Article

Monitoring *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) infestation in soybean using hyperspectral remote sensing

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Simple Summary: The whitefly Bemisia tabaci became a primary pest in soybean fields in Brazil in the last decades causing losses in the yield. Its reduced size and fast population growth make monitoring a challenge. The use of hyperspectral remote sensing is a tool that allows the identification of arthropod infested areas without contact with the plants. This optimizes the time spent on crop monitoring, which is important for large cultivation areas such as soybean fields. In this study, we investigated differences in the responses obtained from leaves of soybean plants, non-infested and infested with Bemisia tabaci in different levels, with the aim of differentiating the plants using hyperspectral remote sensing. The leaves were collected from soybean plants to obtain hyperspectral curves in the laboratory. The hyperspectral curves of infested and non infested leaves were then differentiated with good accuracy by the responses of the curves related to photosynthesis and water content of the leaves. The results obtained here can be helpful in improving the monitoring of Bemisia tabaci in the field, which is important in the decision making of integrated pest management program for this key pest.

Abstract: Although monitoring and observing insect pest populations in the fields is essential in crop management, it is still a laborious and sometimes ineffective process. High infestation levels may diminish the photosynthetic activity of soybean plants, affecting their development and reducing the yield. An imprecise decision making in integrated pest management program may lead to an ineffective control in infested areas or the excessive use of insecticides. In order to reach a more efficient control of arthropods population it is important to evaluate the infestation in time to mitigate its negative effects on the crop and remote sensing is an important tool for monitoring. It was proposed that infested soybean areas could be identified, and the arthropods quantified from non-infested areas in a field by hyperspectral remote 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 remote sensing data. Therefore, samples of infested and non-infested soybean leaves were collected and transported to the laboratory to obtain the hyperspectral curves. The results obtained allowed to discriminate the different levels of infestation and to separate healthy from whitefly infested soybean leaves based on their reflectance.

Keywords: glycine max; sampling; pest management; spectroradiometer

1. **Introduction**

According to the USDA [1], world soybean production in the 2018/2019 season was 358.65 million tons. The United States produced 120.52 million tons, followed by Brazil, which produced 117 million tons in the 2019/2020 season. Brazil is projected to be the largest producer of soybeans in the world followed by the United States and Argentina.

In Brazil, the 117 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, Brazilian average yield is projected to be 3.39 tons per hectare (3.9% increase in yield), in 36.9 million hectares (2.7% increase in area) [2].

Although this is the most important agricultural product in Brazil today, the monoculture in wide fields has consequences, such as greater vulnerability to insect pests, causing reduction in yield [3]. Therefore, knowing and monitoring the main pests present in the soybean ecosystem, using a variety of sampling methods, is extremely important for the decision making to be taken at the right time, avoiding yield losses [4].

There are parameters that allow decisions to be taken at the right time, resulting in better control like the Economic Injury Level, which represents the smallest pest population that will cause yield losses equal to the insect management costs [5]. Additionally, the Economic Threshold is the pest population at which actions should be taken, to prevent such population to reach the Economic Injury Threshold [6]. Economic threshold is always lower than the Economic Injury level due to the time required for the control method to be effective.

These levels are already established for the main pests that infest soybean plants. 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 Economic Injury thresholds are still being investigated [7].

To acquire data to 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, which was initially introduced in the United States [8]. Later, this method was modified for the conditions of Brazilian agriculture [9], and introduced in Brazil in the decade of 1970 [10]. Other sampling methods can be used, depending on the insects' habits, such as visually examining the plants for monitoring borer and gall-inducing insects, and soil sampling for insects that live in the soil and feed on the plant's roots.

The sampling methods currently in use are usually practically challenging considering the vast extension of soybean fields in the Midwest region of Brazil [11]. Also, they are time-consuming and expensive, due to the necessity of workers scouting the field [12,13]. Moreover, there is still a lack of reliable sampling methods for some species, especially those too small to see with naked eye, or the ones that inhabit the soil. This scenario promotes the implementation of remote sensing technologies and their benefits, especially the potential time saved by automatizing crop monitoring [13-15].

Abiotic stress, 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, with most studies referring to the 400–1,000 nm range [16]. Hence, advanced remote sensing technologies can be used to detect changes in reflectance from soybean plants, as a non-invasive monitoring method [11].

Remote sensing has been used to detect stress caused by arthropod herbivory in a variety of plant species, such as maize [17], soybean [4], rice [18,19], wheat [20], peach trees [12,21], cotton [22] and potato [23].

However, one of the biggest challenges is the analysis of a large number of bands, from hyperspectral sensors. This analysis is complex and time-consuming, using special algorithms to select a group of bands sensitive to arthropod infestation in each plant species [16]. According to Hair et al. [24], currently, one of the most used statistical procedures to reduce the amount of data without losing

important information is multivariate analysis. Thus, this study aims to develop models to discriminate the levels of whitefly infestation in soybean fields, using proximal hyperspectral remote sensing.

2.   Material and Methods

2.1 Local

The assay was carried out 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 CWa (humid subtropical climates, with dry winter and hot summer), according to Köppen classification [25]. The average year pluviosity is 12800 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 in 11/28/2018, in an area of 1.5 hectares. The field was tilled and fertilized. The soil is classified as dystrophic red-yellow latosol.

2.2 Insect rearing

The rearing of whitefly, *Bemisia 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 [26]. The plants are replaced in the greenhouse when necessary, in order to keep the insect population adequate for the development of bioassays.

2.3 Bioassay

The assay began on 12/13/2018, when the plants reached the phenological stage V3 [27]. The treatments were distributed in a randomized block design, made of four blocks and four treatments (Low, Medium, High and Control) consisting of different *Bemisia 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), supported by bamboo poles and covered with an anti-aphid screen that allows air flow and prevents infestation by unwanted arthropods. The cages were installed on 12/12/2018, 2 m apart from each other.

On 12/19/2018, the cages were manually infested, releasing in each cage one pot with one kale plant and the amount of insect correspondent to each treatment. The treatments were: 1. Control (no insects); 2. Low (ca. 300 adults); 3. Medium (ca. 600 adults); and 4. High (1200 adults).

2.4 Data collection

To collect reflectance data, ten soybean leaflets were collected from the middle third of the plant and stored in plastic bags with identification tags. The leaflets were collected in 01/10/2019, 01/17/2019, 01/24/2019, 01/31/2019, 02/07/2019, 02/14/2019, 02/21/2019, and 02/28/2019. 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 (FieldSpec 3, Analytical Spectral Devices, Boulder, CO, USA). This sensor operates in the spectral range of 350-2500 nm, with 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, designed for non-destructive 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 Sulfate 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.

After calibrating the spectroradiometer, according to the manufacturer's recommendation, all leaf samples were collected in a short period, to allow comparison. The central region of the leaflet was evaluated in a circle of 2.1 cm in diameter (area of 3.5 cm²). In total, 160 spectral samples were collected.

2.5 Data analysis

The large number of information 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. [28], 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 [16], 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 [29] 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 [24].

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. Results and Discussion

The occurrence and damage of the whitefly on soybeans is alarming. High densities can cause losses between 30% and 70% in yield. In addition, this pest is able to tolerate the action of some insecticides, with rapid selection of resistant populations. Therefore, it is necessary to develop techniques to monitor this pest in the field, in the early stages of infestation, to ensure greater efficiency in control.

Recently, geotechnology has been gaining adherents in Brazil, especially in sugarcane fields [30,31], and later in grain fields, such as soybean [32]. Although precision tools currently are more used 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 [33]. 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 are based on collecting data from plants.

To obtain 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 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 [34,35].

In the models generated in each data collection, being 8 in total, some bands were observed more frequently in the models. In figure 1, we can see the frequency of distribution of the bands with the greatest weight in all the models generated.
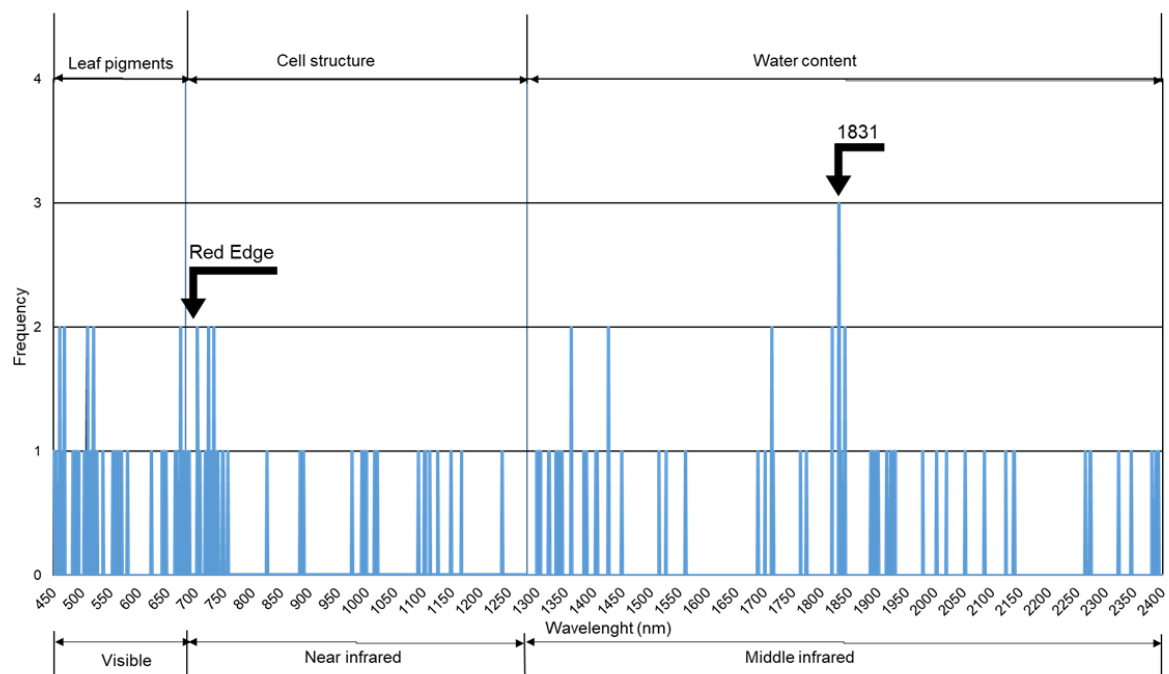

Figure 1. Frequency of appearance of bands (wavelengths) in the statistical models.

The discriminant analysis had its peak of differentiation between treatments (75.50%) on January 31st, at the reproductive stage R4 (Figure 2). By analyzing the infestation data together with the meteorological data, it is possible to observe that the period was dry and hot, boosting the development of whitefly populations in the field.
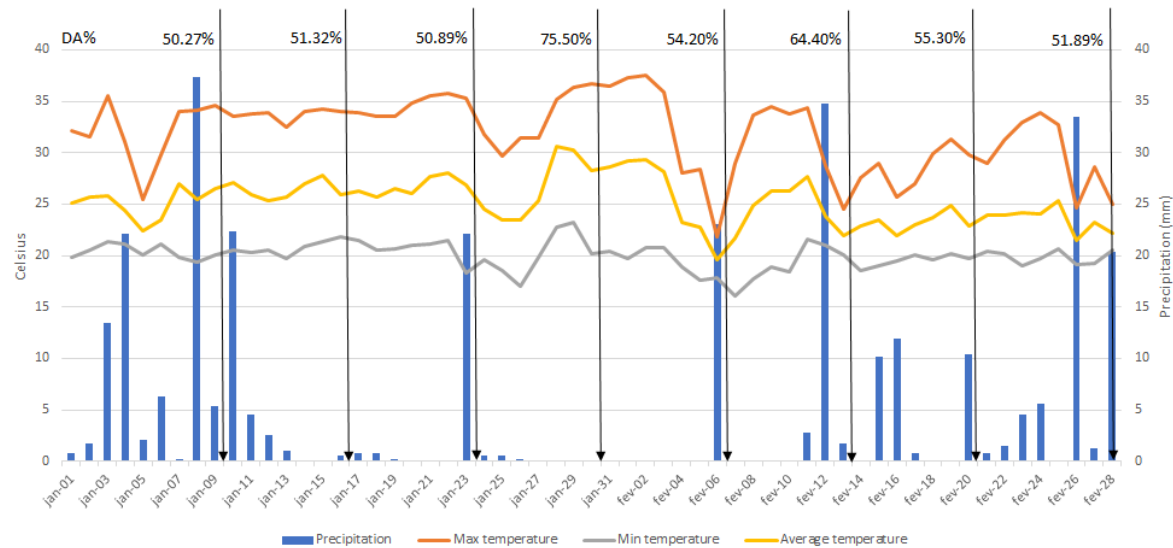
Figure 2. Meteorological data and discriminant analysis accuracy (%).

Therefore, only the data collected on January 31st were 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 all over the analyzed electromagnetic spectrum, compared to the other levels.
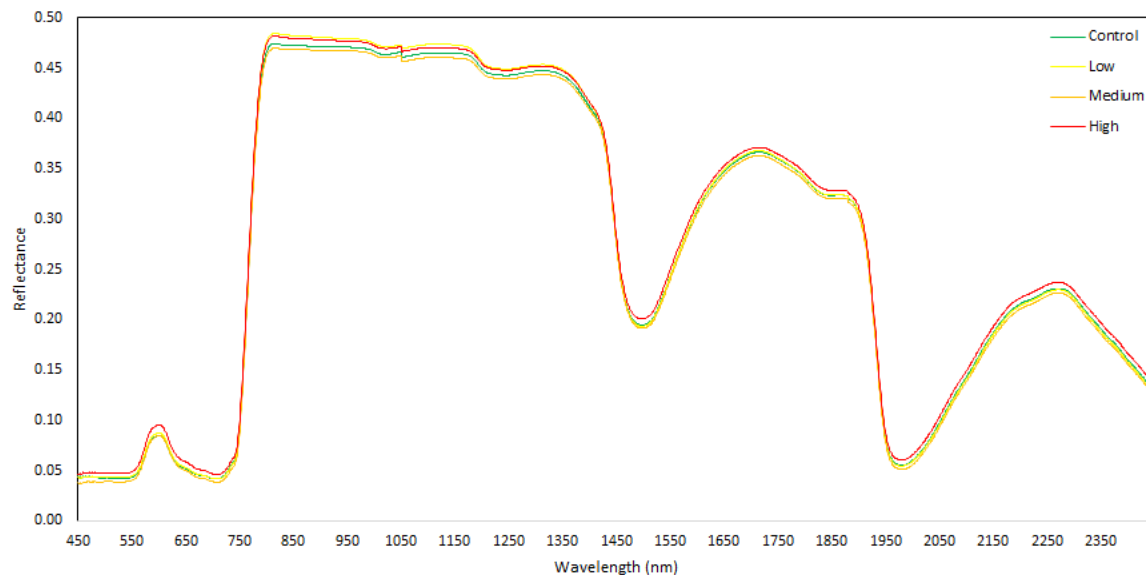

Figure 3. Average spectral curves (450-2400 nm) of soybean leaves under different levels of whitefly infestation.

The first population outbreaks of whitefly in Brazil were reported in the state of São Paulo this insect, in the nymphal stage, excretes a high volume of a sugar-rich watery fluid called honeydew [36]. This fluid is a substrate for the development of fungi of the genus *Ascomycete*, which produce 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.
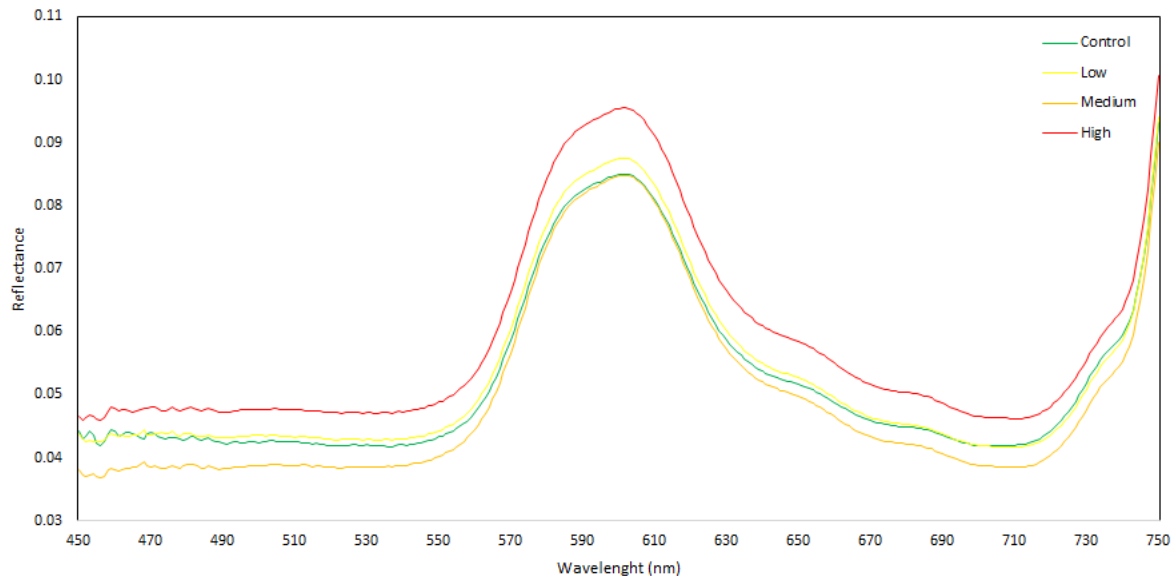

Figure 4. Average spectral curves (450-750 nm) of soybean leaves under different levels of whitefly infestation.

The results shown in the spectral curves, in the wavelengths $405 – 700$ nm, indicate low reflectance (around 10%), with slight increase in the region correspondent to green light (550 nm). The reduction in reflectance is often associated to absorption of foliar pigments due to the presence of chlorophyll. In the spectral region 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 absorbing energy near 645 nm [36].

Thus, it is possible to observe in figure 4 that the treatment with least photosynthetically activity (high infestation) presented higher reflectance in all the visible region spectrum. This might have occurred because of how whitefly infestation affects the plant physiology, altering water balance, photosynthesis, chlorophyll content and metabolites associated with physiological stress [37,38].

Most of the processes mentioned, which alter the leaves' and plants' physiology are observable in the spectral signature of such plants. 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 significative for each of these processes.

Hence, observing the discriminant analysis in the reproductive stage R4 (Figure 5), 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%.
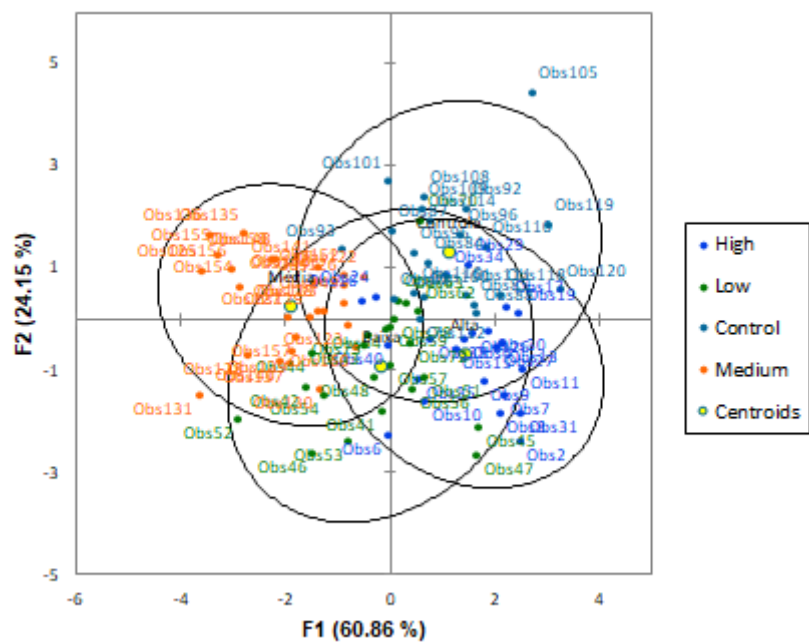
Figure 5. Discriminant Analysis of hyperspectral data (450-2400 nm) of soybean leaves under different levels of whitefly infestation.

After performing the discriminant analysis with 70% of the samples, the next step was the validation of the model with the 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 total accuracy rate of 75.48% (Table 1). The plants with medium infestation were classified more accurately (85.71%) while most of the error in classification occurred in plants with 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 was collected, the average number of nymphs per leaflet in the medium infestation was double of the number 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 [39], 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 a more complex scenario, whereas the average number of nymphs per leaflet in the high infestation were almost 8 times higher than the medium infestation.

Table 1. Linear discriminant classification of parasitized host eggs.

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%)

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.

Analyzing all the bands selected, we have 17 wavelengths, being 5 in the visible region (450-682 nm), 6 in the near-infrared region (716-1167 nm), and 6 in medium-infrared region (1321-2265 nm). These values are presented in table 2.

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.

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 NIR shoulder at approximately 780 nm. This region indicates the sharp increase in reflectance from the VIS to NIR regions associated with strong chlorophyll absorptions and internal leaf structure.

The 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.

Table 2. Discriminant equations as a function of foliar reflectance of plants under different levels of infestation.

	High	Low	Control	Medium
Intercept	-1102.33	-1098.13	-1119.65	-1096.49
450	-10865.5	-10404.6	-11702.4	-10500
484	11974.36	10209.63	12855.01	11117.2
516	-58935.3	-51094.7	-56854.6	-54050.4
520	49430.5	42788.03	46405.43	44677.59
682	4322.146	3613.893	4759.895	3734.77
716	-2335.89	-2303.37	-2084.76	-2421.18

739	1674.114	3989.261	2873.146	5409.076
748	2707.008	434.5705	1256.311	-1149.96
975	74.32199	208.433	970.4353	474.2767
1112	-8089.84	-7762.87	-7194.17	-9892.21
1167	-14279.4	-14584.1	-16181.1	-11723.6
1321	25909.97	25822.36	25812.28	25385.85
1426	974.6417	2207.818	2080.898	2169.166
1832	90.80342	415.9712	968.7407	643.3495
1892	-5491.52	-6498.19	-6911.74	-6030.51
1915	5026.022	5903.34	6016.831	5823.88
2265	-1675.51	-2764.42	-2593.08	-3627.17

Thus, the intervals that had higher representativity were the visible and medium infrared regions. 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 [26].

Phloem is a vegetal tissue made of sieve elements, and sclerenchyma and parenchyma cells. The main functions of this plant tissue are to transport water, minerals and 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.

These results can be used for the implementation of IPM programs, to determine where and when control methods are required for managing the pest. Future research is necessary to validate the results obtained, specifically 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.

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

4. Conclusions

It is possible to separate healthy, whitefly-infested soy leaves based on their 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 near and medium infrared regions were the most important for the model, as they are directly related to photosynthesis and water content in the leaves.

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