do ar e a umidade relativa média do ar. A RNA teve alta capacidade para prever a intensidade de ataque da praga em diferentes anos, estágios fenológicos das plantas e biomas ($r_v=0,962$). No plano de amostragem foi utilizada a técnica de armadilha adesiva amarela. Primeiramente foi verificada a correlação entre as densidades de *D. maidis* na armadilha e em plantas de milho. Foi calculado a variância relativa (VR) das densidades. Foi avaliada a distribuição de frequência dos dados e estabelecido o número de amostras. Foram calculados o tempo e o custo de amostragem. Essa técnica possibilitou amostragens precisas ($VR<25\%$) e rápidas (<2 min/amostra). As densidades avaliadas pelas armadilhas apresentaram correlações significativas com a intensidade de ataque da praga às plantas. Os dados se ajustaram a distribuição de probabilidade binomial negativa. O plano de amostragem consistiu na instalação de uma armadilha a cada dois hectares. O custo e tempo de execução do plano de amostragem foi de até US\$/ha 0,50 e até 2,5 min/ha. Portanto, o modelo de previsão e o plano de amostragem determinados são promissores para serem incorporados a programas de manejo integrado de *D. maidis*.

Palavras-chave: Cigarrinha do milho. Modelo de previsão. Plano de amostragem.

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1 GENERAL INTRODUCTION

2 Corn (*Zea mays*) is a primary staple for both human consumption and livestock
3 feed (Tanumihardjo et al. 2020). In 2022, 1.16 billion tons of this grain were produced
4 on 203.47 million hectares (FAO 2022). Corn is the most produced grain crop in the
5 world. This has allowed the presence of corn plants in the field practically throughout
6 the year, favoring the continuity of pest and pathogen cycles, causing phytosanitary
7 problems for the crop (Summers et al. 2004; Moya-Raygoza et al. 2007; Oliveira and
8 Frizzas 2022).

9 The corn leafhopper, *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera:
10 Cicadellidae), is one of the main pests of corn crops, capable of causing considerable
11 declines in productivity (Waquil et al. 1999; Virla et al. 2021; Oliveira and Frizzas 2022).
12 The expansion of planted areas, successive corn cultivation, and improper
13 management of the corn leafhopper have contributed to the increased incidence of this
14 pest in fields (Summers et al. 2004; Moya-Raygoza et al. 2007; Oliveira and Frizzas
15 2022). *Dalbulus maidis* is native to Mexico and uses corn and teosinte as hosts (Moya-
16 Raygoza et al. 2007; Oliveira and Frizzas 2022). The main damage caused by this
17 pest is its ability to transmit pathogens to corn plants. *Dalbulus maidis* is a vector of
18 *Spiroplasma kunkelii*, which causes corn stunt spiroplasma (CSS), the phytoplasma
19 causing maize bushy stunt (MBSP), and maize rayado fino virus (MRFV) (Waquil et al.
20 1999; Oliveira and Frizzas 2022). Direct damage to plants is due to sap suction and
21 toxin injection (Waquil et al. 1999).

22 Decision-making systems are essential parts of an integrated pest management
23 program (Pedigo 1989; Lima et al. 2017). Seasonal pest dynamics models enable the
24 planned use of decision-making systems. Insect pest populations generally fluctuate
25 throughout the year. Knowing when population peaks occur allows for the
26 concentration of sampling efforts and thus the adoption of control measures at the
27 appropriate time (Pedigo 1989; Farias et al. 2022; Arcanjo et al. 2024).

28 Pest population dynamics can be affected by climate variables and host plants
29 (Pereira et al. 2007; Farias et al. 2020; Krechemer and Foerster 2020). Climate
30 variables are the main factors in pest regulation, as they affect survival, reproduction,
31 and behavior (Pereira et al. 2007; Soares et al. 2020; Farias et al. 2022; Oliveira and
32 Frizzas 2022). In addition, they act indirectly on pests as they influence the availability
33 of host plants and the population of natural enemies. Insect pest populations are also
34 affected by the presence of the host plant, its nutritional quality, and the age of the

plant (Pereira et al. 2007; Soares et al. 2020; Farias et al. 2022; Oliveira and Frizzas 2022).

Machine learning algorithms can be utilized to build predictive models of the seasonal dynamics of insect pests (Watts and Worner 2008; Aparecido et al. 2020; Farias et al. 2022). Artificial neural networks (ANNs) are computational systems designed to reflect the architecture of the human brain and have applications in modeling insect populations (Tarca et al. 2007). They can produce predictive models that account for nonlinear relationships between the variables and fit complex models with multiple predictors (Watts and Worner 2008; Aparecido et al. 2020). These networks consist of artificial neurons that can learn from pre-existing data (Tarca et al. 2007).

Sampling plans are important instruments for making decisions within integrated pest management programs (Higley and Pedigo 1996; Castle and Naranjo 2009; Rosado et al. 2014; Santos et al. 2023). Pest density monitoring in crops is carried out through sampling plans. This density is compared with decision-making indices to make the decision to control or not control (Pedigo et al. 1986; Fernandes et al. 2011; Rosado et al. 2014; Costa et al. 2019; Arcanjo et al. 2021). A conventional sampling plan establishes the methodology to assess pest populations, the number of samples, and the time and cost required for sampling (Gonring et al. 2020; Mota et al. 2022; Arcanjo et al. 2024). Sampling plans should be simple, representative, accurate, fast, and low-cost (Higley and Pedigo 1996; Macêdo et al. 2019; Pereira et al. 2023).

Sampling plans that use traps are desirable for monitoring insect pests where this technique can be used (Summers et al. 2004; Campbell and Hanula 2007; Vastrad et al. 2010; Infante 2018). This technique is time-saving, easy to use, and allows the capture of insects. Trap sampling plans should include an adequate number of samples to allow rapid, affordable, and accurate sampling (Southwood 1978; Fernandes et al. 2015; Mota et al. 2022). However, traps can only be used for sampling when the density of insects captured correlates with the density of insects found in the crop (Campbell and Hanula 2007; Böckmann et al. 2015; Fernandes et al. 2015).

This thesis was developed in two of the largest corn producing areas in Brazil. The evaluation encompassed in the Brazilian tropical savannah and in the Atlantic Forest biome. Notably, these biomes exhibit divergent weather conditions, providing a dataset that allows us to identify the primary factors influencing the population size of *D. maidis* and to develop a feasible sampling plan.

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CHAPTER 1:

Artificial Neural Networks for Predicting the Seasonal Dynamics of *Dalbulus maidis* (Hemiptera: Cicadellidae)

Abstract: This study addresses the challenge of predicting *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae) density in cornfields by developing an artificial neural network (ANN). Over two years, we collected data on meteorological variables (atmospheric pressure, air temperature, dew point, rainfall, relative humidity, solar irradiance, and wind speed), plant age, and density of *D. maidis* in cornfields located in two Brazilian biomes (Atlantic Forest and Brazilian Tropical Savannah). Out of 1056 ANNs tested, the neural network featuring a 30-day time lag, six neurons, logistic activation, and resilient propagation demonstrated the lowest root mean squared error (0.057) and a high correlation (0.919) with observed *D. maidis* densities. This ANN exhibited an exceptional fit in low-density (Atlantic Forest) and high-density (Brazilian Tropical Savannah) scenarios for *D. maidis*. Critical factors influencing *D. maidis* seasonal dynamics, including corn plant age, rainfall, average air temperature, and relative humidity, were identified. This study highlights the potential of the ANN as a promising tool for precise predictions of pest seasonal dynamics, positioning it as a valuable asset for integrated pest management programs targeting *D. maidis*.

Keywords: corn leafhopper; machine learning; population ecology; precision agriculture; pest management.

1. INTRODUCTION

Forecasting the seasonal dynamics of pests stands as the cornerstone for developing robust integrated pest management (IPM) programs (Barzman et al. 2015). Understanding the occurrence and abundance of pests is essential for effective sampling planning and control measures, which is fundamental in reducing economic losses (Morgan 2000; Zalucki et al. 2009; Farias et al. 2022). This significance is further underscored in cases where pests are vectors of pathogens, increasing the risk and need for crop protection actions (Oliveira and Frizzas 2022).

In the neotropical region, the intensification of agricultural practices has prompted shifts in the pest landscape of corn crops. For instance, until 2015, the corn leafhopper *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae) held a secondary pest status. However, continuous and expanded cultivation seasons have provided favorable conditions for the sustained survival of this pest year-round, resulting in population rises (Moya-Raygoza et al. 2007; Virla et al. 2021; Oliveira and Frizzas 2022). Consequently, *D. maidis* has become the primary pest of corn in Latin America, causing substantial losses, reaching up to 70% (Waquil et al. 1999; Virla et al. 2021; Oliveira and Frizzas 2022).

Significantly, these losses are attributed to *D. maidis* acting as a vector for maize-stunting pathogens, including corn stunt Spiroplasma, maize bushy stunt phytoplasma, and maize rayado fino virus (Nault 1980; Gonzalez et al. 2018; Oliveira and Frizzas 2022). Thus, forecasting the seasonal dynamics of *D. maidis* is indispensable, given its status as a significant threat to corn crops and its role as a vector of pathogens.

Weather conditions and host plants primarily affect the population size of *D. maidis* (Foresti et al. 2022; Cunha et al. 2023). Temperature, a key determinant in pest population dynamics, positively correlates with increased population size (Krechmer and Foerster 2020; Foresti et al. 2022; Cunha et al. 2023). In contrast, rainfall reduces the population, inducing pest mortality (Naranjo and Ellsworth 2005; Meneses et al. 2016; Foresti et al. 2022). *Dalbulus maidis* initiates colonization of corn early in the crop season, reaching peak abundance at maturity and subsequently reducing (Moya-Raygoza et al. 2007; Gonzalez et al. 2018; Oliveira and Frizzas 2022). Interestingly, given its exclusive association with corn, *D. maidis* migrates to colonize new corn crops upon the maturation of the current one (Purcell 1988; Moya-Raygoza et al. 2007; Oliveira et al. 2013). This intricate interplay between temperature, rainfall, and crop

phenology implies a non-linear association, highlighting the need to develop reliable models to predict this pest's seasonal dynamics accurately (Noriyuki et al. 2022; Prabhakar et al. 2022).

Forecasting pest and pathogens dynamics often involves linear and non-linear regression models (Tonnang et al. 2017; Prabhakar et al. 2022). However, these conventional analysis tools rely on a series of assumptions, which can challenge field data (Tonnang et al. 2017; Farias et al. 2022; Prabhakar et al. 2022). Machine learning (ML) algorithms, particularly artificial neural networks, are well-suited for modeling complex associations with multiple predictors, encompassing non-linear relationships between input and output variables (Paul et al. 2019; Carmo et al. 2020; Farias et al. 2022). Modeled after human brain functioning, ANNs consist of interconnected artificial neurons organized in layers. Weights, offsets, and activation functions characterize neurons. These weights and offsets undergo iterative updates during training using algorithms to enhance ANN performance and reduce predictive error (Tarca et al. 2007). Consequently, ANN can effectively model multiple dependent variables without assuming a specific statistical data distribution. This attribute makes ANN a reliable tool for forecasting seasonal pest dynamics (Farias et al. 2022).

Despite the potential for applying ANN in insect population modeling, its use in integrated pest management for predicting pest dynamics has recently gained increasing community attention and applications (Tonnang et al. 2017; Skawsang et al. 2019; Farias et al. 2022). While ANN and other related neural networks have found extensive use in pest identification (Tonnang et al. 2017; Carmo et al. 2020), the same cannot be said for forecasting seasonal pest dynamics. Consequently, this study aims to explore the capability of ANN to predict the seasonal dynamics of *D. maidis* in corn crops from two regions in Brazil, utilizing weather data and the age of corn.

2. MATERIALS AND METHODS

2.1. Data collection

We monitored *D. maidis* density in eight commercial corn fields throughout the 2020/2021 and 2021/2022 crop seasons. The evaluation encompassed four fields in the Brazilian tropical savannah (Gurupi, Tocantins, Brazil; AF) and four in the Atlantic Forest biome (Viçosa, Minas Gerais, Brazil; BTS), representative of the central regions of corn cultivation in the country (Table 1). Notably, these biomes exhibit divergent

weather conditions, providing a dataset that allows us to identify the primary factors influencing the population size of *D. maidis*.

The commercial fields adhered to standard cultural practices, including fertilizer application based on soil analysis and using pesticides and herbicides for pest, pathogen, and weed control. Irrigation was not implemented. Field assessments started seven days after planting and were repeated every seven days until harvest. Each evaluation involved the random selection of 50 plants, subjected to thorough inspection by counting the number of *D. maidis* on each leaf. Additionally, the age of plants (days after planting) was recorded during assessments. Meteorological data for each field (atmospheric pressure, air temperature, dew point, rainfall, relative humidity, solar irradiance, and wind speed) were retrieved from weather stations from the Brazilian National Institute of Meteorology (INMET 2023).

2.2. Data pre-processing

All analyses and graphic design were conducted using R (version 4.1.3) and RStudio (R Core Team 2022). To streamline the analysis and mitigate multicollinearity effects, a Pearson correlation analysis ($\alpha = 0.05$) was performed among weather variables to reduce the number of predictors and address data dimensionality. Variables with a correlation coefficient $|\geq 0.50|$ were excluded (Table 2). Consequently, four variables were retained for analysis: average air temperature, rainfall, relative humidity, and wind speed (Table 2). The final set of input variables for the model included these four weather variables and plant age (days after planting).

We assessed the impact of different time lags in selected weather variables on the performance of the ANN. Eight-day moving averages of weather variables were employed to achieve this, spaced at intervals of 0, 7, 10, 20, 30, 40, 50, and 60 days from field samplings. Before ANN modeling, the data were filtered based on these time lags, resulting in eight distinct datasets. Each dataset underwent shuffling, and the data were randomly split into a training set (70%) and a validation set (30%) using the "sample.split" function from the "caTools" package (Tuszynski 2019).

The input variables underwent centering (subtracting the mean) and scaling (conversion to z-scores) using the "scale" function from the "stats" package. This crucial step in ANN models prevents overweighting more extensive measurements during

learning, facilitating model convergence. Additionally, the density of *D. maidis* (response variable) was rescaled from zero to one using the "rescale" function from the "scales" package (Wickham 2018).

2.3. Data analysis

ANNs were constructed using the "mlp" function from the RSNNS package (Bergmeir and Benítez 2012). Four activation functions (exponential, hyperbolic tangent, identity, and logistic) and three learning algorithms (resilient propagation, scaled conjugated gradient, and standard propagation) were systematically tested. The maximum number of iterations was limited to 100, and the size of the ANN (the number of neurons in the hidden layer) varied between 1 and 11. The initial ANN weights were randomly generated using the "Randomize_Weights" initialization function, with the remaining arguments set to default values.

A total of 1056 ANNs were constructed, encompassing 4 activation functions, 3 learning algorithms, 11 ANN sizes, 1 response variable, and 8 intervals of weather variables lagged from the field assessments. The root mean squared error (RMSE_t) and Pearson correlations (*r_t*) were computed between the predictions of each model and the *D. maidis* density data used in the training stage. The primary aim was identifying the optimal ANN configuration characterized by low RMSE_t, high *r_t*, and minimal neurons in the hidden layer. Additionally, the lag time of this selected ANN needed to align with the duration of the life cycle of *D. maidis*, representing the period when the insect was exposed to climatic elements (Farias et al. 2022).

Subsequently, the root mean squared error (RMSE_v) and Pearson correlations (*r_v*) were calculated between the predictions of the chosen ANN and the *D. maidis* density data used in the validation stage. This step aimed to assess whether the determined ANN avoided errors associated with overfitting (Bilbao and Bilbao 2017).

The significance of each initial predictor in the selected ANN was evaluated using the Olden algorithm, implemented through the "olden" function in the "NeuralNetTools" package. This method calculates the relative importance of predictors by considering the product of the weights of hidden input and output connections and the sum of these products across all hidden neurons (Olden et al. 2004). The winning ANN underwent 250 runs, each with a distinct random set of initial weights. Olden estimates were recorded and aggregated to compute the 95%

confidence interval (CI95), where a predictor was deemed significant if the CI95 thresholds did not include zero (Beck 2018).

Subsequently, sensitivity analyses were conducted using partial dependence graphs of significant predictors concerning the response variable, implemented through the “partial” function of the “pdp” package. These graphs illustrate the relationship between the response variable and a specific predictor, considering the average effect of other factors (Greenwell 2017). For each crop, seasonal dynamics curves of *D. maidis* populations were generated, incorporating data on observed densities of this insect and densities predicted by the selected ANN (Farias et al. 2022).

3. RESULTS

From 1056 ANNs, the selected ANNs for each time lag demonstrated robust correlations ($r_t \geq 0.829$) between their predictions and the observed densities of *D. maidis* in both the training and validation stages (Table 3). The optimal ANN featured a 30-day time lag before scouting, six neurons in the hidden layer, a logistic activation function, and a resilient propagation learning algorithm. This ANN demonstrated one of the lowest root mean squared errors (RMSE_t = 0.057) and a substantial correlation ($r_t = 0.919$) between its predictions and the observed densities of *D. maidis* used in the model training stage (Table 3). Moreover, in the model validation stage, the selected ANN predictions exhibited a high correlation ($r_v = 0.962$) with the densities of *D. maidis* in the corn crops utilized in this study phase.

The winning ANN comprises an input layer with five neurons, where the initial predictors include the age of corn plants (I1), average air temperature (I2), average relative humidity (I3), total rainfall (I4), and average wind speed (I5) (Figure 1). It features a hidden layer with six neurons (H1, H2, H3, H4, H5, and H6) and an output layer with a single neuron (O1) containing the prediction of *D. maidis* densities in corn crops. In the diagram, black and gray lines depict positive and negative weights between layers, with the thickness of the line indicating the relative magnitude of each weight.

The 95% confidence intervals for the effects of the initial predictors—age of corn plants, total rainfall, average air temperature, and average relative humidity—did not encompass the origin, indicating that these four predictors significantly influenced the selected ANN describing the seasonal dynamics of *D. maidis*. Conversely, the 95% confidence interval for the effect of the initial predictor average wind speed passed

through the origin, suggesting that average wind speed did not significantly impact the chosen ANN characterizing the seasonal dynamics of *D. maidis* (Figure 2).

Among the predictors with a significant effect on the selected ANN, it was observed that average air temperature positively influenced the densities of *D. maidis*. In contrast, total rainfall and average relative humidity negatively affected insect densities. The density curve of *D. maidis* concerning the age of corn plants displayed a distinctive pattern, showcasing a peak point. In the initial phase of corn development, *D. maidis* densities increased, reaching a maximum. Subsequently, there was a decline in insect densities as the age of the corn plants continued to grow (Figure 3).

In the Atlantic Forest biome, we observed lower densities (average $\pm$ standard deviation) of *D. maidis* (0.11 ± 0.02 insects/plant) compared to the Brazilian tropical savannah biome (6.15 ± 0.99 insects/plant). In both biomes, the density of *D. maidis* increased initially and then reached a population peak. Towards the end of corn cultivation, there was a decline in *D. maidis* densities (Figures 4A and 5A).

Specifically, in the Atlantic Forest biome, *D. maidis* densities were higher (average $\pm$ standard deviation) in the second planting season (0.20 ± 0.05 insects/plant) compared to the first planting season (0.04 ± 0.01 insects/plant) (Figure 4A). In the Brazilian tropical savannah biome, during the first year, *D. maidis* densities in the first planting season (1.50 ± 0.38 insects/plant) were lower than in the second season (10.50 ± 3.12 insects/plant). In the second year in this biome, the density of this insect in the first season (9.69 ± 1.18 insects/plant) was higher than in the second planting season (2.45 ± 0.51 insects/plant) (Figure 5A). Notably, in all these scenarios, the densities of *D. maidis* predicted by the ANN model closely matched the observed crop densities (Figure 5A).

Climatic conditions in crops exhibited variations between biomes and planting times (Figures 4B and 5B). The average air temperature was higher (average $\pm$ standard deviation) in crops conducted in the Brazilian tropical savannah biome (26.43 ± 0.20 °C) compared to those in the Atlantic Forest biome (20.14 ± 0.38 °C). Conversely, relative humidity displayed the opposite pattern (Atlantic Forest: $82.24 \pm 0.18\%$; Brazilian tropical savannah: $77.23 \pm 1.05\%$). Rainfall levels were similar between the two biomes (Atlantic Forest: 5.14 ± 0.77 mm/day; Brazilian tropical savannah: 6.68 ± 0.84 mm/day).

Further distinctions were observed between the first and second planting seasons. The average air temperature was higher (average $\pm$ standard deviation) in the

first season (Atlantic Forest: 21.89 ± 0.56 °C; Brazilian tropical savannah: 25.99 ± 0.49 °C) than in the second season (Atlantic Forest: 17.23 ± 0.76 °C; Brazilian tropical savannah: 23.85 ± 0.50 °C). Similarly, rainfall was more significant in the first season (Atlantic Forest: $\bar{x} = 1.89 \pm 1.45$ mm/day; Brazilian tropical savannah: 8.72 ± 3.68 mm/day) compared to the second season (Atlantic Forest: 0.52 ± 0.46 mm/day; Brazilian tropical savannah: 2.83 ± 0.91 mm/day). The average relative humidity, however, remained similar in each biome between the two planting seasons (Figures 4B and 5B).

4. DISCUSSION

Forecasting the seasonal dynamics of pests is vital for effective management strategies. However, accurate predictions necessitate incorporating intricate interactions among input variables, accounting for the diverse field scenarios (Paul et al. 2019; Santos et al. 2021; Farias et al. 2022). In this study, we introduce an ANN designed to predict the density of *D. maidis* 30 days in advance, utilizing a dataset from the two biomes where corn is cultivated in Brazil. Our optimal ANN incorporates four climate variables and plant age as inputs. Average air temperature, rainfall, and relative humidity emerge as the climatic factors influencing the seasonal dynamics of *D. maidis*.

Artificial Neural Networks (ANNs) can exhibit two types of errors: underfitting and overfitting. Underfitting occurs when the ANN is too simplistic, leading to low correlation (r_t) between its predictions and the data used in the model training stage (Jabbar and Khan 2015; Bilbao and Bilbao 2017). The selected ANN in this study, with a high r_t of 0.919, experienced minimal error due to underfitting. On the other hand, overfitting arises when the ANN is overly complex and excels in training but performs poorly in the validation stage (Bilbao and Bilbao 2017). In this work, the chosen ANN demonstrated low error due to overfitting, as evidenced by its high correlation ($r_v = 0.962$) with *D. maidis* densities in the model validation stage. This favorable outcome could be attributed to the selected ANN having a reasonable number of neurons (six) in the hidden layer and a low root mean square error (RMSE_t = 0.057). ANNs with lower values of RMSE_t and fewer neurons in the hidden layer typically yield predictions with minimal underfitting and overfitting errors (Bilbao and Bilbao 2017; Alemu et al. 2018).

The inclusion of meteorological data in the winning ANN from the 30 days preceding density assessments up to the sampling period, mirroring the life cycle of *D.*

maidis in the fields (Oliveira et al. 2013; Cunha et al. 2023), underscores the dependence of ANN predictions on biological data influencing pest dynamics (Tonnang et al. 2017). In this study, the ANN was explicitly designed to incorporate climatic elements throughout the entire life cycle of *D. maidis* in corn crops (Oliveira et al. 2013; Cunha et al. 2023). Throughout the life cycle of insect pests, climatic elements play a crucial role in shaping the biological characteristics of these organisms (Farias et al. 2020; Santos et al. 2021).

Populations of *D. maidis* were influenced by climatic elements and the age of corn plants. Notably, air temperature, rainfall, and relative humidity emerged as significant factors affecting the populations of this insect. Air temperature, in particular, can impact *D. maidis* survival, development, growth, and reproduction (Meneses et al. 2016), with the optimal temperature development estimated at 29 °C (Foresti et al. 2022). Higher temperatures were predominantly observed in the Brazilian tropical savannah biome compared to the Atlantic Forest. Consequently, the anticipation was for higher insect densities in the former, given the positive association between temperature and *D. maidis* density predicted by the ANN. This aligns with higher densities under elevated temperatures, as evidenced in the Brazilian tropical savannah biome.

Our model predicted a negative correlation between precipitation and *D. maidis* density, aligning with the established understanding that rainfall negatively impacts pest population dynamics in tropical areas (Santos et al. 2021; Farias et al. 2022). Directly, rain induces mortality through its impact on insects and indirectly hampers pests' development, mating, and feeding. Additionally, humid conditions can enhance the virulence of pathogens, such as fungi, thereby reducing population size (Tahira et al. 2014; Rossini et al. 2021; Souza et al. 2021; Oliveira and Frizzas 2022). Furthermore, our model indicated a negative relationship between relative air humidity and *D. maidis* densities. Higher relative air humidity can adversely affect insect populations as it leads to increased mortality caused by entomopathogenic fungi during periods of elevated humidity (Tahira et al. 2014).

Conversely, the age of corn plants exhibited a non-linear association with the density of *D. maidis*. In the initial stages, field corn was colonized by *D. maidis*, primarily originating from neighboring fields. Subsequently, the density of *D. maidis* increased, reaching a peak at the onset of the reproductive phase. Around day 27, a second generation becomes evident in the field, coinciding with the conclusion of the

reproductive phase. The density decreases as the availability of nutrients tends to decline with the aging of the plant (Oliveira et al. 2013; Rossini et al. 2021).

The winning ANN developed in this study is a valuable tool for future predictions of *D. maidis* in cornfields and can be effectively integrated into Integrated Pest Management (IPM) programs for this pest. Its biological relevance is underscored by the inclusion of factors influencing the *D. maidis* population, highlighting the reliability of ANNs as a machine-learning tool for predicting seasonal pest dynamics. Moreover, the user-friendly nature of the model allows other practitioners to make predictions by inputting four climate variables (air temperature, relative humidity, precipitation, and wind speed) along with corn plant age, facilitating predictions of pest density 30 days in advance.

5. CONCLUSIONS

This study employed an ANN to predict the density of *D. maidis* in cornfields, utilizing weather variables and plant age as inputs. With a 30-day time lag before scouting, the winning ANN demonstrated high predictive accuracy, reflected in a low root mean squared error and a strong correlation with observed pest densities. The model showcased versatility, effectively fitting common and higher pest density scenarios in distinct Brazilian biomes. Climatic factors such as average air temperature, rainfall, and relative humidity, alongside the age of corn plants, emerged as crucial influencers of *D. maidis* seasonal dynamics. The study's findings provide a valuable tool for further pest predictions in corn fields and offer insights into integrated pest management strategies, emphasizing the significance of considering complex biological interactions and climatic elements in forecasting pest dynamics.

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Table 1. Geographical information, area, and evaluation period of corn crop fields in the Atlantic Forest (Viçosa, Minas Gerais, Brazil) and Brazilian tropical savannah (Gurupi, Tocantins, Brazil).

Biome	Field	Latitude	Longitude	Area (ha)	Period
Atlantic Forest	1	-20.74607	-42.84286	2.75	10/21/2020 - 3/18/2021
	2	-20.7462	-42.84226	2.8	10/28/2021 - 3/28/2022
	3	- 20.777859	-42.813692	1.92	3/27/2022 - 8/17/2022
	4	- 20.775052	-42.818961	2.49	3/27/2022 - 8/19/2022
Brazilian	1	- 11.832192	-48.984128	16	12/19/2020 - 5/17/2021
Tropical savannah	2	- 11.756362	-48.85585	18	3/4/2021 - 7/26/2021
	3	-11.83532	-48.999266	4	12/18/2021 - 5/17/2022
	4	- 11.752713	-48.847683	8	2/14/2022 - 7/6/2022

Table 2. Pearson pairwise correlation between weather variables. * Variables that were not selected because they correlated greater than 0.500 with other variables. Abbreviations: AP = atmospheric pressure; DP = dew point; RA = rainfall; RH = relative humidity; SI = solar irradiation; Tavg = average temperature; Tmin = minimum temperature; Tmax = maximum temperature; W = winds.

Variable	RA	RH	DP	SI*	Tavg	Tmin*	Tmax*	W
AP*	0.127	0.166	0.053	-0.003	0.176	0.128	0.165	-0.873
RA		0.309	0.225	-0.277	0.018	0.175	-0.053	0.181
RH			0.025	-0.538	-0.470	-0.146	-0.470	-0.283
DP*				0.045	0.864	0.966	0.443	0.269
SI					0.324	0.075	0.351	0.089
Tavg						0.912	0.646	0.165
Tmin							0.504	0.201
Tmax								0.053

Table 3. Topology (number of neurons, activation function, and learning algorithm) and performance in the training stage (rt= Pearson correlations between observations and predictions and mean squares of errors (RMSEt) and validation stage (rv and RMSEv) of artificial neural networks (ANN) designed to predict the seasonality of the the corn leafhopper *Dalbulus maidis*. The selected network is in bold.

Lag	Neurons	Activation function	Learning function	rt	RMSEt	rv
0	10	Hyperbolic	Rprop	0.829	0.079	0.849
7	3	Hyperbolic	Rprop	0.884	0.065	0.872
10	10	Logistic	Rprop	0.949	0.046	0.935
20	3	Logistic	Rprop	0.924	0.053	0.896
30	6	Logistic	Rprop	0.919	0.057	0.962
40	4	Hyperbolic	Rprop	0.901	0.06	0.952
50	9	Sigmoid	Rprop	0.978	0.029	0.939
60	9	Logistic	Rprop	0.945	0.046	0.944

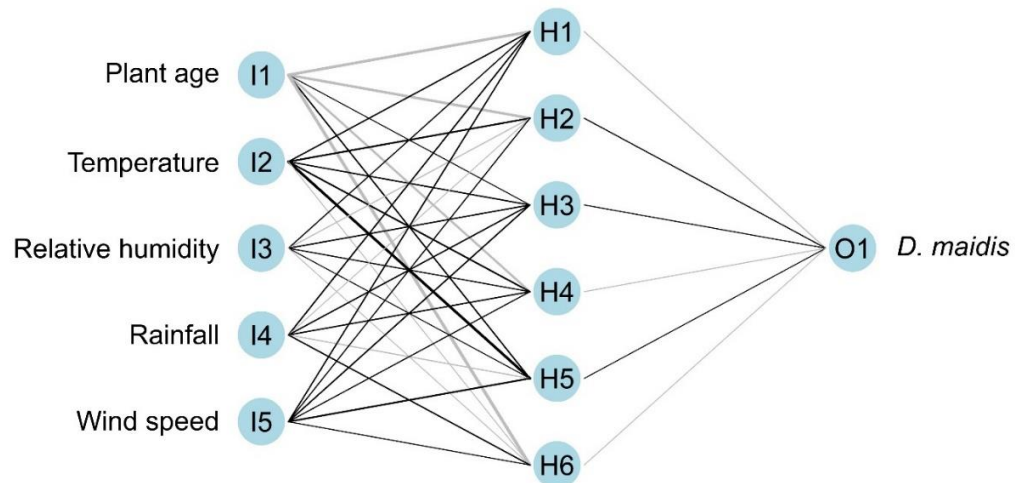


Figure 1. Diagram of the winning artificial neural network for determining the prediction model of the seasonal dynamics of *Dalbulus maidis* in corn crops. This ANN has an input layer with five neurons (I1 to I5), a hidden layer with six neurons (H1 to H6), and an output layer with a single neuron (O1). The black and gray lines represent positive and negative weights between layers. The thickness of the line corresponds to the relative magnitude of each weight.

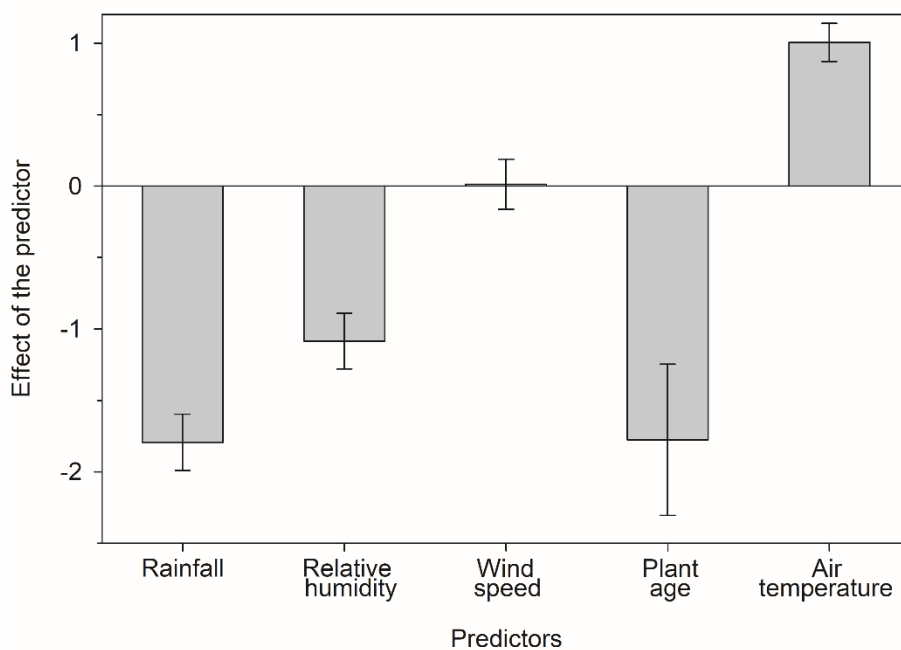


Figure 2. Mean $\pm$ confidence interval at 95% probability of the effects of the predictors on the density of *Dalbulus maidis* in corn crops. These determinations were made using the Olden method.

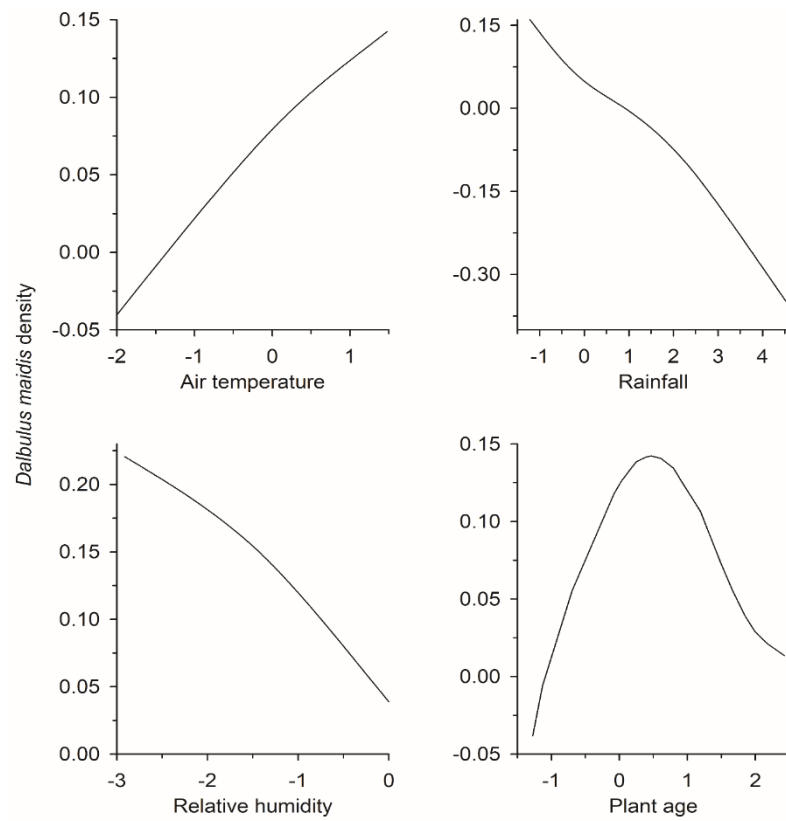


Figure 3. Partial dependence plots of the winning network depicting the variation in *Dalbulus maidis* density against levels of the variables: average air temperature, rainfall, average relative humidity, and plant age.

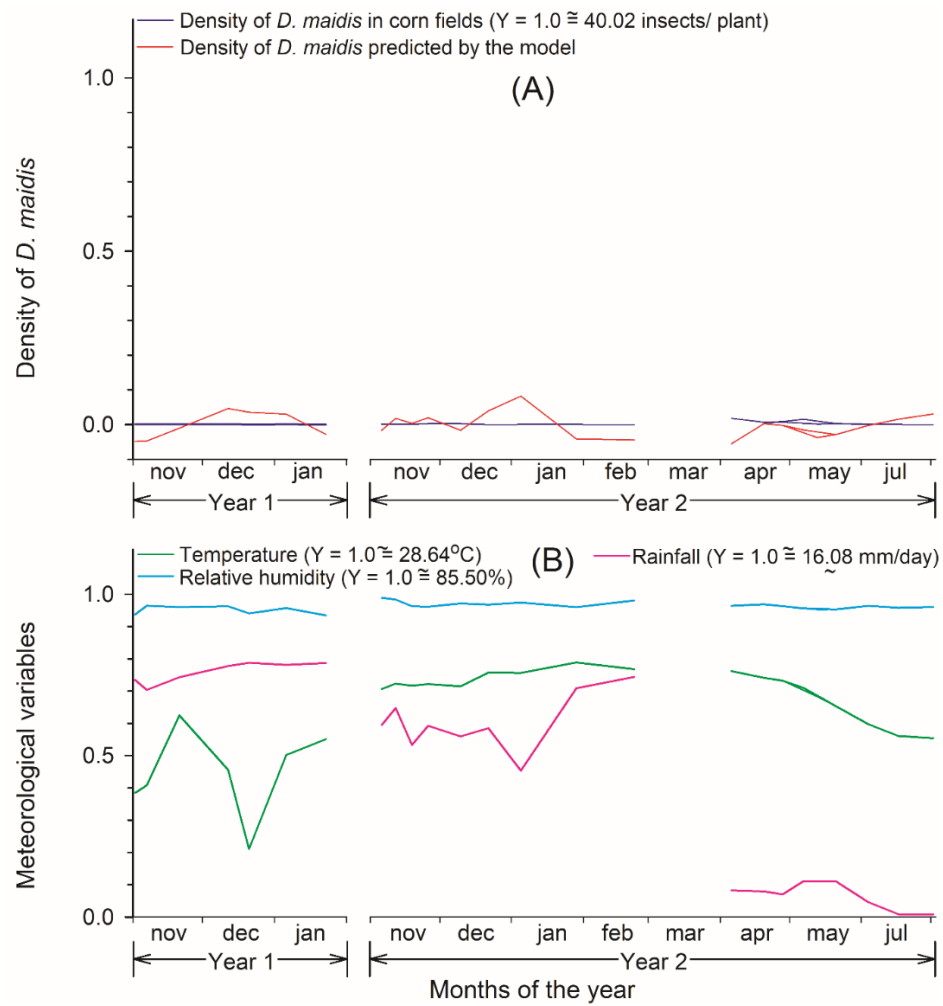


Figure 4. (A) Predicted and observed densities of *Dalbulus maidis* and (B) variation meteorological variables in corn fields in the Atlantic Forest biome. The y-variables were rescaled from 0 to 1, and their actual ranges are given in the line legends.

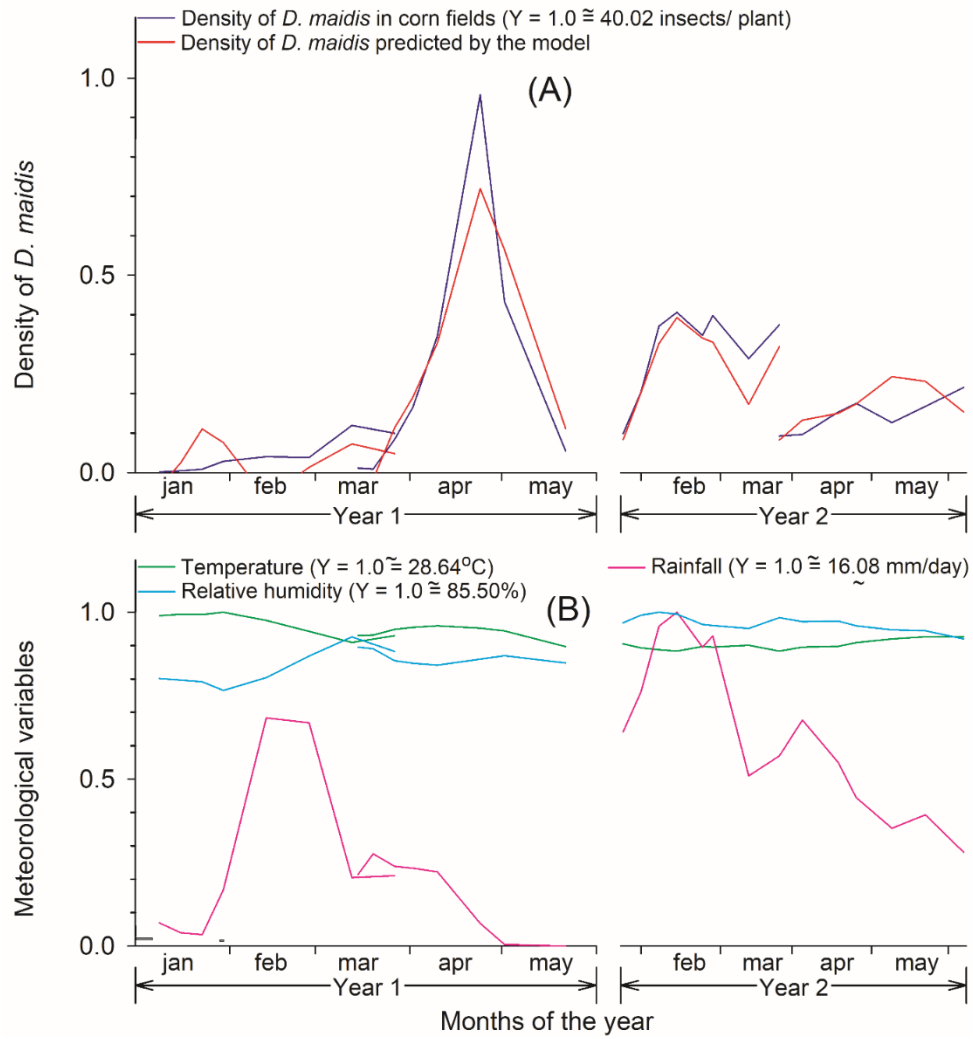


Figure 5. (A) Predicted and observed densities of *Dalbulus maidis* and (B) variation meteorological variables in corn crops in the Brazilian tropical savannah biome. The y-variables were rescaled from 0 to 1, and their actual ranges are given in the line legends.

CHAPTER 2:

The first standardized sampling plan designed to scout *Dalbulus maidis* (Hemiptera: Cicadellidae) adults in corn crops using yellow sticky traps

Abstract

Dalbulus maidis (Hemiptera: Cicadellidae) is a significant pest of corn crops, potentially causing yield losses of up to 70%. *D. maidis* primarily damages corn by transmitting pathogens. Direct plant damage is caused by sap suction and toxin injection. Sampling corn fields to determine the *D. maidis* density for decision-making systems can be challenging due to field size, time, and cost. Yellow sticky traps (YST) are commonly employed to determine the presence and density of *D. maidis* because they are easy to use and allow quick and low-cost assessment. This study aimed to propose and evaluate a new sampling plan for *D. maidis* in corn crops using YST. This research was carried out in commercial corn crops in the Brazilian Atlantic Forest and Cerrado biomes for three years. The pest density in traps and on plants were moderately ($r = 0.54-0.65$) and significantly ($p < 0.0001$) correlated. The density evaluated in the traps showed relative variance of less than 25% and sampling time of up to 2 min per sample unit. The negative binomial distribution was adequate to represent the *D. maidis* density probability distribution for the YST sampling method with K common (2.8979) among all the fields evaluated. The sampling plan consisted of installing one trap every two hectares. The plan's total cost ranged from US\$/ha 0.41 to 0.50, with a total sampling time of up to 2.5 min/ha. We propose this new sampling plan for *D. maidis*, which is suitable for incorporation into management programs in corn crops, as it is representative, accurate, fast, and low-cost.

Key words: corn leafhopper, decision-making, sampling cost, sampling time, negative binomial.

1. INTRODUCTION

Corn (*Zea mays*) is one of the main sources of human food and animal production (Tanumihardjo et al. 2020). In 2022, 1.16 billion tons of this grain were produced on 203.47 million hectares (FAO 2022). Corn is the most produced grain crop in the world. In Brazil, the cultivation of this cereal is carried out in three crops: first crop (September/October), second crop (February/March), and third crop (May), combined with seed production, silage, and small subsistence crops. This has allowed the presence of corn plants in the field practically throughout the year, favoring the continuity of pest and pathogen cycles, causing phytosanitary problems for the crop (Summers et al. 2004; Moya-Raygoza et al. 2007; Oliveira and Frizzas 2022).

The corn leafhopper, *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae), is one of the main pests of corn crops, capable of causing yield reductions of up to 70% (Waquil et al. 1999; Virla et al. 2021; Oliveira and Frizzas 2022). The expansion of planted areas, successive corn cultivation, and improper management of the corn leafhopper have contributed to the increased incidence of this pest in fields (Summers et al. 2004; Moya-Raygoza et al. 2007; Oliveira and Frizzas 2022). *Dalbulus maidis* is native to Mexico and uses corn and teosinte as hosts (Moya-Raygoza et al. 2007; Oliveira and Frizzas 2022). The main damage caused by this pest is its ability to transmit pathogens to corn plants. *Dalbulus maidis* is a vector of *Spiroplasma kunkelii*, which causes corn stunt spiroplasma (CSS), the phytoplasma causing maize bushy stunt (MBSP), and maize rayado fino virus (MRFV) (Waquil et al. 1999; Oliveira and Frizzas 2022). Direct damage to plants is due to sap suction and toxin injection (Waquil et al. 1999).

Sampling plans are essential decision-making tools in integrated pest management programs (Higley and Pedigo 1996; Castle and Naranjo 2009; Santos et al. 2023). In sampling plans, pest densities in fields are determined and compared with decision-making control thresholds (Rosado et al. 2014; Arcanjo et al. 2024). The first step in developing a new decision-making system for pest control is determining a conventional sampling plan (Bacci et al. 2006; Silva et al. 2019; Arcanjo et al. 2024). Sampling technique, number of samples per field, sampling time, and cost are determined in this plan (Bacci et al. 2008; Rosado et al. 2014). Furthermore, these plans are used to establish decision-making thresholds, validate sequential sampling plans, and can be incorporated into smartphone applications (Bacci et al. 2006; Araújo

et al. 2020). A practical sampling plan should be accurate, representative, cost-effective, and quick to execute (Bacci et al. 2006; Paes et al. 2019; Mota et al. 2022).

The evaluation of *D. maidis* density in corn crops has been conducted using direct counting techniques and yellow sticky traps (Böckmann et al. 2015; Canale et al. 2023; Pinto et al. 2023, 2024). Sampling plans employing traps are desirable due to their ease of use, allowing for quick and cost-effective assessment of pest populations in fields (Böckmann et al. 2015; Fernandes et al. 2015). The reliability of trap usage in pest monitoring depends on the correlation between the number of captured insects and pest densities on plants in the fields (Gillespie and Quiring 1987; Pinto-Zevallos and Vänninen 2013).

Therefore, there is a need for studies on sampling plans for *D. maidis* in corn fields. Thus, the objective of this study was to establish a practical sampling plan for *D. maidis* in corn fields using yellow sticky traps.

2. MATERIAL AND METHODS

2.1. Experiment settings

This research was conducted in commercial corn fields in the Atlantic Forest and Cerrado biomes from 2021 to 2023. The fields in the Atlantic Forest biome were located in Viçosa, Minas Gerais state, Brazil (20°45'17"S, 42°52'57"W; altitude = 648 m). Fields in the Cerrado biome were located in Gurupi, Tocantins state, Brazil (11°45'19"S, 48°50'29"W; altitude = 265 m). These locations were selected due to Brazil's main corn cultivation areas and their different environmental characteristics. The climate of the Atlantic forest is characterized by a tropical high-altitude climate, with hot, humid summers and milder, drier winters (Peel et al. 2007). Average annual temperatures vary between 17°C and 23°C. The climate of the Cerrado biome is classified as humid tropical, with a rainy season from November to April and a dry season from May to October (Peel et al. 2007). Temperatures are higher, with annual averages varying between 24°C and 27°C. Agriculture is more extensive in the Cerrado, with large areas dedicated to grain production (IBGE 2023).

The evaluation of *D. maidis* density was conducted using yellow sticky traps (25 cm x 10 cm). The traps were installed at the height of the corn plants according to the phenological stage evaluated (Figures 1A, 1B, and 1C) (Meneses et al. 2016). On each evaluation date, the traps remained in the field for seven days as this period is suitable

for evaluating *D. maidis* populations in each phenological stage (Summers et al. 2004; Canale et al. 2023; Moya-Raygoza and Chacón-Torres 2023). This was done because it was found that using this time period, the *D. maidis* density in the traps correlated with the one estimated by counting insects on the corn plants. Subsequently, the traps were collected and taken to the laboratory for counting the number of leafhoppers captured (Figure 1D).

This work was conducted in four stages: (i) evaluation of the correlation between the *D. maidis* density obtained by the two sampling method; (ii) determination of the sample size (number of traps/ha) and (iii) sample technique evaluation in relation to the traditional one with respect to accuracy and cost-effectiveness. All statistical analyses were conducted using the packages of the R software (R Core Team 2022).

2.2. Correlation between *D. maidis* densities obtained via the two sample techniques

The reliability of trap usage in pest monitoring depends on the magnitude of the correlation between the number of captured insects using traps and pest density estimated by counting insects directly on plants in the corn fields (Gillespie and Quiring 1987; Pinto-Zevallos and Vänninen 2013; Fernandes et al. 2015). For this, five corn fields were evaluated, two located in Viçosa, Minas Gerais, Brazil, and three in Gurupi, Tocantins, Brazil.

In each field, 10 traps were installed 100 m apart when the plants were at different developmental stages: the vegetative (V2 and V4) and reproductive (R1 and R4) stages. *Dalbulus maidis* densities were assessed in these traps and on corn plants. Corn plants were evaluated on the day the traps were installed in the field. The evaluated plants were located at 10, 30, and 50 m distances from each trap. At each distance, four corn plants were selected for insect counting. Each of the four plants was positioned north, south, east, and west of the trap (Figure 1E). The pest density was evaluated in the whorl of the plants (Figure 1F), as it is the most suitable sampling unit for evaluating *D. maidis* densities using direct counting techniques (Pinto et al. 2023).

Dalbulus maidis density were subjected to the Lilliefors test to verify the normality of the data (Thode 2002) and to the Levene test to verify the homogeneity of the variance of the residuals. As these data did not present a normal distribution and/or their residuals did not present homogeneity of variance, they were subjected to nonparametric analyses. Thus, the densities in the traps and in the plants at different

distances were subjected to nonparametric Spearman correlation analyses and their significance was assessed by the nonparametric z test.

2.3. Sample technique evaluation

The choice of the best sampling technique to estimate the insect density should consider the precision, sampling cost, and evaluation time to allow sound and cheaper decision-making (Gusmão et al. 2005; Bacci et al. 2008; Pinto et al. 2023). This stage of the work was carried out in six approximately five-hectare corn fields. Three were in Viçosa and three in Gurupi. The corn plants were sampled at vegetative (V4, V6, V8, and V10) and reproductive stages (R2 and R4). Twenty yellow sticky traps were installed in each field. These traps were distributed in a regular grid pattern to avoid directional biases in density assessments (Gusmão et al. 2005; Bacci et al. 2008; Santos et al. 2023). The number of leafhoppers captured in each trap was evaluated. The time spent on assessments conducted at each trap was also recorded with a chronometer. Mean density and respective standard error (SE) and the mean sampling time at each trap (mean $\pm$ SE) were calculated. The precision criterion used to select the sampling unit was the relative variance (RV) of *D. maidis* density estimate using the following equation (1) (Southwood 1978; Gusmão et al. 2005; Bacci et al. 2008):

$$(1) \quad RV_{ij} = 100 \times (SE/\bar{x}),$$

where RV is the relative variance (%), i is each vegetative or reproductive stage, j is the biome, SE is the standard error of *D. maidis* densities and $\bar{x}$ is the average insect density. Following the chosen precision criteria, components of sampling plans are selected when evaluated densities show a relative variance is lower than 25% (Southwood 1978; Young and Young 1998; Gusmão et al. 2005; Pinto et al. 2023).

2.4. Determining the number of traps for the sampling plan.

The number of samples per area unit or per field is an essential part of pest sampling plans. This number should enable relative quick assessments (with relative fewer samples) and with the lowest possible standard error associated with the mean density estimate (Young and Young 1998; Fernandes et al. 2015; Mota et al. 2022). In a conventional plan a fixed sample size is determined at a previously established precision and confidence level required for the insect density estimate. This stage of the work was conducted in 22 commercial corn fields ranging from 18 to 20 hectares, with plants at vegetative stages (V2, V3, V4, V5, V6, and V8) and reproductive stages

(VT, R1, R2, R4, R5, and R6). Among the 22 fields evaluated, eight were located in Viçosa, and fourteen in Gurupi. Fifty traps were installed in each field and stage of plants. The traps were distributed in a regular grid pattern across the field areas for the reasons previously mentioned. The means and standard errors of *D. maidis* densities in each field were calculated. After, it was determined which frequency distributions (negative binomial, Poisson, or positive binomial) the data of *D. maidis* densities in each field fit. The goodness-of-fit of the proposed distributions fit o each density field data, in each vegetative or reproductive stage was assessed by the Chi-square test at the 0.05 significance level. The selected frequency distribution was the one that best fit to the pest density data in most crop fields in different phenological stages (Young and Young 1998; Rosado et al. 2014; Gonring et al. 2020).

The value of the common dispersion parameter (k common) is necessary for determining the number of samples. The existence of K common value aiming to have a dispersion parameter that reflects the variability of all crops and was independent of density. For this purpose, the value of the aggregation parameter (k) was calculated for each of the 22 fields using according to equation (2) (Young and Young, 1998):

$$(2) k = \bar{x}^2 / (S^2 - \bar{x}) ,$$

where k = tthe negative binomial distribution parameter, $\bar{x}$ = mean, and S^2 = variance of the density of *D. maidis* in the field.

To verify the existence of a common aggregation parameter (K common) representing all corn fields, the value of k for each field was subjected to simple linear regression analysis using the procedure described by Bliss and Owens (1958). This analysis considers that the corn fields have a K common when this regression shows a significant slope and a non-significant intercept according to the F test at $\alpha = 0.05$ (Bliss and Owen 1958).

The number of traps for the *D. maidis* sampling plan in corn crops was estimated. The number of samples was calculated according to equation (3) (Young and Young 1998):

$$(3) NA = \frac{1}{C^2} \left(\frac{1}{\bar{x}} + \frac{1}{k_c} \right),$$

where NA = number of samples, C = admitted error, $\bar{x}$ = population mean, and k_c = common aggregation parameter of the negative binomial frequency distribution. These calculations employed using precision error values of 0.05, 0.1, 0.15, 0.20, and 0.25.

These values were used because they define the error range used in the calculations of estimated mean insect densities in decision-making systems of integrate pest management programs (Southwood 1978).

A graph of the number of traps (Y-axis) versus the sampling error (X-axis) was plotted using this data. The error adopted in the calculation was the one at which the number of traps in the sampling plan stabilized. Thus, using this error, the value of K common was calculated to determine the number of traps to be used in the *D. maidis* sampling plan (Moura et al. 2007; Bacci et al. 2008; Mota et al. 2022; Pinto et al. 2023).

2.5. Determining the time and cost of the sampling plan as a function of field size.

This stage of the work was carried out in six commercial corn fields with areas of 2, 6, 10, 30, 50, and 100 hectares. The plants in these fields were at the vegetative stage (V4). In each field, the number of traps determined in the previous stage of this work was installed (one trap per two hectares). These traps were distributed in a regular grid pattern across the field areas to avoid directional biases in density assessments (Gusmão et al. 2005; Bacci et al. 2008; Santos et al. 2023). For each field, the times (installation, assessment, and total) and costs (installation, assessment, materials, and total) of sampling were recorded. Times and costs per hectare and total were calculated for each field. Labor and material costs (pencils, paper, yellow sticky traps) were included in the cost calculations (Bacci et al. 2006; Fernandes et al. 2015; Mota et al. 2022).

3. RESULTS

3.1. Relationships between *D. maidis* densities on corn plants and evaluated by sticky trap

Positive and significant correlations ($p < 0.0001$) were observed between *D. maidis* densities on sticky traps and those on corn plants at 10, 30, and 50 m from the traps, with Spearman correlation coefficients ranging from 0.31 to 0.65 (Table 1).

3.2. Sample technique evaluation

The densities of *D. maidis* assessed in the corn fields by yellow sticky traps ranged from 1.10 (field with plants at stage V6) to 104.55 (field with plants at stage V10) insects per trap (Figure 2A). In all these situations, the densities of *D. maidis* assessed using yellow sticky traps showed relative variances lower than the threshold of 25% (Figure 2B). The sampling times of *D. maidis* using yellow sticky traps ranged

from 1.19 min (field with plants at stage V6) to 2.04 min (field with plants at stage V10) per trap assessed (Figure 2C).

3.3. Number of samples for the sampling plan

Dalbulus maidis densities fitted the negative binomial frequency distribution in 18 out of the 22 evaluated corn fields (81.81%) using yellow sticky traps. This was evident because in these 18 fields, the chi-square values were not significant ($p > 0.05$). In none of the corn fields did *D. maidis* densities fit the Poisson frequency distribution. However, in four of the fields, *D. maidis* densities fit the positive binomial distribution (Table 2). Since more than 70% of the fields had densities fitting the negative binomial distribution, the number of samples for the *D. maidis* sampling plan was calculated using the formula appropriate for this frequency distribution.

The insect densities in the corn fields assessed using yellow sticky traps exhibited a regression curve of the aggregation parameter (K common) as a function of the parameter K of each field with a significant slope ($p < 0.05$) and a non-significant intercept ($p > 0.05$) (Table 3). Thus, a common aggregation parameter was established (K common = 2.8979) among the *D. maidis* densities assessed using yellow sticky traps in all corn fields.

The number of samples for the sampling plan stabilized at an error rate of 20%. Thus, a 20% error rate was adopted in calculating the number of samples for the sampling plan. Using a maximum error of 20%, the number of traps needed to evaluate *D. maidis* populations in corn fields measuring 18 to 20 hectares was nine samples (Figure 3).

3.4. Time and cost of sampling plan as a function of field size

In corn fields measuring 2, 6, 10, 30, 50, and 100 hectares, the total times spent on trap installation and assessment per hectare were 2.47, 1.74, 1.51, 1.19, 1.08, and 0.98 minutes, respectively. The total sampling time per field was higher in larger fields. However, in larger fields, the time spent per hectare sampled decreased. In fields up to 10 hectares, most of the time was spent on trap installation. In fields measuring 30, 50, and 100 hectares, most of the time was spent on trap assessment. The total installation cost ranged from US\$/ha 0.41 to US\$/ha 0.50. Although the total sampling cost was higher in larger corn fields, the sampling cost per hectare was lower in larger

fields. Most of the sampling cost was attributed to the materials used, especially the cost of purchasing yellow sticky traps (Table 4).

4. DISCUSSION

The sampling plan determined in this study was practical because it was representative, allowing for quick sampling (from 0.98 to 2.47 minutes per hectare), low-cost (from 0.41 to 0.50 US\$/ha), and with a low error (maximum error of 20%). Determining pest densities using yellow sticky traps enabled representative sampling of field conditions because the densities assessed by this technique were highly correlated with insect densities on corn plants. The high significance ($p < 0.0001$) of this correlation when corn plants were at different stages demonstrates that the yellow sticky trap technique is representative of corn field conditions throughout the plant development period (Böckmann et al. 2015; Fernandes et al. 2015).

The fact that *D. maidis* densities assessed by yellow sticky traps showed a relative variance of less than 25% demonstrates that this technique enables accurate sampling (Bacci et al. 2006; Lopes et al. 2019). This characteristic is associated with determining sampling plans with fewer samples, which reduces the cost and time of sampling (Lopes et al. 2019; Silva et al. 2020). This is important for implementing control measures at the right time, thereby avoiding economic losses (Rosado et al. 2014; Pinto et al. 2017).

The densities of *D. maidis* fit the negative binomial distribution in the vast majority of the evaluated fields because the variances of the density data were greater than their means (Taylor 1961; Barrigossi et al. 2001; Gusmão et al. 2005). The frequency distribution model is essential for selecting the formula to be used in calculating the number of samples in the sampling plan (Gusmão et al. 2005; Pinto et al. 2017). However, the frequency distribution of insect densities does not provide information about the spatial distribution of insects in the cultivation fields, as this determination does not consider the location of samples in the areas (Young and Young 1990; Barrigossi et al. 2001; Araújo et al. 2019).

When densities in the fields present different values from the aggregation parameter of the negative binomial distribution, it requires calculating the number of samples for each field, making it difficult to determine a sampling plan that can be used in different situations (Young and Young 1990; Barrigossi et al. 2001; Araújo et al. 2019). However, in the fields evaluated in this study, *D. maidis* densities exhibited a

common aggregation parameter among all corn fields. This indicates that the sampling plan determined in this study can be used in different corn fields (Bliss and Owen 1958; Rosado et al. 2014; Arcanjo et al. 2024). Another important aspect is that this common aggregation parameter includes fields with both vegetative and reproductive stage corn plants. Furthermore, these corn fields belong to crops conducted in the Atlantic Forest and Brazilian tropical savannah biomes, which are the main corn-producing regions in Brazil. Therefore, the sampling plan determined in this study is representative of the various situations in corn fields where *D. maidis* has caused damage. This fact makes this sampling plan practical and therefore more likely to be adopted by farmers.

According to Southwood (1978), the largest error value allowed in the densities determined by sampling plans is 25%. In determining the sampling plan, a 20% error was used because it was the lowest value for this characteristic from which there was no significant increase in the number of samples to be used in this plan. Therefore, the use of a 20% error allowed the densities of *D. maidis* to be assessed accurately and a practicable sampling plan to be determined (Southwood 1978; Macêdo et al. 2019; Silva et al. 2020). Therefore, this is yet another characteristic that makes the sampling plan determined in this work practicable since using technical-scientific criteria it has the smallest number of samples and the smallest possible error associated with its determinations.

The time required to walk between samples should be considered in a sampling plan. In this study, we developed a sampling plan in which the maximum time spent sampling *D. maidis* in corn fields was 2.47 minutes per hectare. We observed that the time for trap installation and evaluation increases in larger fields. However, in larger fields, the total time per hectare decreases. Additionally, the cost of sampling is also important and should be considerably lower than the cost spent on pest control (Moura et al. 2007; Araújo et al. 2019; Arcanjo et al. 2024). As the field size increased, there was also an increase in the cost per sampled field. However, there was a reduction in cost per hectare with the increase in the size of the sampled fields. This is due to the lower cost of trap installation in larger fields. In this context, the trap installation and evaluation time was 2.47 minutes per hectare in 2-hectare fields and \$0.98 per minute per hectare in 100-hectare fields.

In conclusion, the sampling plan determined in this work can be incorporated into integrated *D. maidis* management programs in corn crops. This is because this

sampling plan is robust and provides essential information for farmers to correctly use the yellow sticky trap technique when sampling *D. maidis* populations in corn crops.

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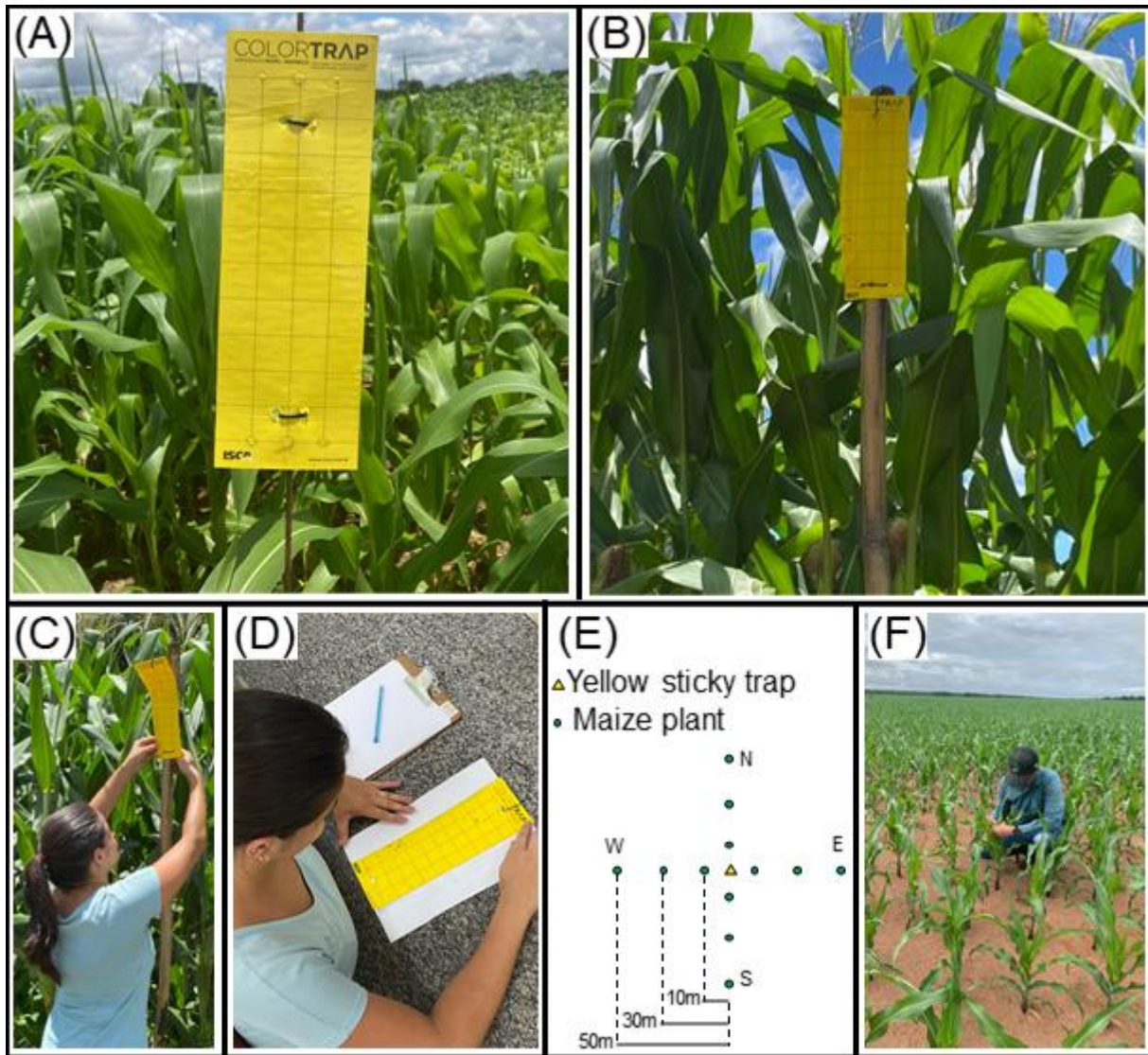


Figure 1. Sampling of *Dalbulus maidis* by yellow sticky trap in (A) fields with plants in the (A) vegetative and (B) reproductive stages, (C) installation and (D) trap evaluation, (E) spatial distribution of plants used in determining the relationships between densities in plants and in traps and (F) sampling by direct counting.

Table 1. Lilliefors test to verify data normality, Levene test to verify homogeneity of residual variance and nonparametric Spearman correlations (r) between *Dalbulus maidis* densities assessed by yellow sticky trap and the densities of this insect in corn plants in the vegetative (V2 and V4) and reproductive (R1 and R4) stages at different distances from the traps. The significance (p) of the correlations was assessed using the nonparametric z test.

Distance (m)	Lilliefors test	Levene test	r	z test	p
V2					
10	0.18*	44.57*	0.54	3.80	0.0001
30	0.17*	43.95*	0.49	3.41	0.0003
50	0.21*	51.16*	0.43	3.03	0.0012
V4					
10	0.42*	27.92*	0.45	3.16	0.0008
30	0.42*	28.22*	0.46	3.21	0.0007
50	0.42*	26.78*	0.31	2.17	0.0149
R1					
10	0.12ns	24.59*	0.64	4.46	<0.0001
30	0.11ns	45.03*	0.65	4.52	<0.0001
50	0.12ns	48.46*	0.56	3.90	<0.0001
R4					
10	0.08ns	101.42*	0.60	4.19	<0.0001
30	0.12ns	104.44*	0.58	4.08	<0.0001
50	0.13*	103.84*	0.51	3.59	0.0002

*, ns = test values significant or not according to the z test at $p < 0.05$, respectively.

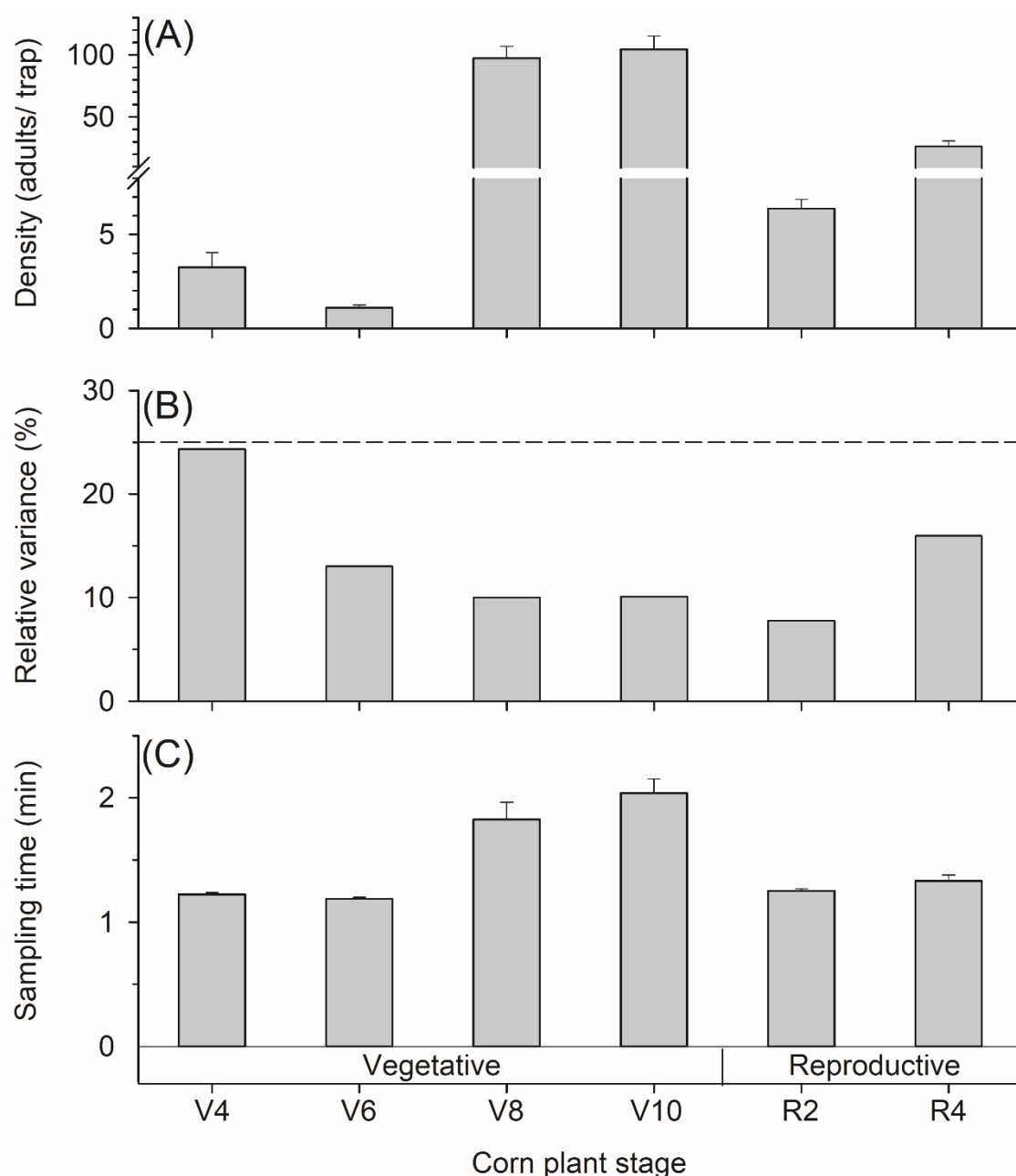


Figure 2. (A) Densities (mean $\pm$ standard error), (B) relative variance and (C) sampling time (mean $\pm$ standard error) of *Dalbulus maidis* densities assessed by yellow sticky trap in corn fields with plants in the vegetative stage (V4, V6, V8 and V10) and reproductive (R2 and R4).

Table 2. Frequency distribution of *Dalbulus maidis* densities assessed by yellow sticky trap in 22 corn fields.

Fd	Bm	St	Density ($\bar{x} \pm \text{SE}$)	χ^2 (df = 1)		
				Negative binomial	Poisson	Positive binomial
1	AT	V8	10.30 $\pm$ 1.84	1.32 $\times 10^1$ *	1.00 $\times 10^{17}$ *	2.97 $\times 10^3$ *
2	AT	R1	12.25 $\pm$ 0.07	7.64 $\times 10^{-2}$ ns	1.00 $\times 10^{25}$ *	3.62 $\times 10^4$ *
3	AT	V4	13.00 $\pm$ 4.04	2.42 ns	4.55 $\times 10^{29}$ *	1.77 $\times 10^5$ *
4	AT	R2	13.17 $\pm$ 2.49	1.08 $\times 10^{-1}$ ns	1.56 $\times 10^2$ *	8.50 $\times 10^{-3}$ ns
5	AT	VT	14.63 $\pm$ 4.31	3.58 ns	1.0 $\times 10^{42}$ *	2.19 $\times 10^5$ *
6	AT	R4	14.90 $\pm$ 3.37	5.05 $\times 10^{-1}$ ns	1.51 $\times 10$ *	2.21 $\times 10^{-13}$ ns
7	AT	V5	18.90 $\pm$ 1.85	1.04 $\times 10^{-2}$ ns	3.40 $\times 10^{19}$ *	1.38 $\times 10^4$ *
8	AT	V3	19.10 $\pm$ 2.72	4.50 $\times 10^2$ *	1.00 $\times 10^{31}$ *	2.47 $\times 10^7$ *
9	BTS	V8	29.32 $\pm$ 3.11	1.99 ns	7.30 $\times 10^{220}$ *	1.08 $\times 10^{11}$ *
10	BTS	V2	31.96 $\pm$ 2.63	5.11 $\times 10^1$ *	5.80 $\times 10^{146}$ *	1.56 $\times 10^{12}$ *
11	BTS	R5	33.25 $\pm$ 6.48	3.95 $\times 10^{-3}$ ns	1.47 $\times 10^{10}$ *	1.05 $\times 10^{-30}$ ns
12	BTS	R4	34.33 $\pm$ 4.45	2.74 $\times 10^{-3}$ ns	1.51 $\times 10^{34}$ *	1.62 $\times 10^{19}$ *
13	BTS	V3	35.67 $\pm$ 4.27	1.78 $\times 10^{-1}$ ns	1.88 $\times 10^{32}$ *	4.56 $\times 10^{18}$ *
14	BTS	V2	38.46 $\pm$ 3.16	3.18 $\times 10^{-2}$ ns	1.00 $\times 10^{158}$ *	4.54 $\times 10^{26}$ *
15	BTS	V6	49.92 $\pm$ 4.52	2.35 $\times 10^{-1}$ ns	4.34 $\times 10^{19}$ *	4.39 $\times 10^4$ *
16	BTS	V3	56.76 $\pm$ 5.09	5.77 $\times 10^{-1}$ ns	6.80 $\times 10^{183}$ *	8.95 $\times 10^{22}$ *
17	BTS	V4	64.83 $\pm$ 12.04	4.52 $\times 10^{-2}$ ns	1.82 $\times 10^{24}$ *	2.63 $\times 10^{29}$ *
18	BTS	R6	71.88 $\pm$ 4.79	5.32 $\times 10^{-2}$ ns	2.40 $\times 10^9$ *	1.24 $\times 10^{27}$ *
19	BTS	V6	101.00 $\pm$ 20.31	8.42 $\times 10^{-3}$ ns	2.92 $\times 10^{16}$ *	4.00 $\times 10^{-1}$ ns
20	BTS	R1	155.75 $\pm$ 15.45	6.68 $\times 10^{-2}$ ns	6.60 $\times 10^{116}$ *	1.87 $\times 10^{20}$ *
21	BTS	VT	14.62 $\pm$ 1.59	5.91 $\times 10^1$ *	3.79 $\times 10^{35}$ *	2.32 $\times 10^6$ *
22	BTS	R1	160.28 $\pm$ 10.90	2.40 $\times 10^{-2}$ ns	1.62 $\times 10^{54}$ *	1.01 $\times 10^{28}$ *

Fd = field, St = corn plant stage, AT = Atlantic forest biome and BTS = Brazilian tropical savannah biome.

* and ^{ns} χ^2 significant and non-significant ($\alpha = 0.05$), respectively.

Table 3. Analysis of variance of *Dalbulus maidis* density data evaluated by yellow sticky trap in corn fields to verify the existence of a common aggregation parameter (K common) using a negative binomial distribution.

Factors	Degrees of freedom	Sum of squares	Mean squares	F
Slope $1/k_{\text{common}}$	1	150.09	150.09	94.41*
Intercept	1	4.47	4.47	2.81 ^{ns}
Residual	19	30.20	1.59	
$K_{\text{common}} = 2.8979$				

^{ns} Não-significativo

*Significativo a 5% de probabilidade pelo teste F.

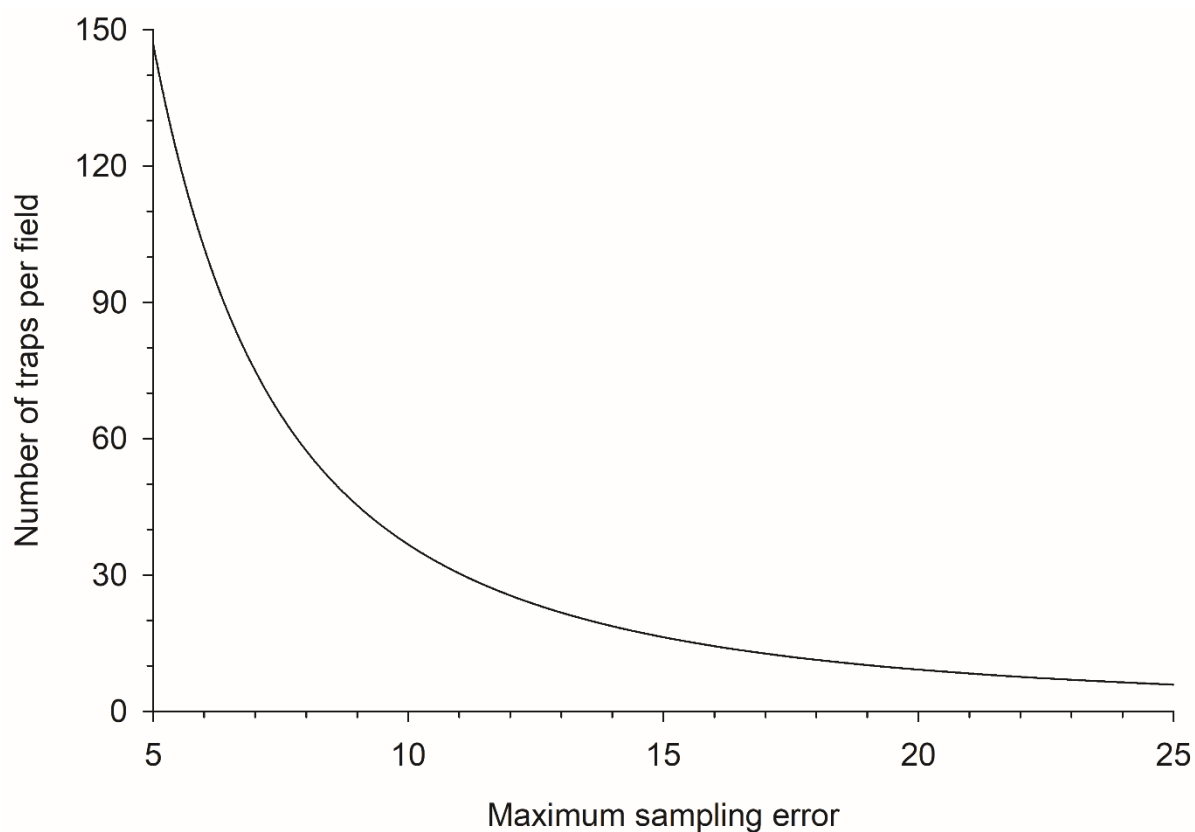


Figure 3. Number of yellow sticky traps to be used in evaluating *Dalbulus maidis* densities as a function of the maximum error allowed in this sampling.

Table 4. Sampling time and costs to evaluate *Dalbulus maidis* densities by yellow sticky traps in corn fields of different sizes.

Field size			Time*				Partial costs						Total cost	
(ha)	Installation		Evaluation		Total		Installation		Evaluation		Materials		(US\$/field)	(US\$/ha)
	(min)	(%)	(min)	(%)	(min/field)	(min/ha)	(US\$)	(%)	(US\$)	(%)	(US\$)	(%)		
2	3.47	70.15	1.48	29.85	4.95	2.47	0.19	18.71	0.08	7.96	0.74	73.33	1.01	0.50
6	6.03	57.64	4.43	42.36	10.46	1.74	0.33	12.03	0.24	8.84	2.16	79.13	2.72	0.45
10	7.77	51.27	7.38	48.73	15.15	1.51	0.42	9.60	0.40	9.13	3.57	81.27	4.39	0.44
30	13.49	37.85	22.15	62.15	35.63	1.19	0.73	5.82	1.20	9.56	10.65	84.62	12.59	0.42
50	17.06	31.60	36.91	68.40	53.97	1.08	0.93	4.49	2.01	9.71	17.73	85.81	20.67	0.41
100	24.38	24.83	73.82	75.17	98.20	0.98	1.33	3.25	4.01	9.84	35.44	86.91	40.78	0.41

* Use of nine yellow sticky traps every two hectares.

GENERAL CONCLUSION

O modelo de previsão e o plano de amostragem determinados são promissores para serem incorporados a programas de manejo integrado de *D. maidis* em cultivos de milho por eles serem robustos, precisos e adequados para prever e tomar decisões