Arthropod-Plant Interactions
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ORIGINAL PAPER

Impacts of soybean-induced defenses on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) development

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Abstract As soybean (*Glycine max* [L.] Merrill) is an important crop throughout the world, the action of herbivorous insects responsible for economic and productivity losses is the subject of constant research. *Spodoptera frugiperda* (J.E. Smith) caterpillars can cause extensive damage to soybean culture; this work investigated possible harmful effects on these caterpillars associated with the possible induced defenses of soybean plants. For this purpose, we assessed the biology of the insect (leaf consumption and performance traits) and chemical composition of the soybean leaves by ultraviolet–visible spectroscopy and chemometrics of three treatments: control, mechanically wounded soybean leaves, and *S. frugiperda*-damaged soybean leaves. The results reveal that both types of injuries induce changes in soybean metabolism regarding the production of phenolic substances, although only the herbivore-damaged plants provoke negative effects on insect biology. Variations in carotenoid production during the circadian cycle were also found in the control group. These results confirm that the soybean plants could endure and activate chemical defense mechanisms that impair the developmental lifecycle of the insect, suggesting possibilities for sustainable control strategies.

Keywords *Spodoptera frugiperda* · Soybean · Plant–insect interaction · Insect biology · UV–Vis · Chemometrics

Introduction

Plants, even as sessile organisms, can recognize herbivory and defend themselves by various morphological, biochemical, and molecular mechanisms intended to impair herbivores (Mithöfer and Boland 2012; War et al. 2012). Early events in the plant cells start as soon as herbivore damage begins: changes in the transmembrane potential (depolarization), followed by changes in the cytosolic calcium concentrations, generation of ROS (reactive oxygen species) in an oxidative burst, the initiation of a kinase cascade, generation of jasmonic acid and related compounds, and finally, gene activation followed by metabolic changes toward explicit defense responses with the production of bioactive specialized compounds, such as phenolic compounds, alkaloids, and cyanogenic glycosides, that can be toxic to the herbivore (Fürstenberg-Hägg et al. 2013). On the other hand, the herbivorous insects try to deal with these plant-derivative xenobiotics though rapid excretion, metabolization in non-toxic compounds, or sequestration for own protection against predators (Heidel-Fischer and Vogel 2015).

Caterpillars from the *Spodoptera* genus, which belong to the Noctuidae family (moths), are distributed throughout the world, mainly in tropical and subtropical areas, and can

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damage crops (Formentini et al. 2015; Oliveira et al. 2014; Pogue 2002; Sá et al. 2009; Santos et al. 2009). *Spodoptera frugiperda* (J.E. Smith) can eat more than 80 species of plants, including maize, cotton, and soybean (Barros et al. 2010a, b; Capineira 2001, 2008; Cruz 1995; Pogue 2002). It has been considered a primary pest in maize and cotton, generating high levels of defoliation on crops of the entire Americas (Sá et al. 2009). As a moth, *S. frugiperda* is a strong flier that can survive strong winters, migrating long distances in the Northern hemisphere looking for warmer areas and food (Capineira 2001; Cruz 1995). In tropical areas, such as Brazilian crop fields, it can remain in the same area because of the favorable climate and food resources in close proximity (Barros et al. 2010a, b). As a polyphagous herbivore, it can take advantage of the crop rotation and double crop system (winter crop). *S. frugiperda* can adapt itself to other alternative hosts, increasing the risk of insect outbreaks and economic losses (Barros et al. 2010a, b; Boregas et al. 2013; Pogue 2002; Sá et al. 2009). The potential crop losses to pests can be especially elevated in crops growing under high productivity conditions, large-scale productions, and tropical and subtropical climatic conditions (Oerke 2004; Oerke 2006). This is the case with soybean (*Glycine max* [L.] Merrill) crops in the Americas.

Soybean complex (soybean grain, soybean meal, and soybean oil) is one of the most important Brazilian agribusiness commodities, and it accounted for more than 50% of the planted area in Brazil in the 2015/2016 harvest (Conab 2016). Brazil and the USA are the largest soybean producers in the world, and both countries employ strategies to manage weeds and insect pests since they want to maintain high levels of productivity and economic profit (Bueno et al. 2012; Hartman et al. 2011; Oerke 2004, 2006; Oliveira et al. 2014).

The importance of the management of *S. frugiperda* in soybean crops has increased since it can be equally or more damaging than *Anticarsia gemmatalis* Hübner, one of the major caterpillar pests in Brazilian soybean (Bueno et al. 2011); *S. frugiperda* has low susceptibility to *Cry1Ac* protein (GM technology available to control caterpillars in soybean) (Bernardi et al. 2014; Santos et al. 2009); it can be non-selective (Barros et al. 2010b) and has high rates of survival in alternative host crops (Santos et al. 2009). In addition to this, *S. frugiperda* was indicated to be one of the most difficult pests to manage among 31 pests targeted by Bt crops in Latin American (Blanco et al. 2016). All of this added to the double-cropped system (winter crop + summer crop) largely adopted in the Brazilian Midwest region causes an increase in the risk of insect outbreaks (Barros et al. 2010a, b; Santos et al. 2009).

The aim of this work was to investigate whether the herbivory of *S. frugiperda* can induce defense mechanisms in soybean plants that are different from ordinary mechanical injury and determine whether herbivore-damaged and mechanically wounded soybean plants can impair the next *S. frugiperda* caterpillars that fed on them through the analyses of some performance traits, such as leaf consumption, biology, and reproduction (pupal and larval development, pupal weight, average number and viability of eggs, and sex ratio). In addition, we compared the chemical profiles of soybean leaf extracts before and after injuries.

Materials and methods

The bioassays of this study were performed with the assessment of the *S. frugiperda* biology and chemical analyses of soybean leaves from three treatments: *S. frugiperda*-damaged, mechanically wounded, and control leaf groups. The caterpillars were reared in the laboratory, and the soybean plants were grown in a greenhouse. The analyses of caterpillar performance traits included leaf consumption, biology, and reproduction (pupal and larval development, pupal weight, average number and viability of eggs, and sex ratio). The chemical analyses were performed by ultraviolet–visible spectrophotometry (UV–Vis) of leaf extracts followed by chemometric analyses.

Rearing *S. frugiperda*

The larvae initially collected from a maize crop (C-strain) were replicated and maintained in the laboratory at 25 ± 2 °C, 12 h photophase, and 60 ± 10% relative humidity (RH). The larvae were fed an artificial diet (Greene et al. 1976) adapted by Parra (1986), and adults were fed an aqueous diet of 10% honey. The caterpillars were used for bioassays from the 10-day-old (3rd–4th instar) F1 generation in the laboratory. Any caterpillars that were fed soybean leaves at any point were not reused in this experiment.

Greenhouse

BRS 284 soybean plants, a non-transgenic cultivar cropped in Brazil which allows for earlier planting and has high productivity, were grown in a greenhouse, latitude 20°23′15.04″S, longitude 54°36′2487″W, and altitude 646 m, during January and February, 2014. Fertilization was carried out as recommended for culture after soil analysis with ammonium sulfate, super simple phosphate, potassium chloride, and correction with dolomitic limestone (Broch and Ranno 2012; Roscoe and Gitti 2012). Plants were sprinkler-irrigated six times per day. Each plant in the V6 vegetative stage (Fehr and Caviness 1977),

approximately 40 days after seeding, was assigned to one handmade cage (soft fine silk approximately 30 Mesh, 22 × 16 cm, velcro fastener) placed on the third trifoliate at the petiole.

Control, mechanically wounded, and herbivore-damaged treatments began at the same time at 7 am. The control treatment (CONT) only received one cage per plant in the beginning of the experiment and subsequently underwent no further experimental interventions. The mechanically wounded treatment (MEC) was performed by making one wound with tweezers (0.5 cm lesion) in the third trifoliate inside the cage every hour until 7 pm (12 h of damage). The herbivore-damaged treatment (SPD) received one 10-day-old and after 20 h fasting period *S. frugiperda* caterpillar per cage in the third trifoliate.

Biological analyses were conducted with the second and fourth soybean trifoliates collected daily after the first 24 h of the experiment; they were used to feed caterpillars with prior herbivore-damaged, mechanically wounded, and control plants at the laboratory.

For chemical analyses, the leaf samples (from the third trifoliate) in each treatment were collected at 0, 5, 10, and 24 h after the beginning of the experiment. The caterpillars and all cages were then removed and the last collect time occurred 24 h after their removal, which is called a recovery time point in this paper considering that the plant was allowed to recover from previous damage. Seven plants (biological replicates) were used for each treatment in each time point (7 plants × 3 treatments × 5 collect times = 105 trifoliates for chemical analyses). Each plant was collected only once. After collection, the leaves were submerged in liquid nitrogen to stop the metabolism, and they were kept in a freezer until extraction.

Leaf consumption

A total of 25 replicates of caterpillars rearing in the laboratory and excised leaf material from the three prior treatments were placed together in Petri dishes lined with filter paper (25 caterpillars × 1 leaf × 3 treatments = 75 measures). The leaves were photographed before and after 24 h of consumption. The leaf area consumed was quantified using the electronic leaf area meter using a Sony Alpha DSLR-A350 camera using the maximum resolution of 14.2 megapixels and no flash, which was supported with a common tripod and positioned looking down at 30 cm from the table. In the table, the soybean leaves were placed on the white sheet of paper with a printed dotted frame, where the focus of the camera was adjusted. Above the frame, a rectangle (3 × 0.5 cm) was used to establish the equivalence between pixel and centimeters. The leaf consumption analysis was carried out by processing the photos in Java-based software using an ImageJ library (developed in our group).

Biology and reproduction of *S. frugiperda*

In total, 50 repetitions for each treatment were used to evaluate the pupal and larval development, pupal weight, average number and viability of eggs, and sex ratio. Each laboratory-reared caterpillar was placed in a plastic container lined with filter paper, including a piece of cotton moistened with distilled water and one leaflet per container. Daily the larvae development was checked out, the excrements were removed, and the leaves were substituted.

The 24-h-old pupae were sexed and weighed. The sex ratio was calculated by dividing the number of females by the total number of individuals (male + female) (Silveira-Neto et al. 1976). For each treatment, the pupae were divided into ten couples. Adults were fed a 10% honey solution renewed every 2 days.

The egg production and viability were measured for each individual mother and evaluated daily using a stereomicroscope. Similarly, the number of hatched caterpillars was counted to determine the viability of eggs per treatment.

Bartlett and Shapiro–Wilk tests were employed to check for homoscedasticity (variance) and normal (residuals), respectively. The average number of viable eggs was transformed into $\sqrt{X + 0.5}$. The complete dataset was submitted to analysis of variance and the averages were compared by Tukey's test with 5% probability.

Chemical analysis by UV–Vis spectrophotometry

The extracts were prepared using approximately 2.0–2.5 g of third trifoliates from a pool of 7 soybean plants in each treatment and time point (7 plants × 3 treatments × 5 collect times = 105 trifoliates for chemical analyses); the leaves were macerated in triplicate for 5 min with approximately 45 mL of methanol (analytical grade), ultrasonicated for 20 min, filtered through a piece of cotton, centrifuged for 5 min (2500 rpm) followed by evaporation in a fume hood. For UV–Vis analysis the dried extracts were resuspended in methanol to prepare a solution of 0.25 mg/mL. The absorbances were measured at 200–800 nm in spectrophotometer model U3000 Scan (Hitachi).

Chemometric analysis

The chemometric analysis from the UV–Vis spectra was performed using a principal components analysis (PCA) algorithm for data reduction (Pirouette software, v.4.0, Infometrix, USA). The wavelengths from 200–230 to 576–800 nm were excluded to avoid the excessive contribution of noise to the variance of the system. The UV–Vis

data from each treatment (control, mechanically injured, and herbivore-injured groups) were organized in a data matrix that was submitted to PCA after preprocessing by mean centering and normalization.

Results and discussion

Leaf consumption and biology of the *S. frugiperda* caterpillars

The caterpillars were fed leaves from herbivore-damaged soybean plants and consumed 51.40% less leaves than the control treatment and 45.21% less than leaves from mechanically wounded plants (Fig. 1). This reduction in consumption indicates deterrents to feeding, probably due to the production of compounds related to plant defenses (Schoonhoven et al. 2005).

According to Table 1, the extension of the larval period indicates that herbivore-damaged soybean plants caused difficulties in the development of the *S. frugiperda*. In general, the extension of the larval period is caused by the reduction of food intake due to the existence of one or more inhibitors in food, the nutritional inadequacy of the food substrate (Martinez and Van Emden 2001), or production of defense metabolites impairing the development (Schoonhoven et al. 2005; Isman 2002).

For the pupal stage, we could observe lower average pupal weight from caterpillars that fed on herbivore-damaged rather than the mechanically wounded or control plants, although no change in the extension of pupal development stage was observed (Table 1).

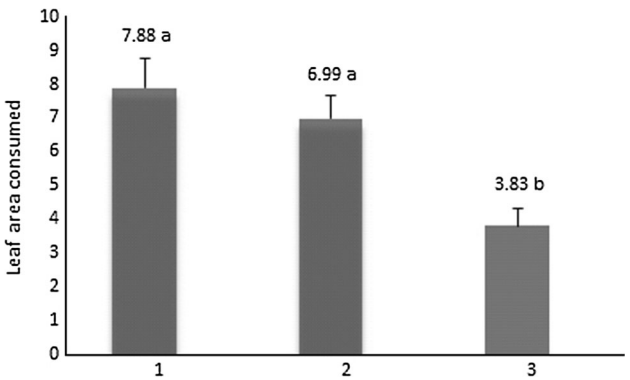

Fig. 1 Leaf area (cm^2) consumed by caterpillars of *S. frugiperda*-fed control (**a**), mechanically wounded (**b**), and *S. frugiperda*-damaged (**c**) soybean leaves during 24 h at 25 ± 2 °C, 60 ± 10% RH, and a photoperiod of 12 h. Means followed by the same letter are not significantly different from each other. Tukey's test at 5% probability. Number of observations: 25. (CV% = 57.05, $F = 8.98$, $P = 0.0003$, DF = 2). The bars indicate the standard error (1, 0.90; 2, 0.67; 3, 0.51). Caterpillars that were fed *S. frugiperda*-injured soybean leaves reduced the leaf area consumption

Bortoli et al. (2012) also found a similar trend in pupal stage duration and weight in their studies with *Anticarsia gemmatalis* caterpillars and soybean. These results suggest the caterpillar development after herbivore-damaged soybean plants food intake can be impaired due to the activation of defense mechanisms in soybean.

There was no significant deleterious effect on the reproductive rate of females from caterpillars that were fed herbivore-damaged and mechanically wounded soybean plants, although there was a decreasing trend in egg viability and an increasing trend in infertile females for the herbivore-damaged treatment (Table 1). These results suggest that there is a cost to the fitness of *S. frugiperda* when it feeds on soybeans damaged by a herbivore. Such a finding would be helpful in understanding how the defenses that soybean has against this herbivore work against it and possibly other herbivores. On the other hand, the sex ratio was changed in the herbivore-damaged treatment, indicating an increase of male individuals, and this suggests a shift in insect reproductive strategy since some insect orders can increase the contribution of the male insects regarding the quality of nuptial gifts in the event of poor host plant quality, including possible high levels of defensive compounds (Awmack and Leather 2002).

Chemical analyses by Ultraviolet–Visible spectrophotometry of soybean leaf extracts

The acquisition of a fingerprint from soybean leaf extracts using ultraviolet–visible spectrophotometry (UV–Vis) and chemometric tools provides important information for complex samples (Azcarate et al. 2013; Casale et al. 2010; Sârbu et al. 2012). The samples analyzed by UV–Vis were collected at five different time points (0 h, after 5, 10, 24 h, and recovery) and divided into three different treatments: control, mechanically injured, and herbivore-injured.

Figure 2 shows the overlapped representative UV–Vis spectra for each treatment in the time point 24 h in the wavelength range analyzed (231–575 nm). These spectra suggest the presence of isoflavones, glycosylated flavonols, and phenolic acids as well as carotenoids.

Flavonoids exhibit two major absorption bands in the UV–Vis spectra; band I absorbs between 320 and 385 nm, which represents the B-ring; band II absorbs between 250 and 285 nm, representing the absorption of the A-ring. Variation in the substitution of the A- and B-rings, an increased or decreased number of hydroxyl or methoxy groups, or even the introduction of sugar units can shift the absorption bands to higher or lower wavelengths (Markham 1982). Phenolic acids absorb in the range of 270–330 nm (Robards et al. 1999), and carotenoids absorb in the visible, from 400 to 500 nm (Rodriguez-Amaya 2001).

Table 1 Biology and reproduction parameters of *S. frugiperda*-fed soybean plants from control, mechanically wounded, and herbivore-damaged treatments

Parameters	Control	Mechanically wounded	Herbivore damaged	CV%	*F*	*P*
Larval development (days)	19.36 ± 0.19b (45)	19.69 ± 0.24b (39)	21.34 ± 0.33a (41)	8.25	17.18	<0.001
Pupal development (days)	10.18 ± 0.12a (44)	9.74 ± 0.18a (39)	10.28 ± 0.24a (41)	11.72	2.31	0.104
Pupal weight (mg)	191.33 ± 4.32a (44)	180.26 ± 3.36a (39)	161.95 ± 4.25b (41)	14.76	13.54	<0.001
Average number of eggs per female*	399.40 ± 74.52a	397.90 ± 47.69a	361.00 ± 63.23a	31.20	0.27	0.768
Egg viability (%)	64	62	33	–	–	–
Infertile females	10	10	40	–	–	–

25 ± 2 °C, 60 ± 10% RH, and a 12 h photoperiod. The means (X ± standard error) in the same line followed by the same letters are not significantly different at the P value described in the line. Values between brackets express the number of observations

* Data were transformed in $\sqrt{X + 0.5}$ for statistical analysis. Degrees of freedom = 2

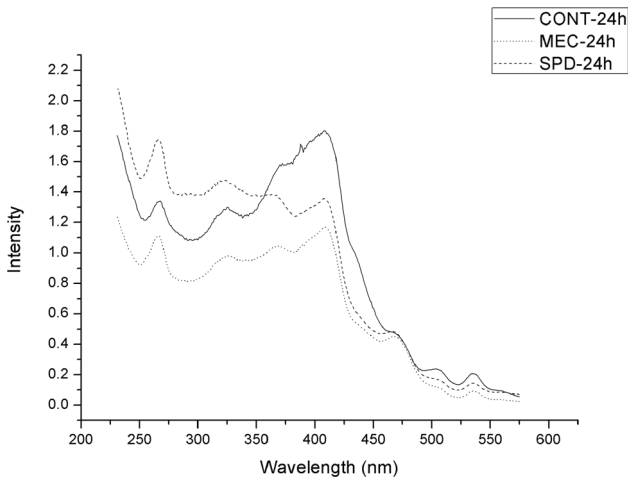

Fig. 2 Overlapped representative UV–Vis spectra from soybean leaf extracts of the control (CONT-24 h), mechanically wounded (MEC-24 h), and herbivore-damaged (SPD-24 h) treatments at the time point 24 h over the wavelength range analyzed (231–575 nm). UV–Vis spectra data are complex and suggest the presence of flavonoids, phenolic acids, and carotenoids

Considering that the UV–Vis spectra for the samples have complex and very similar data, PCA was applied as an exploratory analysis to perform the data reduction (Beebe et al. 1998).

Chemometric analyses by PCA from UV–Vis spectra

Control leaf samples

The PCA algorithm performs data reduction through the transformation of the original coordinate system into a new coordinate system, which is composed of principal components or factors. The projection of the samples in these new coordinates generates the score plot, while the projection of the variables (UV–Vis wavelengths, in this case) generates the loading plot. The new generated axes (principal components) express the variance of the system in decreasing order. The first principal component contains the greatest explained variance and the similarities and/or differences appearing in this axis contain the major source of variation of the set samples. The analysis of score and loading plots together allows us to extract chemical information about the system (Beebe 1998).

The score plot of the UV–Vis spectra obtained from control samples without any injury (either mechanically or caused by *Spodoptera frugiperda*), evaluated at 0, 5, 10, 24 h, and recovery time points showed two groups of samples: one on the positive PC1 axis (CONT-0 h and CONT-24 h) and another near zero and negative PC1 axis (CONT-5 h, CONT-10 h, and CONT-Rec) (Fig. 3a). On the PC2 axis, the explained variance was very small and was considered insignificant to the interpretation of the data.

The group CONT-0 h and CONT-24 h was observed on the positive PC1 because the loadings correspond to absorption from 361 to 575 nm (Fig. 3a, b). This range of wavelength suggests that the absorption is due to certain types of flavonoids or pigments such as carotenoids. The sample collection schedules between 7:00 and 8:00 am for these samples. Considering the light incidence that reaches the surface of the Earth, both UV-A and UV-B (280–400 nm) account for 9% of the total, of which 95% is UV-A (320–400 nm). Although at this collection time the incident light of these wavelengths is limited, according to Jahnke (1999), studies with *Dunaliella bardawil* observed a high and rapid production of carotenoids after UV-A irradiation, which only requires a low intensity of UV-A to double the level of carotenoids. Therefore, a low quantity of UV-A can be useful to produce carotenoids in the leaves, which agree with the grouping of CONT-0 h and CONT-24 h samples on the positive PC1 axis.

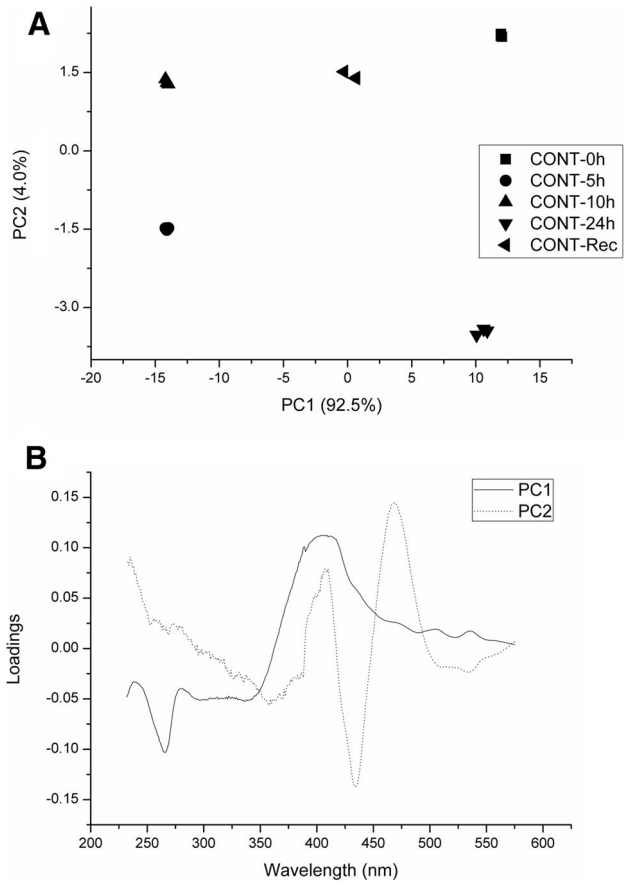

Fig. 3 Chemometric analyses from UV–Vis spectra of the soybean leaf extracts of the control treatment at the 0 h, after 5 h, 10 h, 24 h, and 1 day recovery time points. **a** PCA score plot shows the distribution of samples according to the collect time. **b** PCA loading plot for the first (PC1) and the second (PC2) principal components suggests the importance of carotenoids for sample distinction. Control samples showed changes related to circadian cycle

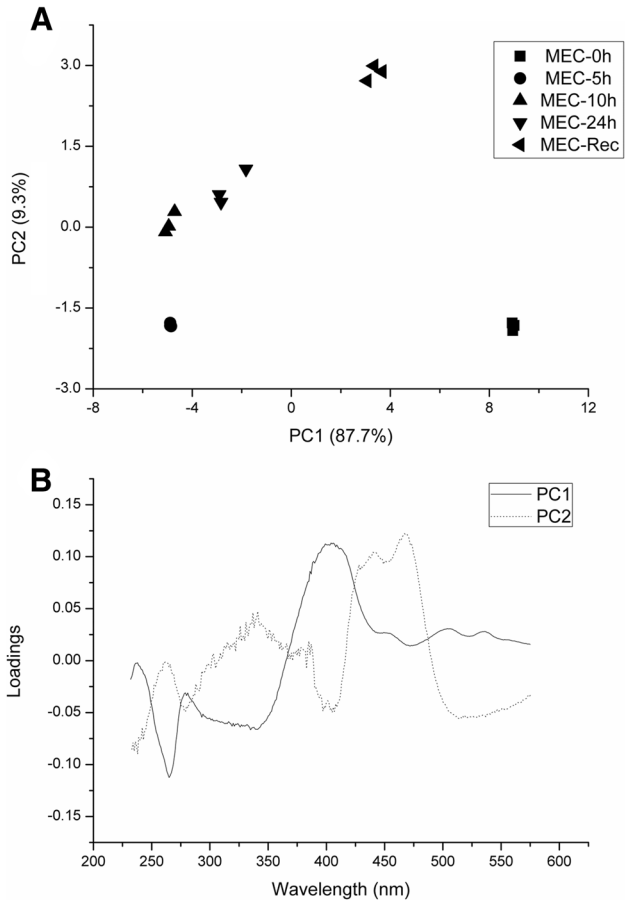

Fig. 4 Chemometric analyses from UV–Vis spectra of the soybean leaf extracts of the mechanically wounded treatment at the 0 h, after 5 h, 10 h, 24 h, and 1 day recovery time points. **a** PCA score plot shows the mechanically wounded samples allocated on the negative PC1 scores, farther from recovery and without injury (MEC-0 h) samples. **b** PCA loading plot for the first (PC1) and the second (PC2) principal components suggests the importance of phenolic compounds for sample distinction. The plant response decreased after the mechanical wound stress was ceased

The samples CONT-5 h, CONT-10 h, and CONT-Rec were allocated near zero and on the negative PC1 axis because the collection time was approximately midday for CONT-5 h and CONT-Rec and 5:00 pm for the CONT-10 h samples, a period of the day during which the incidences of UV-A and UV-B are larger. The reduction of carotenoids was important to this grouping, as previous studies have shown that UV-B radiation inhibits photosynthesis (Jahnke 1999). Thus, there is a decrease of the pigments responsible for this grouping, such as carotenoids (Victorio et al. 2007). For these samples, the important loadings were the wavelengths from 231 to 360 nm that can be correlated to phenolic compounds in general.

Mechanically wounded leaf samples

The score and loading plots for soybean leaf samples with mechanical wound (Fig. 4a, b) show the grouping of MEC-5 h, MEC-10 h, and MEC-24 h samples on the negative PC1 scores, with the main loadings from 231 to 360 nm, and farther from MEC-0 h and MEC-Rec samples on the positive PC1 scores, with loadings between 361 and 575 nm.

These results show that mechanical wound led to the plant response because in the MEC-5 h, MEC-10 h, and MEC-24 h samples, the loading plot suggests the presence of phenolic compounds such as flavonoids and phenolic acids, indicating an increase in the production of secondary metabolites. 24 h after the beginning of the experiment, i.e., 14 h after the mechanical wound had ceased, the loadings at 231–360 nm still were important to negative PC1 such as for MEC-5 h and MEC-10 h, indicating that the MEC-24 h samples continued producing these metabolites. Furthermore, even though the collection times of MEC-24 h and MEC-0 h samples were both between 7

and 8 am, MEC-24 h were still under stress with the production of secondary metabolites; this is different from the MEC-0 h, which was subject to only the variation of the circadian cycle. Thereafter, these samples are distinguished in the score plot. Nevertheless, the MEC-Rec samples were allocated on more positive PC1 axis closer to MEC-0 h, where the most important loadings are 400–500 nm due to the presence of carotenoids, indicating the chemical similarity between these samples and suggesting the recovery process of the plant (Fig. 4a, b).

According to the literature, mechanical stressors (abiotic stress) may influence the expression of secondary metabolism and affect their circadian cycle in plants (Gobbo-Neto and Lopes 2007), where responses are short or long term relative to the damage of plant tissues, in addition to local and systemic responses, increasing the production and the accumulation of secondary metabolites in the plant (Gobbo-Neto and Lopes 2007; Taiz and Zeiger 2009; Vázquez-Flota et al. 2004).

Spodoptera frugiperda-damaged leaf samples

Figure 5a shows the score plot from UV–Vis spectra obtained from the extracts of the herbivore-damaged soybean leaves. SPD-5 h, SPD-10, SPD-24, and SPD-Rec samples were allocated on the most negative PC1 scores, far from SPD-0 h. Although the PC2 axis only explains 5% of the variance, it allows for the separation of SPD-Rec samples to more positive score values, SPD-5 h samples to more negative score values, and the other samples can be grouped together close to zero.

The projection of the SPD-0 h samples showed high positive score values on PC1 due to the wavelength bands ranging from 366 to 575 nm (Fig. 5b), corresponding to carotenoid absorptions. The loadings for SPD-5 h, SPD-10 h, SPD-24 h, and SPD-Rec samples were the wavelengths between 231 and 365 nm on the negative PC1 axis, suggesting the importance of phenolic compounds for these samples as induced responses of plant defense.

The recovery herbivore-damaged samples (SPD-Rec) were allocated close to SDP-5 h, SPD-10 h, and SPD-24 h on more negative PC1 scores (Fig. 5a), differently from recovery mechanically wounded samples (MEC-Rec), highlighting differences in the recovery process of the plant after herbivory, indicating the maintenance of the defense responses even after herbivory was finished.

When we compared the effects of the three treatments on the UV–Vis chemical profile together in a single PCA, especially 24 h after the start of the damage (Fig. 5c), we could reinforce an important difference between damaged and control samples on PC1 axis, where phenolic compounds were responsible for damaged plant distinction through wavelengths between 231 and 365 nm on the negative PC1 axis (Fig. 5d). PC1 explains 83.1% of the total variance and on this axis we could observe the major dispersion of the three treatments. Herbivore-damaged and mechanically wounded leaves were displayed on negative PC1 axis, while control leaves were allocated on positive PC1 axis, corroborating the differences of these treatments considering the importance of the phenolic compounds for this separation. In addition to this, we could observe a proximity of CONT-0 h and CONT-24 h soybean leaves on positive PC1 axis and a distance on PC2 axis. CONT-24 h resembles CONT-0 h in PC1 since both leaves did not suffer damages although the minor distinction on PC2, with only 8.6% of explained variance, could be associated with aging of the plant.

We could observe that the carotenoids can vary during the circadian cycle and such variation was predominantly found in the control group treatment. This variation also happened in all treatments since a higher level of carotenoids was observed at the beginning of the experiment. However, in samples under mechanical wounding or prior to caterpillar feeding the variation in phenolic compounds was more important over time corresponding to the secondary metabolites that negatively affect the caterpillar feeding as induced defense.

Secondary metabolites may be synthesized by the plant as a biochemical response to the presence of microorganisms or insect herbivores when attacked, in addition to other types of injury, such as mechanical or another type of stress (nutrient, water, etc.). The presence of inhibitors or elicitors located on the surface of microorganisms or in the saliva of insects interact with receptors present in plants by the recognition phenomenon, which induces the production of these metabolites through the activation of specific genes and increased enzymatic activity. These metabolites are produced in specialized vesicles associated with the endoplasmic reticulum, which allows them to be stored in vesicles after their production either in their original form or associated with sugar units as phenolic substances, as they are mostly toxic and must be maintained in their reduced form. Thus, when the plant is subjected to any type of biotic or abiotic stress, these substances are changed from a non-toxic form in which they were reduced and stored in small organelles to a toxic form wherein they are oxidized and are no longer stored in organelles. Phenolic substances that remain free in the cytoplasm can have toxic effects that result in the delay of infection and the developmental delay of insects and that elicit effects on the plant cells, contributing to hypersensitivity reactions (Mithöfer and Boland 2012; Mithöfer and Maffei 2016; Silva et al. 2008; Taiz and Zeiger 2009; War et al. 2012).

Some authors confirm that there is a difference between the two types of stress studied here. Mechanical wounds on leaves could not mimic the chewing insect attack because

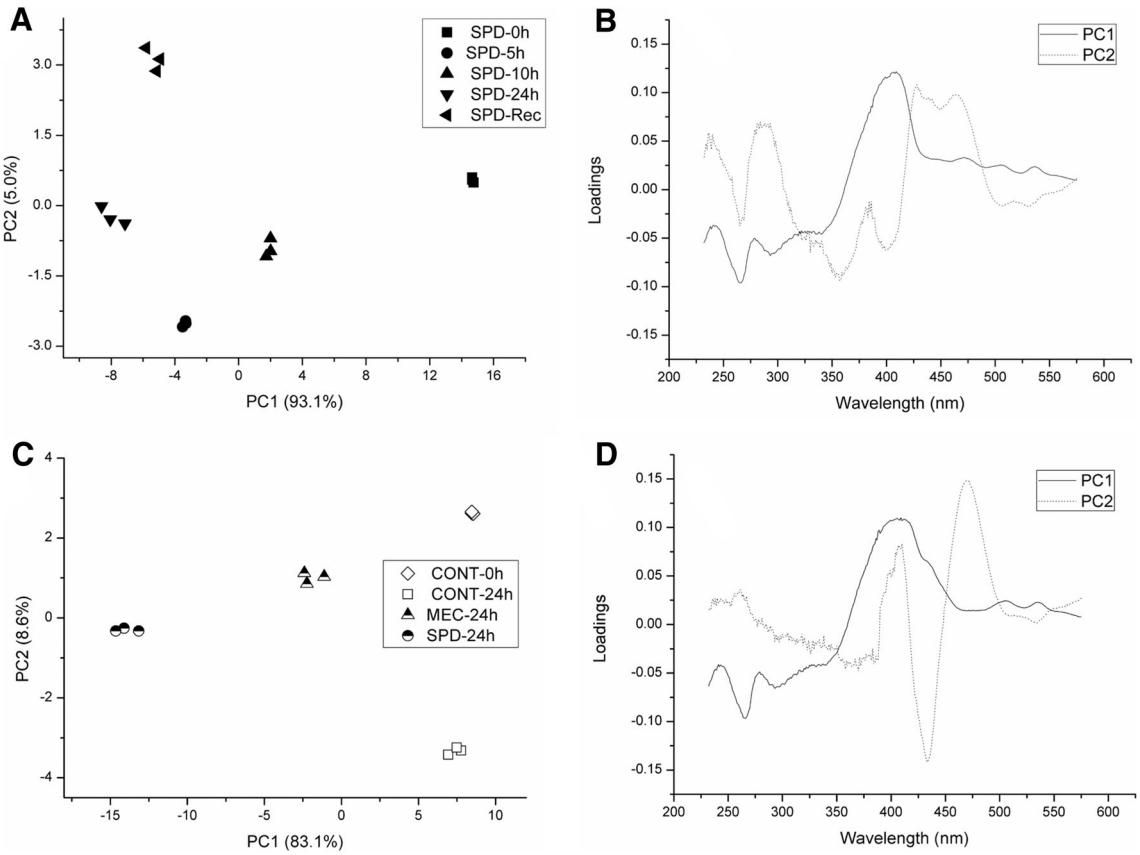

Fig. 5 Chemometric analyses from UV–Vis spectra of the soybean leaf extracts of the *S. frugiperda*-damaged treatment at the 0 h, after 5 h, 10 h, 24 h, and 1-day recovery time points, and comparison between the three treatments (control, mechanically wounded, and *S. frugiperda* treatments) 24 h after the beginning of the experiment. **a** PCA score plot shows the herbivore-damaged and recovery samples allocated on the most negative PC1 scores, far from samples without injury (SPD-0 h). **b** PCA loading plot for the first (PC1) and the second (PC2) principal components suggests the importance of phenolic compounds for sample distinction. The plant response is maintained even after the *S. frugiperda* damage stress was ceased. **c** PCA score plot for the three treatments (control, mechanically wounded, and *S. frugiperda* treatments) 24 h after the beginning of the experiment shows the separation between damaged and control leaves on PC1 scores. **d** PCA loading plot for the first (PC1) and the second (PC2) principal components reinforces the importance of phenolic compounds as chemical response for damaged plant distinction, since wavelengths between 231 and 365 nm on the negative PC1 axis correspond to damaged samples in PCA score plot

the insects release substances that can be relevant inductors of plant hormones that activate defense genes. An example is the work performed in *Arabidopsis thaliana* plants under biotic and abiotic stresses, which showed that only one defense gene is induced by insects and not by mechanical wound (Reymond et al. 2000).

Another study showed that tomatoes when exposed to mechanical wound had an RNase enzyme increased in the first 10 h only at the location of injury. The function of this enzyme revealed that it was involved in the senescence process, suggesting that an alternative signaling pathway could be related to injury caused by a cut or wound, independently of the other hormones (Groβ et al. 2004).

Our results obtained from biological and chemical analyses indicated that the presence of caterpillars trigger plant responses against herbivory causing deleterious effects on larvae and pupae. *Spodoptera frugiperda* caterpillars-fed herbivore-damaged soybean plants resulted in an increased duration of larval development period and a decrease in pupal weight and leaf consumption in the herbivore-damaged treatment compared to the others, suggesting the activation of a defense mechanism and production of metabolites after the caterpillar attack. Although the mechanically wounded leaves have resulted in changes in the spectral profile (UV) viewed by chemometric analyses, no significant changes in the *S. frugiperda* caterpillar biology that were fed these mechanically wounded plants were observed.

The literature reports the presence of isoflavones, among them, genistein and daidzein in soybean leaves and grains, along with flavonol-derivative glycosides of kaempferol and phenolic acids, such as caffeic acid, salicylic acid, ferulic acid, cinnamic acid (Porter et al. 1986; Osman and Fett 1983; Ho et al. 2002). Ho et al. (2002) compared the

differences in the chemical profiles of soybean seeds and leaves. The seeds showed large amounts of malonyl genistin, followed by malonyl daidzein, genistein, daidzin, genistein, and daidzein. In contrast, the leaves showed traces of malonyl genistein and genistin, but six flavonoid glycosides of kaempferol derivatives were isolated and identified. According to a recent study (Murakami et al. 2014), isoflavones were considered elicitors in soybean leaves with previous herbivory by *Spodoptera litura*.

Bortoli et al. (2012) observed that *Anticarsia gemmatalis*-damaged soybean leaves had differences in chemical composition, with an increase of the concentration of isoflavonoids, probably due to an induced response related to defense mechanisms. Furthermore, isoflavonoid compounds may have pre- and post-ingestive effects on herbivores, hindering the development of insects, as observed by Piubelli et al. (2005). Clear negative effects on the biology of *A. gemmatalis*, including high mortality, reduced weights of both caterpillars and pupae, and lengthened life cycles were found in injured soybean genotypes.

Conclusion

Based on our results, both herbivore damages and mechanical wounds induce changes in the soybean metabolism with the production of phenolic substances. However, only the caterpillars-fed herbivore-damaged plants experienced harmful effects on their biology. The chemical analysis of the control leaf samples showed differences between the collection periods of the day, highlighting variations in carotenoid production during the circadian cycle. The chemometric analyses also showed that the recovery of the plant was different, depending on the type of injury. Mechanically wounded recovery samples became more similar to the plants at the beginning of the experiment (MEC-0 h) in contrast to herbivore-damaged recovery samples (Figs. 4a and 5a, respectively), suggesting that the herbivore-damaged defenses were maintained. These findings can be useful in understanding how the defenses that soybean produces against *S. frugiperda* could impair it and possibly other herbivores.

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References

Awmack CS, Leather SR (2002) Host plan quality and fecundity in herbivorous insect. Annu Rev Entomol 47:817–844

Azcarate SM, Cantarelli MA, Pellerano RG, Marchevsky EJ, Camiña M (2013) Classification of Argentinean Sauvignon blanc wines by UV spectroscopy and chemometric methods. J Food Sci 78(3):427–432

Barros EM, Torres JB, Ruberson JR, Oliveira MD (2010a) Development of *Spodoptera frugiperda* on different hosts and damage to reproductive structures in cotton. Entomol Exp Appl 137:237–245

Barros EM, Torres JB, Bueno AF (2010b) Oviposição, desenvolvimento e reprodução de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) em diferentes hospedeiros de importância econômica. Neotrop Entomol 39(6):996–1001

Beebe KR, Pell RJ, Seasholtz MB (1998) Chemometrics: a practical guide. Wiley, New York

Bernardi O, Sorgatto RJ, Barbosa AD, Domingues FA, Dourado PM, Carvalho RA et al (2014) Low suceptibility of *Spodoptera* cosmioides, *Spodoptera eridania* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to genetically-modified soybean expressing Cry1Ac protein. Crop Prot 58:33–40

Blanco CA, Chiaravalle W, Dalla-Ria M, Farias JR, Garía-Degano MF, Gastaminza G, Mota-Sánchez D, Murúa MG, Omoto C, Pieralisi BK, Rodríguez J, Rodríguez-Maciel JC, Terá-Santofimio H, Terán-Vargas AP, Valencia SJ, Willink E (2016) Current situation of pests targeted by Bt crops in Latin America. Curr Opin Insect Sci 15:131–138

Boregas KGB, Mendes SM, Waquil JM, Fernandades GW (2013) Estágio de adaptação de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) em hospedeiros alternativos. Bragantia 77(1):61–70 Portuguese

Bortoli SA, Murata AT, Vacari AM, Bortoli CP, Ramalho DG (2012) Herbivoria em soja: efeito na composição química das folhas e na biologia da lagarta da soja e do percevejo verde pequeno. Comun Sci 3(3):192–198 Portuguese

Broch DL, Ranno SK (2012) Fertilidade do solo, adubação e nutrição da cultura da soja. In: Tecnologia e Produção: Soja e Milho 2011/2012. Fundação MS, Maracajú. [cited 2016 Nov 22], [about 37 p.]. Portuguese. Available from: http://www.fundacaoms.org.br/base/www/fundacaoms.org.br/media/attachments/15/15/5385dbdd42bd6c653952352ab872eed8dd643a607266e_02-fertilidade-do-solo-adubacao-e-nutricao-da-cultura-de-soja_543353833.pdf

Bueno AF, Panizzi AR, Corrêa-Ferreira BS, Hoffmann-Campo CB, Sosa-Gómez DR, Gazzoni DL (2012) Histórico e evolução do manejo integrado de pragas da soja no Brasil. In: Hoffmann-Campo CB, Corrêa-Ferreira BS, Moscardi F (eds) Soja: manejo integrado de insetos e outros artrópodes-praga. Embrapa, Brasília, pp 37–74

Bueno RCOF, Bueno AF, Moscardi F, Parra JRP, Hoffmann-Campo CB (2011) Lepidopteran larva consumption of soybean foliage: basis for developing multiple-species economic thresholds for pest management decisions. Pest Manag Sci 67(2):170–174

Capineira JL (2001) Handbook of vegetable pests. Academic press, San Diego

Capineira JL (2008) Encyclopedia of entomology, 2nd edn. Springer, New York

Casale M, Oliveri P, Armanino C, Lanteri S, Forina M (2010) NIR and UV-Vis spectroscopy, artificial nose and tongue: comparision of four fingerprinting techniques for the characterisation of Italian red wines. Anal Chim Acta 668:143–148

CONAB—Companhia Nacional de Abastecimento (2016) Acompanhamento da safra brasileira de grãos—safra 2015/2016. 3(10),

Julho 2016. Available at http://www.conab.gov.br. Accessed 4 Aug 2016

Cruz I (1995) A lagarta-do-cartucho na cultura do milho. Circular técnica 21. Embrapa, Sete Lagoas

Fehr WR, Caviness CE (1977) Stages of soybean development [monograph] Special Report 80: Iowa State University Cooperative Extension Service.

Formentini AC, Sosa-Gómez DR, Paula-Moraes SV, Barros NM, Specht A (2015) Lepidoptera (Insecta) associated with soybeans in Argentina, Brazil,Chile and Uruguai. Cienc Rural 45(12):2113–2120

Fürstenberg-Hägg J, Sagrobelny M, Bak S (2013) Plant defense against insect herbivores. Int J Mol Sci 14(5):10242–10297

Gobbo-Neto L, Lopes N (2007) Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quim Nova 30:374–381 Portuguese

Greene GL, Leppla NC, Dickerson WA (1976) Velvetbean caterpillar: a rearing procedure and artificial medium. J Econ Entomol 69:487–488

Groβ N, Wasternack C, Köck M (2004) Wound-induced RNaseLE expression is jasmonate and systemin independent and occurs only locally in tomato (*Lycopersicon esculentum* cv. Lukullus). Phytochemistry 65:1343–1350

Hartman GL, West ED, Herman TK (2011) Crops that feed the World 2. Soybean—worldwide production and constraints caused by pathogens. Food Secur 3(1):5–17

Heidel-Fischer HM, Vogel H (2015) Molecular mechanisms of insect adaptation to plant secondary compounds. Curr Opin Insect Sci 8:8–14

Ho HM, Chen RY, Leun LK, Chan FL, Huang Y, Chen XY (2002) Difference in flavonoid and isoflavone profile between soybean and soy leaf. Biomed Pharmacother 56:289–295

Isman M (2002) Insect antifeedants. Pestic. Outlook 13:152–157. doi:10.1039/B206507J

Jahnke LS (1999) Massive carotenoid accumulation in *Dunaliella bardawil* induced by ultraviolet-A radiation. J Photochem Photobiol B 48:68–74

Markham KR (1982) Techniques of flavonoids identification. Academic Press, Tokyo

Martinez SS, Van Emden HF (2001) Redução do crescimento, deformidades e mortalidade de *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) causadas por azadiractina. Neotrop Entomol 30(1):113–125

Mithöfer A, Boland W (2012) Plant defense against herbivores: chemical aspects. Annu Rev Plant Biol 63:431–450

Mithöfer A, Maffei ME (2016) General mechanisms of plant defense and plant toxins. In: Gopalakrishnakone P, Carlini CR, Ligabue-Braun R (eds) Plant toxins. Springer, Netherlands. doi:10.1007/978-94-007-6728-7

Murakami S, Nakata R, Aboshi T, Yoshinaga N, Teraishi M, Okumoto Y et al (2014) Insect-induced daidzein, formononetin and their conjugates in soybean leaves. Metabolites 4:532–546

Oerke EC (2006) Crop losses to pests. J Agric Sci 144(1):31–43

Oerke EC, Dehne HW (2004) Safeguarding production—losses in major crops and the role of crop protection. Crop Prot 23(4):275–285

Oliveira CM, Auad AM, Mendes SM, Frizzas MR (2014) Crop losses and the economic impact of insects pest on Brazilian agriculture. Crop Prot 56:50–54

Osman SF, Fett WF (1983) Isoflavone glucoside stress metabolites of soybean leaves. Phytochemistry. 22(9):1921–1923

Parra JRP (1986) Criação de insetos para estudos com patógenos. In: Alves SB (ed) Controle microbiano de insetos. Manole, São Paulo, pp 348–373

Piubelli GC, Hoffmann-Campo CB, Moscardi F, Miyakubo SH, Oliveira CN (2005) Are chemical compounds important for soybean resistance to *Anticarsia gemmatalis*? J Chem Ecol 31(7):1509–1525

Pogue MG (2002) A world revision of the Genus *Spodoptera* Guenée (Lepidoptera: Noctuidae). Mem Am Entomol Soc 43:1–202

Porter PM, Banmiairt WL, Hassett JJ (1986) Phenolic acids and flavonoids in soybean root and leaf extracts. Environ Exp Bot 26(1):65–73

Reymond P, Weber H, Damond M, Farmer EE (2000) Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. Plant Cell 12(5):707–720

Robards K, Prenzler PD, Tucker G, Swatsitang P, Glover W (1999) Phenolic compounds and their role in oxidative processes in fruits. Food Chem 66:401–436

Rodriguez-Amaya DB (2001) A guide to carotenoid analysis in foods. ILSI Press, Washington

Roscoe R, Gitti DC (2012) Manejo e Fertilidade do solo para a cultura da soja. In: Tecnologia e produção de soja 2013/2014. Fundação MS, Maracajú, pp 16–44. [cited 2016 Nov 22] Portuguese. Available from http://www.fundacaoms.org.br/base/www/fundacaoms.org.br/media/attachments/144/144/55ad57541853c97f65214d32ca0f45913585c9514bb71_01-manejo-e-fertilidade-do-solo-para-a-cultura-da-soja.pdf

Sá VGM, Fonseca BVC, Boregas KGB, Waquil JM (2009) Sobrevivência e Desenvolvimento Larval de *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) em Hospedeiros Alternativos. Neotrop Entomol 38(1):108–115

Santos KB, Neves P, Meneguim AM, Santos RB, Santos WJ, Boas GV et al (2009) Selections and characterization of the *Bacillus thuringiensis* strains toxic to *Spodoptera eridania* (Cramer), *Spodoptera coismioides* (Walker) and *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). Biol Control 50(2):157–163

Sârbu C, Naşcu-Briciu RD, Kot-Wasik A, Gorinstein S, Wasik A, Gorinstein S et al (2012) Classification and fingerprint of kiwi and pomelo fruits by multivariate analysis of chromatographic and spectroscopic data. Food Chem 130:994–1002

Schoonhoven LM, van Loon JJA, Dicke M (2005) Insect-plant biology, 2nd edn. Oxford University Press, Great Britain

Silva RA, Reis VM, Baldani Olivares FL (2008) Defesa de Plantas contra Ataque de Fitopatógenos. Embrapa Agrobiologia, Seropédica **Portuguese**

Silveira-Neto S, Nakano O, Barbin D, Villa-Nova NA (1976) Manual de ecologia dos insetos. Agronômica Ceres, Piracicaba Portuguese

Taiz L, Zeiger E (2009) Plant physiology, 4th edn. Sinauer, Sunderland

Vázquez-Flota F, Carrilo-Pech M, Minero-Garcia Y, Miranda-Ham ML (2004) Alkaloid metabolism in wounded *Catharanthus roseus* seedlings. Plant Physiol Biochem 42:623–628

Victorio CP, Kuster RM, Lage CLS (2007) Qualidade de Luz e Produção de Pigmentos Fotossintéticos em Plantas In Vitro de *Phyllanthus tenellus* Roxb. Rev Bras Biocienc 5(2):213–215

War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S, Sharma HC (2012) Mechanisms of plant defense against insect herbivores. Plant Signal Behav 7(10):1306–1320