### Chapter 4 Phytosanitation: A Novel Approach Toward Disease Management



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Abstract For millennia, man has been producing food, using agriculture, but with increasing cultivated areas, due to the increasing need for food, problems related to production, especially the increase of insect pests, diseases of plants and interferences with weed plants also multiplied. The evolution of plants, through a better genetic approach, transformed the terrestrial environment, making them a very valuable resource for the herbivore community. In ecosystems, plants and insects are just some of the living organisms that continually interact in complex ways and may be the most complex relationships observed in nature. The generated effects of this interaction may be beneficial or harmful to both. To avoid insect attack, plants have developed different mechanisms, such as physical and chemical barriers, defense proteins, volatile substances, secondary metabolism, and trichomes. On the other hand, the insects developed different patterns of associations with host plants, together with different feeding strategies necessary for the exploration of the hosts. Herbivorous insects present complementary adaptations as a response to each defense adaptation in host plants. It is clear that insects are successful in terms of number of species and size of population and as the chemical composition of plants is variable, this represents a challenge for insect feeding. However, insects possess a powerful set of enzymes that constitute the defense against toxic chemicals produced by plants.

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#### 4.1 Introduction

Plants constantly undergo adverse situations, managing to modulate defense responses to overcome these conditions and return to normal development. Knowing how plants protect themselves is essential to increase plant production and quality.

The factors that can affect overall plant development are considered to be stress conditions, characterized as different conditions that affect growth, development of plants. The factors involved can be biotic, by organisms, or abiotic, due physical or chemical environment.

With trophobiosis being intimately related to physiological stress mechanisms, capable of leading to a state in which free amino acids and reducing sugars are available to feed insects, it is important to observe the factors that promote stress, as well as the agricultural practices capable of minimizing it.

Stress in plants is defined as any condition that affects or blocks metabolism, growth, and plant development (Lichtenthaler 1996). It is the state whereby an increase in cellular metabolic demand leads to the initial destabilization of the functions followed by the normalization and increase of resistance. Thus, the tolerance limit can be changed, and as a consequence, an extra adaptive capacity will be demanded. The result can be permanent damage or even death of the plant (Larcher 2000). Plant stress involves a range of biological and environmental factors. These factors include cold, heat, cohabitation with weeds, insects, and diseases caused by viruses, fungi, bacteria, etc. (Siedow 1995).

A series of natural environmental conditions can cause plant stress, which can be divided into biotic and abiotic factors (Larcher 2000). Among these stress factors, capable of promoting metabolic imbalances that act on proteosynthesis and, therefore, plant resistance, the following are prominent (Chaboussou 1999):

- Biotic factors: spacing, pests and diseases, genetic constitution of the plant (the species and variety, phenological age).
- Abiotic factors: climate (solar energy, temperature, humidity, precipitation, cosmic influences).
- Cultivation factors: soil (chemical composition, structure, aeration), fertilization (organic and mineral), grafting (influence of the rootstock on the physiology of the graft and reciprocally), treatment with pesticides.

Plants generally respond to environmental stress in a similar manner, firstly reducing their growth rate and the rate of resource intake. This is true both for plants adapted via evolution to environments with scarce resources and for any plant that physiologically adjusts to any limiting factor (Pires et al. 1998).

Generally, stress is related to diverse responses in plants and may encompass changes in gene expression and cellular metabolism to changes in growth and productivity rates. Plant responses to stress depend on the duration, severity, amount of exposure, and combination of stress factors, as well as the type of organ or tissue, age of development, and genotype. Some responses allow plants to cope with stress, while others are not so apparent (Kacperska 2004).

Cases in nature where a stress factor acts in isolation are rare. Frequently, multiple stresses are involved, in a combination of factors (Larcher 2000). Some plant species are more tolerant to stress than others are, while others are much less so. Air temperature is one of the most stressful factors, being able to manifest in minutes (both highs and lows); soil humidity can take days, while mineral deficiencies in the soil can take months to manifest themselves (Taiz and Zeiger 2004). To the extent that a plant tolerates more stress, it can adapt. However, it is not adapted, since adaptation involves the level of resistance determined by genetic makeup. Therefore, adaptation to stress results in anatomical, morphological, cellular, biochemical, and molecular aspects (Lopes et al. 2011).

#### 4.2 Plant Stress and Colonization by Pests

In general, plants subjected to abiotic stress factors are more susceptible to herbivorous insects (Larsson 1989), the increase in the concentration of free nitrogen in the leaves, which produces higher amounts of amino acids and results in greater growth, development, survival, fecundity, and, therefore, greater abundance of insects (White 1984), which leads to greater herbivory.

The hypothesis of plant stress presupposes that plants under stress are more vulnerable to insect attacks, because they will be richer in nutrients and less protected by chemical defenses (White 1984). Biotic stress leads to alterations in the pattern of protein expression of plants, with inhibition of the induction of biosynthesis of protein constituents. There is induction of proteinase inhibitors, as a possible defense mechanism against insects (Green and Ryan 1972). Methyl jasmonate action also occurs in the alteration of rubisco enzyme levels and of other proteins (Cavalcante et al. 1999). There are also alterations in rubisco levels with the senescence of *Brassica napus* Linnaeus (Brassicaceae) (Ghosh et al. 2001).

Generally, one can propose two theories to explain the relation between plant stress and pest attacks (Angelo and Dalmolin 2007). The first is that hydric stress is a more significant factor in insect population explosions, given that population increases of diverse insect species occur after periods of drought (Mattson and Haack 1987; Angelo and Dalmolin 2007).

One of the initial studies that presented this hypothesis was produced by White (1970), involving *Cardiaspina densitexta* (Taylor 1962) (Hemiptera: Psyllidae) regarding *Eucalyptus fasciculosa* (F. Muell.) (Myrtaceae) in Australia. The stress hypothesis was expanded by Rhoades (1979), who argued that plants produce toxins under stress, reducing the production of allelochemicals of high metabolic cost and redirecting resources to produce cheaper allelochemicals. Therefore, the production of toxins is increased and the production of compounds that reduce digestibility

reduced. The literature contains a large number of citations presenting the hypothesis of stress in plants in relation to herbivores. Examples can be found in Austarå and Midtgaard (1987), with *Neodiprion sertifer* (Geoffrey) (Hymenoptera: Diprionidae) on *Pinus sylvestris* L. (Pinaceae) after acid rain; Cates et al. (1983), with *Choristoneura occidentalis* (Freeman) (Lepidoptera: Tortricidae) on *Pseudotsuga menziesii* (Mirbel) Franco (Pinaceae) after exposure to drought; and Coleman and Jones (1988), with *Plagiodera versicolora* (Laicharting) (Coleoptera: Chrysomelidae) on *Populus deltoides* Bartr. ex Marsh (Salicaceae) after exposure to ozone, among others.

The second theory refers to the balance in the allocation of resources that takes place between the processes related to growth and differentiation under determined environmental conditions. The growth of roots, branches and leaves, or any process requires cell division. However, cell differentiation is the maturation and specialization of existing tissues (Angelo and Dalmolin 2007). Therefore, the allocation of carbon to these different functions cannot occur simultaneously, and equilibrium in the processes of growth and differentiation interacts in the herbivory competition and thus can define the strategies of defense of plants (Herms and Mattson 1992).

Competition in resource-rich environments leads to strategies directed toward growth, while stress from poorer environments leads to differentiation strategies (Stamp 2003), such as products related to differentiation obtained via secondary metabolism, as well as the production of trichomes and the enrichment of leaf cuticles (Herms and Mattson 1992). This involves a cost for enzyme production and the transport and storage structures involved in defense (Angelo and Dalmolin 2007).

#### 4.3 **Biotic Factors and Pest Attacks**

#### 4.3.1 Density

Plant density during cultivation can contribute to the establishment of a microclimate favorable to pest attacks, though this is not the rule. In coffee plants, where increased planting density allows the obtainment of greater production per unit area, there is a significant increase of phytosanitary problems, mainly from attacks by the coffee borer [*Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae)] (Braccini et al. 2008).

However, in denser spacing, the incidence of mining bug is reduced, and problems with the coffee borer are aggravated. This results from the microclimate formed, which provides, in the closer spacing, greater humidity in the environment (Braccini et al. 2008).

#### 4.3.2 Phenological Age

The hypothesis of phenological age of the host plant predicts that herbivores prefer and/or develop better in younger plants, due to having better nutritional quality, than in older plants. Plant age interferes in the abundance of sucking insects in citruses, such as *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae) and *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae). The abundance of *A. floccosus* and *T. citricida* is greater in 1-year old plants than in older plants (3, 5, 10, and 20 years).

#### 4.3.3 Variety

The identification of the varieties to be cultivated is one of the most important steps for a farmer, given that not applying the necessary practices for the variety chosen can lead to greater plant stress. In this case, there are various aspects of management to be noted, and up until the present moment, there is no variety resistant to all types of stress that a plant can confront, either biotic or abiotic. Additionally, one should consider that accesses or lineages with different morphological and physiological characteristics can possibly contribute to variations in stress resistance factors.

The use of resistant varieties, selected by traditional genetic improvement programs, contributed considerably to integrated pest management programs. Due to the low cost and better environmental preservation, the use of resistant materials is a highly desirable strategy for insect control.

Some varieties have a certain degree of resistance to insects, and the biosynthesis and regulation of chemical compounds associated with these defenses by plants have been studied for some time now. Currently, it is known that these defenses are found in various plant tissues and among these compounds, antibiotics, alkaloids, terpenes, and proteins can be found, among others. Among the proteins, enzymes such as chitinases, lectins, and digestive enzyme inhibitors are found (Ryan 1990).

#### 4.3.4 Pest Attacks

Herbivory is an interaction between plants and different organisms with important ecological and evolutionary repercussions. This interaction is determined by variation in local biotic and abiotic conditions that affect the quality and quantity of the resources offered by the host plant. Therefore, the intensity of herbivory depends on innumerous characteristics of the plants, including leaf thickness, the carbonnutrient relation present in the tissues, the concentration of secondary compounds, the water content contained in the plants, etc. (De Moraes et al. 2001).

To avoid injuries caused by herbivores, plants developed defensive strategies based on the presence of chemical compounds, mechanical barriers, or biological associations. Among the defense mechanisms utilized by plants against pathogens and herbivores, the production of secondary metabolites stands out, such as tannin, flavonoids, terpenes, alkaloids, etc. (Moraes 2009).

#### 4.4 Stresses and Species Reactive to Oxygen (EROs)

When submitted to stress, plants show various physiological and biochemical responses. Sources promoting stress are diverse and can be biotic (pathogens, pests) and abiotic (extreme temperatures, hydric availability – excess or lack, wind, radiation, UV, salinity, heavy metal, etc.), requiring adaptations for survival and production (Scansalios 2005).

Stress becomes a challenge for agricultural production compromising food production. Crops are constantly submitted to factors that promote imbalances, which in turn promote the production of antioxidant defenses as a response, inducing the formation of oxygen reactive species (EROs) (Scandalios 1997).

EROs are cellular stress indicators or secondary messengers involved in the translation pathways for signals in response to stress (Mittler 2002; Foyer and Noctor 2005). The main source of EROs is photorespiration, in which neutral and nonreactive molecular oxygen ( $O_2$ ) during the metabolic process of transference and transport of electrons can produce free radicals, resulting in radical superoxide ( $O_2 \cdot -$ ), radical hydroxyl (OH-), and radical hydrogen peroxide ( $H_2O_2$ ) (Foyer and Noctor 2009).

When radicals are at high concentrations, they are toxic to the plant. To reduce the damage caused by the EROs, aerobic agents develop nonenzymatic and enzymatic methods to combat them. The nonenzymatic means involve the production of  $\beta$ -caroteno and vitamins C and E. However, the enzymatic defense induces the production of enzymes capable of neutralizing the radicals and/or intermediary oxygen counting on the superoxide dismutase (SOD), peroxidase (PPO), catalase (CAT), polyphenol oxidase (POD), and phenylalanine (PAL), among others (Scandalios 2005).

The EROs can interact with lipids from the membranes, fragment the peptide chain, modify amino acids, cause deletion and mutation in the DNA, degrade the nucleotide bases, and lead to cellular death. Each organelle has a potential target for the damage or accumulation of EROs, and enzymes attempt to maintain cellular homeostasis, which can lead to the activation or disconnection of some genes (Munné-Bosch et al. 2013).

# 4.5 Stresses and the Production of Species Reactive to Oxygen (EROs) in Soy Plants

Even with growing territorial expansion and agricultural production, soy cultivation, as with many others, its potential for output and quality is influenced by internal and external factors during cultivation, such as chemical and physical characteristics of the soil, climatic and edaphic components, genetic characteristics, and phytosanitary management. The adaptation to the tropical climate and low nodulation are examples of factors referred to as negatives of productivity (Hartman et al. 1991). However, another important factor in this sense is the fairly diversified entomofauna of this culture, which contains an elevated number of insect species, with those that cause serious damage to the culture being considered the main pests. Others, considered secondary pests, occur at lower levels and only under special conditions lead to economic liabilities. A third group corresponds to the beneficial insects that feed on insect pests and therefore act as natural control agents (Carneiro et al. 2010).

To increase soy productivity therefore, that is, to produce more without increasing the plantation area, the improvement of the technology used in the management of the culture is necessary, mainly when it influences factors such as disease incidence, pest populations, and agricultural characteristics that can result in the alteration of production potential. The greater expression of the potential of soy cultivars, however, depends on the conditions of the environment where the plants develop. Therefore, changes in plant population reduce or increase yield because of plant density and spacing (Tourinho et al. 2002).

However, population increases or alterations in the sowing system can favor undesirable factors, such as inter- and intraspecific competition of the plants for environmental resources, especially water and nutrients, morphophysiological changes, damage to optimal soil conditions due to excessive movement of agricultural machinery in the cultivation area, as well as shading between plants caused by an increase in the leaf area index (IAF) (Argenta et al. 2001), which is high in the soy culture that is greater than what the plants really need to carry out photosynthesis and generate energy for development (Truble et al. 1993; Haile et al. 1998; Gazzoni and Moscardi 1998). However, in heavy infestations of defoliating caterpillars, the increase of IAF is an advantage, since leaf loss caused by the caterpillars permits the entry of light through the canopy and therefore guarantees the development of vegetation at the base and middle of the plant, promoting better grain production.

The increase in population density of plants or alteration in the sowing system can further affect plant-arthropod relations, since with a greater number of plants by area, there will be modifications to the environment in which they are located such as changes in intensity of solar radiation that reaches the leaves of the lower third and middle, influencing the microclimate (humidity and temperature) that is a severe limiting factor for the development of a pest population (Rodrigues et al. 2010).

#### 4.6 Influence of the Different Sowing Systems on Soy Culture

Soy cultures have gone through many changes with the use of new technology. The introduction of cultivars with a tendency for undetermined growth with predominant characteristics such as greater precocity, new plant architecture, greater

potential for production, and smaller leaflets with a more vertical inclination has raised various questions in terms of the management of soy cultivation leading to research seeking a scientific basis to show which planting system is better for these new cultivars (Procópio et al. 2013).

The sowing system with less intraspecific competition allows better utilization of available resources for the growth and production of soybean grains (Rambo et al. 2003). Reducing the distance between the lines is a worldwide trend and reduces the time for the crop to reach 95% of the solar radiation (Shaw and Weber 1967).

Cross-soybean cultivation has been a common choice among producers. The cross-sowing consists of sowing along parallel lines, followed by a distribution of semenstes in lines forming an angle of  $90^{\circ}$  in relation to the previous ones; thus a grid of lines is over the cultivation area (Lima et al. 2012).

The double-row system or skip row also seeks to optimize the use of resources and consequently reduce production costs. This type of arrangement is based on two between rows, one internal and the other external (Chiavegato et al. 2010). In the soy culture, the seeds are sown in double rows with a spacing between the internal rows of 0.20 m, while with the external between rows, the spacing utilized is 0.40 or 0.60 m. Changing the spacing configuration for sowing by removing of one or more rows is a technique of the planting system that can favor a higher penetration of light and agrochemicals into the canopy. This improves the rate of photosynthesis and the health and longevity of the leaves nearer to the soil, which, finally, can maximize the productivity of grains. The lack of seeders, however, is a significant barrier to the development of this arrangement.

In the denser cultivation system, the spacing between the rows is reduced and can lead to modifications in the quantity of dry material accumulated by the plants and reduction of the area of the between rows (Scott and Aldrich 1975) and the leaf area and index, which can result in an increase in grain output (Pires et al. 1998).

Some advantages in terms of the conventional system can be considered in the densified system, such as optimization of the factors of soil, machines, tools, and inputs, less degradation of the area used, better weed control, more efficient water use, greater capture of photosynthetically active radiation, and earlier harvesting. However, there are also risks with a greater possibility of incidence of pests and diseases, a lower number of fruits per plant and lower weight per 1000 grains (Chiavegato et al. 2010).

The aim of agricultural practice from the physiological point of view is to maximize the photosynthetic efficiency of the cultures and seek gains in productivity and quality of the final product, highlighting the importance of seeking information regarding  $CO_2$  assimilation (Brandão Filho et al. 2003). The respiratory and photosynthetic variation of soy occurs due to development, resulting from the alteration of the drainage force, in the architecture and leaf structure (Porras et al. 1997; Pereira 2002). The photosynthetic and respiratory rate of the plants from this culture progressively increases from the vegetative to the reproductive stage and reaches maximum values during the period of grain filling. Starting from the moment in which the demand for photosynthates increases, there is an increase in photosynthesis, which can be observed during the stage of grain growth, considered primary drains for the plant (Pereira 2002). The canopy architecture is considered a determining factor in the photosynthetic capacity of the soy culture (Wells 1991). This is characterized by a dense upper layer of leaves that hampers the penetration of light into the lower strata, such that at the beginning of the reproductive period, around 50% of the liquid radiation reaches the soil surface; however, in the R5 stage (beginning of the grain filling) and R6 (maximum grain volume), 20% reaches the middle part of the plant community and only 10% the lower part. Therefore, even with the increase in leaf area index, there will only be an increase in radiation interception up to a certain point (Pengelly et al. 1999), since, it implies an increase in self-shading, leading to growth and an increase in the coefficient of luminous extinction (Pengelly et al. 1999).

Stomata function is another limiting factor on photosynthetic rate, given that it controls  $CO_2$  absorption (Costa and Marenco 2007). The stomatal pores permit water vapor loss into the atmosphere during transpiration and the entry of  $CO_2$  that takes place from photosynthetic fixation of carbon (Vavasseur and Raghavendra 2005). Generally, when there is competition for water or hydric deficits, plants reduce the degree of stomatal opening, thereby reducing stomatal conductance, to reduce water loss and maintain hydric balance. Therefore, the greater the hydric deficit, the lower the degree of openness of the stomata and, consequently, the greater the resistance to the entry of atmospheric  $CO_2$  (Kerbauy 2004). In terms of transpiration, stomatal conductance diminishes in relation to the water fraction available for the plant and for the incidence of photosynthetically active radiation (Bergonci and Pereira 2002).

Some morphophysiological characteristics (branches per plant, length of branches, and number of fertile nodes) have a relation to the productive potential of the soy plant, representing a greater photosynthetic surface and potential productivity due to the number of sites for the emergence of reproductive buds. However, the number and length of branches can also represent an additional demand redirecting photoassimilates that, in another way, might be used in fixation and in the production of reproductive structures (Navarro Júnior and Costa 2002).

#### 4.7 Biochemical Response of Soy Plants to Sowing Arrangements and to Insect Attack

Sowing arrangements can also influence the plant-arthropod arrangement as a result of modifications in the microclimate caused by greater exposure to solar rays that will reach the leaves of the lower third and middle, which is a limiting factor on the development of a pest-insect population (Rodrigues et al. 2010).

As consequence of the alterations in the soy plant morphology and of insect population fluctuations, the increase in productivity of species reactive to oxygen (EROs) can occur. This is an important metabolic alteration for plants under biotic and abiotic stress conditions (Van Bbreusegem et al. 2001; Apel and Hirt 2004; Foyer and Noctor 2005). The elimination of the EROs and the protection against oxidative damage in plants take place via enzymatic and nonenzymatic antioxidant defense systems. The first group was represented by the enzymes superoxide dismutase (SOD), catalases (CAT), peroxidases (POD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione S-transferase (GSTs). The second was represented by nonenzymatic compounds such as vitamin C and glutathione (GSH),  $\beta$ -carotene, phenolic compounds, tocopherols, and polyamines (Hernández et al. 2001; Blokhina et al. 2003; Scandalios 2005).

Therefore, it is possible to observe that in cultures sown in crossed and reduced planting systems, there is a lower occurrence of defoliating Lepidoptera (Carvalho 2014), and given this, fewer insecticide applications were required for the control of caterpillars, consequently increasing the liquid output of the production (Higley and Peterson 1996). The CO<sub>2</sub> assimilation rate (A,  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (gs, mol m<sup>-2</sup> s<sup>-1</sup>), transpiration rate (E, mmol m<sup>-2</sup> s<sup>-1</sup>), and internal CO<sub>2</sub> concentration in the leaf (Ci,  $\mu$ mol mol<sup>-1</sup>) were greater in the conventional and double-row planting systems than in the crossed and reduced planting. The CO<sub>2</sub> assimilation rate is greater in these sowing arrangements, since the rate of photosynthesis of the leaves increases with the development of the plant (Rosa et al. 2007; Pereira 2002). This relationship is observed because during transpiration, the stomatal pores permit the loss of water vapor into the atmosphere and the entry of CO<sub>2</sub>, through the photosynthetic fixing of carbon (Vavasseur and Raghavendra 2005), resulting in greater internal concentrations.

The reflex of this alteration is related to greater intraspecific competition that can occur due to competition for essential sources such as water, light, and nutrients (Raventós and Silva 1995). Under these conditions, plants reduce the degree of stomatal opening, in this way reducing stomatal conductance, diminishing water loss, and maintaining hydric balance. The greater the competition and consequently the hydric deficit, the lower the degree of stomatal opening and, therefore, the greater the resistance to the entry of atmospheric  $CO_2$  (Kerbauy 2004).

In the crossed and reduced planting system, there is a formation of areas with high intraspecific regions, particularly in the intersection of the sowing rows. This means that greater competition between plants in these systems leads to a reduction of stomatal conductance and implies a lower rate of  $CO_2$  assimilation, a lower rate of transpiration, and lower internal concentration of  $CO_2$ . Consequently, there is a lower incidence of defoliating caterpillars, since the insects prefer physiologically healthier plants. The quantity and quality of food have a direct effect on host preference, as well as affecting the growth rate, the development time, body weight, and survival as well as fecundity, longevity, movement, and the competition capacity of adults (Panizzi and Parra 2009).

However, even with more competition between the plants sown in crossed and reduced planting systems, there is no difference in the leaf area ratio  $(dm^2.g^{-1})$ , which occurs due to the greater efficiency of the photosynthetic in soy plants, independent of leaf area (Campos et al. 2008). In other words, even with the lower rate of CO<sub>2</sub> assimilation in sowing systems that promotes competition, there is no reflection of this stress in the emission of leaf area and consequently in soy plant production. The defoliation of around 30%, which is the control level for the soy culture (Hoffmann-Campo et al. 2012), shows the need to control these insects, which

increases production costs. Defoliation in soy plants causes a loss of 10.7 bags.ha<sup>-1</sup> for sequential defoliation, in the vegetative (33%) and reproductive (17%) stages (Reichert and Costa 2003).

In terms of enzymatic activity, the peroxidase and polyphenol oxidase enzymes are more active in sowing arrangements with greater competition between plants. This generates more stress for soy plants, in this way provoking an increase of reactive species (EROs) and consequently the increase of the activity of these enzymes. However, with the increase of stress caused by the increase of pest populations in plant arrangements, there is an inversion in enzymatic activity due to the stress caused by the feeding on the soy plants.

There are numerous studies that demonstrate the relationship between enzymes and the reaction process to insects (Lattanzio et al. 2006; Frazen et al. 2007; Gustche et al. 2009; Pierson et al. 2011; Marchi-Werle et al. 2014; Timbó et al. 2014; Cruz et al. 2016). Therefore, changes to the oxidative enzyme levels generally occur in response to population fluctuations and feeding by insects, since the greater the insect population density in the arrangements of double sowing rows, the greater the peroxidase and polyphenol oxidase enzyme activity.

Therefore, it is important to underline the importance of sampling pest-insects in the context of Integrated Pest Management (IPM) in soy. The identification of the correct moment for pulverization of the insecticide results in a reduction of the phytosanitary control costs and lower environmental interference in sowing arrangements in which the insect populations do not go beyond control levels.

#### 4.8 Biochemical Response of Corn Plants Under Hydric Stress and Insect Attack

Productivity in corn cultivation is highly prejudicial to elevated occurrence of pests. Among these, the species *Spodoptera frugiperda* (J.E. Smith 1797) (Lepidoptera: Noctuidae) stands out, which is considered a key pest for the culture in Brazil. This caterpillar mainly causes damage in the leaves of the plants. Additionally, high-density infestations can also occur on the ground, attacking the base of the plants, as the dark sword-grass *Agrotisipsilon* (Hufnagel 1766) (Lepidoptera: Noctuidae) habitually does, or feeding on the reproductive structures, as the species *Helicoverpa zea* (Boddie 1850) (Lepidoptera: Noctuidae) does.

Corn genetically modified for insect control (*Bt* corn) is one of the main control tools for *S. frugiperda*. However, in areas with the adoption of technology, which accounts for 80% of the crops in Brazil (Isaaa 2016), the 2013/2014 crop required the realization of on average two applications of insecticide, due to elevated *S. frugiperda* infestations, even in *Bt* corn plantations, in diverse regions of Brazil (Farias et al. 2014).

Initially, the need for additional control was attributed to the loss of resistance of the *Bt* technology; however, it was also found that abiotic factors cause interference in plant physiology and these disturbances are related to the expression of the insec-

ticidal proteins. Physiological alterations are mainly related to the activation of stress enzymes that act in photosynthetic inhibition, reduction of respiration, cell wall breakdown, reduction of leaf expansion, reduction of metabolic and cellular activities, and cell death (Sorg 2004).

The responses of cultures to hydric stress can be complex and vary according to their duration (Liu et al. 2010). When there are hydric deficits, the first metabolic responses to reduce water loss into the environment are the closure of the stomata and a reduction of transpiration. With this, the process of assimilation of carbon and other nutrients is affected, meaning that the development of the culture is slower even leading to smaller size plants. This reduces the distance between nodes and the leaf area and with a smaller leaf expansion; there is a reduction in the liquid photosynthesis rate and in quantities of photosystems present in the leaf. This also induces the formation of species reactive to oxygen.

The hydric stress caused by the greater competition between plants affects the photosynthetic activity, through stomatal closure and the consequent reduction of  $CO_2$  absorption. However, only more severe deficits affect the photosynthetic process of carbon reduction; moderate deficits do not affect the photosynthetic reactions in the chloroplasts (Farias et al. 2007).

The morphological characteristic to avoid water loss is the closure and rolling of the leaves during the hottest hours of the day (Taiz and Zeiger 2004; Ge et al. 2012; Terzi et al. 2010; Farooq et al. 2009). When plants are submitted to hydric deficits, the aerial part has lower development, investing the photoassimilates in root growth and expansion for greater water absorption (De Souza et al. 2016).

The imposition of hydric deficits on corn plants during the vegetative stage promotes biochemical alterations initiating the production of secondary compounds and EROs, such as the accumulation of peroxide in chloroplasts and in the mesophile (Zhao et al. 2016). In addition to the EROs, the secondary compounds formed, such as phenols, have the primary function in metabolism of protecting the plants, such as protecting the plants when attacked by insects, releasing these compounds to deter herbivores. This can also be correlated with stress enzymes such as peroxidase and polyphenol oxidase presenting a synergistic effect with the defense response.

The hydric factor affects overall plant growth and development. The frequency and intensity of hydric stress constitute factors of primary importance for the limitation of global agricultural production (Ortolani and Camargo 1987). Lack or excess leads to disastrous effects on plant development given that various physiological aspects such as openness and closure of stomata, photosynthesis, and leaf growth and expansion can undergo alterations when the plant is submitted to hydric stress. This can consequently generate alterations to secondary metabolism (Moraes 2009).

There are optimal limits for humidity for plant development. The water intake by the root system means that one of the fundamental problems of agriculture is encountered in the water balance of the soil-root system. Excess water in the soil can alter chemical and biological processes, limiting the quantity of oxygen and accelerating the formation of compounds toxic to the roots. On the other hand, the intense percolation of the water provokes the removal of nutrients and the inhibition of the normal growth of the plant. Hydric surpluses, though significant, cause less problems than drought. Hydric deficits, characterized by different forms and intensities, are the main cause of loss of productivity; however, it presents a correlation in the concentration of secondary metabolites, which are important in the insect-plant relationship, acting as allelochemicals toxic to the insects (Moraes 2009).

In the coffee plant, there are variations in secondary metabolite levels, reducing the viability of the *Leucoptera coffeella* eggs (Guérin-Méneville) (Lepidoptera: Lyonetiidae), causing physiological disturbances in the larvae and pupa, and increasing insect mortality (Awmack and Leather 2002). Hydric stress in plants has been considered one of the main factors for attack by insect herbivores. *Eucalyptus* sp. plants, for example, when submitted to a period of hydric stress become more susceptible to attack by the psyllid *Cardiaspina densitexta* (Taylor) (Hemiptera: Psyllidae).

During hydric deficits, in addition to an increase in the concentration of nutritional compounds, turgor pressure, and a reduction in water content in the plants, there is an elevation in the quantity of allelochemicals. Under these conditions, the suckers can benefit from the greater concentration of nitrogen induced by stress, given that they can effectively extract it.

Another example is the interaction of corn plants that express *Bt* proteins, stressed from being submitted to hydric deficits and infested by caterpillars. This is related to the release of phenols, by the synergistic effect to abiotic and biotic damage, which correlates with the fragility of the technology when exposed to hydric deficits, being potentialized when caterpillar attacks occur.

With the oxidative explosion caused by various sources of stress, the abiotic and/ or biotic factors promote the increase of the activity of the superoxide dismutase enzyme (SOD), which is instantly activated, since it is considered the first enzyme on the defense line. SOD is responsible for the dismutation of the radical  $O_2^{\bullet}$ , generating  $H_2O_2$  and  $O_2$  (Breusegem et al. 2001). The increase of SOD in the corn hybrids stressed from hydric deficits and caterpillar infestations activates the plant defense system (Świątek et al. 2014) and also elevates the rate of production of SOD. SOD action produces the substrate for the activation of other enzymes, such as peroxidase, that breaks down the peroxide of the hydrogen. The activity of this enzyme is characterized by plant-insect interaction, being one of the principal means of plant defense (Van Loon et al. 2006; Kehr 2006).

With all the production of stress enzymes to reduce and control the adverse effects of the EROs in plant metabolism, the production of proteins in the plant is affected. Due to this, there is a failure in the control of the caterpillars, even with Bt plants. The plants are restricted in the synthesis of protein and begin to increase enzyme activity related to the combat of EROs, resultant on the transition of the metabolism of the protein synthesis to hydrolysis (Bilgin et al. 2010). As well as insect attacks, in transgenic cultivars under abiotic stress such as hydric deficits, there is a reduction of soluble proteins contained in the leaves affecting the production of Bt proteins (Dong and Li 2007). The expression of proteins can also vary according to plant age and is more active in green tissues. Therefore, they decrease during the reproductive phase (Rao 2005).

Change to the physiological processes (photosynthesis, respiration, stomatal conductance, and transpiration) of the plants in response to feeding by arthropods is an important step to understand plant-arthropod interaction (Peterson and Higley 1993). There is a direct relation between the increase of stress with stomatal closure, because with the stomata closed, there is a reduction in the absorbance of carbon (CO<sub>2</sub>) by the plant (Larcher 2006), therefore reducing the rate of CO<sub>2</sub> assimilation and the internal CO<sub>2</sub> concentration as well as a reduction in transpiration.

Plants in interaction with biotic factors can also emit molecular signs in this manner activating nuclear genes involved in plant defense responses. This results in an increase of activity of enzymes directly or indirectly associated with plant stress. This takes place through plant-insect interactions wherein the plant is not a passive entity but an active organism, shown through evolutionary processes to be developing protection mechanisms that interfere in its exploitation by insects (Vendramim and Guzzo 2009).

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