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Damage assessment and monitoring of the whitefly *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) in soybean

Inana Xavier Schutze

Thesis presented to obtain the degree of Doctor in Science. Area: Entomology

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
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1. Mosca-branca 2. Condições de campo 3. Sensoriamento proximal 4. Teor de proteína 5. Teor de açúcares 6. *Glycine max* 7. Amostragem I. Título

DEDICATION

This thesis is dedicated to all the women and girls who once,

like me,

dreamed of becoming scientists.

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EPIGRAPH

"When you want something all the universe conspires in helping you to achieve it".

The alchemist

SUMMARY

RESUMO

Avaliação de danos e monitoramento da mosca-branca *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) na cultura da soja

Nas últimas décadas, *Bemisia tabaci* MEAM1 tornou-se importante praga para a soja brasileira, causando danos diretos e indiretos, entretanto, o nível populacional que efetivamente causa perdas de produtividade na cultura ainda é desconhecida. Seu tamanho reduzido e rápido crescimento populacional tornam o monitoramento um desafio e, como espécie sugadora, torna-se mais difícil medir os danos causados, sendo este inferido indiretamente por perdas de produção. O objetivo neste trabalho foi entender como *B. tabaci* influencia na cultura da soja e identificar novas abordagens para melhorar seu manejo. A temperatura influencia no desenvolvimento de *B. tabaci* afetando a duração do ciclo de vida, o tamanho da população e o rendimento da cultura. Um aumento de 3 ºC afetou a densidade populacional de *B. tabaci*, promovendo um ciclo de vida mais curto e, como consequência, mais gerações da mosca-branca. *Bemisia tabaci* pode causar perdas acima de uma tonelada em uma lavoura de soja o que, em temperaturas de aproximadamente 25 ºC, representou 30% da produção total. Foi observada ainda redução de 33 g no peso de mil grãos e, embora não tenham sido observadas diferenças significativas no vigor dos grãos, as perdas estimadas foram de até 440 kg ha^{-1} no teor de proteína. O monitoramento de insetos-praga nos campos é essencial, mas trabalhoso e às vezes ineficaz. O uso de sensoriamento proximal hiperespectral (PS) permite a identificação de áreas infestadas por artrópodes sem contato com as plantas, otimizando o tempo gasto no monitoramento da cultura, importante para grandes áreas de cultivo. O PS hiperespectral encontrou diferenças nas folhas de soja, não infestadas e infestadas por *B. tabaci*, com boa acurácia pelas respostas das bandas relacionadas à fotossíntese e teor de água, permitindo discriminar os diferentes níveis de infestação e separar as folhas de soja saudáveis das infestadas com base em sua refletância. A tomada de decisão imprecisa em um programa de manejo integrado de pragas pode levar a um controle ineficaz. Além disso, altos níveis de infestação podem diminuir a atividade fotossintética da soja, reduzindo seu desenvolvimento e produtividade. Uma alternativa para medir diretamente os danos causados por *B. tabaci* é acompanhar como a alimentação do inseto altera a composição química da folha e a fotossíntese de plantas infestadas. A composição química afeta o desempenho das moscas-brancas, plantas com maiores teores de açúcares apresentam melhores condições para o desenvolvimento de *B. tabaci*, também tem sido relatada redução da taxa fotossintética causada pela infestação da mosca-branca em muitas lavouras causando perdas de produtividade, associadas à diminuição do teor de clorofila. Redes de correlação foram criadas a partir de dados sobre teor de açúcares, amido e parâmetros fotossintéticos conectando esses fatores ao número de ninfas. A alimentação de *B. tabaci* afetou a fisiologia da planta e sua interação foi refletida pelas relações entre os parâmetros fotossintéticos, bem como os níveis de açúcares e amido. Os resultados encontrados aqui são úteis para melhor entender algumas condições em que a soja é mais vulnerável a *B. tabaci*, fornecendo ferramentas para melhorar o monitoramento e controle desta praga-chave.

Palavras-chave: Mosca-branca, Condições de campo, Sensoriamento proximal, Teor de proteína, Teor de açúcares, *Glycine max*, Amostragem

ABSTRACT

Damage assessment and monitoring of the whitefly *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) in soybean

In recent decades, *Bemisia tabaci* MEAM1 has become a serious pest for soybean in Brazil, causing direct and indirect damage, however, the population level that effectively causes productivity losses in soybean is not known. *Bemisia tabaci* reduced size and fast population growth make monitoring a challenge, and since whiteflies are sucking species, it is more difficult to measure the damage caused by this pest, so generally, the damage is indirectly inferred by yield losses. The goal here was to better understand how *B. tabaci* influences the soybean crop and to identify new approaches for improving the management of this pest. Temperature influences *B. tabaci* development affecting the lifecycle length, population size, and crop yield. An increase of 3 ºC in temperature affected the population density of *B. tabaci*. The higher temperature promoted a shorter lifecycle and, as consequence, more *B. tabaci* generations. *Bemisia tabaci* can cause yield losses of over a ton in a soybean field and, in temperatures around 25 ºC, it represented 30% of total production. A decrease of 33 g in the weight of thousand grains was also observed, and although no significant differences were observed among the grains' vigor quality, estimated losses were up to 440 kg ha^{-1} in protein content. Monitoring insect pest populations in the fields is essential, but laborious and sometimes ineffective. The use of hyperspectral proximal sensing (PS) is a tool that allows the identification of arthropod-infested areas without contact with the plants. This optimizes the time spent on crop monitoring, important for large cultivation areas. The hyper-spectral PS was used to find differences in the responses obtained from *B. tabaci* soybean non-infested and infested leaves, with good accuracy by the responses of the bands related to photosynthesis and water content, which allowed us to discriminate the different levels of infestation and to separate healthy from whitefly infested soybean leaves based on their reflectance. Imprecise decision-making in an integrated pest management program may lead to ineffective control. Also, high infestation levels may diminish the photosynthetic activity of soybean, reducing their development and yield. An alternative to directly measuring the damage caused by *B. tabaci* is following how the insect feeding alters leaf chemical composition and photosynthesis of infested plants. Leaf chemical composition affects the performance of whiteflies. Plants with a higher level of sugars present better conditions for the development of *B. tabaci*, also reduction of the photosynthetic rate caused by whitefly infestation has been reported for many crops causing yield losses, associated with decreases in chlorophyll content. Correlation networks were created from data on sugar content, starch, and photosynthetic parameters connecting these factors to the number of nymphs. *Bemisia tabaci* feeding affected the plant's physiology and its interaction were reflected in part by the relationships among photosynthetic parameters as well as the levels of sugars and starch. The results found here are helpful for better understanding some conditions in which soybean is more vulnerable to *B. tabaci*, providing tools for improving the monitoring and control of this key pest.

Keywords: Silverleaf whitefly, Field conditions, Hyperspectral proximal sensing, Protein content, Sugar content, *Glycine max*, Sampling

1. GENERAL INTRODUCTION

The Economic Injury Level has not been studied in recent years, especially for hemipterans. Among these, *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) represents a key pest for several economically important crops such as soybean (*Glycine max* (L.) Merril) (Pozebon et al., 2020).

The *B. tabaci* complex, is composed of highly polyphagous species, with more than 600 hosts (Oliveira, Henneberry and Anderson, 2001). Currently, several biotypes of *B. tabaci* are known, among these biotypes B and Q, also described within the whitefly complex as Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) species, respectively, are those of greater economic importance in the world (Ansari et al., 2017), in Brazil, biotype B (species MEAM1) is more widely distributed today (de Moraes et al., 2018). This species is considered one of the most invasive and destructive pests, including soybean (Baldin et al., 2017).

In Brazil, the pest's first occurrence was biotype A and dates from 1928 in western Bahia. However, economic losses were reported only in 1968 in cotton, in the states of São Paulo and Paraná (Costa et al., 1975). The biotype B was identified in these states in 1991, and studies indicate that biotype A was being replaced by biotype B, which became dominant (Lourenção and Nagai 1994), presenting a greater number of hosts (Perring, 2001) and high potential reproductive (Gulluoglu, Arioglu and Kur, 2010). *Bemisia tabaci* was considered a secondary soybean pest in Brazil until the 2000s and currently represents a species of economic importance in the crop (Vieira et al., 2016).

The damage caused by the whitefly to soybean is the result of feeding and can be classified as direct and indirect damage. Direct damage is related to phloem sap suction, which can cause plant death in high infestations (Vieira et al., 2016). Indirect damage can occur either by favoring the growth of the fungus *Capnodium* sp., also known as sooty mold, on the leaf surface of plants causing loss of photosynthetic capacity or by the transmission of the CpMMV (Cowpea mild mottle virus), responsible for causing the disease of stem necrosis, reported in soybeans in the country in the early 2000s (Almeida et al., 2002, Belay et al., 2012, Vieira et al., 2016).

Despite spending most of the cycle in the sessile phase in the plant (Walker et al., 2010), complicating factors such as the location on the leaf's abaxial surface and the waxy layer that covers its body (Güllüoğlu et al., 2010) hinder the contact of the insecticide with the whitefly, thus prioritizing the use of systemic insecticides for control. Also, the fact that *B.*

tabaci MEAM1 is one of the most polyphagous species (Perring, 2001) favors the insect's permanence in the environment, making it difficult to control.

Another factor is the complexity in quantifying the damage caused by the whitefly and the lack of an established level of damage, which leads the producer to carry out preventive applications, only due to the presence or absence of the insect, without considering a tolerated density, leading to a greater number of applications in the field (Vieira et al., 2013).

The damage caused by *B. tabaci* is estimated by loss in production. However, other strategies can be used to predict the losses, like interference in the photosynthesis and chemical composition of the leaves, such as sugars, the main component in the phloem sap and honeydew. Interactions between the chemical composition of leaves and the performance of whiteflies reported that varieties with a higher glucose and sucrose concentrations provide better conditions for the development of *B. tabaci* (Hasanuzzaman et al., 2018). A positive correlation between carbohydrate levels and whitefly populations has also been reported (Hegab et al., 2014), and that higher amounts of sugars, as glucose and fructose, also contribute to higher densities of whiteflies in cotton (Bi et al., 2005). In regards to photosynthesis, the reduction in the photosynthetic rate caused by *B. tabaci* infestation has been reported for many crops (Lin et al., 1999; Chen et al., 2004; Islam and Ren, 2009), and reduction in the net photosynthetic rate (Chen et al., 2004; Lin et al., 1999) induced by *B. tabaci* infestation, associated with decreases in photosynthetic capacity and stomatal conductance (Buntin et al., 1993).

The main control method used in the management of *B. tabaci* is chemical control with the use of insecticides (Baldin et al., 2017). In Brazil, cases of resistance have been reported for many insecticides such as acetamiprid, imidacloprid, thiamethoxam, endosulfan, chlorpyrifos, cartap, chlorantraniliprole, among others (Silva et al., 2009; Dângelo et al., 2018), making it necessary greater attention to the correct use of such products, to preserve molecules and consequently maintain valid tools for insect control.

In addition to representing higher costs to the producer, the abuse of insecticides ignores the fact that the *B. tabaci* MEAM1 species currently presents several cases of resistance to insecticidal molecules (APRD, 2017), which may result in a rapid loss of product efficiency, restricting options in some cases, and increasing the relevance of defining the best time to use the technique, to preserve them. Both biological and behavioral species characteristics favor the probability of selecting resistance to commercial insecticides, such as rapid development, high reproductive, and dispersal capacities (do Valle and Lourenção,

2002). Population outbreaks of whitefly in the soybean crop in recent years may even be related to the abusive use of non-selective insecticides to natural enemies (Vieira et al., 2012).

The soybean crop was one of the pioneers in the establishment of Integrated Pest Management (MIP), today it has more than 40 years of implantation in Brazil (Moscardi et al., 2012). The IPM was suggested as a change in the strategy of pest control, with interest in sustainability and environmental quality, with a focus on ecology and economic reality in pest control programs, and a greater emphasis on tactics to be used than on tactics themselves (Higley and Pedigo, 1996).

In Brazil soybean is mainly cultivate in large areas, which demand efficient monitoring and decision-making regarding pest control. One of the strategies that have been gaining prominence is the use of remote sensing to achieve this efficiency in large crops, such as those found in soybean in Brazil (Dara, 2019). Remote sensing is an important tool for monitoring, especially small pests hard to identify in the field such as whiteflies. This scenario promotes the implementation of remote or proximal sensing technologies and their benefits, especially the potential time saved by automatizing crop monitoring (Carrière et al., 2006, Backoulou et al., 2011). Remote/proximal sensing has been used to detect stress caused by arthropod her-bivory in a variety of plant species, including soybean (Huang et al., 2013). The most promising results were achieved in studies based on hemipteran pests because their feeding activity (sucking) indirectly affects the infested plants' physiology, and therefore, their reflectance profiles (Iost Filho et al., 2020). In most of these studies, infested and non-infested plants were discriminated against with good accuracy.

Currently, Brazil is the largest producer of soybeans in the world, in 2021 it is expected to produce approximately 134 million tons (CONAB, 2021). The crop occupies the largest planted area in the country, totaling approximately 34 million hectares, while corn, the second-largest planted area, occupying only half of this area (17.3 million ha) (CONAB, 2017).

Establishing a level of control for a pest requires not only measuring the damage it causes to the crop, but also knowing its interactions, such as how it affects the plant, when the plant is most vulnerable. Thus, the goal of this study was to find ways to provide a better understanding of how *B. tabaci* influences the crop and to identify new approaches for improving monitoring and the management of this pest in soybean.

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2. DAMAGE ASSESSEMENT OF *Bemisia tabaci* MEAM1 (HEMIPTERA: ALEYRODIDAE) ON SOYBEAN UNDER FIELD CONDITIONSN

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Abstract

Bemisia tabaci MEAM1 (Hemiptera: Aleyrodidae) is a key insect pest in soybean fields in Brazil. Temperature influences its development affecting the lifecycle length, population size, and crop yield. This study investigated different densities of *B. tabaci* that can affect soybean yield and change in vigor and protein content of soybean grains. Differences in temperature in the two harvest years affected the population density of *B. tabaci*, reaching 413 nymphs per leaflet in the first year, and 179 the second year, when the average temperature was 3 ºC higher. The higher temperature promoted a shorter lifecycle and, as consequence, more *B. tabaci* generations with less overlap in the leaves. Yield was affected with losses up to 500 kg ha^{-1} in 2017/2018 and 1,147 kg ha^{-1} in 2018/2019. We also observed a decrease in the weight of thousand grains of 18 g in the first year and 33 g in the second. Although no significant differences were observed among the grains' vigor quality, estimated losses were up to 440 kg ha^{-1} in protein content. *Bemisia tabaci* MEAM1 can cause yield losses over a ton in a soybean field and, in temperatures around 25 ºC it represented 30% of total production. Although the vigor parameters of soybean grains were not affected, the infestations resulted in losses in the grains' protein content. Establishing IPM programs is a challenge, and there is a constant need for improvement. Results, as shown in this study, can be helpful in the decision making to control the whitefly in soybean areas.

Keywords: *Glycine max*, Field condition, IPM, Yield, Grain weight, Temperature, Silverleaf whitefly

2.1. Introduction

Bemisia tabaci Middle East-Asia Minor 1 (MEAM1) (Gennadius, 1889) (Hemiptera: Aleyrodidae) is a widely spread pest, known for causing damages to diverse species of cultivated crops like cotton, potato, tomato, and recently gaining more attention, soybean (Baldin et al., 2017; Oliveira et al., 2001). The whitefly *B. tabaci* MEAM1 was first reported in Brazil in 1991 (Lourenção and Nagai, 1994), and its importance on soybean related in

2000's (Moscardi et al., 2012; Vieira et al., 2016). Three more *B. tabaci* species are present in Brazil, the two native species from the New World (NW1 and NW2) group (Marubayashi et al., 2013) and the Mediterranean (MED) (Barbosa et al., 2015). However, MEAM1 is the predominant species in the country and open fields, present in all soybean producer regions. MEAM1 is also the only species detected in Central West of Brazil. Although MED species is found in the South and Southeast mainly infesting ornamentals plants and greenhouses (de Moraes et al., 2018; Pozebon et al., 2020), it was found colonizing soybean plants in open fields in São Paulo and Paraná States in Brazil (Bello et al., 2021).

Soybean is a major vegetable protein source, and thirty percent of its grain is protein (USDA, 2019a). Soybean is the fifth most produced crop in the world (FAO, 2017), and also the fourth most produced cereal, Brazil and United States are the main soybean producers, representing 70% of world production, with an expected production of 136 and 112 million tons respectively in 2021 (USDA, 2019b). The damages caused by *B. tabaci* MEAM1 include losses up to 30% in soybean yield (Vieira et al., 2013), control costs of US$ 75 ha^{-1}, and more than US$ 3 billion on Brazil's agriculture since the establishment of the pest (Pozebon et al., 2020). Despite the importance of soybean and the relevance of *B. tabaci*, there is no Economic Injury Level (EIL) stablished to this pest in the crop.

Climatic factors play an important role in the performance and development both on crops and pests. Agricultural zoning was established in Brazil to assist farmers in making decisions regarding the best sowing time and cultivars that best adapt to their region in relation to climatic conditions, such as temperature (MAPA, 2020a). Central West of Brazil is the main soybean producer in the country, and the average annual temperature in the region is one of the factors that makes it an excellent location for crops such as soybean.

The temperature has influence not only on the crop, but also in the insect's lifecycle length. In the case of *B. tabaci,* the lifecycle can vary from 70 days at 15 °C to 21 days at 30 °C using soybean as host (Albergaria and Cividanes, 2002). These variations in the lifecycle length can affect the whitefly density, which would lead to consequences in the yield.

The aim of this study was to evaluate the damages caused by different densities of *B. tabaci* on soybean at field conditions, in order to quantify the population level capable of causing damage to soybean yield and to verify changes caused by the pest attack to the plants on vigor and protein content of the grains.

2.2. Material & Methods

Insects

The initial *Bemisia tabaci* MEAM1 population was obtained from the colony of Agronomic Institute of Campinas (IAC) in Campinas, São Paulo, Brazil in 2017, which was previously identified as *B. tabaci* MEAM1. Molecular characterization of the insects was made periodically during the study, for confirmation of the insect biotype (De Barro et al. 2003). The *B. tabaci* was maintained in greenhouse conditions (approximately 13 hours photoperiod at 29 °C and 40 ± 10% RH) on cabbage (*Brassica oleracea* L.) at the Dept. of Entomology and Acarology of Luiz de Queiroz College of Agriculture (ESALQ). For bioassays, adults were collected selecting paired couples to obtain an approximate 1:1 sex ratio (Byrne and Bellows, 1991).

Determination of population level of *B. tabaci* MEAM1 on field

Experiments were conducted in two soybean (BRS 232) seasons, 2017/2018 and 2018/2019, from mid-December to early March. The first was conducted at the experimental field in the Federal University of São Carlos (UFSCar), Buri, São Paulo, Brazil, under geographical coordinates of W 48°32’59.54” and S 23°36’28.07” and the second at the experimental field at ESALQ, Piracicaba, São Paulo, Brazil, under geographical coordinates of W 47°37’24.7” and S 22°42’55.4”. The fields were tilled and fertilized with nitrogen, phosphorus and potassium, following the standard procedures (Malavolta, 1992). The soil is classified as dystrophic red-yellow latosol. The experiments had a block design with randomized plots, four treatments, consisting of three levels of infestation and a control treatment (no infestation) (Table 1). Each plot was represented by a field whitefly-proof cage (C 2.0 × W 1.7 × H 1.6 m) supported by four bamboo stems (2 m), covering three lines with an average of 25 soybean plants per line, spaced 50 cm between lines and 7cm between plants, around 75 plants per cage. There were four replication per treatment, totaling 16 plots, each year. Assessments were made weekly as the culture developed for two months.

The number of adults released inside the cages was determined based on previous works to obtain a certain number of nymphs per leaflet throughout development of the crop to reach an expected population density (Table 1) (Vieira et al., 2013, 2016). All the 16 cages were placed when soybean was in V1 phenological stage (Fehr et al., 1971) and the

infestations performed when the soybean was in V2. The experiment was conducted throughout the development of the soybean.

The infestation level in each plot was evaluated weekly, counting the number of adults present on 10 leaflets between the middle and upper thirds of random plants inside the cage. Leaflets were collected and evaluated in the laboratory, where the number of eggs and nymphs of *B. tabaci* MEAM1 on each leaflet were countedand the leaflet area was measured using a Leaf Area Meter (LI-3000C, LI-COR Inc.).

Table 1 Infestation levels of treatments, corresponding to the number of adults of *Bemisia tabaci* MEAM1 released on soybean and expected population density of nymphs per leaflet in field experiment

Treatment	Infestation level	Adults released	Expected population density (nymphs/leaflet)
T1	Control	0	0
T2	Low	300	60
T3	Medium	600	120
T4	High	1200	240

When the expected population density was reached (Table 1) the soybean plants from treatments were sprayed with cyantraniliprole (Benevia^{TM}) at the dose of 125 g a.i./ha using a backpack sprayer.

Field cage infestation

To transport the specimens to the field cages, acetate cages enclosed on the top with “voile” fabric were used, which covered potted cabbage seedlings. The adults of *B. tabaci* were collected from the rearing plants in a greenhouse, with a sucker made with 5/16” clear plastic hose, “voile” fabric and 10 mL pipette tip. The specimens were quantified and transferred to the acetate cages 24 hours before infestations. The acetate cages were taken to the field and removed from the pots inside the field cages, leaving the cabbage seedlings exposed and the whiteflies free to disperse in the field cage.

Determination of damage caused by *B. tabaci* MEAM1 on soybean yield

At the end of the ripening stage (R8), at the ideal time of harvest, the pods were collected and stored in paper bags for drying (up to 13% humidity) using air circulation at 40 ºC to evaluate the yield (kg ha^{-1}) and weight of 1000 grains (g).

Grain evaluation

These analyses were conducted using the grains from the second season (2018/2019), harvested they were stored in a cooling chamber (T 20 °C and 40% RH) until the time of analysis.

Moisture content levels. The samples were weighed to an average of 22 g of soybean seeds per plot and placed in aluminum containers. Each container and its lid were previously weighed and identified. The samples were then evenly distributed in the containers and placed in the oven on their respective lids and kept at 105° C for 24 hours. After the drying period, the samples were removed from the oven, quickly covered, and placed in a desiccator with silica gel until it cooled and then weighed again, following the recommendations contained in the Rules for Seed Analysis (RAS) (MAPA, 2020b). For each sample, two repetitions were performed. Equation (1) was used to calculate the moisture percentage based on the wet weight of the grains.

$$\%\ \text{Moisture (M)} = 100(\text{P} - \text{p})/\text{P} - \text{p} \qquad (1)$$

Where:

P = initial weight, weight of the container and respective lid plus the weight of the wet sample;

p = final weight, weight of the container and respective lid plus the weight of the dry sample;

t = tare weight of the container and its lid.

The final humidity was obtained by the arithmetic mean of the percentages of each repetition.

Germination test. For the germination test, two repetitions were performed, consisting of 50 seeds of each plot per repetition. In each repetition, the seeds were homogeneously distributed through a 50-cell seed tray on two sheets of paper for seed germination (28 × 38 cm) moistened with an amount of water equivalent to 2.5 times the weight of the substrate,

after sowing the seeds were covered with another sheet of paper under the same conditions. Subsequently, individual rolls were made for each repetition and then grouped into four rolls joined by elastic at the upper and lower ends. The rolls were then placed in a germination chamber at 25 °C, and the evaluations were carried out by four and seven days after sowing (DAS), according to RAS (MAPA, 2020b). The evaluations consisted of counting the normal germinated seeds, the abnormal germinated seeds, the seedlings, and the dead seeds. Germinated seeds were considered normal when all essential structures such as the hypocotyl and primary root have developed, cotyledons with less than 50% of damage, the primary leaves are green, and the apical bud is in expansion. All these structures should be developed, complete, proportionate, and healthy. Seeds that did not have such characteristics were considered abnormal. The seeds that did not germinate 4 DAS were kept in the rolls for evaluation at 7 DAS. The results were expressed as a percentage of normal seedlings in each plot.

Grain vigor evaluation

Accelerated aging test. Seeds were distributed in a single layer on a wire mesh in gerbox type plastic boxes (11 × 11 × 3.5 cm) containing 40 mL of water, keeping the seeds out of contact with water. The boxes were transferred to a BOD incubator at 41 °C, and maintained for 48 hours, after the germination test was performed at 25 °C, as described in the previous experiment, during 4 DAS. After this period the percentage of normal seedlings of each plot was counted. In the accelerated aging test (AAT), two repetitions of each plot were made, each repetition consisting of 50 seeds.

Electrical conductivity. Two repetitions were performed per plot, containing 50 seeds weighed per repetition. The samples were placed in disposable plastic cups with a capacity of 200 mL, containing 75 mL of distilled water. The cups were then kept in a BOD incubator at 25 °C for 24 hours. After conductivity meter readings were performed, the readings are expressed in $\mu Scm^{-1}g^{-1}$. Equation (2) was used to calculate the mean electrical conductivity between repetitions.

$$\text{Electrical conductivity (EC)} = \left(\left(\frac{\text{reading 1}}{\text{weight 1}}\right) + \left(\frac{\text{reading 2}}{\text{weight 2}}\right)\right)/2 \quad (2)$$

Where:

Reading = reading of the conductivity meter ($\mu Scm^{-1}g^{-1}$) for each repetition;

Weight = weight (g) of each repetition.

Grain protein content evaluation

Sample preparation. Two repetitions of approximately 15 g of each field plot were separated. The samples were ground in an electric knife mill until they acquired the consistency of flour. Then, the samples were stored individually in a universal collector with a lid.

Moisture. The moisture content was determined by grinding the soybean in an IKA mill model: A11BS32. Then, the fresh mass (around 5 g) was weighed in a watch glass, previously dried for 60 minutes in an oven at 40 °C. After weighing, the ground soybeans were placed in a ventilated oven at a temperature of 60 °C overnight. After being cooled in a desiccator, they were subjected to new weightings until a constant weight was obtained. These measurements were performed with two repetitions for each sample. The percentage of humidity (U%) was obtained by equation (3) (Araújo et al., 2006).

$$U\% = (M_f - M_d) * 100/M_f \quad (3)$$

Where:

M_f = initial fresh mass,

M_d = dry mass after drying in an oven.

For the moisture, glass plates were labeled with a sample number, had their weight recorded, then 5 g of each sample was weighed onto the respective plates. The plates containing the samples went to the oven at 60 °C, where they stayed for approximately 14 hours for drying. After removing it from the oven, the plates were placed inside the desiccator, where they remained until they reached room temperature. Then the plates were removed and already weighed, avoiding the accumulation of moisture.

After all the plates were weighed, they returned to the oven for another hour, then a second weighing was carried out following the same criteria.

The humidity was calculated according to the percentage of humidity in relation to the wet and dry mass.

Crude protein content determination. The crude protein content was determined by the Kjeldhal method (Bataglia et al., 1983). The ground soybeans were weighed and transferred to a test tube where approximately 0.5 g of potassium sulfate catalyst and 3 mL of concentrated sulfuric acid were added. The digestion process starts when the temperature of the digesting plate reached the temperature of 350 ºC, this takes around 12 hours to be complete. After cooling the tubes, 2 mL of hydrogen peroxide was added, and the acid solution was reheated at a temperature of 70 ºC until the residual hydrogen peroxide evaporates. Nitrogen was distilled in a basic medium with 15 mL of sodium hydroxide at 30%, collecting the distilled ammonia in boric acid solution in the presence of the indicators. The nitrogen content was determined by titrating the samples in boric acid with 0.1 N hydrochloric acid solution until the color change from greenish blue to pink. Equation (4) was used to calculate the protein contain.

$$\%\ \text{protein} = \frac{(V_{HCl} - V_{blank}) * N_{HCl} * 14 * 100 * 6.25}{DM_g} * 1000 \qquad (4)$$

Where:

V_{HCl} = volume of HCl solution spent on sample titration;

V_{blank} = volume of HCl solution spent titrating solution with no sample added;

N_{HCl} = normality of HCl; DM = dry mass.

Statistical analysis

Data were subjected to exploratory analyzes to verify normality, homogeneity, and suitability to the Shapiro-Wilk (Shapiro and Wilk, 1965) and Bartlett (Bartlett, 1952) models for further analysis of variance (ANOVA) and multiple comparisons of means by Tukey's test. Due to the variability of natural events in the field, statistical significance was set at $p \leq 0.1$ for field bioassays and $p \leq 0.05$ for laboratory bioassays (SAS Institute, 2001).

2.3. Results

Determination of population level of *B. tabaci* MEAM1 on field

In the first season, in Buri, the population of *B. tabaci* reached higher levels for all the whitefly stages evaluated: eggs (Fig. 1), nymphs (Fig. 2), and adults (Fig. 3) compared to the second season, in Piracicaba.

The initial egg density of *B. tabaci* in both years was similar, presenting a drop in the number in the first weeks, and a fast increase at the beginning of soybean reproductive stage (R1-R2), reaching 6.9 eggs cm^{-2} of leaf in the first year and 2.6 eggs cm^{-2} in the second (Fig. 1). The number of eggs remained high for weeks, enough to maintain the population levels along the soybean cycle.

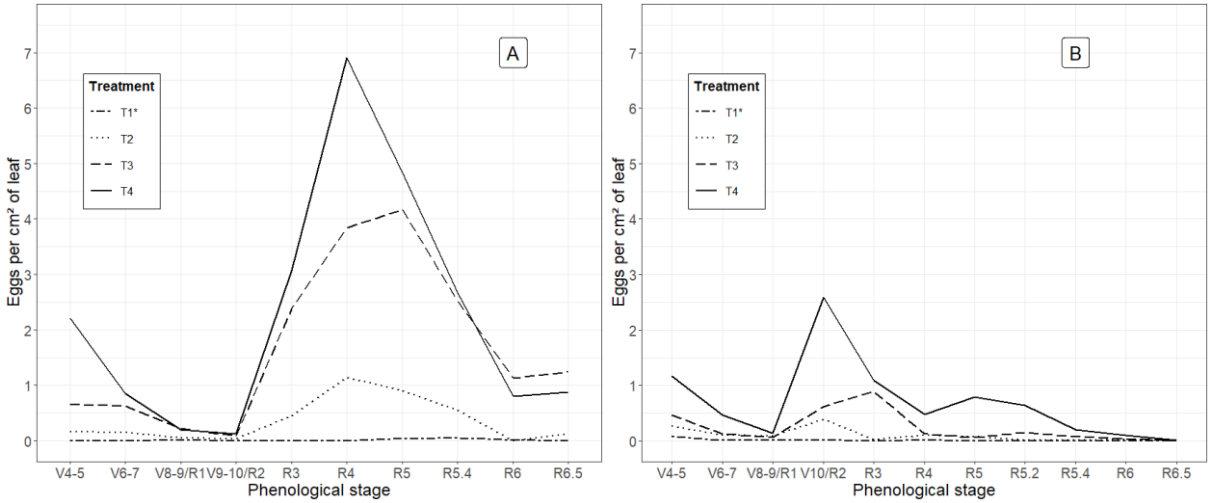

Figure 1. *Bemisia tabaci* MEAM1 eggs per square centimeter of leaflet over the phenological stages of soybean in two crop seasons A (2017/2018) and B (2018/2019). Treatments: control with no infestation ($T1^{*}$), low infestation (T2), medium infestation (T3) and high infestation (T4) levels.

An increase in the number of nymphs was observed in the soybean reproductive stage (Fig. 2). The highest densities occurred on R5 in both years, reaching 7.4 nymphs cm^{-2} of leaf in crop season 2017/2018 and 2.8 nymphs cm^{-2} in 2018/2019. Both population densities then began to decline, coinciding with the end of the soybean cycle, when fewer leaves were available.

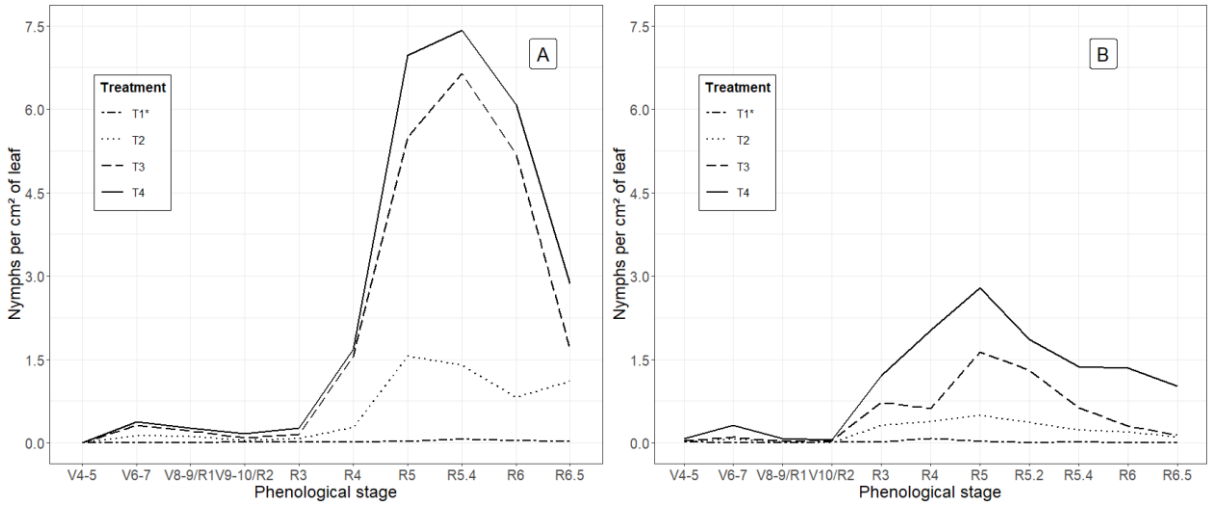

Figure 2. *Bemisia tabaci* MEAM1 nymphs per centimeter square of leaflet over the phenological stages of soybean in two crop seasons A (2017/2018) B (2018/2019) control with no infestation (T1*), low infestation (T2), medium infestation (T3) and high infestation (T4) levels.

The adults of *B. tabaci* who initially infested the cages laid the first eggs in the soybean and remained in the crop for the first weeks, with few specimens present until the emergence of a new generation of adults after 3-4 weeks (Fig. 3). In season 2017/2018, the highest number of adults reached 0.43 cm^{-2} per leaf, while in 2018/2019 this number was 0.16 cm^{-2}.

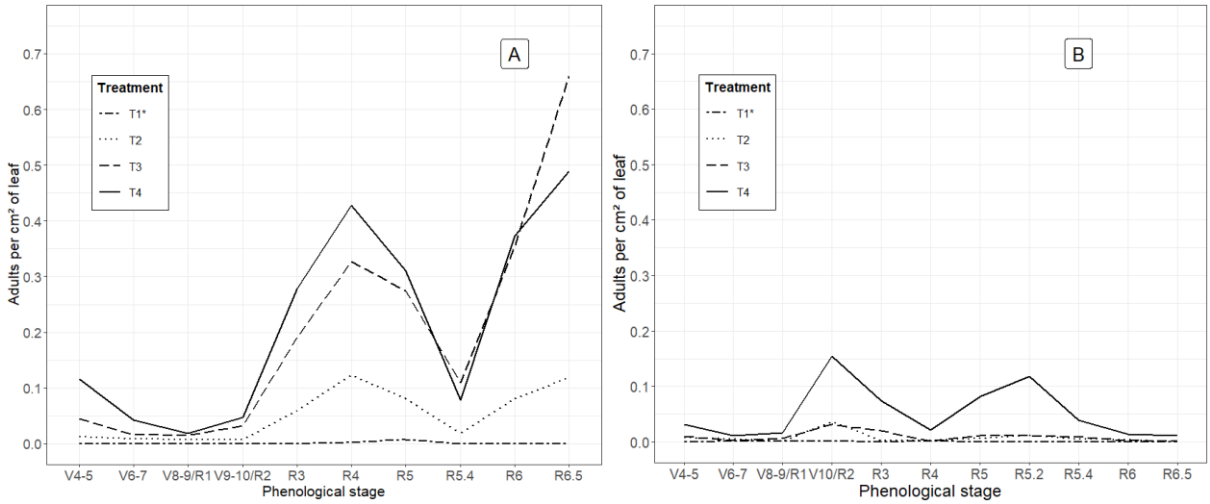

Figure 3. *Bemisia tabaci* MEAM1 adults per centimeter square of leaflet over the phenological stages of soybean in two crop seasons A (2017/2018) B (2018/2019) control with no infestation (T1*), low infestation (T2), medium infestation (T3) and high infestation (T4) levels.

The rains in January, typical in this time of the year in the Southeast of Brazil, affected the density of adults in the trials on R5.4 in 2017/2018 and R4 in 2018/2019 (Fig. 4), reducing the number of all stage of *B. tabaci* (Figs. 1, 2, and 3).

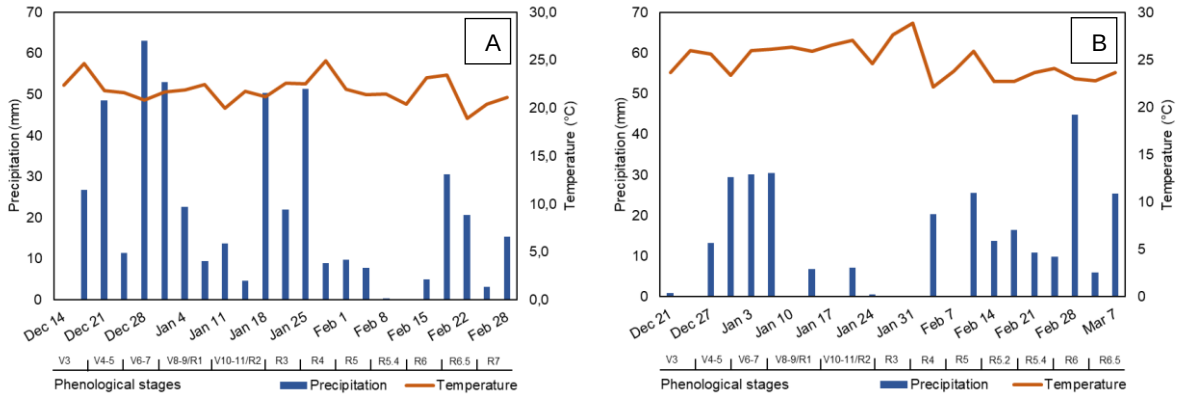

Figure 4. Precipitation (mm) and average temperature (°C) in the municipality of Buri, São Paulo, Brazil, during the crop season 2017/2018 (A), and Piracicaba, São Paulo, Brazil, during the crop season 2018/2019 (B).

Comparing *B. tabaci* population dynamic between the treatments, we can observe a similar development (Figs. 1, 2). All the infested treatments had the population peak occurring on R4 and R5 stages for eggs and adults, and R5 and R5.4 stages for nymphs, coinciding with the seven days required for the hatching of the nymphs at 25 °C (Albergaria and Cividanes, 2002).

In the second year, however, the time required for nymphs to hatch was longer. The peak of eggs occurred around two weeks before the nymphs, which could be related to the occurrence of rainfall in this period of the year, bringing the temperature down (Fig. 1B).

The population of *B. tabaci* observed in the first year of trial was almost two times higher than in the second year for infested treatments. In 2017/2018, the total mean number of nymphs per cm^2 leaflet were 5.52 (T2), 21.34 (T3), and 26.09 (T4) (Fig. 2), while in 2018/2019 these numbers were 2.16 (T2), 5.56 (T3), and 12.11 (T4) (Fig. 2). The control treatment (T1), where no insects were release, had a similar occurrence of *B. tabaci* nymphs in both years, 0.21 (2017/2018), and 0.15 (2018/2019).

The peak in the number of nymphs in the first year occurred in R5.4 (T1= 0.06; T2= 1.39; T3= 6.64 and T4= 7.42 nymphs per cm^2 leaflet), eight weeks after infestation with adults of *B. tabaci* MEAM1. During this same week, there was the occurrence of rainfall, that might have kept the number of nymphs from growing (Fig. 2).

Similar occurred in the second year of trial, the highest density of nymphs was observed when soybean was in R5 (T1= 0.02; T2= 0.49; T3= 1.63 and T4= 2.78 nymphs per cm^2 leaflet), coinciding with rainfall (Fig 2).

All the treatments reached the highest populational density level during the reproductive phenological stage in both years, R5.4 in 2017/2018, and R5 in 2018/2019.

Determination of damage caused by *B. tabaci* MEAM1 on soybean yield

In the first year, 1,947 kg ha^{-1} of soybean yield was obtained in the control treatment (T1), in the second, the soybean yield was 3,787 kg ha^{-1}, almost two times higher than the first year. Similar results were observed in the infested treatments despite the higher density of *B. tabaci* present in all the treatments on the first year of trial (Fig. 5).

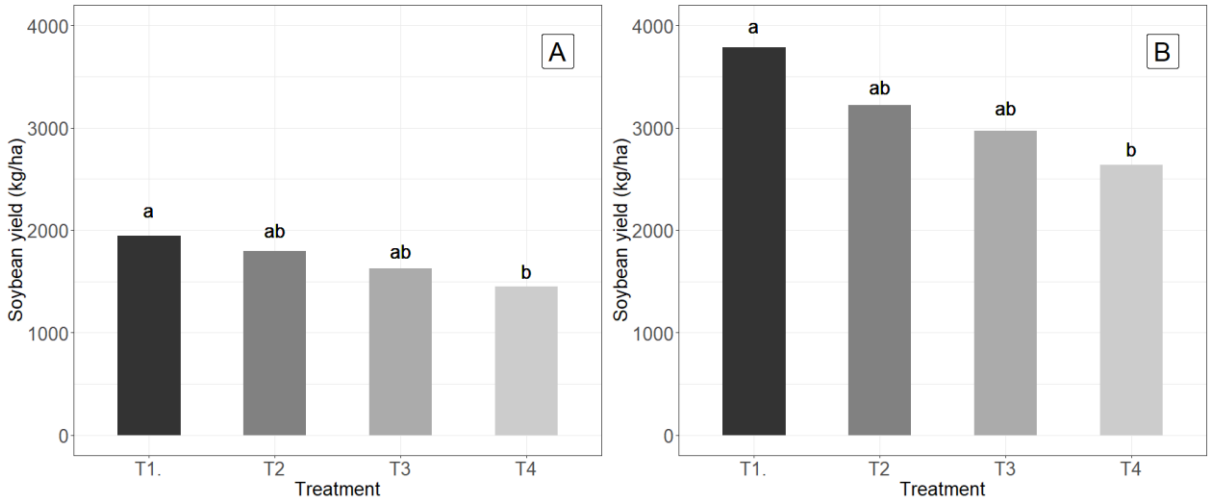

Figure 5. Yield of soybean (kg ha^{-1}) exposed to different *Bemisia tabaci* MEAM1 infestation levels (T1 control, no infestation, T2 low, T3 medium, T4 high infestation) in two crop seasons A (2017/2018) ($p < 0.10$) and B (2018/2019) ($p < 0.05$).

Although the densities of *B. tabaci* in the first year were higher, the control treatment (T1), where no whitefly was released, also resulted in lower production (1,947 kg ha^{-1}), and lighter weight of thousand grains (128.75 g) (Figs. 5, 6), compared to the second year. However, in the second year, when a lower density of whitefly was observed in the infested treatments, a greater production (3,787 kg ha^{-1}) and weight of thousand grains (185.1 g) also were obtained (Figs. 5, 6) in the control.

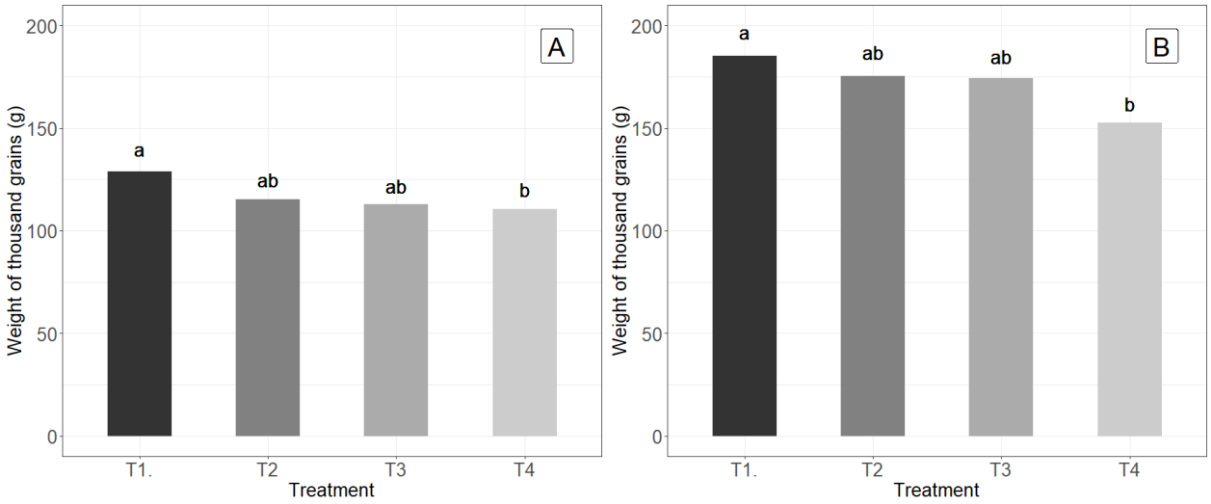

Figure 6. Weight of thousand grains of soybean (g) exposed to different *Bemisia tabaci* MEAM1 infestation levels (T1 control, no infestation, T2 low, T3 medium, T4 high infestation) in two crop seasons A (2017/2018) ($p < 0.10$) and B (2018/2019) ($p < 0.05$).

In both years it was possible to observe differences in the yield and weight of thousand grains between the control (T1) and the high infestation treatment (T4). In the second year, (2018/2019) this difference was more pronounced, with a significance level of 5%, high for field trials, which might be a consequence of the higher production in that year.

The population density in the first year had higher numbers later in the season. The number of eggs picked on the sixth week of evaluation had averages of 7 eggs cm^{-2} per leaf. Two weeks later, the number of nymphs reached the highest density of 7.5 nymphs cm^{-2}, and after two more weeks, the peak of adults was observed, with 0.65 adults cm^{-2}, all in the higher infestation cages. While these values were 2.5 eggs cm^{-2} on the fourth week of evaluation, 2.75 nymphs cm^{-2} on the seventh week, and 0.15 adults cm^{-2} on the second year of trial, that represents around 64% fewer eggs and nymphs compared to the first year when temperatures were higher. For adults, this reduction was even greater, with density 77% lower.

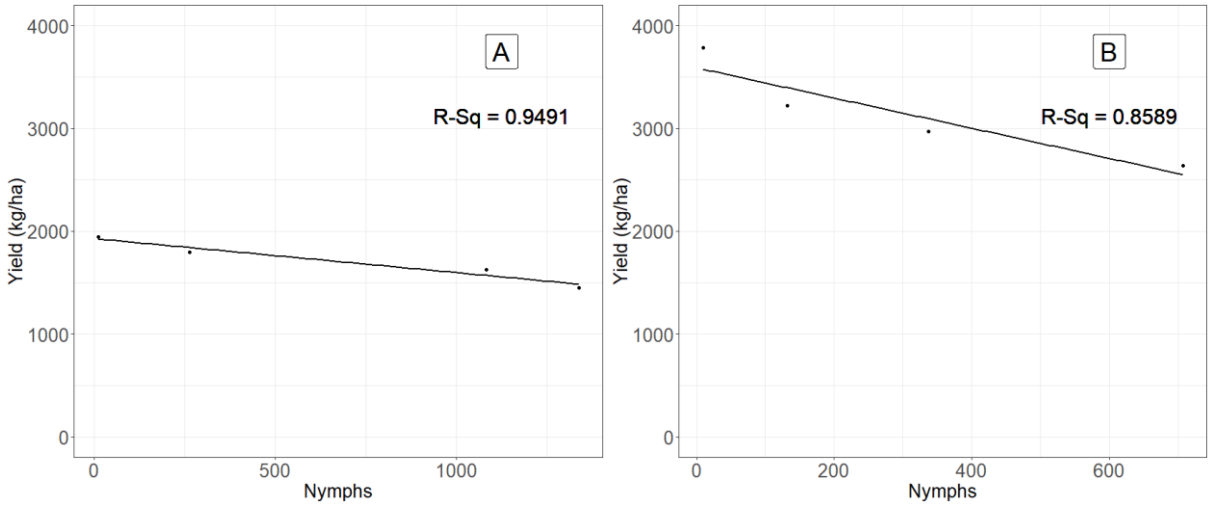

Figure 7. Regression of total number of nymphs of *Bemisia tabaci* MEAM1 per leaflet vs yield of soybean (kg ha^{-1}) in two crop seasons A (2017/2018) ($F_{18.15}$ = 37.30, p = 0.02) and B (2018/2019) ($F_{18.15}$ = 12.18, p = 0.05).

Comparing the number of nymphs and adults of *B. tabaci* MEAM1 with soybean yield, we could observe a negative correlation, as the number of nymphs and/or adults increased, the soybean yield decreased in the two years of trial, 2017/2018 (Fig. 7) and 2018/2019 (Fig. 8).

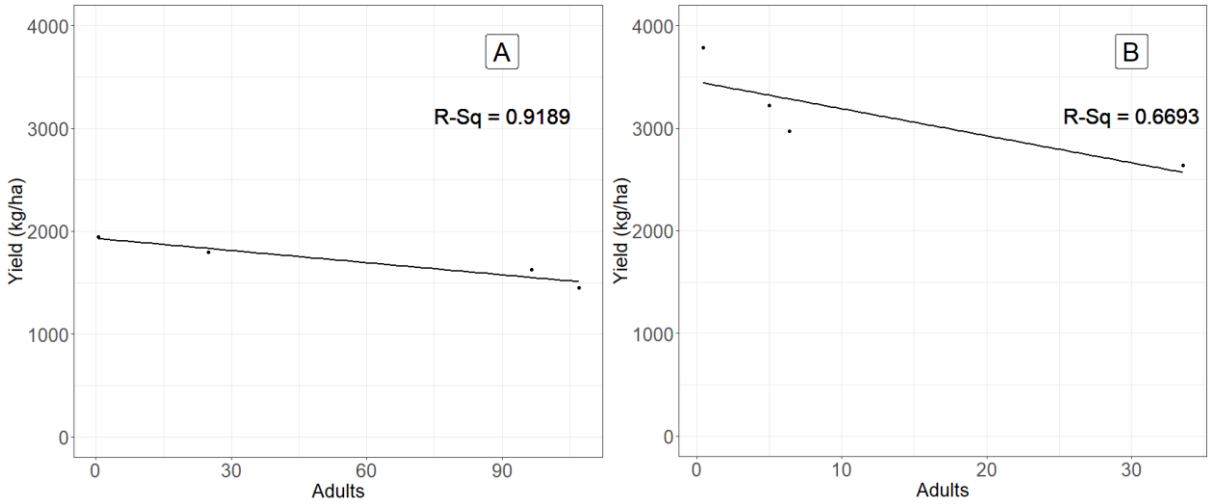

Figure 8. Regression of total number of adults of *Bemisia tabaci* MEAM1 per leaflet vs yield of soybean (kg ha^{-1}) in two crop seasons A (2017/2018) ($F_{18.15}$ = 22.66, p = 0.03) and B (2018/2019) ($F_{18.15}$ = 4.05, p = 0.15).

In the 2017/2018 trial, when the density of nymphs and adults was higher, we could observe a stronger correlation for nymphs (R^2= 0.9491) and adults (R^2= 0.9189) (Fig. 7) versus soybean yield comparing to 2018/2019, when the densities where lower, resulting in a lower R^2 value for nymphs (0.8589) and adults (0.6693) (Fig. 7).

Grain quality and vigor evaluation

The soybean seeds from the field treatments were subjected to the assessment of moisture content levels, germination, germination after accelerated aging process, and electrical conductivity (Table 2). There were no significant differences in the quality and vigor between treatments (Tukey's test $p < 0.05$).

Table 2 Quality and vigor of soybean grains from plants previously exposed to *Bemisia tabaci* MEAM1 infestation (T1 no infestation, T2 low infestation, T3 medium infestation, T4 high infestation level)

Treatment	Moisture content (%)	Germination (%)	Germ. Accelerated Aging (%)	Electrical conductivity ($\mu s\ g^{-1}\ cm^{-1}$)
T1[†]	7,3[ns]	98,25[ns]	96,75[ns]	82,8[ns]
T2	7,3	99,00	97,25	86,8
T3	7,1	97,00	98,00	78,9
T4	7,1	98,00	95,00	75,8

[†] Control.

Grain protein content

Although we could not observe significant differences in the protein content in the grains between treatments (Tukey's test $p < 0.05$) (Table 3), when estimating the total amount of protein considering the yield of each treatment, we can observe a significant reduction of up to 440 kg of protein per hectare between control (T1) and high infestation (T4) treatments.

Table 3 Protein content of soybean seeds and total protein in soybean yield from plants previously exposed to *Bemisia tabaci* MEAM1 infestation (T1 no infestation, T2 low infestation, T3 medium infestation, T4 high infestation level)

Treatment	Grain protein content (%)	Total protein (kg) in soybean yield
T1[†]	36.11[ns]	1365.96[a]
T2	34.85	1120.22[ab]
T3	35.58	1057.43[ab]
T4	34.58	925.92[b]

[†] Control. Means followed by the same letter within a column are not significantly different ($p < 0.05$) in the Tukey test.

2.4. Discussion

Soybean is a suitable reproductive host for *B. tabaci* (Musa and Ren, 2005). The increase in the planted area with soybean was the main factor driving the development of large populations of *B. tabaci* in Brazil (Costa, 1975; Morales, 2006), and critical damages on the crop were observed decades later (Bortolotto et al., 2015; Vieira et al., 2011).

The number of eggs is directly affected by the density of adults (Naranjo and Ellsworth, 2005). The number of eggs in the first weeks was similar in both years, but the abrupt drop of adults caused by the early rain in the 2019 soybean cycle led to a lower number of eggs, while the rain fell later in the crop cycle of 2018. Heavy rain at the end of January in the region of Buri in 2018 (INMET, 2020) and in Piracicaba in 2019 (LEB, 2020) may have provoked an accented decline in the number of adults present on the leaves. Although immobile stages such as eggs and nymphs were not affected, fewer eggs would be laid due to the reduction in the number of adults, thereby resulting in a lower density of nymphs. Previous studies have shown a decrease in *B. tabaci* population after rainfall in the field for soybean (Lima et al., 2002), cotton (Kataria et al., 2019), tomato (Chaudhuri et al., 2001), and chili (Chaubey and Mishra, 2018). Rainfall is the main adverse factor for this pest population, which can affect, in prolonged periods of rain, the survival of the eggs and nymphs (Villas Bôas et al., 1997).

The first evaluation in the 2017/2018 trial occurred in the first half of December, while in 2018/2019, it took place in the second half of December. This could explain the one-week difference in the population growth between the two years, probably caused by the differences in temperature that affect the development of whitefly (Nava-Camberos et al., 2001). The

damage caused by *B. tabaci* depends on its density, which is related to environmental temperature (Butler et al., 1983; Nava-Camberos et al., 2001). Rainfall has a negative correlation to the *B. tabaci* population, and it is positively correlated with maximum temperatures (Kataria et al., 2019).

Temperatures around 30 °C meet the perfect thermal requirements for *B. tabaci* development, which has a complete life cycle within 21 days and a survival rate of 90% at this temperature (Albergaria and Cividanes, 2002). The state of Mato Grosso, Central-West of Brazil, is the main soybean producer region and also one of the hottest in the country. The annual average maximum temperature reaches between 29 and 33 °C (INMET, 2020), making it a very suitable place for whitefly, a primary soybean pest in that area (Pozebon et al., 2020).

In the state of São Paulo, where the trials were conducted, the annual maximum temperature ranges from 27 to 31 °C (INMET, 2020). We could observe differences in the two crop seasons production, and the density of *B. tabaci*, due to these temperatures. The ectothermic physiology of *B. tabaci* makes it highly sensitive to changes in temperature (Zidon et al., 2016), which leads to fluctuations in its population density (Munyuli et al., 2017). In the first year, lower temperatures (± 22 °C) were observed during the period of the trial, while in the second year, the average temperature was about 3 °C higher (± 25 °C). Despite the apparently subtle difference in the temperature between the years, it could cause a large difference in the number of whiteflies present in the plants.

A temperature study made with soybean as host showed that at the temperature of 20 °C, *B. tabaci* lifecycle demands around 40 days to be completed, with a survival rate of 76,5%. Whereas at 25 °C, it decreases to 28 days, with 82% of survival (Albergaria and Cividanes, 2002). This agrees with the density of adults observed in this study. In the first crop season, the highest number of adults occurred at the sixth week of evaluation, 42 days after infestation. While in the second year, the population peaked 28 days after infestation, the peak of *B. tabaci* population occurred during the reproductive stage of soybean in both years, R5.4 (2017/2018) and R5 (2018/2019), and also reported in previous studies (Czepak et al., 2018; Vieira et al., 2013).

In the first year of the trial, the highest density of nymphs was observed in the reproductive stage R5.4, with an average of 7.4 nymphs cm^{-2} of leaf (413.3 nymphs per leaflet). Whereas in the second year, the peak occurred on R5, with 2.8 nymphs cm^{-2} of leaf (179.7 nymphs per leaflet). The densities obtained in our study, conducted in Southeast of Brazil, are higher than the observations made in field trials in the Central-West states of Goiás and Mato Grosso do Sul, where densities topped on 136.3 nymphs per leaflet (Vieira et al.,

2013) and 7.1 nymphs per leaflet (Da Silva Oliveira et al., 2018) respectively, both observed on plants at reproductive stage R2, with a decrease in the number of nymphs until the end of soybean cycle. However, these experiments were carried out in the open field, where plants and whitefly were exposed to natural enemies, that could be acting as biocontrol agents, as well as competition with other pests (Naranjo and Ellsworth, 2005). Furthermore, they were dependent upon the natural occurrence of *B. tabaci*, and also, the pest was able to disperse. On the other hand, in this experiment, we performed infestations and had plants protected from these interferences by field cages. In greenhouse conditions, densities of 79.5 and 84.4 nymphs per leaflet were observed in soybean (Suekane et al., 2013; Vieira et al., 2011).

Regarding eggs and adults, the outbreaks were also observed during the reproductive stage, with densities of 201.1 for eggs and 52.6 for adults per leaflet (do Valle et al., 2012) The number of eggs observed in the present study was higher in the first crop season, 370.6 eggs per leaflet (6.9 eggs cm^{-2} per leaf), but lower in the second, 155.0 (2.6 eggs cm^{-2} per leaf). However, the numbers of adults were lower, 22.9 adults per leaflet (0.4 adults cm^{-2} per leaf) in the first year, and 9.2 (0.2 adults cm^{-2} per leaf) in the second.

The yield and weight of thousand grains observed in the second year were higher than the first year in all treatments, regardless of densities. A significant difference between control and high infestation level treatments, on yield and weight of thousand grains, in both crop seasons were observed, even the first trial presenting a higher population density, the losses in the second were more pronounced. The difference was 500 kg ha^{-1} in the first crop season and 1,147 kg ha^{-1} in the second for yield. For the weight of a thousand grains, the difference was 18 g in the first year and 32.5 g in the second. A difference of 1,505 kg ha^{-1} between control (4,992 kg ha^{-1}) and weekly sprayed (3,486 kg ha^{-1}) treatments was obtained in the soybean yield exposed to *B. tabaci* in a previous field study (Vieira et al., 2013), 30% higher than found here. Although the weight of the grains was not affected in that trial, the number of whiteflies was around three times lower than the observed in the present study, indicating that the high temperatures in Goiás, where that study was conducted, intensified the damage caused by *B. tabaci*, contributing to a greater number of generations.

The higher the soybean yield, the more sensitive to *B. tabaci* soybean plants appears to be. In both years, a high density of nymphs was observed during the seed fill stage (R5-R6), considered the most critical for soybean seed evaluation due to the impact of the movement of nutrients from leaves to seeds (Bellaloui, 2013). To maintain the nutritional intake, *B. tabaci* actively controls the feeding rate, and thus, the developmental rate (Isaacs et al., 1998). In the second year, when the greater yield was obtained, although fewer nymphs were feeding on the

plants, the impact on yield was greater. Probably due to the competition between *B. tabaci* feeding and the movement of nutrients to fill the seeds, reducing the concentration of nutrients in the leaves, and increasing the feeding rate of the nymphs.

When the average number of nymphs per leaflet was 133.75 in the high infestation treatment (T4) at the first year of trial, and the yield in the control treatment (T1) was 1,947 kg ha^{-1}, the loss, comparing T1 and T4, was 500 kg ha^{-1}. In the second trial, the average of nymphs was 64.28 per leaflet in T4, the yield in T1 3,787 kg ha^{-1}, and the loss was 1,147 kg ha^{-1}. According to previous results, where the average number of *B. tabaci* nymphs per leaflet was 58.48 in the weekly sprayed treatment, and the yield in the control was 4,992 kg ha^{-1}, the loss was 1,505 kg ha^{-1} (Vieira et al., 2013).

The differences found here did not affect the quality of the grains regarding the vigor parameters evaluated, soybean plants were able to compensate for the loss of production capacity by reducing the weight of the grains but maintaining the qualitative characteristics evaluated. A study on *B. tabaci* feeding showed that plants tend to concentrate their nutrients, maintaining a similar total nutritional content but increasing the nutritional value per unit by reducing water (Isaacs et al., 1998).

Although there were no significant changes in the percentage of the protein content of the grains, the reduction in the yield and weight resulted in great losses in protein.

B. tabaci recently became a primary pest to soybean fields in Brazil (Pozebon et al., 2020), and challenges for establishing IPM programs are still being improved. Results as shown in this study, can be helpful in the decision making to control the whitefly *B. tabaci* MEAM1 in soybean areas.

2.5. Conclusions

Our results indicate that the *B. tabaci* MEAM1 population level capable of causing damage to soybean yield depends on the climatic factors, such as rainfall and temperature, that directly affect the population development. In high densities (3 nymphs/cm^2), *B. tabaci* MEAM1 can cause yield losses over a ton in a soybean field, and, even though the vigor parameters of soybean grains were not affected, the infestations resulted in losses in the protein content of grains.

Authors Contribution

Inana Schutze: Conceptualization, Methodology, Formal analysis, Data curation, Writing- Original draft preparation and Editing, Investigation. **Pedro Yamamoto:** Conceptualization, Visualization, Supervision, Writing- Reviewing, Funding acquisition.

Conflic of Interest Declaration

All authors have read and approved this version of the article and declare no conflict of interest.

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3. MONITORING *Bemisia tabaci* (GENNADIUS) (HEMIPTERA: ALEYRODIDAE) INFESTATION IN SOYBEAN BY PROXIMAL SENSING

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Abstract

Although monitoring insect pest populations in the fields is essential in crop management, it is still a laborious and sometimes ineffective process. Imprecise decision-making in an integrated pest management program may lead to ineffective control in infested areas or the excessive use of insecticides. In addition, high infestation levels may diminish the photosynthetic activity of soybean, reducing their development and yield. Therefore, we proposed that levels of infested soybean areas could be identified and classified in a field using hyperspectral proximal sensing. Thus, the goals of this study were to investigate and discriminate the reflectance characteristics of soybean non-infested and infested with *Bemisia tabaci* using hyperspectral sensing data. Therefore, cages were placed over soybean plants in a commercial field and artificial whitefly infestations were created. Later, samples of infested and non-infested soybean leaves were collected and transported to the laboratory to obtain the hyperspectral curves. The results allowed us to discriminate the different levels of infestation and to separate healthy from whitefly infested soybean leaves based on their reflectance. In conclusion, these results show that hyperspectral sensing can potentially be used to monitor whitefly populations in soybean fields.

Keywords: *Glycine max*; Sampling; Pest management; Spectroradiometer

3.1. Introduction

According to the United States Department of Agriculture (USDA) [1], world soybean (*Glycine max* (L.) Merril) production in the 2018/2019 season was 361.06 million tons. Brazil is projected to be the largest producer of soybeans in the world by 2021 [2]. In

Brazil, the 119.70 million tons harvested in 2018/2019 were grown in around 35.90 million hectares, meaning that the average yield was 3.26 tons per hectare. In the 2019/2020 season, the average Brazilian yield is projected to increase by 3.9% and a 2.7% increase in the area [3]. Although this is the main product of Brazilian agribusiness today, representing a quarter of the gross production value of agriculture in Brazil [4], the monoculture in wide fields has consequences, such as greater vulnerability to insect pests, causing a reduction in yield [5].

Therefore, knowing and monitoring the main pests present in the soybean ecosystem, using a variety of sampling methods, is extremely important for the decisionmaking to be taken at the right time, avoiding yield losses [6]. The occurrence and damage of the whitefly *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) in soybean fields are alarming; in high densities, it can cause losses between 12% and 30% in yield [7]. In addition, this pest is able to tolerate the action of some insecticides, with a rapid selection of resistant populations [8].

There are parameters that allow decisions to be taken at the right time, resulting in better control like the economic injury level (EIL) [9] and the economic threshold (ET) [10], the pest population at which actions should be taken, prevent such population from reaching the EIL [11].

These levels are already established for the main pests that infest soybean plants [12]. However, for some species that became more important recently, such as whiteflies (Hemiptera: Aleyrodidae), spider mites (Acari: Tetranychidae) and even pod-eating caterpillars (Lepidoptera: Noctuidae), the EIL and ET are still being investigated [13]. To acquire data and calculate these levels, crop fields must be regularly checked for pests. In soybean fields, the sampling method most used for monitoring insects that inhabit the aerial part of the plants is the beat cloth, initially introduced in the United States [14], this method was later modified and introduced in conditions of Brazilian agriculture [15,16].

The sampling methods currently in use are challenging, considering the vast extension of soybean fields in the Midwest region of Brazil [17]. In addition, they are time-consuming and expensive due to the necessity of workers scouting the field [18,19]. Moreover, there is still a lack of reliable sampling methods for some species, especially those too small to see with the naked eye or the ones that inhabit the soil. This scenario promotes the implementation of remote or proximal sensing technologies and their benefits, especially the potential time saved by automatizing crop monitoring [19–21].

Recently, proximal sensing has been gaining adherents in Brazil, especially in sugarcane fields [22,23], and later in grain fields, such as soybean [24]. Although precision

tools currently are used more for planting/sowing operations, fertilization and weed control, there is a growing interest of researchers in providing tools to be used in insect and plant pathogen (disease) management [25]. The main difference between those operations and insect/disease management is that the former is based on collecting data from the soil, while the latter is based on collecting spectral data from plants.

To obtain spectral data from plants, it is necessary to understand that, when light reaches the leaves (canopy), part of that energy is reflected back to the observer. The amount of energy reflected at each wavelength is called the reflectance spectrum, sometimes shortened to spectrum or reflectance. Reflectance depends on the properties of the leaf surface and its internal structure, as well as the concentration and distribution of biochemical components [26,27]. Moreover, biotic stresses, such as herbivory by arthropod pests, induce physiological responses in plants that impair their ability to perform photosynthesis, leading to changes in leaf reflectance in some parts of the spectrum. On this matter, most studies refer to the 400–2500 nm range, especially with hyperspectral sensors [28]. Hence, advanced sensing technologies can be used to detect changes in reflectance from soybean plants as a non-invasive monitoring method [17].

Remote/proximal sensing has been used to detect stress caused by arthropod herbivory in a variety of plant species, such as maize [29], soybean [6], rice [30,31], wheat [32], peach trees [18,33], cotton [34] and potato [35]. The most promising results were achieved in studies based on hemipteran pests because their feeding activity (sucking) indirectly affects the infested plants' physiology, and therefore, their reflectance profiles [36]. In most of these studies, infested and non-infested plants were discriminated against with good accuracy.

In the search for responses more detailed than infestation vs. non-infestation, a variety of approaches have been used to analyze reflectance data from plants infested with different pest densities. In general, the best correlation indexes were achieved when infestation levels (classes, not the absolute number of insects per plant) and narrow-band wavelengths (not individual wavelengths) were compared [37]. However, it is still necessary to study how different analytical approaches interfere in the quality and usability of such information remotely extracted from infested plants.

Hence, it can be said that one of the biggest challenges regarding hyperspectral remote sensing is the analysis of a large number of bands. This analysis is complex and time-consuming, using special algorithms to select a group of bands sensitive to arthropod infestation in each plant species [28]. According to Hair et al. [38], currently, one of the most

used statistical procedures to reduce the amount of data without losing important information is multivariate analysis. One example of multivariate analysis is the discriminant analysis that is done with the objective of separating the observations into groups [39]. In addition, classification analysis is done to assign observations whose group memberships are unknown to the established groups based on *p* measured values [29]. This association is only possible if part of the observations from each group is previously available. Thus, this study aimed to develop models to discriminate the levels of whitefly infestation in soybean fields, using hyperspectral proximal sensing.

3.2. Material and Methods

Local

The bioassay was carried out in the experimental field at the College of Agriculture "Luiz de Queiroz", from the University of São Paulo, located in Piracicaba, Sao Paulo state, Brazil. The area is located at the following coordinates: Datum (SIRGAS 2000): 22°42'16" S Lat.; 47°37'23" W Long.; approximated altitude 532 m.

The climate is humid subtropical climates, with dry winter and hot summer (CWa), according to Köppen classification [40]. The average year pluviosity is 1,280 mm, and the average temperature is 22 °C, with the average temperature in the hottest month of 25 °C and 18 °C in the coldest month.

Conventional soybean, variety BRS 232, was sown on 28 November 28 th, 2018, in an area of 1.5 hectar. The field was tilled and fertilized with nitrogen, phosphorus and potassium, following the standard procedures used by the grower in the cultivated area. The soil is classified as dystrophic red-yellow latosol.

Insect Rearing

The rearing of whitefly, *B. tabaci* biotype B (Gennadius, 1889) (Hemiptera: Aleyrodidae), started from a population acquired at the Agronomic Institute of Campinas. The population is maintained in kale plants and kept in a greenhouse covered with an anti-aphid screen [41]. The plants are replaced in the greenhouse, when necessary, in order to keep the insect population adequate for the development of bioassays.

Bioassay

The bioassay began on 13 December 13th, 2018, when the soybean plants reached the phenological stage V3 (third node, two fully expanded trifoliate) [42]. The treatments were distributed in a randomized block design, made of four blocks and four treatments (low, medium, high and control) consisting of different *B. tabaci* infestations, totalizing 16 experimental units. Each experimental unit consisted of a cage (2.0 m long, 1.7 m large, and 1.6 m high) set up over the crop in the field. The cages were supported by bamboo poles and covered with an anti-aphid screen that allows airflow and prevents infestation by unwanted arthropods. The cages were installed on 12 December 12th, 2018, 2 m apart from each other, and comprised about 75 plants each one.

On 19 December 19th, 2018, the cages were manually infested, releasing in each cage one pot with one kale plant and the amount of insect corresponding to each treatment. The treatments were: 1 - control (no insects); 2 - low (approximately (ca.) 300 adults); 3 - medium (ca. 600 adults); and 4 - high (ca. 1200 adults), the number of adults released was intended to reach densities of nymphs enough to differentiate the treatments from each other in a period of weeks. The insects continued feeding on the plant along the soybean crop cycle.

Data Collection

To collect reflectance data, ten leaflets from the middle third of the soybean plants were collected from each cage and stored in plastic bags with identification tags, a total of 160 leaflets per collection. The leaflets were collected on 10 January 2019, 17 January 2019, 24 January 2019, 31 January 2019, 7 February 2019, 14 February 2019, 21 February 2019, and 28 February 2019. Then, the samples were taken to the laboratory in a thermal box with ice cubes to maintain the turgidity of the leaves during the collection of spectral data.

Spectral data were collected from each leaflet using a spectroradiometer (Field-Spec 3, Analytical Spectral Devices, Boulder, CO, USA). This sensor operates in the spectral range of 350–2500 nm, with a spectral resolution of 1.4 nm in the range of 350–1050 nm and 2 nm in the range of 1051–2500 nm. The sensor was connected to the ASD Leaf Clip accessory (Analytical Spectral Devices, Boulder, CO, USA), designed for nondestructive spectral measurements, without interference from external light, minimizing errors associated with diffuse light. This accessory has a halogen light source (4.5 W) with an incidence light of 45° for the sample, which allows the measurement of the directional reflectance of the light directly from the sample.

A Barium plate that reflects 100% of the light was used as a reflectance standard. The spectral data were stored by the system for posterior determination of the samples' reflectance factor, which was multiplied by the readings of each sample.

The central region of each leaflet was evaluated in a circle of 2.1 cm in diameter (area of 3.5 cm2), resulting in one spectral sample per leaflet.

There was a total of eight sampling dates. At each sampling date, 10 leaflets from each one of the 16 cages were sampled, in a total of 160 spectral samples. All leaflets were collected in an interval of less than an hour to allow comparison. After obtaining the spectral data, the nymphs of each leaflet were counted in a stereoscopic microscope (40× magnification) to obtain the infestation data.

The meteorological data were obtained from the weather station of the University of Sao Paulo [43]. The information collected from the website was the maximum, average and minimum temperature (°C) and precipitation (mm).

Data Analysis

A large amount of data in a spectral curve makes it difficult to group samples into different classes based on visual criteria alone. In addition, according to Bauriegel et al. [44], the reflectance in the same spectrum presents high collinearity, producing a large number of redundant information. Therefore, a multivariate analysis was used to reduce the dimensionality of the data and to determine the effects of treatments more clearly.

According to Nansen and Elliot [28], the use of multivariate statistics is the best tool to interpret the spectral behavior of vegetation under stress, allowing interpretations that would not be possible using univariate statistics.

The software XLSTAT [45] was used to analyze the data matrix of 1950 wavelengths (range of 450–2400 nm). A discriminant analysis was carried out to develop and validate a method to determine infestation levels using spectral data. Thus, the spectral curve was condensed into a single point, along with its discriminatory value. By calculating the average value of discriminant points from a group, we obtain the group's average, known as centroid. The verification of the significance of the discriminant functions is a generalized measure of the distance between the groups' centroids. Therefore, if the distribution of the discriminating scores in each group shows little overlap, the discriminating function separates the groups well [38].

To do the discriminant analysis, a simulation was carried out with 70% of the samples to generate a discriminant model, which was tested in the 30% remaining samples. The ratio selection was random, as well as the selection of which samples would be part of the model (70%) or the test (30%).

3.3. Results and Discussion

In the discriminant models generated for each of the eight sampling dates, some bands were observed more frequently than others (Figure 1). The frequency of distribution of the bands with the greatest weight in all the eight models generated can be observed. Some bands in the visible region (461, 469, 510, 520 and 673 nm), near-infrared, NIR region (703, 722 and 732 nm), and shortwave infrared (SWIR) (1360, 1426, 1713, 1819 and 1842 nm) were observed in two of the eight discriminant models. The individual band 1831 nm was observed in three of the eight models.

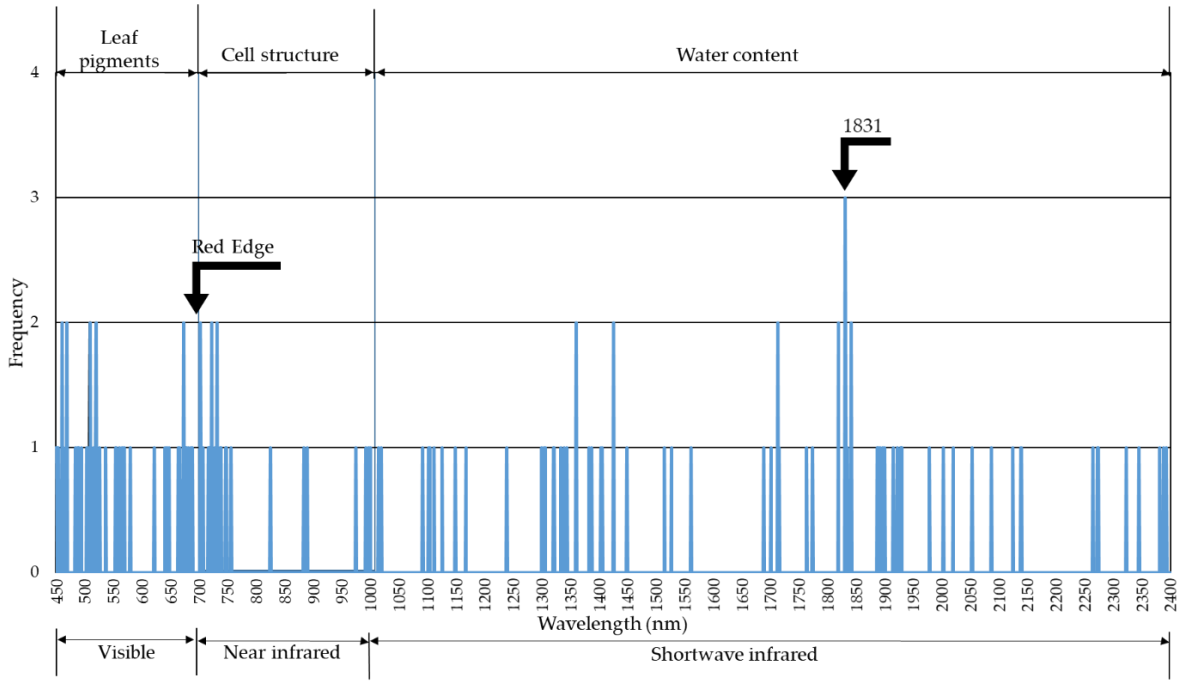

Figure 1. Frequency of appearance of individual bands (wavelengths) in the eight discriminant models.

Regarding the differentiation between treatments (low, medium, high infestations, and control) based on the discriminant analysis, the best results were achieved on 31 January, with 75.50% accuracy (Figure 2), when the soybean was in the reproductive stage R4. Such

accuracy was obtained in the cross-validation analysis, where part of the samples was provided to the machine as a learning set, and the rest of the samples (validation set) were classified by the machine based on the learning set. In this case, accuracy (%) means how much treatment classification by the machine was similar to the real treatments in the field.

By analyzing the infestation data together with the meteorological data, it is possible to observe that the period was dry and hot (Figure 2), boosting the development of whitefly populations in the field.

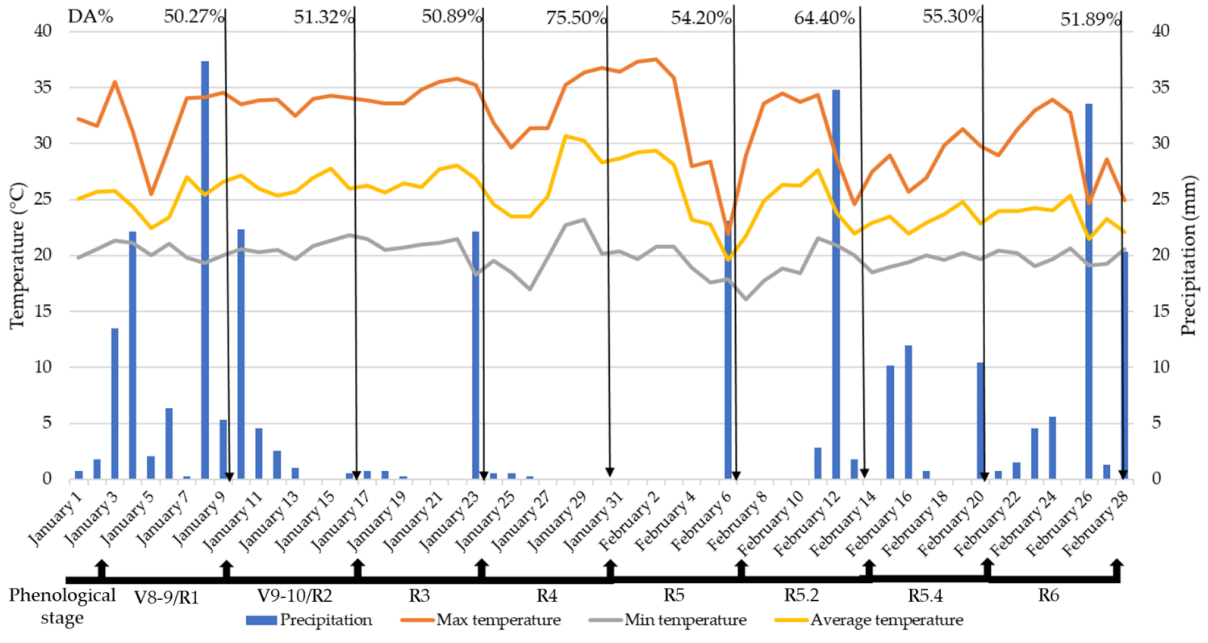

Figure 2. Meteorological data and discriminant analysis (DA) accuracy (%). Letter "V" stands for vegetative stages and "R" for reproductive stages.

Therefore, only the data collected on 31 January was used for a more detailed analysis. Evaluating the spectral curves that represent the average reflection of each infestation level, we could observe a difference in the reflectance intensity (Figure 3). More specifically, the high level of infestation showed greater reflectance across the analyzed electromagnetic spectrum compared to the other levels.

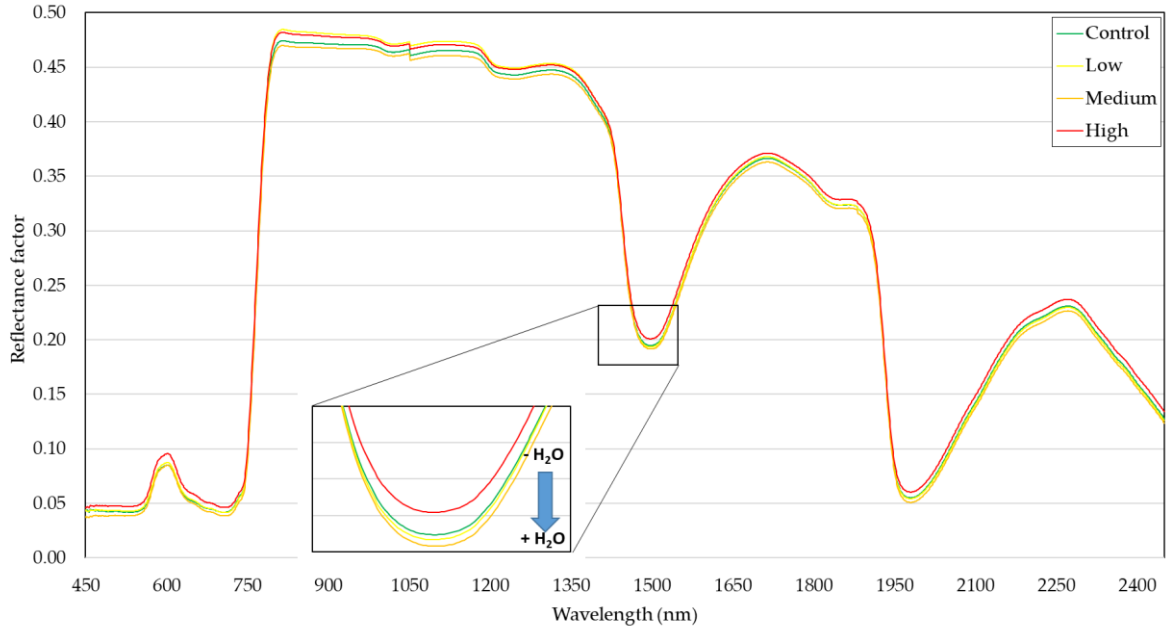

Figure 3. Average spectral curves (450–2400 nm) of soybean leaves under different levels of whitefly infestation.

The water bands, highlighted in Figure 3, occur when the energy in these wavelengths interacts with OH of the water molecules, causing a vibrational effect. The effect absorbs the energy of this wavelength, not reflecting it. With no reflection, the absorption feature occurs. In these bands, it is observed that the most infested area has less water. This is indicative of water stress precisely because the plant is not managing to keep the turgidity; this could be related to the infested leaves not being able to transport water due to damages caused by *B. tabaci* feeding, affecting xylem on the vascular bundles, where phloem is also located [46].

In the range of the NIR 800–1000 nm, the band related to the leaf structure, a higher intensity was observed in the most infested plants. This was a notorious fact because the better the structure is, the greater the reflectance intensity is expected. However, an anatomical investigation showed that, although *B. tabaci* impacts the leaf anatomy, they occur on the abaxial surface, where the vascular bundles are located, while we used the adaxial surface to perform the data collection. The differences found here are more related to the physiology of the leaf rather than its anatomy [46].

High densities of *B. tabaci* causes the occurrence of honeydew. In the nymphal stage, they excrete a high volume of this sugar-rich watery fluid [47], which is a substrate for the development of fungi of the genus Ascomycete that produces the symptom known as sooty mold. This symptom turns the foliar surface to black, causing more solar radiation to be

absorbed, resulting in burns and falls. This pathosystem can be limiting for photosynthesis and, therefore, reduce plant production. This situation was observed in the visible region (Figure 4), where the high level of infestation presented higher reflectance intensity that is directly related to photosynthetic pigments. With the lower photosynthesis, the plant does not absorb wavelengths at the blue and red ranges, and thus, reflection gets higher.

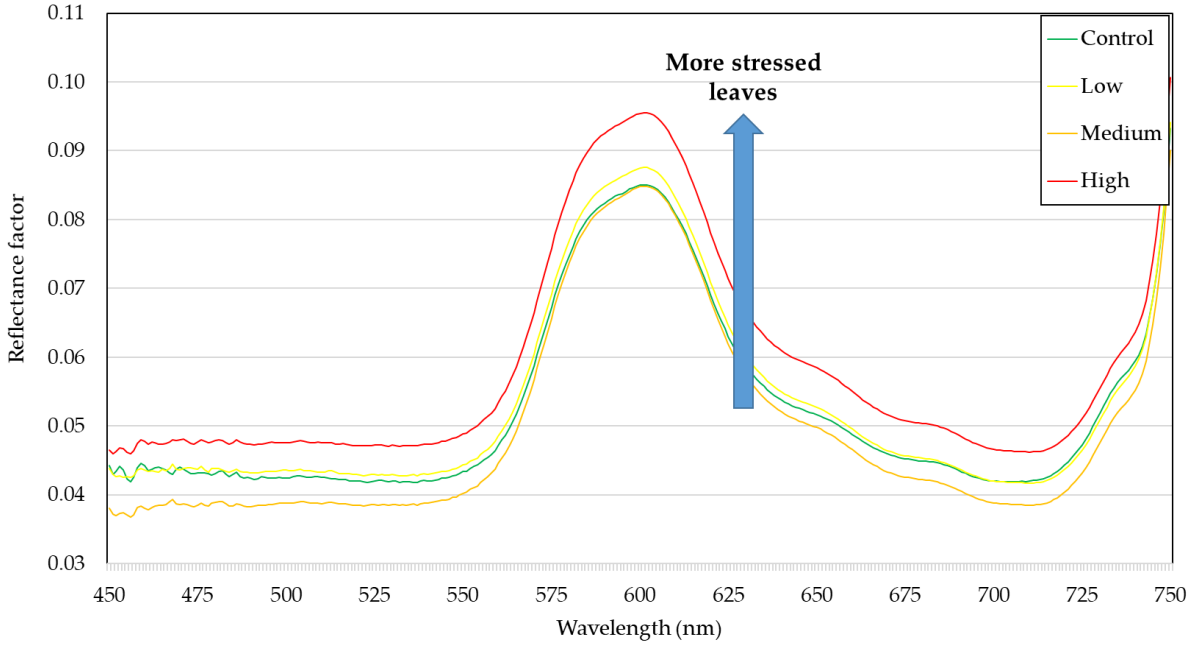

Figure 4. Average spectral curves (450–750 nm) of soybean leaves under different levels of whitefly infestation.

The results shown in the spectral curves in the wavelengths 450–750 nm indicate low reflectance (around 10%), with a slight increase in the region correspondent to green light (550 nm) (Figure 4). The reduction in reflectance is often associated with the absorption of foliar pigments due to the presence of chlorophyll. In the spectral r-gion correspondent to blue light, the absorption occurs near the wavelength 460 nm and is related to the presence of xanthophyll, carotenes, and chlorophyll pigments a and b. In the red-light region, chlorophyll acts as absorbing energy near 645 nm [47].

Thus, it is possible to observe (Figure 4) that the treatment with the least photosynthetically activity (high infestation) presented higher reflectance in all the visible region spectrum. This may have occurred because of how whitefly infestation affects the plant physiology, altering water balance, photosynthesis, chlorophyll content and metabolites associated with physiological stress [48,49].

Most of the processes mentioned, which alter the leaves' and plants' physiology are observable in the spectral signature of such plants (Figure 4). However, advanced statistical tools are required to know how much each process is correlated to each wavelength. Moreover, these analyses are necessary to identify which wavelengths are more significant for each of these processes.

Hence, observing the discriminant analysis (Figure 5) in the reproductive stage R4, where each point of the graphic represents one spectral curve from the leaflets collected from the cages, we can see that the first function explains 60.86% of the data variability, while the second function explains 24.15%.

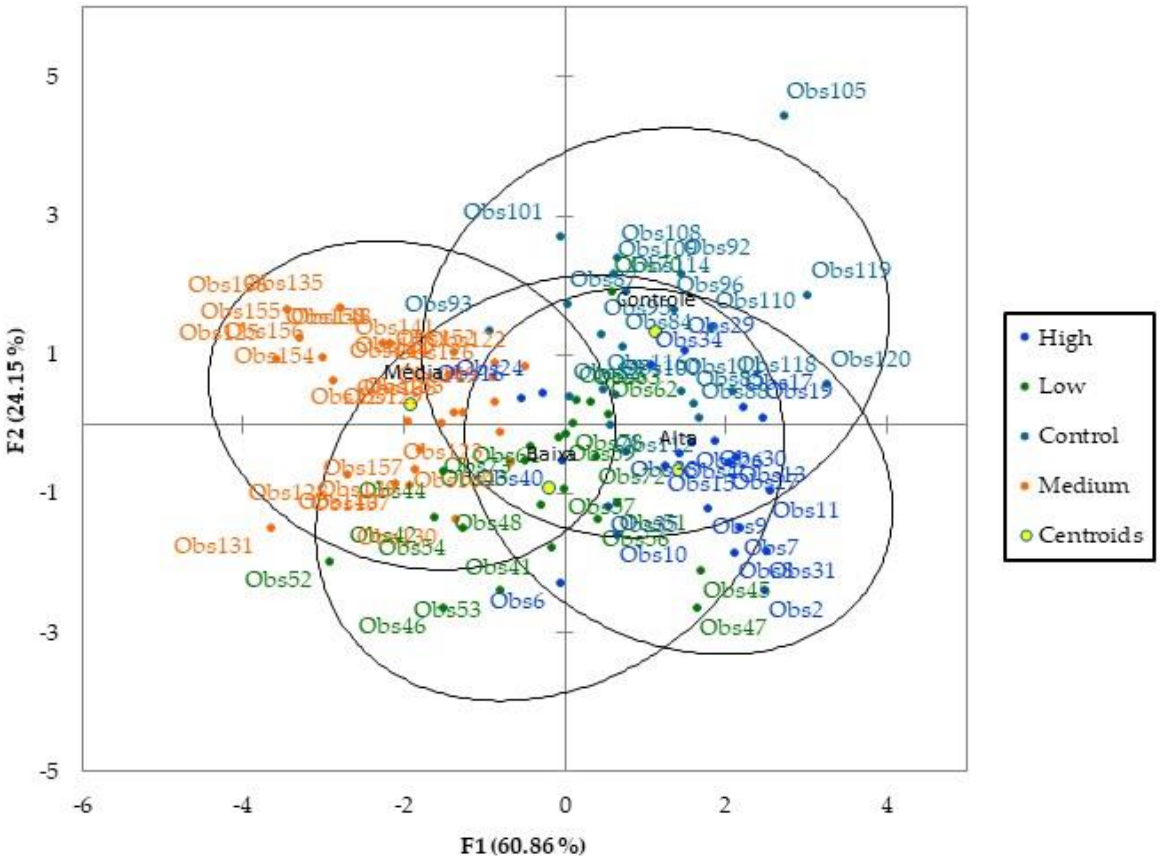

Figure 5. Discriminant analysis of hyperspectral data (450–2400 nm) of soybean leaves under different levels of whitefly infestation. Obs stands for observation, and F1 and F2 for function.

After performing the discriminant analysis (DA) with 70% of the samples, the next step was the validation of the model, with 30% of the samples left. With the discriminant function obtained, the cross-validation was performed, and the samples were identified in their correspondent infestation level, with a total accuracy rate of 75.48% (Table 1). The

plants with medium infestation were classified more accurately (85.71%), while most of the errors in classification occurred in plants with a low infestation (69.57%). The difficulty with this discrimination is the lack of visible symptoms early in the season and connecting the factors causing the biophysical/chemical changes in the plants. At the date when the data were collected, the average number of nymphs per leaflet in the medium infestation was two times the number of nymphs in the low infestation treatment, which could make it more difficult to separate the low infestation from the control group. Soybean plants have a good water compensation [50], which could provide a good response against sucking pests until certain levels; by raising the level of whitefly infestation, the amount of water consumed by this pest which feeds on the phloem vessels also increases, allowing to see a better distinction between the treatments. On the other hand, as the number of whiteflies present in the leaves goes up, the occurrence of the sooty mold also increases, which harms a more accurate reading of the data due to a more complex scenario, whereas the average number of nymphs per leaflet in the high infestation was almost eight times higher than the medium infestation.

Table 1. Linear discriminant classification of hyperspectral data (450–2400 nm) of whitefly infested soybean leaves (n = 160). Independent validation was carried out with 48 samples and classified with 75.48% accuracy.

Actual Class	Assigned Class by Training Model			
	High	Low	Control	Medium
High	17 (73.91%)	3	2	1
Low	2	16 (69.57%)	3	2
Control	1	3	16 (72.73%)	2
Medium	0	4	0	24 (85.71%)

Analyzing all the bands selected, we have 17 wavelengths, 5 in the visible region (450–682 nm), 6 in the near-infrared region (716–1167 nm), and 6 in the shortwave infrared region (1321–2265 nm).

Blue wavelengths (450 and 499 nm were selected in DA) are strongly influenced by chlorophyll absorption, along with carotenoid absorption features present in the 450–499 nm region. Carotenoids have proven important for the discrimination of senescent leaves when

the decay of chlorophyll and the diminishing of the strong chlorophyll absorption feature reveal the carotenoid absorption feature [46].

The red edge (682, 716, 739, 748 nm were selected in DA) encompasses the region from the red reflectance minimum around 680 nm to the near-infrared (NIR) shoulder at approximately 780 nm. This region indicates a sharp increase in reflectance from the visible (VIS) to NIR regions associated with strong chlorophyll absorptions and internal leaf structure [46].

The focused shortwave infrared (FSWIR) (2265 nm was selected in DA) has the lowest average band selection rate, with its highest selection at bin 2250–2299 nm most likely associated with the weak absorption features of cellulose and lignin present at 2270 nm [46].

Thus, the intervals that had higher representativity were visible and SWIR. One possible reason for this result is the fact that these regions are related to photosynthesis, light absorption for this process, and water absorption. Moreover, the feeding behavior of whitefly can affect all the three processes mentioned above. Both nymphs and adults feed on phloem using their stylets [41].

Phloem is a vegetal tissue made of sieve elements and sclerenchyma and parenchyma cells. The main functions of these parts are to transport photoassimilates (organic compounds produced by photosynthesis). These functions are related to the wavelengths mentioned, and best represented the interaction between whitefly infestation and the spectral curves. This is due to the fact that this tissue is the most affected by this pest.

To sum up, our results show that, in the conditions tested, it is possible to separate healthy and whitefly infested soybean plants based on foliar reflectance. In addition, we can separate the levels of infestation (low, medium and high) with good accuracy, using classification analysis. The uniqueness of this technique is related to the plant data acquisition with remote sensors, which could be used in commercial fields to improve pest monitoring in the future. In the specific case of whitefly, this approach is extremely relevant because of the difficulty in visually monitoring very small insects in large fields. Hence, the use of monitoring systems based on plant reflectance is a very promising tool.

These results show that future research needs to be done in larger areas and natural infestation levels to validate the sampling technique proposed in this study, using other sensors and conditions. More specifically, it is necessary to understand the spectral behavior of soybean plants out of the experimental cages used in this study, as well as to analyze the efficiency of sensors attached to terrestrial or aerial platforms. After being validated, this

technique can be used to increase the implementation of IPM programs, to determine where and when control methods are required for managing the pest.

Hence, the translation of the spatial, spectral, and radiometric information obtained by hyperspectral spectroradiometers into multispectral sensor resolution demands much attention, being one more feasible way of taking this information into the crop fields in the present.

3.4. Conclusions

It is possible to separate healthy and whitefly infested soybean leaves based on their spectral reflectance. In addition, the results obtained by the discriminant analysis of the hyperspectral data showed a clear distinction between the different levels of infestation. Finally, the NIR and SWIR were the most important for the model, as they are directly related to photosynthesis and water content in the leaves.

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4. NETWORK CORRELATION TO EVIDENCE THE INFLUENCE OF *Bemisia tabaci* FEEDING ON THE PHOTOSYNTHESIS AND FOLIAR SUGAR AND STARCH COMPOSITION IN SOYBEAN

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Abstract

Bemisia tabaci represents a species of economic importance in soybean. One of the obstacles to the management of *B. tabaci* is to quantify the damage caused by the pest, as these are indirectly inferred through losses in productivity. The goal of this study was to identify the influence of *B. tabaci* feeding on soybean by assessing effects on photosynthetic parameters, and the sugar and starch content of soybean leaves, and try to establish the best parameter to directly quantify its damage on the crop. Correlation networks were created from data on sugar content (fructose, glucose, sucrose and maltose), starch and photosynthetic parameters (initial fluorescence, performance index on absorption basis, and turn-over number), connecting these factors to the number of nymphs at each infestation level (low, medium and high) in two crop phenological stages, vegetative and reproductive. In general, nymphs were more abundant during the vegetative stage. Starch presented the stronger correlations with nymph density. A strong positive correlation between fructose and nymph density was observed for infestation levels at the vegetative stage. Among the photosynthetic parameters, the turn-over number N, was positively correlated with lower numbers of nymphs, and negatively correlated with higher densities. *Bemisia tabaci* feeding affected the plant's physiology and its interaction is reflected in part by the relationships among photosynthetic parameters as well as the levels of sugars and starch.

Keywords: Sweetpotato whitefly, *Glycine max*, Turn-over number, fructose

4.1. Introduction

The whitefly *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) is considered one of the most destructive invasive pests of many crops, including soybean (*Glycine max* (L.) Merril) (Baldin et al., 2017). Whiteflies were considered a secondary pest in soybean until the early 2000s, but today it represents a species of economic importance (Vieira et al., 2016), responsible for causing direct damage through the suction of phloem sap and indirectly favoring formation and growth of the fungus of the genus *Capnodium* sp. that causes sooty mold due the excretion of honeydew.

One of the obstacles to the management of *B. tabaci* is to quantify the damage caused by the pest, as these are indirectly inferred through losses in productivity, which invariably is confounded by the impacts of other pests during the soybean production cycle. An alternative to directly assess the damage caused by *B. tabaci* is to estimate the effects of its feeding on photosynthesis and the chemical composition of the leaves such as sugars and starch.

The interaction between the chemical composition of leaves and the performance of whiteflies has been investigated in eggplant and results show that varieties with a higher glucose and sucrose concentrations provide better conditions for the development of *B. tabaci* (Hasanuzzaman et al., 2018). A positive correlation between carbohydrate levels and whitefly populations has also been reported in eggplant and other solanaceous (Hegab et al., 2014), and higher amounts of sugars, as glucose and fructose, also contribute to higher densities of whiteflies in cotton (Bi et al., 2005). These elements have a role in the development of *B. tabaci* and, might be useful in understanding the influence of *B. tabaci* on soybean at different phenological stages under different population densities.

Another way to investigate the injury caused by *B. tabaci* in soybeans is assessing how the infestation interferes with photosynthesis. The reduction in the photosynthetic rate caused by *B. tabaci* infestation has been reported for many crops, such as cotton, zucchini, eggplant and tobacco (Chen et al., 2004; Islam and Ren, 2009; Lin et al., 1999). *Bemisia tabaci* feeding has been associated with reduction in the net photosynthetic rate in zucchini (Chen et al., 2004; Lin et al., 1999), and a reduction in the photosynthetic rate, induced by *B. tabaci* infestation, is associated with decreases in photosynthetic capacity and stomatal conductance (Buntin et al., 1993). It is known that *B. tabaci* causes damage to soybean (Padilha et al., 2021), resulting in yield losses and reduction in grain weight. The goal of this study was to identify the influence of *B. tabaci* feeding on soybean by assessing effects on photosynthetic parameters, and the sugar and starch content of soybean leaves and try to establish the better parameter to direct quantify its damage in the crop.

4.2. Material and Methods

Bemisia tabaci rearing

Whiteflies were reared in cages in a greenhouse using soybean (*Glycine max* (L.) Merril) as the host. Plants were grown in 2-liter pots in a greenhouse and replaced every 20 days. The initial whitefly population was collected from a cotton field at the United States Department

of Agriculture – Arid Land Research Center (USDA-ALARC) in Maricopa, Arizona, USA, and was previously identified as *B. tabaci* biotype B (MEAM1). Molecular characterization of the insects was made periodically during the study, for confirmation of the insect biotype (De Barro et al. 2003).

Establishment of soybean plants

Soybean (*var.* 01057408) was sown in seed trays containing a general purpose growing medium. When the seedlings reached the V1 phenological stage (Fehr et al., 1971) they were transplanted individually to a 5-liter pot, containing 9 parts growing medium / 5 parts sand, both sterilized. This process was repeated again after two weeks to obtain soybean in two different phenological stages for further infestation with *B. tabaci*. Each plant was covered with an anti-aphid screen attached to the bottom of the pot that served as a cage for eventual whitefly infestation. The experiment was carried out in greenhouse conditions.

Soybean infestation with *Bemisia tabaci*

For the infestation, adults of *B. tabaci* were collected from the rearing culture with an aspirator made with an 8 mm transparent plastic hose, "voil" fabric and a 10 ml pipette tip, placed in Eppendorf® tubes and released into the cages containing the soybean plants.

Soybean at V2 and R1 phenological stages were infested at three different levels on the same date. The control treatment contained no insects (Table 1). The experimental unit was a single cage.

Table 1. Infestation levels of *Bemisia tabaci* released in the cages containing soybean in phenological stages V2 and R1, and the expected population density (nymphs per leaflet) in each treatment.

Treatment	Infestation level	Number of *B. tabaci* adults released	Soybean phenological stage at infestation	Expected population density (nymphs/leaflet)
T1	Control	-	R1	0
T2	Low	20	R1	40
T3	Medium	40	R1	120
T4	High	80	R1	240
T5	Control	-	V2	0
T6	Low	20	V2	40
T7	Medium	40	V2	120
T8	High	80	V2	240

The experiment was carried out in a completely randomized design, consisting of the eight treatments with 12 replications. The experiment was repeated for a total of 24 repetitions per treatment. Samplings of both phenological stages ended simultaneally.

Bemisia tabaci population density

A leaflet from the middle third of each plant was collected every two weeks for the duration of the experiment for a total of six samples, and placed individually in a transparent plastic bag for transport to the laboratory. Samples were refrigerated until they were processed. Counts were made from the leaflet abaxial surface with a stereoscopic microscope (40x magnification). Then the leaf area of the sample was measured with the LI-3100C (LI-COR, Inc., Lincoln, NE, USA).

Photosynthetic parameters by Chlorophyll Fluorescence Induction Kinetics

For the Chlorophyll Fluoresce Induction Kinetics (OJIP) protocol a leaf on the plant was dark-adapted for at least 20 minutes, with detachable leaf clips. Measurements were then performed using the FluorPen FP100 (Photo System Instruments, Czech Republic).

A clip was attached to one of the leaflets in the upper middle third of the plant before the measurements. The device emits a saturation pulse through a beam of light, which when reflected is read by the device. The OJIP protocol is a dark-adapted chlorophyll fluorescence technique, used to measure plant stress. The parameters measured include initial fluorescence (F_0), performance index on absorption basis (Pi_Abs), and turn-over number (N). These assays were performed in situ in the greenhouse weekly.

Sampling and sample preparation

The leaflets were collected every two weeks, stored individually in 50 mL centrifuge tubes and immediately frozen in liquid nitrogen, after which they were stored in a freezer at -80 °C. One leaflet per plant was collected, totaling 12 leaflets per treatment in each evaluation. Samplings were collected for a total of six bi-weekly samples.

The leaflets were individually homogenized in liquid nitrogen using a pestle and a mortar, weighted in aliquots of 20 mg each sample, placed in 2 ml Eppendorf® tubes, and stored in a -80 °C freezer for analyses of chemical composition (sugars and starch).

Chemical composition assessment of soybean leaves

The assessment of the quantity and quality of sugars and starch, present in the infested soybean leaves, was carried out for all the leaf samples previously prepared. The evaluation of the sugar content was carried out by first preparing the samples for extraction of non-structural carbohydrates and then extracting the starch for subsequent chromatographic analysis.

Extraction of non-structural carbohydrates

25 µL of maltose (20 mg / mL of 80% ethanol) and 1 mL of 80% ethanol were added to the tubes containing the samples, the tubes were placed in a water bath at 85 °C for 10 minutes and then centrifuged for 5 min at 10,000 rpm. The supernatant was transferred to a new 2 ml tube. 0.5 ml of 80% ethanol was added to the tubes containing the precipitate, submitting them again to the water bath and centrifugation as described above for two more times, adding the supernatants each time. The tubes containing the total supernatant were adjusted to 2 mL with 80% ethanol and centrifuged once more for 5 min at 10,000 rpm. The samples were then transferred to HPLC vials using a syringe and a 0.45 µm filter. The sugars assessed here were fructose, glucose, sucrose and maltose.

Starch extraction

25 µL of maltose (20 mg / mL in 80% ethanol) and 500 µL of 2M potassium hydroxide were added to the tubes containing the centrifuged precipitate resulting from the extraction of non-structural carbohydrates, placed in a water bath at 100 °C for 10 minutes, and then allowing to cool to room temperature. Then 100 µL of 1M acetic acid (PH = 4.5), 50 µL of Tris 1M buffer solution (PH = 7.2) and 100 µL of alpha-amylase were added. The tubes were placed in a water bath at 85 °C for 30 minutes, and then allowing to cool to room temperature. 100 µL of 1M acetic acid (PH = 4.5) and 500 µL of amyl glucosidase were added, and again tubes were placed in a water bath at 100 °C for 4 minutes. Finally, 75 µL of 2M sodium hydroxide was added and the volume of the tubes adjusted to 2 mL with 100% ethanol for centrifugation at 6,000 rpm for 5 minutes. The sample solution was then transferred to HPLC vials using a syringe and a 0.45 µm filter.

HPLC analyses

The analyses of content were determined in 2 mL of each sample following methods proposed for measurements of nonstructural carbohydrates in plant tissues (Zhao et al., 2010) using a high performance liquid chromatography system (Shimadzu) composed of a Luna 5u NH2 100Å column with dimensions of 250 × 4.6 mm 5 µm column (Phenomenex) and a refractive index detector (Shimadzu).

Statistical analysis

Statistical analyses were performed using the R Core Team (2019) program. Data were checked for normality, homogeneity, and suitability by the Shapiro-Wilk (Shapiro and Wilk, 1965) and Bartlett (Bartlett, 1952) models for further analysis of variance (ANOVA) and multiple comparisons of means by Tukey's test

We conducted a correlation network analysis with the package corrr (Kuhn et al., 2020) to assess correlations among variables. The graphical displays show which variable appear closer together and are joined by stronger paths. The paths were colored by their sign (red for positive and blue for negative). The proximity of the points was determined using multidimensional clustering.

Correlation networks were created from data on sugar content, starch and photosynthetic parameters, connecting these factors to the number of nymphs at each infestation level (low, medium and high) in two phenological stages, vegetative and reproductive. The more solid the color of the line, the stronger the correlation between the factors.

4.3. Results

***Bemisia tabaci* population density**

The number of nymphs was significantly different between the treatments both in the vegetative and reproductive stages, and we observed higher numbers as infestation levels were increased. However, for leaf area difference was observed only in plants infested in the vegetative stage (Table 2).

Table 2. Average number of nymphs of *Bemisia tabaci* biotype B (MEAM1) per leaflet and leaflet area at each infestation level for the vegetative stage (V_2) and reproductive stage (R_2).

Infestation Levels	V_2		R_2	
	mean ± SE			
	Nymphs	Area (cm²)	Nymphs	Area (cm²)
Control	0.00 ± 13.87^{a}	38.43 ± 1.31^{a}	0.00 ± 3.78^{a}	40.38 ± 0.91^{ns}
Low	56.14 ± 13.87^{ab}	37.92 ± 1.39^{a}	12.75 ± 3.78^{ab}	38.93 ± 0.99
Medium	90.53 ± 13.87^{b}	34.96 ± 1.41^{ab}	19.88 ± 3.78^{b}	38.49 ± 0.96
High	208.89 ± 13.87^{c}	32.97 ± 1.47^{b}	46.50 ± 3.78^{c}	38.14 ± 0.99

Within each column values followed by a different letter are significantly different from each other ($p<0.05$; Tukey's test).

Photosynthetic parameters, sugars, and starch composition

The objective of correlation networks was to observe how the interactions between the factors evaluated were altered by different densities of nymphs. Because the control treatments were not infested with *B. tabaci*, they are not included in this correlation network analysis.

In the low infestation, reproductive stage, the greatest positive correlation occurred between nymphs and N (Fig. 1). This correlation was negative for low infestation in the vegetative stage (Fig. 2). The same inversion also was observed for all the other levels of infestation (Figs. 3 and 4, and 5 and 6).

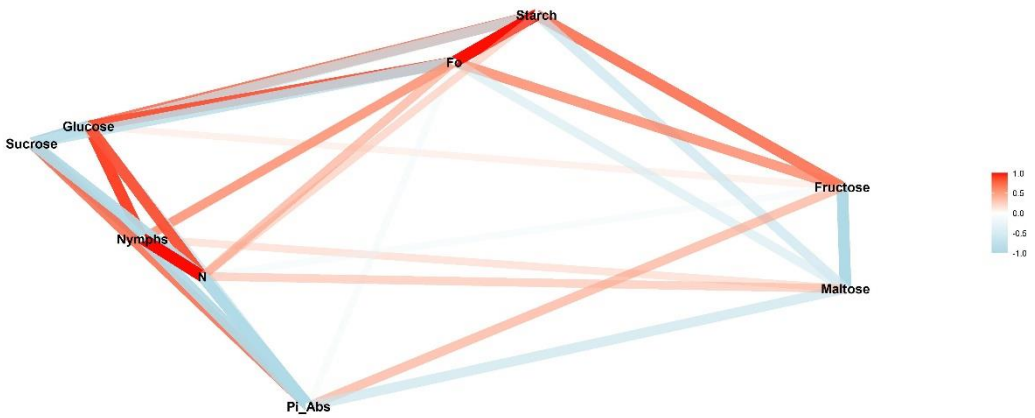

Figure 1. Correlation between nymphs, in the low infestation treatment (T2), and photosynthetic parameters [initial fluorescence (F_0), performance index on absorption basis (Pi_Abs), and turn-over number (N)], sugars (Glucose, Fructose, Maltose, and Sucrose) and starch in the soybean reproductive stage.

In the reproductive stage, the correlation of nymphs and Pi_Abs was negative but in the vegetative stage, these correlations were positive. This same pattern was observed between fructose and nymphs. The networks also show that Pi_Abs and fructose are positively correlated in all the treatments.

Another correlation observed for all the infestation levels was between F_0 and starch. In the reproductive stage a strong positive correlation was observed (Figs. 1, 3, and 5), this correlation also appeared strong, but negative in the vegetative stage (Figs. 2, 4, and 6).

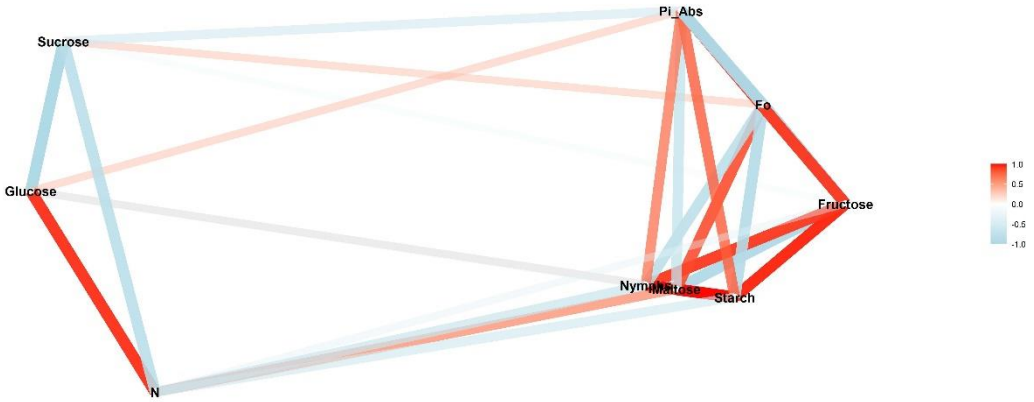

Figure 2. Correlation between nymphs in the low infestation treatment (T6) and photosynthetic parameters [initial fluorescence (F_0), performance index on absorption basis (Pi_Abs) and turn-over number (N)], and sugars (Glucose, Fructose, Maltose, and Sucrose) and starch in the soybean vegetative stage.

We observed different patterns of correlation between the reproductive and vegetative stages (Figs. 1 and 2). In the reproductive stage, positive correlations were observed between nymphs and glucose, maltose, and starch, and negative correlations with sucrose and fructose (Fig. 1). In the vegetative stage, these correlations were mostly positive, except for maltose and glucose, which were negatively correlated (Fig. 2).

While phenological stages altered the correlation network, the different infestation treatments also affected the relationships between the parameters in the correlation network. For medium infestation in the reproductive stage, we observed a strong negative correlation between nymphs and fructose, and nymphs and starch (Fig. 3) but a positive correlation between nymphs and glucose and maltose. The correlations between nymphs and F_0 and Pi_Abs were both negative.

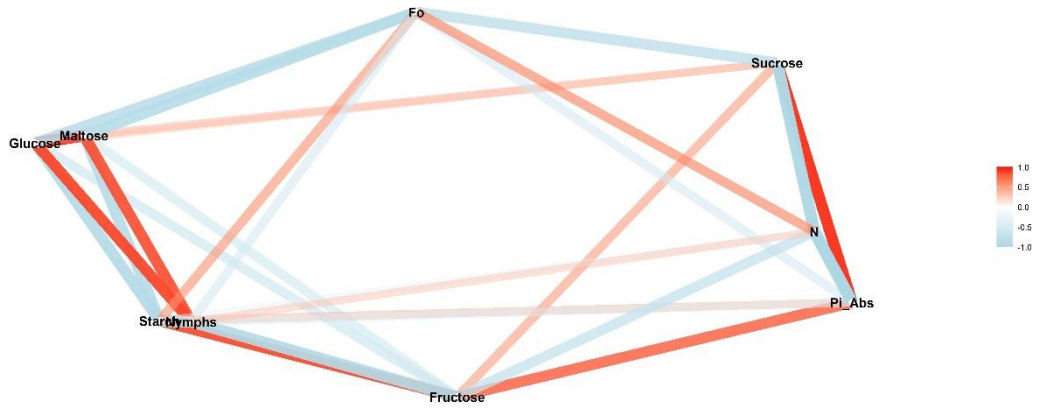

Figure 3. Correlation between nymphs in the medium infestation treatments (T3) and photosynthetic parameters [initial fluorescence (F_0), performance index on absorption basis (Pi_Abs) and turn-over number (N)], sugars (Glucose, Fructose, Maltose, and Sucrose) and starch in reproductive soybean stage.

In the vegetative stage, the correlations of nymphs with fructose and starch were positive, (Fig. 4). For the photosynthetic parameters, the correlations were mostly negative, only Pi_Abs did not significantly correlate with nymphs.

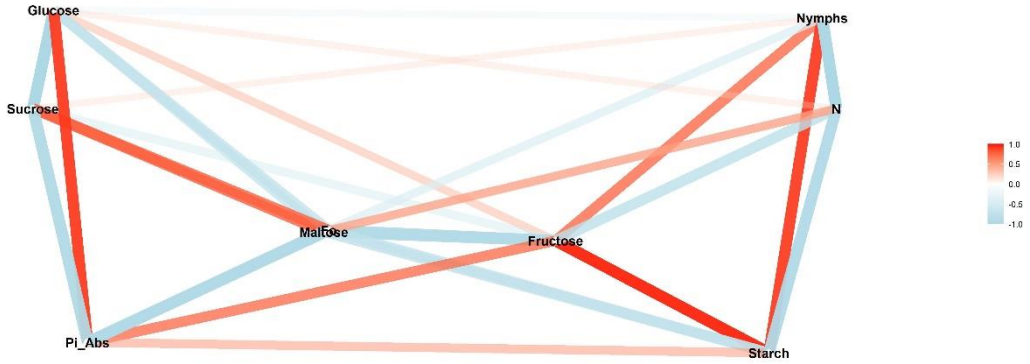

Figure 4. Correlation between nymphs in the medium infestation treatments (T7) and photosynthetic parameters [initial fluorescence (F_0), performance index on absorption basis (Pi_Abs) and turn-over number (N)], sugars (Glucose, Fructose, Maltose, and Sucrose) and starch in the vegetative soybean stage.

In the high infestation, reproductive stage, there was a negative correlation between nymphs and maltose, and nymphs and glucose, and a strong positive correlation between nymphs and starch (Fig. 5), which indicates that this might be one of the main factors

responsible for increasing the density of nymphs. A similar pattern was observed in all the treatments with medium and high density of nymphs (Figs. 3, 4, and 6). For photosynthetic parameters, F_0 showed a negative correlation with nymphs, but again no significant correlation was observed with Pi_Abs.

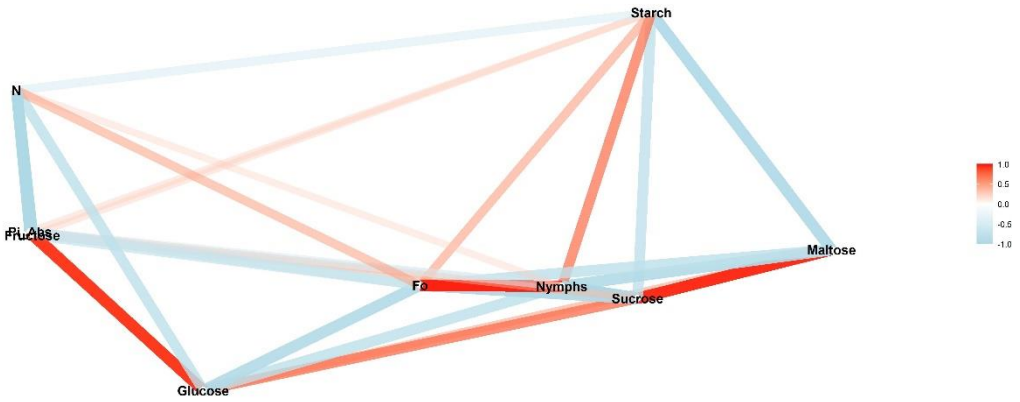

Figure 5. Correlation between nymphs in the high infestation treatment (T4) and photosynthetic parameters [initial fluorescence (F_0), performance index on absorption basis (Pi_Abs) and turn-over number (N)], sugars (Glucose, Fructose, Maltose, and Sucrose) and starch in the reproductive soybean stage.

In the high infestation, vegetative stage treatment, the correlations of nymphs with sugars were positive for fructose and sucrose, and negative for maltose and starch. No significant correlation was observed for glucose (Fig. 6). For photosynthetic parameters correlation strengths were lower; Pi_Abs was positively, and F_0 negatively correlated, while N was again negative, following the same pattern described for the low infestation treatment.

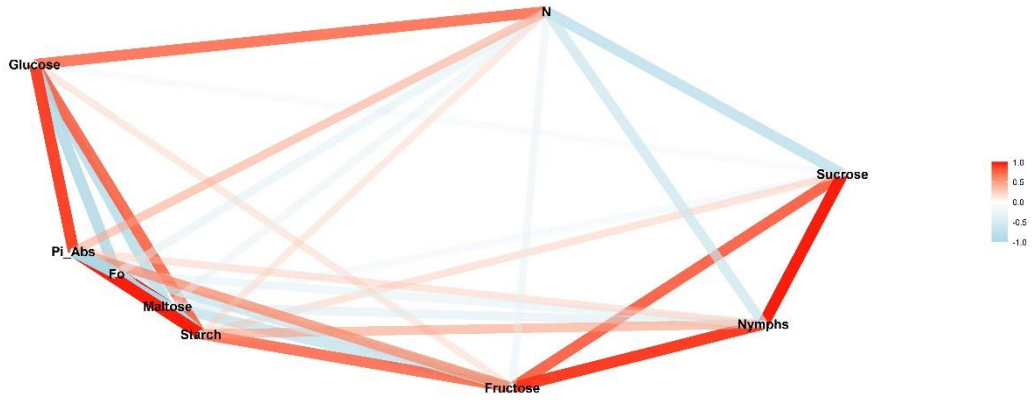

Figure 7. Correlation between nymphs in the high infestation treatments (T8) and photosynthetic parameters [initial fluorescence (F_0), performance index on absorption basis (Pi_Abs) and turn-over number (N)], sugars (Glucose, Fructose, Maltose, and Sucrose) and starch in the vegetative soybean stage.

4.4. Discussion

Despite its size, *B. tabaci* can cause damage that affects not only the yield production directly, but also the physiology of the plant (Oliveira et al., 2001). Unlike defoliating pests like caterpillars, which reduce the photosynthetic area of the leaves, or stinkbugs, that cause direct damage to grains, the whitefly affects plant performance by feeding directly from its energy sources.

When soybean was infested in the vegetative stage nymphal density increased faster than with infestation in the reproductive stage. It is possible that during the vegetative stage more nutrients are available in the leaves as the plant is allocating photosynthate to the promotion of plant growth, consequently providing better conditions for the development of *B. tabaci*. After reaching the reproductive stage these resources are reallocated to production of flowers and grains (Bellaloui et al., 2013), with fewer nutrients available in the leaves, thus there are perhaps less nutrients available to promote *B. tabaci* growth rates.

Although infestations occurred at the same time, the number of nymphs in the vegetative stage was more than four times higher than in the reproductive stage, which also affected the leaf area in the high infestation treatment, representing a very high density of nymphs per cm^2 (Table 2).

The calculated data exported by the OJIP protocol show the changes in chlorophyll fluorescence that occur during the exposure of a photosynthetic organism to high irradiation

can be used as a sensitive signature of photosynthesis (Stirbet et al., 2018). At time 0 (t_0) a super pulse is emitted at an intensity of 80% to induce the maximum fluorescence of chlorophyll (F_m). Here the photosynthetic organism is represented by soybean leaflets exposed to the pulse of irradiation emitted by the FluorPen. The data obtained represent a measure of plant stress, in our study, caused by the *B. tabaci* feeding.

Ideally, in a healthy plant, the initial fluorescence (F_0) should be lower compared with treatments that subject the plant to some type of stress (Strasser et al., 2000). This means that a positive correlation should be observed between nymphal density and F_0. This pattern was observed in the low and high infestations, at the reproductive stage, but not in the medium infestation, which showed a negative correlation. The same pattern of correlations was observed for starch in these treatments.

The F_0 also showed correlation with starch. The correlation between these parameters was positive in the reproductive stage and negative in the vegetative stage, this pattern was observed for all the treatments, but was strongest under low infestation. Starch is an energy reservoir, synthesized in photosynthetic cells, and degraded to provide substrates for photosynthesis and sucrose for the plant (Zeeman et al., 2010). Higher nymph density in the vegetative stage, and the positive correlation between starch and F_0 also was observed. At the same time, the content of sucrose was inversely proportional to starch. Higher concentration of starch in the leaves indicates a healthier plant, capable of providing higher content of sucrose for plant growth, and as consequence better conditions for *B. tabaci* development (Hasanuzzaman et al., 2018).

F_0 and starch presented a positive correlation between them in all the treatments at the reproductive stage. Even when these negative correlations occurred in medium infestation, the positive correlation between F_0 and starch was still observed, indicating that the initial fluorescence is influenced not only by stress but also on starch content. In the vegetative stage, a negative correlation between nymphs and F_0 was observed in all the levels of infestation. However, in this stage the correlation between F_0 and starch was negative in all the treatments, supporting the relation between starch and initial fluorescence.

In the case of the absorption flow performance index (Pi_Abs), the expected response is the inverse of that expected for F_0. When the plants are healthy and free from stress, they are expected to perform better in the absorption of light by chlorophyll pigments in the leaves. Non-stressed plant should present higher indexes of absorption, while plants subject to stress through higher insects infestations, should show a negative correlation, corresponding to a lower vitality of the plant (Strasser et al., 2000). Treatments in the reproductive stage showed

a negative correlation as expected. No significant correlation was observed for medium and high infestation in the vegetative stage, probably due the high density of nymphs interfering with the absorption of light by the leaves. However, a positive correlation was observed in the low infestation at the vegetative stage. This could be explained due the negative correlation of Pi_Abs with N. While Pi_Abs indicates the capacity of the plant to absorb light energy, N expresses the rate at this light energy is converted into energy to the plant. In this case, soybean would be absorbing more light than converting in an attempt to compensate for the stress caused by the presence of the nymphs (Isaacs et al., 1998). This was more likely to occur at the vegetative stage since there are more resources available in the leaves than at the reproductive.

The N, or turn-over number, represents how many times the primary quinone acceptor (Q^A) was reduced to Q^{A-} in the interval from time zero (t_0) to reach maximum fluorescence (F_m) after receiving electrons from water using light energy. This direct electron transfer is essential in the process of converting light energy into chemical energy (Kato et al., 2016; Strasser et al., 2000). An increase in the absorption index would be reflected in a lower reduction rate of the quinone acceptor (Q^A), such that the plant would be absorbing more light but not converting this light energy to photoassimilates. Previous study have shown that increases in F_0, which is inverse to Pi_Abs, represents more Q^{A-} formation (Kato et al., 2016), i.e., the apparently higher light absorption does not mean the plant has better photosynthetic performance. In other words, plants infested with *B. tabaci* nymphs could have a good absorption rate, but the photoassimilates produced would be consumed by them instead of being distributed in the plant.

Whiteflies are phloem-feeders, and phloem is responsible for transporting photoassimilates, generally high concentrations of sugars (Walker et al., 2010). The primary sugars associated with the high occurrences of whiteflies are sucrose and glucose, responsible for improving survival and adult longevity and fecundity, and promoting feeding and oviposition preference, and immature development (Hasanuzzaman et al., 2018). As these sugars are also related to photosynthesis, a negative relationship between *B. tabaci* infestation and photosystem activity is expected. Here that situation resulted in poorer plant development as indicated by smaller leaves in soybean under high insect infestation.

The vegetative stage appears to provide better conditions for the insects' development, as evidenced by the faster growth of nymphs during this stage. Differing from that observed for the other sugars, the correlations between maltose and glucose with nymphs were both negative. Maltose and glucose are both products of starch breakdown, being the two major

forms of carbon exported from chloroplasts during its degradation (Lu and Sharkey, 2006). Maltose, negatively correlated with starch in all the treatments, and was present in higher concentrations during the vegetative stage.

Even though glucose also is a product of starch breakdown, it did not present the same negative correlation with starch. First, it could be due to maltose provide an energetic advantage over export of glucose, representing two-thirds of the carbon export, while glucose represents the rest (Lu and Sharkey, 2006). And second, representing a smaller part in the leaf composition, it could have been affected by a reduction in the leaflet's size, especially in the high infestation treatment. In the high infestation treatment, there was no significant correlation between glucose and nymphs.

One of the main components of honeydew excreted by whiteflies is fructose (Byrne and Miller, 1990; Henneberry et al., 1996), also reported for other hemipterans (Fischer et al., 2005; Shaaban et al., 2020). A strong positive correlation between fructose and nymphs was observed for all treatments at the vegetative stage when the greatest number of *B. tabaci* nymphs occurred. A similar pattern was observed for sucrose. Varieties with higher content of fructose and sucrose in their leaves were reported to promote higher densities and better conditions for development of *B. tabaci* in eggplants and cotton, respectively (Bi et al., 2005; Hasanuzzaman et al., 2018).

Among the photosynthetic parameters the turn-over number N, was positively correlated in the low infestation treatment, and negatively correlated in higher infestation levels, independent of the phenological stage. The lower the infestation level the stronger the positive correlation. The assessment of the correlation between nymphs and N might represent useful tool for evaluating the impact of *B. tabaci* in the crop, especially in large areas, where monitoring can time consuming, reducing the need of counting the nymphs.

Bemisia tabaci feeding affects the plant's physiology reflected in part by the relationships among photosynthetic parameters as well as the levels of sugars and starch. From our results, the greatest impacts of insect infestation occur during the vegetative phenological stage, when plants are more suitable for *B. tabaci* development, thus leading to plant stress that will have consequences on productivity.

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Supplementary Material

Statistical analysis

All statistical analyses were performed using the R Core Team (2019) program. A two-way ANOVA was used to estimate how the means of the tested variables change according to the levels of factors treatment and stage. We checked the normal distribution and homogeneity of variance. When necessary the data were transformed using the Box-Cox (1964) method using the function boxcox from MASS package (Venables and Ripley, 2002). In addition, we used a multivariate analysis with Permutation test with the package vegan (Oksanen et al., 2019).

Results

In the univariate analyses we observed that no significant interaction between treatment and stage was found to the variables F_0 ($F_{3,24} = 0.16$; $P = 0.91$); fructose ($F_{3,24} = 0.15$; $P = 0.93$); glucose ($F_{3,24} = 0.92$; $P = 0.44$); maltose ($F_{3,24} = 0.31$; $P = 0.81$); N ($F_{3,24} = 1.22$; $P = 0.32$); Pi_Abs ($F_{3,24} = 0.04$; $P = 0.98$); starch ($F_{3,24} = 0.38$; $P = 0.76$) and sucrose ($F_{3,24} = 0.21$; $P = 0.88$). In all these variables there was no difference between the treatments or stages ($P > 0.05$). However, when we considered all variables in the multivariate analysis with Permutation test, we observed that there was significant effect only of the stages (F= 34.239; P= 0.001).

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Treatment	Fructose/Treatment	Glucose/Treatment	Maltose/Treatment	Starch/Treatment	Sucrose/Treatment	Fo/Treatment	Pi_Abs/Treatment	N/Treatment
Control	0.0016±0.0001	0.0003±0.00010	0.0003±0.0002	0.2692±0.0074	0.0009±0.00007	7088.81±199.78	5.27±0.60	750.84±27.42
Low	0.0016±0.0002	0.0002±0.00004	0.0004±0.0001	0.2626±0.0069	0.0009±0.00007	6971.07±211.56	5.33±0.66	723.52±31.88
Medium	0.0018±0.0002	0.0002±0.00006	0.0003±0.0002	0.2637±0.0084	0.0009±0.00007	7116.50±182.24	5.16±0.53	835.00±85.51
High	0.0018±0.0002	0.0003±0.00005	0.0003±0.0001	0.2688±0.0057	0.00012±0.00018	7101.02±206.13	5.05±0.70	748.36±25.35
Stage	**Fructose/Stage**	**Glucose/Stage**	**Maltose/Stage**	**Starch/Stage**	**Sucrose/Stage**	**Fo/Stage**	**Pi_Abs/Stage**	**N/Stage**
Vegetative	0.0017±0.00008	0.0003±0.00002	0.0039±0.00013	0.2671±0.0051	0.0009±0.00009	7168.88±156.79	5.44±0.34	744.86±17.64
Reproductive	0.0017±0.00020	0.0002±0.00006	0.0038±0.00015	0.2650±0.0047	0.0010±0.00005	6969.81±109.34	4.88±0.53	784.00±46.29

There was no difference between the treatments or stages by the *F* test ($P > 0.05$).

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