

Electrical signalling on Bt and non-Bt cotton plants under stress by *Aphis gossypii*

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Abstract

Bt cotton is a genetically modified pest-resistant plant that produces an insecticide from *Bacillus thuringiensis* (Bt) to control Lepidopteran species. Surprisingly, there is no study – yet, that characterizes the signalling mechanisms in transgenic cotton plants attacked by non-target insects, such as aphids. In this study, we characterized the production of electrical signals on Bt and non-Bt cotton plants infested with *Aphis gossypii* and, in addition, we characterized the dispersal behaviour of aphids to correlate this behaviour to plant signalling responses. Electrical signalling of the plants was recorded with an extracellular measurement technique. Impressively, our results showed that both Bt and non-Bt cotton cultivars, when attacked by *A. gossypii*, emitted potential variation-type electrical signals and clearly showed the presence of distinct responses regarding their perception and the behaviour of aphids, with evidence of delay, in terms of signal amount, and almost twice the amount of Cry1F protein was observed on Bt cotton plants at the highest density of insects/plant. We present in our article some hypotheses that are based on plant physiology and insect behaviour to explain the responses found on Bt cotton plants under aphid stress

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Aphis gossypii

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ABSTRACT Plants have developed various mechanisms to respond specifically to each biotrophic attack. It has been shown that the electrical signals emitted by plants are associated with herbivory stress responses and can lead to the activation of multiple defences. Bt cotton is a genetically modified pest-resistant plant that produces an insecticide from *Bacillus thuringiensis* (Bt) to control Lepidopteran species. Surprisingly, there is no study – yet, that characterizes the signalling mechanisms in transgenic cotton plants attacked by non-target insects, such as aphids. In this study, we characterized the production of electrical signals on Bt and non-Bt cotton plants infested with *Aphis gossypii* and, in addition, we characterized the dispersal

behaviour of aphids to correlate this behaviour to plant signalling responses. Electrical signalling of the plants was recorded with an extracellular measurement technique. Impressively, our results showed that both Bt and non-Bt cotton cultivars, when attacked by *A. gossypii*, emitted potential variation-type electrical signals and clearly showed the presence of distinct responses regarding their perception and the behaviour of aphids, with evidence of delay, in terms of signal amount, and almost twice the amount of Cry1F protein was observed on Bt cotton plants at the highest density of insects/plant. We present in our article some hypotheses that are based on plant physiology and insect behaviour to explain the responses found on Bt cotton plants under aphid stress.

KEYWORDS : biotic, response, plant, insect, interaction.

1 INTRODUCTION

An organism's capacity to survive in an ecosystem depends on its ability to respond quickly and efficiently to external stimuli and to develop effective and sustainable defences (Zebelo & Maffei, 2015). For this reason, plants have developed numerous mechanisms to react specifically to each biotrophic attack, and cell-to-cell communication between distant tissues is essential to coordinate activities in response to the environment. Thus, plants need to produce a signalling mechanism to integrate perception, transmission, and response to biotic and abiotic actions that occur in the ecosystem (Baluska et al., 2015; Brenner et al., 2006; Pelagio-Flores et al., 2011; Maffei et al., 2007). Electrical signals have been shown to be associated with responses to herbivory (Pachu, 2020), leading to the activation of multiple organism defences (Maffei et al., 2004). However, studies that better characterize the electrical signalling mechanisms in plants attacked by herbivores, such as aphids, are still scarce.

The aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) is a cotton-damaging pest (Malaquias et al., 2017a) of cotton and one of the most important nontarget species of Bt cotton, which is a genetically modified cultivar expressing proteins derived from *Bacillus thuringiensis* (Berliner) (Bt), which gives them high efficiency against some lepidopteran species (Malaquias et al., 2017b; Malaquias et al., 2020). However, researchers have raised concerns about their potential impact on nontarget organisms such as aphids (Hagenbucher et al., 2013) Studies addressing the impact of Bt cotton on the population dynamics of aphids (Udikeri et al., 2012) or on growth or developmental characteristics (Zhao et al., 2016) have shown that aphid feeding on Bt cotton plants can cause morphological, physiological, biochemical and molecular changes in cotton plants and possibly different biological response patterns between Bt and non-Bt cotton cultivars.

Different environmental stimuli cause specific responses in living cells that are capable of transmitting electrical signals (Lautner et al., 2005). Among the signals involved in electrophysiological responses, variation potentials are characterized by rapid depolarization and subsequent slow repolarization. The amplitude and shape of the variation potentials (VPs) vary with the stimulus intensity. In addition, the magnitude and speed of responses decrease as the signal moves away from the stimulus site, and its induction depends on the type of damage sustained (Stahlberg et al., 2006).

Although the methods used to produce transgenic crops are being continuously improved, it is currently not possible to control the exact stability, integration and expression of the gene inserted in plant genomes. Plant physiological traits may be altered; thus, it is crucial to understand how plants produce different signs of stress and convert them into appropriate specific responses. Therefore, it is essential to characterize the type of electrical signalling of Bt and non-Bt cotton plants as a function of the stress caused by *A. gossypii* and provide insights to understand how plants convert these different signals into appropriate physiological reactions. In our study, we characterized the production of electrical signals on Bt [cultivar WideStrike(r)] cotton plants and their non-Bt isolate [cultivar FM 993] infested with *A. gossypii* in alternating light-dark cycles. The aphid *A. gossypii* was used as a model insect for the study because insect feeding occurs at the phloem level, and the biological interactions between the herbivore and its host plant can be considered unique. Additionally, we characterized *A. gossypii* dispersal behaviour to relate this behaviour to plant signalling responses.

2 MATERIAL AND METHODS

2.1 Characterization of the electrical signalling potential of Bt and non-Bt cotton plants Bioassays to record electrical signalling of cotton plants were conducted in the laboratory. The experimental design was a randomized block design, and each treatment was repeated 10 times. The measurement of electrical signals was made on the plant surface. A technique was used to detect electrical signalling potential differences over long periods. At the time of the electrical signal measurements, the cotton plants were placed in a Faraday cage to ensure electromagnetic isolation of the environment at 26 ± 1 degC, with a relative humidity of $60 \pm 10\%$ and a 12-h photophase.

Measurements were made using electrodes consisting of a 0.25 - 0.5 mm diameter silver lead wire chlorinated in 3 M KCl solution. Five electrodes were used, four of which were inserted in different arrangements along the stem of cotton plants. The fifth electrode is the reference electrode and was inserted at the base or at the stem apex. The electrodes are connected to a four-channel data acquisition system with a built-in amplifier (World Precision Instruments Lab-Trax-4 / 24T model) that is connected to a computer with LabScribe version 3.0 software that decodes the signal (Zawadzki et al., 1995, with adaptations).

The recordings of electrical activities in cotton plants were performed continuously for three days. The following variables were obtained: amplitude and duration of the signal, number of signals generated, time of signal concentration and frequency of signals generated by cotton plants. The electrical signalling profile was contrasted between Bt and non-Bt cotton plants infested with those not infested with aphids (*A. gossypii*). Bt and non-Bt cotton cultivars were planted in plastic pots containing soil conditioning substrate (Forth^(r)) and kept separately in cages under the same climate conditions mentioned before.

2.2 Dispersal pattern of *A. gossypii* in Bt and non-Bt cotton plants Bioassays were performed to study aphid behaviour and associate it with data obtained from electrical signalling bioassays. A randomized block design with four treatments was used: **a1** . Bt cotton plants infested with 30 aphids/plant; **a2** . Bt cotton plants infested with 60 aphids/plant; **a3** . non-Bt cotton plants infested with 30 aphids/plant and **a4** . non-Bt cotton plants infested with 60 aphids/plant, distributed in 10 blocks. Bt and non-Bt cotton cultivars were planted in plastic pots containing soil conditioning substrate (Forth^(r)) and kept in the same climate conditions mentioned before.

Aphid infestations were performed on Bt and non-Bt cotton that reached the six-leaf stage. After infestations of the Bt and non-Bt cotton plants with *A. gossypii*, the number of aphids was recorded in the within-plant regions at 0 (immediately during infestation), 24, 48 and 72 h after infestation. The insect within-plant distribution of each Bt and non-Bt cotton plant was analyzed at three positions: bottom, middle and top.

To evaluate aphid dispersal behaviour as a function of cultivars (Bt and non-Bt cotton) and aphid densities, the negative binomial distribution parameter k was used. There are three basic spatial pattern distributions: random distribution, regular or uniform distribution, and aggregate or contagious distribution. This parameter k is an indicator of uniform distribution, where when k tends to zero, the distribution is highly aggregated, k ranging from 2 to 8 indicates moderate aggregation, and values greater than 8 ($k > 8$) indicate that the distribution is random (39). The k values were estimated by the method of moments.

2.3 Data analyses

2.3.1 Characterization of the electrical signalling potential of Bt and non-Bt cotton plants

Descriptive analyses were conducted with boxplots aiming to characterize the quantiles, medians, maximum and minimum values, and outliers of the variables and time for the emission of VPs (variation potentials) after the infestations with aphids on Bt and non-Bt cotton plants and amplitude of VPs.

Correlation analyses were conducted between the variables VP amplitude and signal emission time after infestation plants within each cotton cultivar. The degree of correlation between the variables in each condition was studied using Spearman's rank correlation coefficient ($P < 0.05$) using the `cor.test` function of the R program.

Data on the number of signals per time interval after infestation of Bt and non-Bt cotton plants with aphids were subjected to deviance analysis, with the purpose of studying the interaction involving cotton cultivar,

aphid / plant density and time interval. A generalized linear model with a quasi-Poisson distribution was used. The goodness of fit of the model was evaluated with a simulated normal envelope using the `hnp` package in the R program (Moral et al., 2017).

Deviance analysis was applied to study the interaction involving cotton cultivar, aphid/plant density and period (photophase / scotophase) in the number of VPs. Data were divided into four sections, three of which corresponded to the data recorded during three days of observation, and the last section corresponded to the accumulated data recorded during the three days of evaluation. Negative binomial generalized linear models were used for approximately the 1st and 2nd evaluation days, while quasi-Poisson models were adopted for data recorded on the 3rd day and total accumulated over the three days of evaluations. We used a half-normal plot with a simulated envelope with the `hnp` package (Moral et al., 2017) to assess the goodness-of-fit of the models.

2.3.2 Dispersal pattern of *A. gossypii* in Bt and non-Bt cotton plants

The parameter k in each cotton cultivar and density was compared by confidence intervals. Confidence intervals were generated from the values of k for each block. We used the nonparametric bootstrap technique, with 10,000 pseudoreplications, and for the resampled parameter in each treatment, we used the R program `boot` package (Angelo et al., 2019).

The probability of aphids occurring within each region of Bt and non-Bt cotton plants in each treatment (cultivar and aphid density) was estimated and compared with a multinomial linear model. The analyses to estimate the probabilities and their comparisons were conducted with `nnet` (Venables et al., 2012) and `emmeans` (Lenth, 2020) packages from R.

3 RESULTS

3.1 Characterization of the electrical signalling potential of Bt and non-Bt cotton plants

In descriptive analysis, it was possible to visualize that Bt cotton plants when infested with *A. gossypii* emitted the first VPs (minimum value in boxplot) between time intervals of 0.31 h (60 aphids/plant) and 0.64 h (30 aphids/plant) (Figure 1). In the absence of aphids, only two Bt cotton plants emitted these electrophysiological signals. Non-Bt cotton plants emitted the first VPs after 0.80 h when kept at 30 aphids/plant and after 1.60 h at the density of 60 aphids/plant, as well as in the control (non-Bt and no aphids) (Fig. 1).

The Bt and non-Bt cotton plants infested with aphids emitted signals after 60 h of aphid infestation (Fig. 1), while in cotton plants used as a control, the maximum signal emission values were observed at 55 and 57 h in non-Bt and Bt cotton plants, respectively (Fig. 1). The boxplot with VP amplitude shows that the response variability (maximum values, not including outliers, and 3rd quantile) (Fig. 2) of non-Bt cotton plants at 30 aphids/plant density was lower than that of the other cotton plants infested with *A. gossypii*. The maximum VP found in the control cotton plants was near 28 mV. In general, the mean VP (points within the boxplots) was near all treatments, ranging from 11.22 mV (non-Bt cotton plants infested with 30 aphids/plant) to 17 mV (Bt – control cotton plants). Outliers (points out of boxplots) occurred for 30 aphid/plant (129 mV)-infested non-Bt cotton plants and 60 aphid/plant (116.60 mV)-infested Bt cotton plants (Fig. 2).

Spearman rank analysis revealed that there was no correlation between the amplitude (mV) of VP and time (h) to emission of signals by cotton plants after aphid infestation at all densities studied within each cultivar (Bt and not Bt), except at the densities of 30 aphids / non-Bt cotton plants ($\rho = -0.2659$; $P = 0.0060$) and 60 aphids/Bt cotton plants ($\rho = -0.3528$; $P = 0.00254$).

Analyzing the amount of VPs emitted by the cotton plants, we observed that infestation-free plants emitted few signals, with an average accumulation of 0.75 (control – Bt cotton) and 2.50 signals (control – non-Bt cotton) over 72 h. Only two Bt cotton plants emitted electrical signals in the absence of aphid stress (Table 1).

In the accumulated emission of VPs over 72 h, it was verified that Bt cotton plants exposed to 60 aphids/plant

density emitted fewer signals compared to the other conditions ($P < 0.05$) under aphid stress. However, by assessing the emission within the intervals, the deviance analysis revealed that the signal emission pattern in each cultivar was influenced by the time interval and aphid density, as there was a significant interaction between these three factors ($P = 0.0488$) (**Table 1**).

The highest number of signals emitted by Bt cotton plants when exposed to aphids occurred in the time interval after infestation of 0-12 h (30 aphids/plant) and 0-12, 36-48 and 60-72 h (60 aphids/plant) (**Table 2, Fig. 3**).

When we compared the signal emission pattern between combined treatments involving aphid densities and cotton cultivars within each time interval, it was possible to verify a delay in terms of the production pattern of signalling on Bt cotton plants under stress with 60 insects/plant because until the time interval of 36 h after infestation, there was a lower signal emission by Bt cotton plants when exposed to 60 aphids/plant in relation to the other conditions of aphid density/Bt or non-Bt cotton cultivar (**Table 2, Fig. 3**).

In the time interval of 36-48 h, the emission of signals by Bt cotton plants was lower only in relation to Bt cotton with 30 aphids/plant. Additionally, in the time interval of 60-72 h, the signal production by cotton plants was higher when the Bt and non-Bt cotton plants were exposed to densities of 60 and 30 aphids, respectively, in relation to other conditions (**Table 2, Fig. 3**).

The deviance analysis on the interaction of the factors aphid density versus cotton cultivar versus light period within each studied day (1st, 2nd or 3rd day) and accumulated over these three days influencing the number of VPs emitted by plants shows that there was no interaction ($P > 0.05$) among the studied factors for the 1st and 2nd day and the accumulated days of exposure of Bt and non-Bt cotton plants to aphids. The factor density [$F_{\text{density}} = 2.29, P_{\text{density}} = 0.1294$ (1st day); $F_{\text{density}} = 0.0070, P_{\text{density}} = 0.95$ (2nd day); $F_{\text{density}} = 0.1734, P_{\text{density}} = 0.6813$ (cumulative total)], cultivar [$F_{\text{cultivate}} = 0.003, P_{\text{cultivate}} = 0.95$ (1st day); $F_{\text{cultivate}} = 3.0896, P_{\text{cultivar}} = 0.07$ (2nd day); $F_{\text{cultivate}} = 2.2726, P_{\text{cultivate}} = 0.1466$ (cumulative total)] and period [$F_{\text{period}} = 1.3882, P_{\text{period}} = 0.2387$ (1st day); $F_{\text{period}} = 1.0805, P_{\text{period}} = 0.3679$ (2nd day); $F_{\text{period}} = 0.2966, P_{\text{period}} = 0.5918$ (cumulative total)] did not affect the isolation of VPs emitted by cotton plants.

There was an interaction between the factors aphid density versus cotton cultivar versus light/dark phase ($F = 7.7295, P = 0.04150$) for the number of VPs observed during the 3rd day of exposure of Bt cotton plants and non-Bt to aphids. It was found that on the third day, there was a higher VP production by non-Bt cotton plants exposed to 30 aphids/plant density than Bt cotton plants exposed to the same density during the photophase (Table 2). In addition, VP production by Bt and non-Bt cotton plants exposed to 60 and 30 aphids/plant, respectively, was higher in the light phase than in the dark phase (**Table 2**).

3.2 Dispersal pattern of *A. gossypii* in Bt and non-Bt cotton plants The behaviour of *A. gossypii*, independent of the factors of exposure time of plants to aphids, aphid density and cotton cultivar, followed a highly within-plant aggregated distribution pattern ($k < 2$) (**Table 3**).

Comparisons of the k index, based on confidence interval values, revealed that the highest k index of aphid aggregation with 30 aphids/non-Bt cotton plants was found at 48 h and 72 h after infestation of cotton plants with *A. gossypii* (**Table 3**). However, non-Bt cotton plants exposed to that density had a lower aphid k aggregation index at 72 h of infestation in relation to Bt cotton with 60 aphids/plant (**Table 3**).

In other words, the dispersal rate of *A. gossypii* was higher on Bt cotton with 60 aphids/plant than on non-Bt cotton plants with 30 aphids/plant at 72 h (**Table 3**). In fact, according to the multinomial distribution in the within-plant distribution of *A. gossypii* (**Fig. 4 a, b, c, d**), it was confirmed that with 30 aphids/non-Bt cotton plants at 72 h, the highest proportions of aphids were on the adaxial (0.18) and abaxial (0.49) regions of the leaf (leaf I); however, there was increased insect dispersal to other positions, such as leaf II, adjacent leaf I and main meristem (**Fig. 4 a**). On the other hand, on Bt cotton plants infested with 60 aphids/plant, we observed the most dispersal pattern with 72 h of infestation, where there was clearly an increased insect dispersal, with 0.16 and 0.41 of aphids found in the adaxial and abaxial regions of leaf I, respectively, and 0.20 in the main meristem of the cotton plant.

No significant difference was observed among the treatments within the infestation times of 0 h, 24 h and 48 h in relation to the k index and multinomial distribution, except for the treatment with 60 aphids/Bt cotton plants within 48 h, which showed the most dispersal behaviour because it reached more regions of the cotton plants (**Fig. 4b**).

In the comparisons of aggregation level among the time intervals within non-Bt cotton exposed to 60 aphids/plant, we perceived that the highest k aggregation index was during the infestation time of 48 h (**Table 3**). With Bt cotton plants at a density of 30 aphids/cotton plants, it was found that there was no change in the aphid dispersal pattern at all time intervals (**Table 3**).

4 DISCUSSION

The results from our study showed that both cotton cultivars (Bt and non-Bt), when attacked by *A. gossypii*, emitted electrical signals of the variation potential type. Abiotic and biotic wounds are perceived differently by plants, as has been shown by other studies on plant-herbivore interactions (Pachu et al., 2020; Mithofer et al., 2019a; Bricchi et al., 2020). Insect damage in plants plays a vital role in recognizing the type of biotic stress to the plant (Wu et al., 2010; Bonaventure et al., 2011). Plants differentiate herbivory from mechanical damage by recognizing compounds present in insect saliva because oral secretion of herbivores can induce ionic flux and promote depolarization of the plant membrane potential (Maischak et al., 2007).

Here, in our research, it was possible to describe how Bt and non-Bt cotton plants react to *A. gossypii* stress by changing the transmembrane potential by recording extracellular electrical signals. Although plant responses to herbivorous attack are complex and involve a number of signals, it is important to note that different types of stimuli caused by insect action against plants trigger characteristic electrical signals evoked by plants with a specific influence on plant physiological processes (Gallé et al., 2015). The cascade of events involved in plant signalling as a function of stress perception begins at the plasma membrane of cells with changes in transmembrane potential or ion flow; these are the first responses of plants to biotic and abiotic stresses (Shabala, 2006). Attack on herbivorous plants is known to promote membrane potential changes that trigger an electrical signal that can travel to the entire plant or even trigger local plant defence mechanisms (Ebel & Mithofer, 1998).

Our results indicate the presence of VP on Bt and non-Bt cotton plants at all assessed interval times. An aphid continuously inserts its buccal apparatus into the phloem vessels, altering the hydrostatic pressure in these vessels and consequently altering the pressure in the xylem. VP is a signal whose propagation properties vary with the intensity and distance of the stimulus site and is probably a local electrical response, which is induced by a hydraulic signal, chemical signal or the combined action of these signals (Vodeneev et al., 2018). The hydraulic signal is a wave that results from increased hydraulic pressure in the plant, which propagates through the xylem and initiates the generation of a VP by triggering mechanosensitive ion channels present in the cells adjacent to the plant xylem vessels (Dziubinska et al., 2001). Therefore, harmful stimuli such as local damage, burning and mechanical injuries can evoke VPs (Dziubinska et al., 2003; Zimmermann et al., 2009). These kinds of electrical signals emitted by plants when under stress are especially important for hazard perception and response; thus, the plant can become able to mount an appropriate defence response (Davies & Stankovic, 2006).

Distinct response patterns were attributed to the perception and response to *A. gossypii* by each cotton cultivar and aphid density used in the research. Although we reported the first emission of signals on Bt cotton plants, there was a delay in terms of the propagated signal amount on Bt cotton plants with 60 aphids of infestation with *A. gossypii*, which produced the smallest numbers of signals between 0 to 36 h. Another important result was the greater dispersal behaviour related to this same treatment, mainly during and after 48 h of infestation. We suggest that the results could be supported by two hypotheses and explained independently or combined.

The first hypothesis is based on the possibility of a trade-off in terms of the defense of the Bt plant; a high dispersal could mean a larger exploitation of food resources by aphids and ease penetration of mouth apparatuses by aphids on Bt cotton plants, which may explain why Bt cotton plants emitted faster electrical

signals than non-Bt cotton plants in the first moment, showing that Bt cotton plants may be more susceptible to aphid stress. Inducibility of a plant stress response is the ability to respond to stress only on demand. This is an astrategy that is considered cost-saving (Hilker and Schmullig, 2019). Therefore, this inducibility of plant defense may indicate a delay in the operation of defensive mechanisms but may also mean a strategy to save energy and prevent self-poisoning (Zhu-Salzman, 2008), how our results show them to save energy with less production of signal until 36 h and producing them later. Since induction of a stress response implies that the plant starts activating resistance mechanisms upon encounter with the stressor, this strategy may lead to delay in mounting an effective response (Hilker et al., 2016; Martinez-Medina et al., 2016), a first stress experience may prime the organism for an improved response to a subsequent stress.

With an electrical penetration graph (EPG) used for monitoring the penetration of the mouth apparatus by aphids on Bt and non-Bt plants and recording the waveforms that reflect different aphid feeding activities, a lower percentage of waveform np (non-penetration) was observed when the aphid was walking or grabbing the food with the rostrum on Bt cotton plants (Liu et al., 2005). This suggests that aphids spend less time finding suitable places for penetration of their mouthparts on Bt cotton plants, probably due to the suitability of the tissue structure of Bt cotton plants to feed these aphids (Liu et al., 2005).

The second hypothesis is that the higher aphid dispersal on Bt cotton plants may indicate that the first signals emitted by the Bt cotton plants, even in smaller numbers than the non-Bt cotton plants, were enough to activate the Bt cotton plants' defence, which prevented or hindered aphid feeding, such as occlusion of phloem sieve elements (SEs), which are the main conductive cells in the phloem, by clogging the sieved pores³⁴(Knoblauch & van Bel, 1998). This is presumed to prevent sap loss (Ever, 1982; Schulz, 1998), and this process is seen as a primary plant defense response (Knoblauch & van Bel, 1998). At the same time, the saliva constituents of sucking insects affect cellular processes (Backus, 2005) and therefore are perceived by cells, leading to the activation of signalling mechanisms, supporting the supposition that local damage induces the propagation of a specific injury substance through the xylem, and this induces the electrical response (Vodeneev et al., 2015). The main candidates for signaling molecules are the H₂O₂ (Demidchik & Shabala, 2018) system (Pearce et al., 1991), jasmonic acid, abscisic acid, glutamate, among others. Both H₂O₂ and glutamate may activate calcium permeable channels, increasing intracellular calcium concentrations in plants (Toyota et al., 2018) and being an important trigger for the generation and propagation of VP in plants (Mousavi et al., 2013).

The observed delay in the quantitative signalling pattern of Bt cotton plants when exposed to 60 aphids/plant could be attributed to self-preservation under stress and may be supported by the inclusion of resource reallocation for the production of metabolites and defensive structures (first and second hypotheses simultaneously). In the supplementary material, we show that almost twice the amount of Cry1F protein was observed on Bt cotton plants in the presence of aphids than in the absence of the insect. Well-known anti-herbivorous defence proteins include proteinase inhibitors (PIs) and polyphenol oxidases (PPOs), both considered to interfere with digestive processes in herbivore intestines (Zhu-Salzman, 2008). Therefore, simultaneous resource reallocation can serve not only to save resources; thus, the plant can subsequently use them for growth and reproduction but also to deprive the herbivore of food and consequently increase aphid dispersal, as observed in our results on Bt cotton with 60 aphids/plant, in order to search for the available food source. These abovementioned hypotheses generate a basis for further studies that seek to highlight what possible defense mechanisms are involved and how effective they may be as a function of Bt and non-Bt cotton cultivars. In conclusion, the stress caused by the aphid *A. gossypii* was sufficient to trigger specific responses on Bt and non-Bt plants. Bt cotton plants were faster to propagate VP signals; however, they produced the signals in a smaller quantity with the highest aphid density, also promoting greatest within-plant aphid dispersal. Our results may guide future studies, which aim to elucidate the factors involved in the resistance to stress and plant defense processes and thus assist in the development of successful strategies in integrated pest management.

CONFLICTS OF INTEREST: The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

JKSP, FCOM, JBM, FSR, RFO and WACG conceived the ideas; JKSP, FCOM and JBM collected the data; JKSP, FCOM, JBM, FSR, RFO and WACG designed the methodology, analysed the data and wrote the manuscript. WAC coordinated the project. All authors contributed critically to the drafts and gave final approval for publication.

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Figure Legends, and Tables.

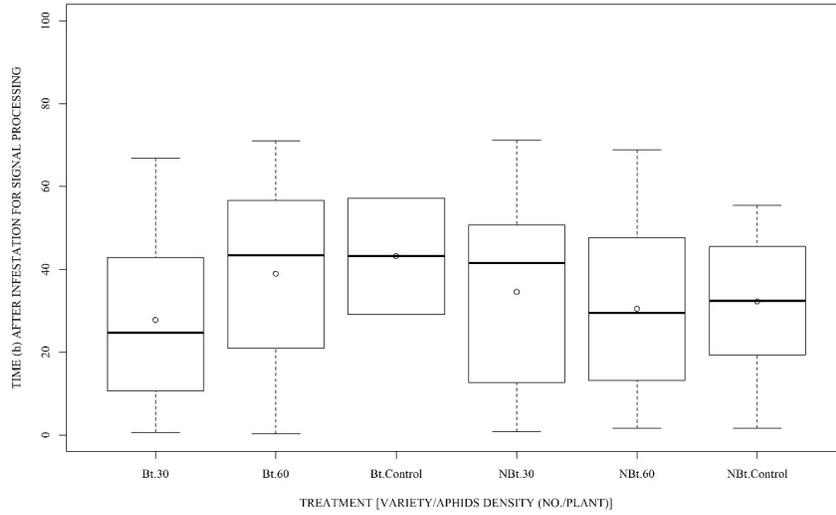


FIGURE 1. Boxplot of time (h) of exposure of Bt and non-Bt cotton plants to different densities of *A. gossypii* emitting potentials of variation (VPs) (mV). Bt and non-Bt cotton plants were infested at densities of 30 and 60 aphids/plant (Bt.30, Bt.60, NBt.30 and NBt.60) and in the absence of aphids (Bt. Control and NBt. Control).

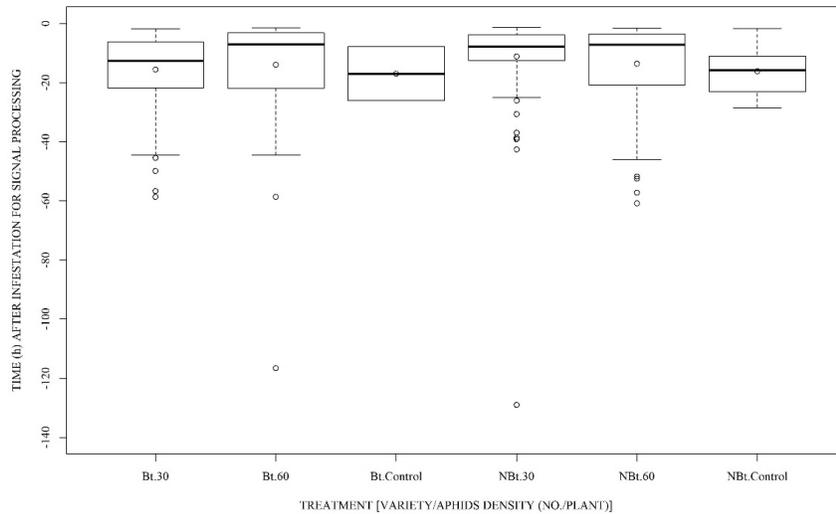


FIGURE 2. Boxplot of amplitude (mV) of variation potentials in Bt and non-Bt (NBt) cotton plants exposed to different densities of *A. gossypii*. Bt and non-Bt cotton plants exposed to densities of 30 and 60 aphids/plant and in the absence of aphids (Bt. Control and NBt. Control).

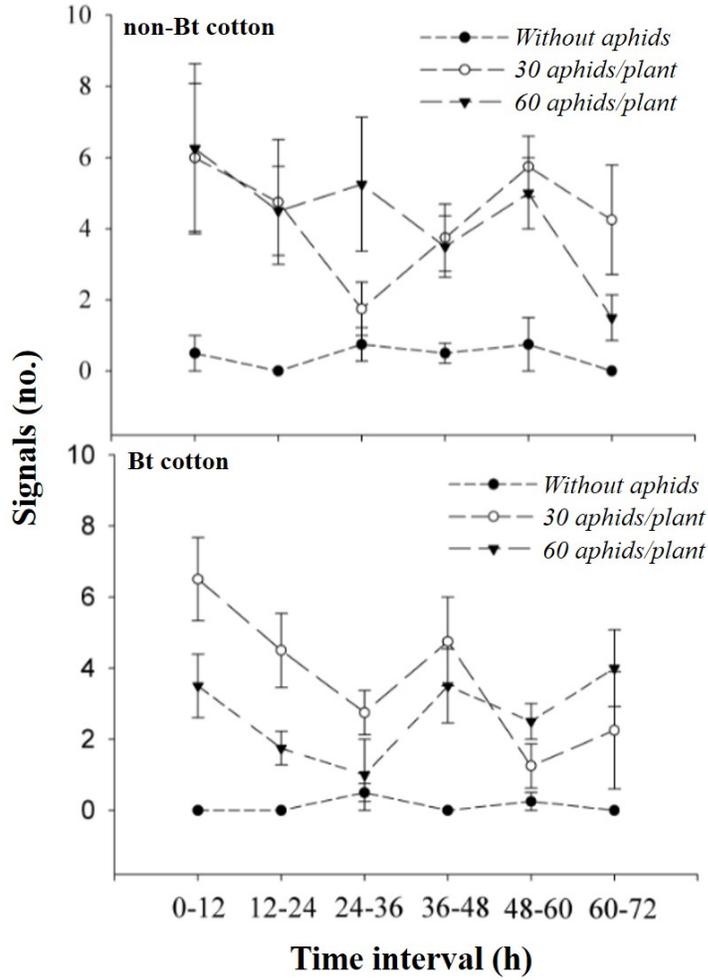


FIGURE 3 . Variation potentials emitted (no.) (MEAN \pm SE) by Bt and non-Bt cotton plants at different time intervals (h) after densities of 30 and 60 aphids / plant and control (absence of aphid). Mean data (points) and error bars (SE) predicted by the generalized linear quasi-Poisson model, except for the plotted values for the control used in both cotton cultivars (Bt or not Bt).

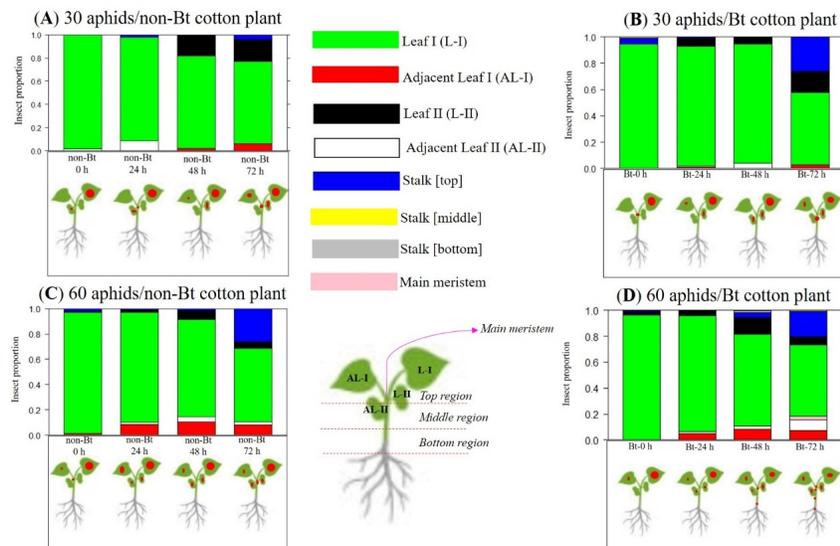


FIGURE 4 . Multinomial distribution with occurrence rate of *A. gossypii* in the regions of non-Bt and Bt cotton plants at infestation times of 0 h, 24, 48 and 72 h with densities of 30 and 60 aphid/cotton plants. The red circle diameter represents the intensity of aphid infestation on cotton plants.

TABLE 1 - Number of variation potentials emitted (mean \pm SE) by Bt and non-Bt cotton plants when subjected to different exposure times and densities of *A. gossypii* /plant.

Time interval (h)	Bt cotton	Bt cotton	Bt cotton	non- Bt cotton	non- Bt cotton	non- Bt cotton
	0 (control)	30	60	0 (control)	30	60
0-12	0,00 \pm 0,00 ^{nia}	6,0 \pm 2,17 A a	3,50 \pm ,89 A b	0,50 \pm 0,50 ^{nia}	6,00 \pm 2,08 A a	6,25 \pm 2,39
12-24	0,00 \pm 0,00 ^{nia}	4,50 \pm 1,04 B a	1,75 \pm 0,47 C b	0,00 \pm 0,00 ^{nia}	4,75 \pm 1,75 AB a	4,50 \pm 1,25
24-36	0,50 \pm 0,25 ^{nia}	2,75 \pm 0,62 C b	1,00 \pm 1,00 D d	0,75 \pm 0,47 ^{nia}	1,75 \pm 0,75 C c	5,25 \pm 1,88
36-48	0,00 \pm 0,00 ^{nia}	4,75 \pm 1,25 B a	3,50 \pm 1,04 A b	0,50 \pm 0,28 ^{nia}	3,75 \pm 0,94 B ab	3,50 \pm 0,86
48-60	0,25 \pm 0,25 ^{nia}	1,25 \pm 0,62 D c	2,50 \pm 0,50 B b	0,75 \pm 0,75 ^{nia}	5,75 \pm 0,85 A a	5,00 \pm 1,00
60-72	0,00 \pm 0,00 ^{nia}	2,25 \pm 1,65 C b	4,00 \pm 0,08 A a	0,00 \pm 0,00 ^{nia}	4,25 \pm 1,54 AB a	1,50 \pm 0,64
acumulated (total)	0,75 \pm 0,25 ^{nia}	22,00 \pm 0,17 a	16,25 \pm 0,13 b	2,50 \pm 0,50 ^{nia}	26,25 \pm 0,21 a	26,00 \pm 0,20

Capital letters compare averages within each column, and lowercase letters compare averages within each row. Means followed by the same letters do not differ from each other by overlapping confidence intervals generated by the quasi-Poisson model ($P < 0.05$).

nia = not incorporated in the analysis.

TABLE 2. Number of variation potentials emitted (mean \pm SE) by Bt and non-Bt cotton plants exposed to different aphid densities / cotton plant and photophase and scotophase on the 3rd assessment day.

Cultivar	Density (aphid/plant)	Photophase	Scotophase
non Bt Cotton	30	6,75 \pm 1,43 A a	2,50 \pm 1,50 A b
Bt cotton		1,50 \pm 0,86 B a	1,75 \pm 1,03 A a
non Bt Cotton	60	3,25 \pm 1,18 AB a	4,50 \pm 1,18 A a
Bt cotton		5,00 \pm 0,70 AB a	2,00 \pm 1,08 A b
non Bt Cotton	0 (control)	0,25 \pm 0,25 ^{nic}	0,25 \pm 0,25 ^{nic}

Cultivar	Density (aphid/plant)	Photophase	Scotophase
Bt cotton		0,00 ± 0,00 <i>nia</i>	0,25 ± 0,25 <i>nia</i>

Uppercase letters compare averages within each column, and lowercase letters compare averages within each row. Means followed by the same letters do not differ from each other by overlapping confidence intervals generated by the quasi-Poisson model ($P < 0.05$).

nia = not incorporated in the analysis.

TABLE 3 . Confidence intervals associated with the *A. gossypii* aggregation index (95% CI) in Bt and non-Bt cotton plants submitted to densities of 30 and 60 aphids/cotton plants quantified at 0, 24, 48 and 72 h after the infestation of cotton plants with aphids.

Density/Cultivar	Time (h)	Time (h)	Time (h)	Time (h)
	0	24	48	72
30 aphids/non-Bt cotton/	0.42 - 0.52 A b	0.47 - 0.74 A ab	0.55 - 0.85 A a	0.58 - 0.77 B a
60 aphids/non-Bt cotton	0.45 - 0.73 A b	0.55 - 1.67 A ab	0.78 - 3.03 A a	0.67 - 1.48 AB ab
30 aphids/Bt cotton	0.45 - 1.16 A a	0.45 - 0.83 A a	0.57 - 0.87 A a	0.77 - 1.55 AB a
60 aphids/Bt cotton	0.42 - 0.90 A b	0.63 - 0.80 A b	0.70 - 1.05 A b	1.12 - 1.46 A a

Uppercase letters compare averages within each column, and lowercase letters compare averages within each row. Means followed by the same letters do not differ from each other by overlapping confidence intervals generated by Bootstrap ($P < 0.05$).