Functional Plant Science and Biotechnology ©2009 Global Science Books

Physiological Alterations in Brazilian Sugarcane Varieties Infected by *Sugarcane yellow leaf virus* (ScYLV)

Sandra M. Scagliusi[1*] • Saikat K. Basu[2] • Jorge Alberto de Gouvea[3] • Jorge Vega[4]

[1] Embrapa Trigo, Rodovia BR 285, Km 294, CEP 99001-970 Passo Fundo, RS, Brazil
[2] Department of Biological Sciences, University of Lethbridge, 4401 University Drive, Lethbridge, AB, T1K 3M4 Canada
[3] Universidade de Passo Fundo (UPF), Rodovia BR 285, Cx. Postal 611, CEP 99052-900 - Passo Fundo, RS, Brazil
[4] Departamento de Fisiologia Vegetal, IB, UNICAMP, CP 6109, CEP 13083-970, Campinas, SP, Brazil

Corresponding author: * **mansur@cnpt.embrapa.br**

ABSTRACT

The objective of this study was to verify the physiological alterations caused by *Sugarcane yellow leaf virus* (ScYLV), one of the most important viral diseases of sugarcane (*Saccharum officinarum* L.). We analyzed the levels of soluble sugars and the activity of peroxidases in different Brazilian sugarcane varieties, with different levels of resistance and susceptibility to the virus. Comparison was also carried out between the leaf blade and the main ribs of +1 and +4 leaves, from healthy and infected plants. Soluble sugars levels (especially sucrose) were found to be higher in leaves of symptomatic infected plants, but not in healthy or asymptomatic infected plants. There was a significant increase in the content of soluble sugars in the main ribs of infected plants, followed by the leaf blade of +1 leaves. The activity of peroxidases was significant higher in symptomatic infected plants compared to healthy or infected plants without symptoms.

Keywords: *Luteovirus*, peroxidases, ScYLV, soluble sugars, sucrose

INTRODUCTION

Sugarcane yellow leaf virus (ScYLV) has become over the years one of the main viral diseases of sugarcane. It was first reported in Hawaii and Brazil (Schenck 1990; Vega *et al*. 1997) and since then, its distribution has been reported in major sugarcane-producing areas of the world (Moonan and Mirkov 2002; Rassaby *et al*. 2004; Abu Ahmad *et al*. 2007). Typical symptoms of the disease in susceptible plants are characterized by prominent yellowing of the midribs, followed by leaf necrosis and often red coloration of the adaxial surface (Vega *et al*. 1997; Izaguirre-Mayoral *et al*. 2002). The yellowing of the midribs can also spread to the leaf blade. Symptoms also include shortening of terminal internodes and sucrose accumulation in the leaves (Vega *et al*. 1997; Gonçalves *et al*. 2005; Lehrer *et al*. 2007). Some sugarcane cultivars infected with ScYLV do not show any disease symptoms, increasing the probability of propagating virus-infected material (Viswanathan and Balamuralikrishnan 2004). Symptoms can also vary according to abiotic factors such as soil conditions and temperatures (Vega *et al*. 1997; Scagliusi and Lockhart 2000). The production of sucrose is severely reduced (Vega *et al*. 1997) and ScYLV has been reported to be responsible for yield losses in some sugarcane varieties (Vega *et al*. 1997; Comstock and Muller 2003; Rassaby *et al*. 2004).

Several studies conducted on pathogen-infected plants have shown important physiological and metabolic alterations leading to several modifications in proteins and carbohydrates and in enzyme synthesis (Bowles 1990; Kombrink and Somssich 1995; Ladygina and Babosha 1996). The establishment of viral infection in healthy plants results in biochemical alterations of the photosynthesis process, carbohydrates translocations and in membrane permeability, frequently causing mosaics, abnormal yellowing, chlorosis or mottling symptoms of systemic infected leaves in susceptible plants (Fraser 1987). All these pathological changes are closely linked to lower rates of photosynthesis. Changes in photosynthetic process, among other factors, are associated with alteration in morphology, metabolism and in the reduction of chloroplast number (Zaitlin and Hull 1987; Balachandran *et al*. 1997; Naderi and Berger 1997; Bertamini *et al*. 2004).

Many studies have already described some of the physiological alterations caused by ScYLV and most of them describe sucrose and starch accumulation in the leaves of infected plants (Lehrer *et al*. 2001; Izaguirre-Mayoral *et al*. 2002; Gonçalves *et al*. 2005; Lehrer *et al*. 2007).

Plants respond to biotic and abiotic stresses by triggering a cascade or network of events that starts with stress perception and ends with the expression of a battery of target genes (Pastori and Foyer 2002). The defense mechanism of plants against pathogen attack is a very good example of a biochemical type of response activated by a biotic factor, eliciting the production of specific types of enzymes (Miteva *et al*. 2005). As part of a mechanism involved in the response of plants against pathogens, a group of enzymes broadly distributed in plants, the peroxidases, plays an important role in the lignification process. Peroxidases (EC 1.11.1.7), among the number of enzymes involved in the plant defense response to pathogen attack, are often the first to show changes in their activity. They are part of non-structural proteins present in the cellular wall, in the plant cell growth mechanisms and they also participate with other enzymes in the lignin biosynthesis (Egea *et al*. 2001).

In our previous work (Scagliusi *et al*. 2008, unpublished data), we analyzed ScYLV concentration in the leaf blade and in the midribs of sugarcane infected plants. Virus concentration was significantly higher in the midribs of younger leaves. So, in the present study, we decided to determine in which part of the plant was occurring carbohydrate accumulation and verify if there was a positive relationship between virus concentration and carbohydrate accumulation in the same leaf tissue.

Considering the importance of peroxidases in the defense mechanisms of plants and to better understand the changes of sucrose assimilation and transport after ScYLV infection, the objectives of this study were to study the

Received: 9 November, 2008. Accepted: 17 July, 2009.

Original Research Paper

levels of soluble sugars (fructose, glucose and sucrose) and the activity of peroxidases in healthy and infected sugarcane varieties, with different levels of resistance and susceptibility to the virus.

MATERIALS AND METHODS

Plant material

Different varieties of sugarcane plants (SP 80-3280, SP 85-3877, SP 80-1842, SP 81-3250, SP 87-344, SP 80-185, SP 80-1816, SP 86-155, SP 87-396, SP 84-2025 e SP 71-6163) proceeding from stalks of healthy and symptomatic infected plants, were grown in pots in a greenhouse under natural sunlight (for enzyme assays). Infection by ScYLV was confirmed by DAS-ELISA (Double Antibody Sandwich – Enzyme Linked ImmunoSorbent Assay) using a specific antiserum raised against the virus. Seedlings were also used as negative controls. Other sugarcane plants cultivated in the field, following general plantation practice, were used for soluble sugar analyses.

Serological analyses

The immunological test used was DAS-ELISA, according to Clark and Adams (1977), with some modifications. ELISA plates were covered with 100 µL ScYLV antiserum (AS-ScYLV), diluted 1:1.000, in carbonate buffer (Merck), pH 9.6. The tested samples were prepared triturating 1.0 g of leaf with liquid nitrogen in 100 mM $NaKPO_4$ (Sigma), pH 6.0, 1% Na_2SO_3 (Sigma), and 0.05% Tween-20 (Bio-Rad). The extracts were centrifuged at 12.000 × *g* for 15 min and 100 µL of the supernatant were used to incubate the plates. Sugarcane seedlings were used as negative control.

Alkaline phosphatase immunoglobulin conjugate (IgG-AP) was diluted to 1:1.000 in Tris/HCl (Bio-Rad) 20 mM, pH 7.4, containing 150 mM of NaCl (Sigma), 0.05% of Tween-20 (Bio-Rad), 0.2% of BSA (Bio-Rad) and 2.0% of skimmed powdered milk (Bio-Rad). All steps were incubated for 2 h at room temperature (21°C) or overnight (4°C). The *p*-nitrophenyl phosphate (PNPP) (Sigma) solution (0.5 mg/mL) was added to the plates and final absorbance was read at 405 nm in an ELISA plate reader (BioRad), starting 1 h after incubation. Samples were considered positive when absorbance values were detected as double than that of the negative controls (seedlings). Different concentrations of purified virus (6.25, 12.5, 25, 50, 100 and 200 ng/mL) were used as a standard to determine virus concentration of tested samples.

Soluble sugar analyses

Eleven sugarcane varieties (SP 80-3280, SP 85-3877, SP 80-1842, SP 81-3250, SP 87-344, SP 80-185, SP 80-1816, SP 86-155, SP 87-396, SP 84-2025 e SP 71-6163) randomly collected in a sugarcane growing area (Centro de Tecnologia Canavieira, Piracicaba, São Paulo, Brazil) were subjected to soluble sugar analyses. The assay was divided in two steps: 1) the first one was carried out on +1 leaves (youngest fully developed leaf, totally expanded) using the leaf blade and the midribs (altogether) and 2) the second one was carried out on +1 and +4 leaves (older leaves), separating the leaf blade from the main ribs. All plants tested were approximately 15 months old (close to harvesting) and three plants of each genotype were used.

The samples were ground in liquid nitrogen using 1.0 g of fresh tissue to 5.0 mL of deionized water. The slurry was centrifuged at 12.000 × *g* for 15 min at 4°C, and the supernatants were filtered through Millipore membrane (0.45 µm). Aliquots of the extract (100 µL) were applied to a Shimadzu LC-10 AD liquid chromatograph (HPLC) equipped with a Shodex Sugar KS-801 column. The most important elution peaks were identified as sucrose, glucose and fructose, using standard solutions for comparisons and the peaks were quantified based on their corresponding area.

Statistical analysis were performed in at least three replicate samples using the analysis of variance (ANOVA) and Tukey's test at the 5% probability level.

Enzyme determinations – peroxidase activity

Peroxidase activity was measured spectrophotometrically according to Peyrano *et al*. (1997), using the phenolic substrates: guaiacol and syringaldazine. The former detects all isoforms of peroxidases and the other later detects peroxidases associated with the cell wall and the lignification processes (Goldberg *et al*. 1983).

Sample extractions were made using +1 leaves from the following sugarcane varieties: SP 84-2025, SP 81-3250 (healthy and ScYLV infected) and from SP 71-6163 (infected with or without symptoms). Liquid nitrogen was used to grind the leaves and 1.0 g of the leaf powder was homogenized with 5.0 mL of 50 mM of phosphate buffer (Merck) at pH 6.0. The extract was centrifuged at 12.000 × *g* for 10 min and the pellet was discarded. The supernatant was used for enzyme determinations.

The reaction mixture contained 5.0 mM guaiacol (Sigma), 5.0 mM H_2O_2 (Merck), 50 mM sodium phosphate buffer (Merck), pH 6.0. For each 3.0 mL of the reaction mixture, 30 µL of the leaf extract were added. Absorbance was measured spectrophotometrically (Beckman DU 40, Instruments Inc., Irvine, CA) and recorded at 470 nm every 10 s, for a 3-min period.

When syringaldazine was used as substrate, the reaction mixture contained 19 µM of syringaldazine (Sigma), 54 µM H_2O_2 (Merck) and 50 mM sodium phosphate buffer (Merck), pH 7.4, and the temperature was maintained at 35°C. For each 3.0 mL of the reaction mixture, 120 µL of the leaf extract were added. Absorbance was measured as above at 530 nm and recorded every 10 s, for a 3-min period.

Statistical analysis were performed in at least three replicate samples using the analysis of variance (ANOVA) and Tukey's test at the 5% probability level.

RESULTS

Soluble sugars analyses

In order to determine the concentration of soluble sugars in the leaves of healthy and infected sugarcane plants, a preliminary test was done to verify which stage of development would be more appropriate to obtain an accurate result. Sugarcane plants infected by ScYLV were compared to genetically identical healthy ones, obtained from meristem tip culture. Our preliminary results showed that only plants close to harvesting (12–15 months old) had enough concentration of soluble sugars in the leaves, which could be measured in a consistent way. So, the healthy plants obtained from meristem tip culture were too young for that kind of analysis. For this reason, sugarcane plants collected in the field were used for these experiments instead, fact that limited this study to the available varieties occurring in the field. Firstly, the plants were tested by DAS-ELISA to evaluate the presence (or absence) of the virus. After obtaining DAS-ELISA results, three groups of plants were formed: 1) healthy, 2) infected without symptoms and 3) infected with symptoms. Soluble sugar analyses were accomplished in +1 leaves (test 1), and in +1 and +4 leaves (test 2) as indicated in each one of the experiments.

The content of sugars in the leaves of infected varieties with symptoms (group 3) was significantly higher than in healthy plants or in infected ones without symptoms (groups 1 and 2, **Fig. 1**). The infected variety SP 71-6163 (with symptoms) showed the highest sugar content in +1 leaves, four times higher than the observed in groups 1 and 2. There was no significant difference among the average of group 1 (healthy plants) and the average of group 2 (infected plants without symptoms). The differences observed in the content of sugars, among the varieties of the same group can be attributed to genetic differences between them. The results obtained from the infected variety SP 71-6163 was 78.6 mg/g fm, which was 50% higher than the ones observed in other varieties of the same group.

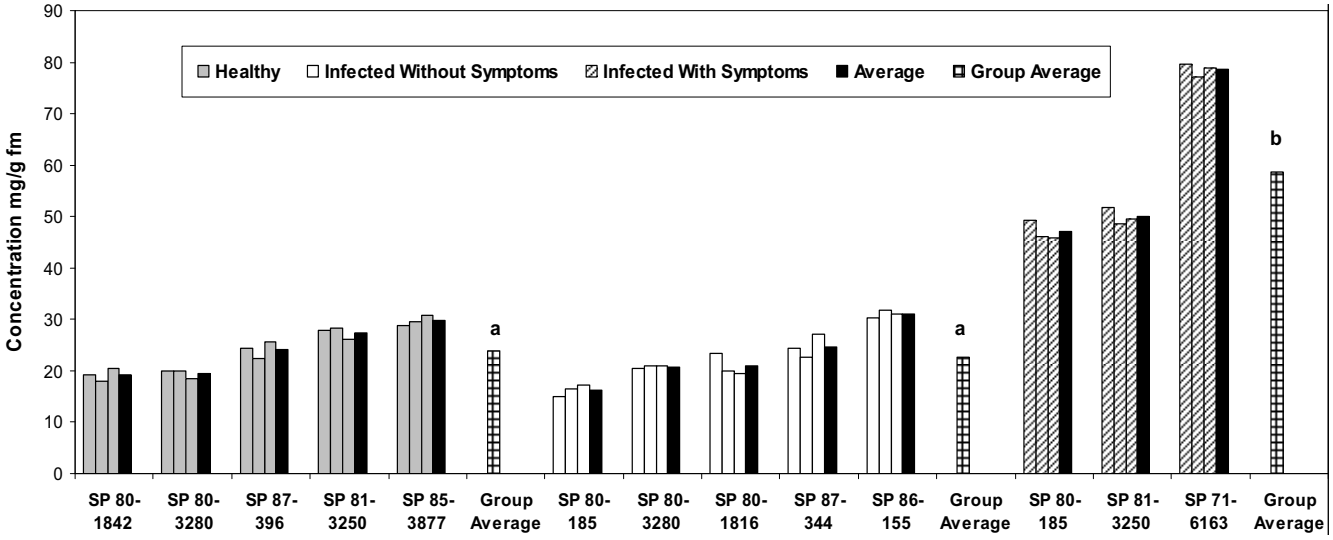

Fig. 1 Soluble sugars concentration (sucrose, glucose and fructose) measured on +1 leaves from sugarcane plants. Isolated columns represent the average of each group. Columns showing the same letters do not differ significantly (Tukey's, 5%).

Soluble sugars distribution in +1 and +4 leaves

In order to determine in which part of the leaf occurred the accumulation of soluble sugars, chromatographic analyses were made separating the leaf blade from the main ribs, only in three sugarcane varieties: SP 84-2025, SP 81-3250 and SP 71-6163. The analyses were made in +1 and +4 leaves, comparing healthy plants with infected ones. All plants from sugarcane variety SP 71-6163 were infected by the virus, so no healthy plants were available. Comparisons were made between infected plants with and without symptoms.

Our results revealed a highly significant increase (140 %) in the main ribs and also an increase of 100% in the leaf blade of +1 leaves (younger leaves) of infected plants (**Fig. 2**). In older leaves (+4 leaves), an increase in the content of sugar of 61% in the leaf blade and 85% in the main ribs was also observed. Although the effect of viral infection on the content of soluble sugars was evident on all sugarcane tested varieties, the increase observed on the leaf blade of +4 leaves of SP 71-6163 and SP 81-3250 varieties (**Fig. 2B, 2C**) did not differ significantly. A significant decrease in the concentration of soluble sugars on +4 leaves was observed compared to +1 leaves, both in the leaf blade and in the main ribs, which was associated with leaf senescence, and it was more evident in infected plants with symptoms (**Fig. 2**).

Peroxidases activity

The results of the enzymatic activity using guaiacol as a substrate are shown in **Fig. 3.** This figure shows the linearity of the enzymatic reaction with a very high R^2 value ($R^2 = 0.999$ for healthy leaves and $R^2 = 0.9934$ for infected leaves). A similar alteration pattern was observed in all tests of guaiacol-peroxidase activity performed in our study. Infected plants exhibited peroxidase activity 50% higher than that of the healthy ones. The same pattern was also observed while comparing infected plants with and without symptoms of the sugarcane variety SP 71-6163 (**Fig. 4**). The results of syringaldazine peroxidase activity are represented in **Fig. 5**, showing a clear pattern of alteration: infected plants with symptoms exhibited higher enzymatic activity (140%) compared to the healthy controls or infected plants without symptoms.

DISCUSSION

The current study demonstrates the impact of ScYLV on the concentration of soluble sugars metabolism in sugarcane leaves. The content of soluble sugars in the leaves of different varieties were organized in three groups: 1) healthy; 2) infected plants without symptoms and 3) infected plants with symptoms, according to the data obtained by DAS-ELISA. Our results showed that the content of sugars in the leaves of infected varieties with symptoms (group 3) was significantly higher than the ones observed in healthy plants or in plants without symptoms, represented by groups 1 and 2. The content of soluble sugars from groups 1 and 2 did not differ significantly. These results indicate that the infection *per se* did not change the concentration of soluble sugars, but a significant increase was observed in infected plants with symptoms.

A significant difference in the content of soluble sugars was also observed among the averages of plants from varieties represented by group 3. We could observe on figure 1 that the variety SP 71-6163 showed concentration of soluble sugars up to 62% higher than the other two varieties (SP 81-3250 and SP 80-185), also infected by the virus and showing symptoms of the disease. Although the accumulation of sugars in the leaves has also been observed in these two varieties, the values observed in variety SP 71-6163 was very superior, justifying the decline in productivity of this variety (Vega *et al.* 1997), fact that did not happen with the variety SP 81-3250.

Our results confirm the accumulation of soluble sugars in the leaves of infected symptomatic plants, suggesting a phloem dysfunction which is associated to the virus infection in these tissues.

Accumulation of carbohydrates in leaves was also observed in potato plants infected by PLRV (*Potato leafroll virus*), modifying the carbohydrate transport for tuber formation (Peters 1987). Similar effects with other viruses also restricted to the phloem were described, as in the case of *Abutilon mosaic virus* in leaves of *Abutilon striatum* (Lohaus *et al*. 2000). Previous reports similar to ours were observed by Gonçalves *et al.* (2005) and Lehrer *et al*. (2007), working with the same virus (ScYLV). Their results showed an increase on the content of total sugars obtained from infected sugarcane leaves, and sucrose was the sugar that accumulated most. However, contrary to our results, the highest carbohydrate concentration was found in infected asymptomatic plants (Lehrer *et al*. 2007). This conclusion, however, do not agree with the decline in productivity in infected symptomatic sugarcane varieties (Vega *et al*. 1997; Rassaby *et al*. 2004), although non-symptomatic plants may sometimes present growth reduction.

Translocation of carbohydrates in sugarcane plants occurs through the leaf blade and leaf sheath, via phloem and then to the center of the stalk and downward to the roots (Alexander 1973). Consequently, a perfect phloem system is a requirement for an appropriate movement of sugars

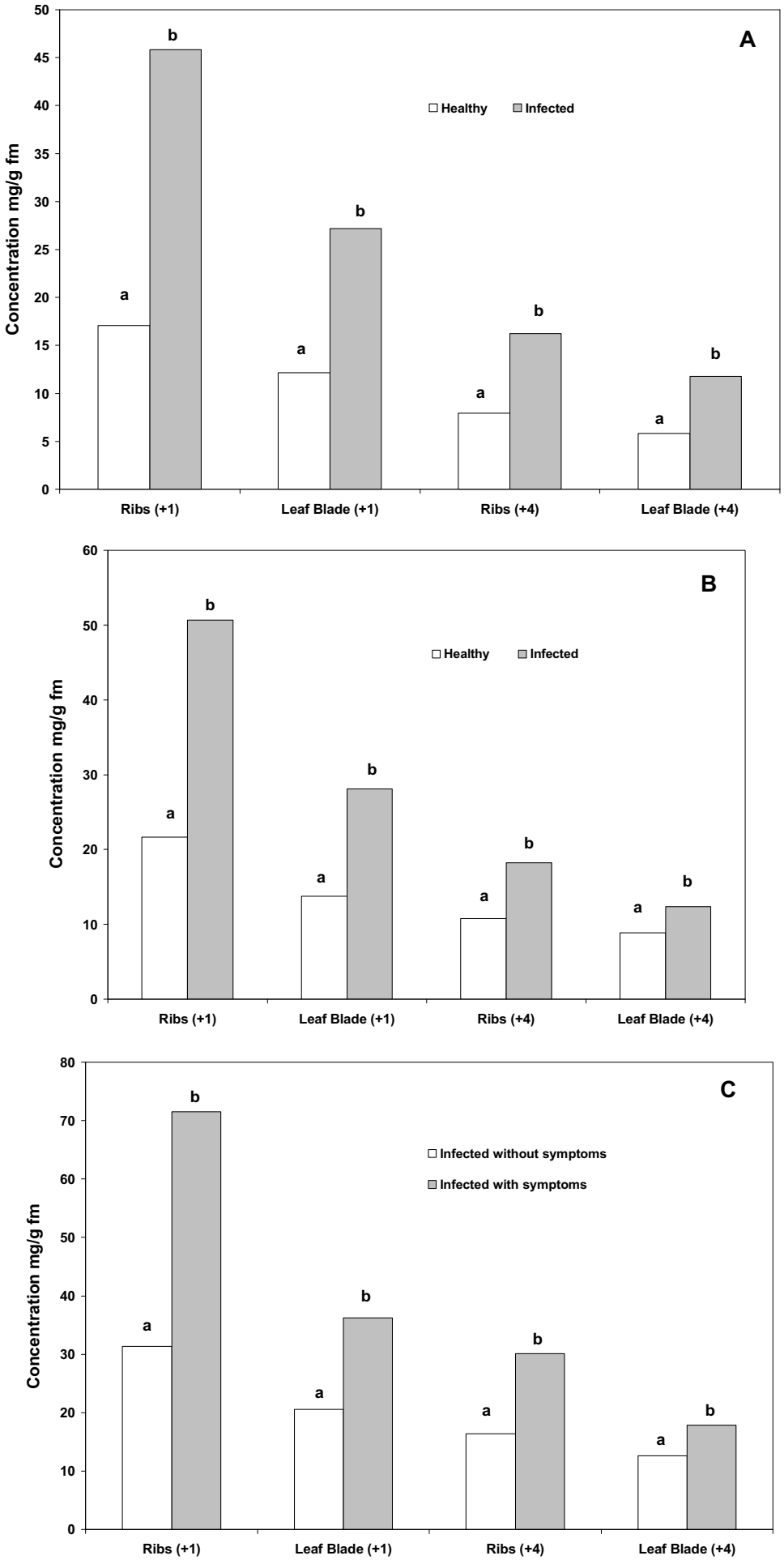

Fig. 2 Soluble sugars distribution in the midribs and in the leaf blade of +1 and +4 leaves of three sugarcane varieties (A, B and C). Columns with the same letters do not differ signicantly, comparison between same leaf and same tissue (Tukey's, 5%)

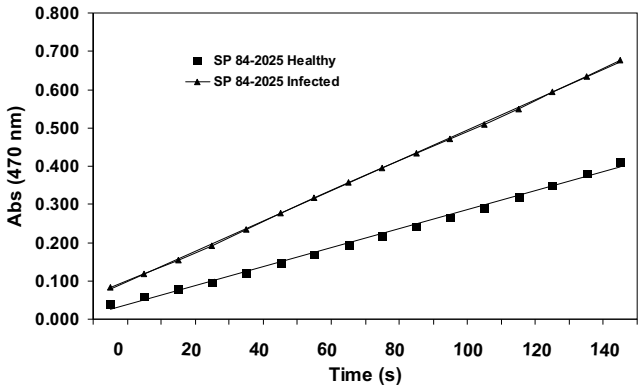

Fig. 3 Peroxidase activity test (using guaiacol as a substrate) in healthy and infected leaves of the sugarcane variety SP 84-2025.

inside the plant (Du *et al*. 2000; Izaguirre-Mayoral *et al*. 2002).

Phloem and companion tissues of virus infected plants are usually modified and become dysfunctional. Vega *et al.* (1997) observed the presence of isometric particles in the phloem companion cells from sugarcane plants infected by ScYLV. These cells presented cytopathic alterations, such as, disintegration of the nuclear membrane, presence of electron-dense material and formation of numerous vesicles. *Luteoviruses* are known by having its replication restricted to the phloem cells, where they tend to accumulate almost exclusively in the sieve elements and in the companion cells of the phloem (Mayo and Ziegler-Graff 1996). In spite of the existence of a large number of plasmodesma connections coming out from the phloem complex, very few *luteovirus* particles are found in the mesophyll or around the vascular bundle cells, suggesting that these viruses are restricted to move through specialized plasmodesma probably due to possible differences in their structure and physiology (Van Bel 1993; Nelson and Van Bel 1998).

The movement of photoassimilates from the mesophyll cells to the sieve tubes can occurs via two contrasting pathways or a combination of both: a symplastic and an apoplastic pathway (Beebe and Evert 1992; Russin *et al*. 1996). Phloem sugar loading in many species occurs via apoplastic pathway; consequently it is unlikely to establish a relationship between plasmodesma size exclusion limit (SEL) and an alteration in the transport of sugars. On the other hand, if sugar loading to the phloem cells in other species occur through the symplastic pathway (Turgeon 1989, 1995; Kim *et al*. 2005), it would be possible to propose that the alterations in the metabolism of plants infected by viruses, resulting in a carbohydrate accumulation, are related to an alteration in the plasmodesmata diameter and their function.

Alterations in the metabolism and in the transport of carbohydrates in plants infected by luteovirus can be better understood when we associated their replication mechanisms to these alterations. The replication process of a luteovirus (single strand RNA positive sense) occurs in the cytoplasm of the host cell (Matthews 1991). After replication, movement proteins coded by the virus start a new process, transporting viral genome into plasmodesmata direction, local where cell-to-cell trafficking of macromolecules happens (Carrington *et al*. 1996; Itaya *et al*. 1998). Movement proteins (MP) can modify cells plasmodesma altering its structure and function (Atkins *et al*. 1991). Previous workers (Herbers *et al*. 1997; Hofius *et al*. 2001) showed the effect of PLRV movement protein (MP17), on the metabolism of carbohydrates and in the photosynthetic process in tobacco transgenic plants. The transformed plants, containing the virus movement protein, developed growth retardation and severe phenotypic changes of source leaves paralleled by a drastic accumulation of soluble sugars and starch (Hofius *et al*. 2001). The increase in carbohydrates concentration, reduced growth and necrosis of leaves of transformed plants, suggest that these plants suffer a limitation in the capacity to export sugars. Ultrathin sections from leaves of infected plants revealed alterations in the plasmodesma of phloem cells, while plasmodesmata from mesophyll cells did not exhibited any modifications. These data favor the hypothesis that the sieve elements and phloem companion cells are the primary targets of movement protein MP17 and that the movement protein could contribute in altering plasmodesmata size exclusion limit (SEL) and carbohydrates metabolism.

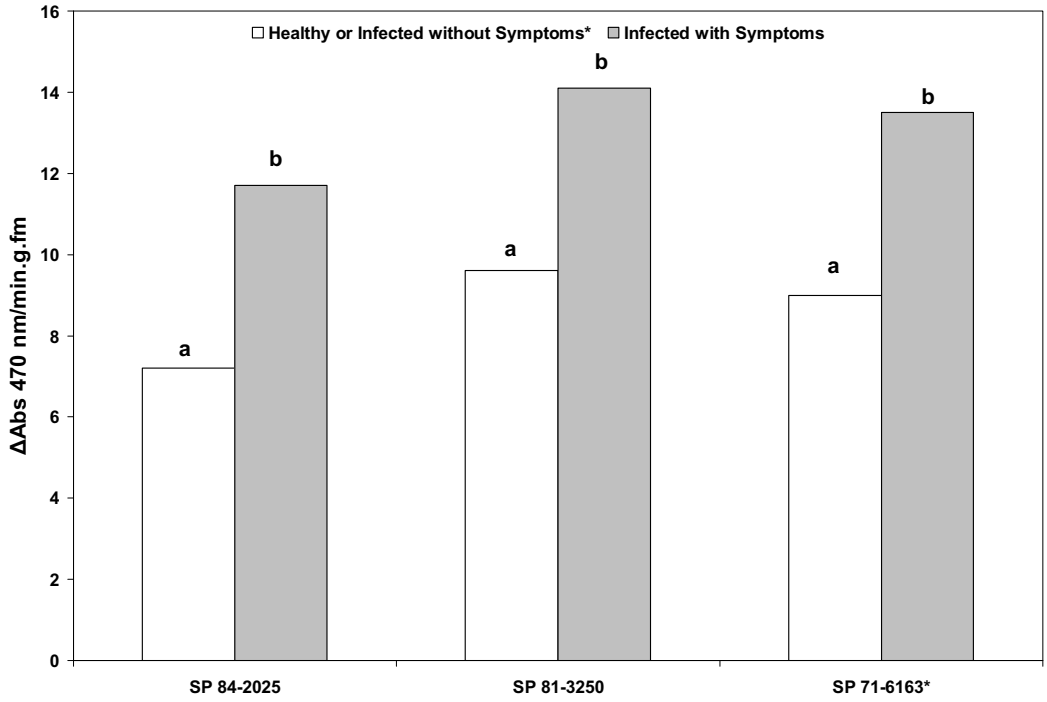

Fig. 4 Guaiacol peroxidase activity in healthy and infected plants. For sugarcane variety SP 71-6163, comparison was made between infected plants without symptoms and with symptoms. Columns showing the same letter do not differ significantly (Tukey's, 5%). Comparisons were made between the same variety. The enzymatic activity (angular coefficient) was calculated in units of Δ_{470}/min/g of fresh matter and the data estimated individually for each of the three tested plants.

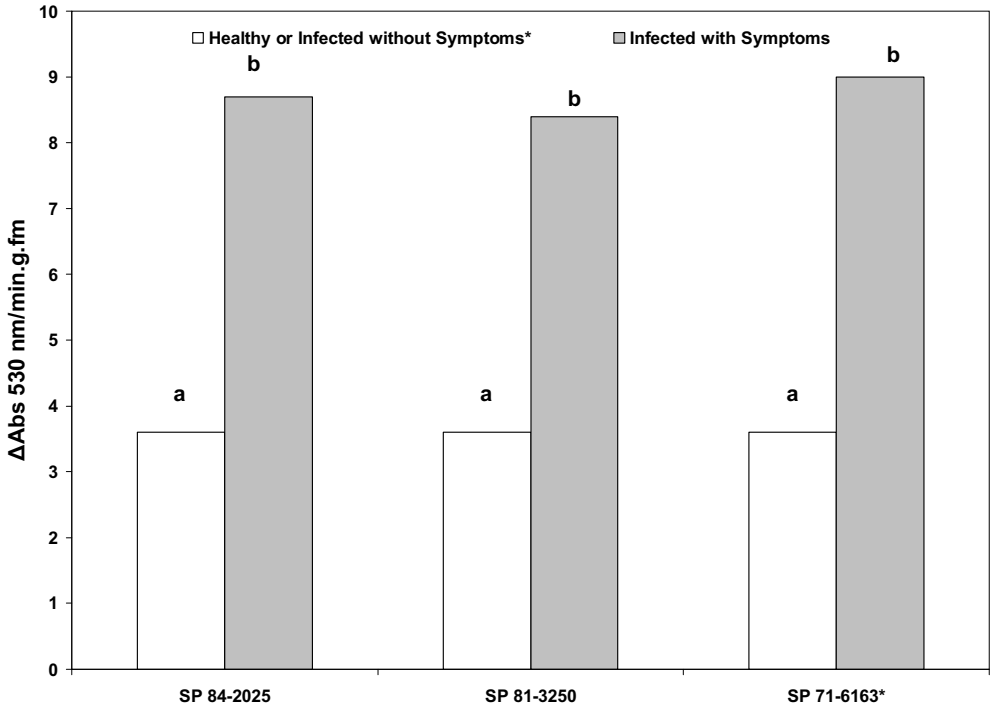

Fig. 5 Syringaldazine peroxidase activity in healthy and infected plants. For sugarcane variety SP 71-6163, comparison was made between infected plants without symptoms and with symptoms. Columns showing the same letter do not differ significantly (Tukey's, 5%). Comparisons were made between the same variety. The enzymatic activity (angular coefficient) was calculated in units of Δ_{530}/min/g of fresh matter and the data estimated individually for each of the three tested plants.

Further investigations on carbohydrate metabolism and its transport in each pathosystems are required to understand the basis of the interaction of a plant pathogen-system with more details.

There are many reasons why a contact with pathogens alter plant primary metabolism. Plants possess several defense mechanisms against biotic and abiotic stresses, both preformed and inducible, promoting the synthesis and accumulation of defense-related secondary metabolites, which may prevent or reduce further damage from pathogens (Van Loon 1976; Van Loon *et al*. 2006). The induction of defense mechanisms is cost-intensive causing an increase of photoassimilates in the plant. Some of the responses involved in a plant-pathogen system are the accumulation of phenolic compounds linked to the cell wall, phytoalexins synthesis and formation of structural proteins and the activation of enzymes involved in the lignification process.

The peroxidases and phenylalanine ammonia lyase (PAL) are some of the enzymes involved in the lignification process, which activities work as a response indicator of plants to stress (Dixon *et al*. 1994; Lee *et al*. 2007). Our results showed a significant increase of peroxidases in symptomatic infected plants, evidencing a high correlation degree among the increase of enzyme activity and manifestation of symptoms. This increase was observed when we used guaiacol and syringaldazine as substrates. Guaiacol detects all peroxidases forms, while syringaldazine detects peroxidases associated with cell wall lignification. Similar results to ours were obtained by Souza *et al.* (1999), who also observed a sharp increase in peroxidases activity in corn plants infected by *Maize common mosaic virus*. The tests were carried out in resistant and susceptible plants, comparing healthy and infected ones. Their results showed an increase in peroxidase activity in two situations: in symptomatic inoculated plants (susceptible) and in healthy plants but resistant to the virus.

The results obtained in the present work suggest that antioxidant enzymes (like peroxidases) can be activated by different types or combinations of stress, triggering some of the cell defense mechanisms against pathogens. Further investigations should be aimed at investigating the role of these antioxidant enzymes induced by pathogenic infections in order to understand their roles under various stress conditions and developmental stages.

REFERENCES

Abu Ahmad Y, Girard JC, Fernandez E, Pauquet J, Lockhart B, Letourmy P, Rott P (2007) Variation in virus populations and growth characteristics of two sugarcane cultivars naturally infected by *Sugarcane yellow leaf virus* in different geographical locations. *Plant Pathology* **56**, 743-754

Alexander AG (1973) *Sugarcane Physiology*, Elsevier Scientific Publishing Co., Amsterdam, pp 25-66

Atkins D, Hull R, Wells B, Roberts K, Moore P, Beachy RN (1991) The *Tobacco mosaic virus* 30 K movement protein in transgenic tobacco plant is localized to plasmodesmata. *Journal of General Virology* **72**, 209-211

Balachandran S, Hurry VM, Kelley SE, Osmond CB, Robinson SA, Rohozinski J, Seaton GR, Sims DA (1997) Concepts of plant biotic stress. Some insights into the stress physiology of virus-infected plants, from the perspective of photosynthesis. *Physiologia Plantarum* **100**, 203-213

Beebe DU, Evert RF (1992) Photoassimilate pathways and phloem loading in the leaf of *Moricandia arvensis* (L.) DC (*Brassicaceae*). *International Journal of Plant Sciences* **153**, 61-77

Bertamini M, Muthuchelian K, Nedunchezhian N (2004) Effect of Grapevine leafroll on the photosynthesis of field grown grapevine plants (*Vitis vinifera* L. cv. Lagrein). *Journal of Phytopathology* **152**, 145-152

Bowles DJ (1990) Defense-related proteins in higher plants. *Annual Review of Biochemistry* **59**, 873-907

Carrington JC, Kasshaw KD, Mahajan SK, Shaad MC (1996) Cell-to-cell and long distance transport of viruses. *Plant Cell* **8**, 1669-1681

Clark MF, Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34**, 475-483

Comstock JC, Muller JD (2003) Incidence and spread of *Sugarcane yellow leaf virus* in sugarcane clones in the CP cultivar development program at Canal Point. *Journal of the American Society of Sugarcane Technologists* **23**, 71-78

Dixon RA, Harrison MJ, Lamb CJ (1994) Early events in the activation of plant defense responses. *Annual Review of Phytopathology* **32**, 479-501

Du YC, Nose A, Kondo K, Wasano K (2000) Diurnal changes in photosynthesis in sugarcane leaves. II. Enzyme activities and metabolite levels relating to sucrose and starch metabolism. *Plant Production Science* **3**, 9-16

Egea C, Ahmed SA, Candela M, Candela ME (2001) Elicitation of peroxidase activity and lignin biosynthesis in pepper suspension cells by *Phytophthora capsici*. *Journal of Plant Physiology* **158**, 151-158

Fraser RS (1987) *Biochemistry of Virus Infected Plants*, Research Studies Press, Letchworth/John Wiley and Sons, New York, USA, 259 pp

Goldberg R, Catesson AM, Czaninski Y (1983) Some properties of syringal-

dazine oxidase, a peroxidase specifically involved in the lignification processes. *Zeitschrift für Pflanzenphysiologie* **110**, 267-279

Gonçalves MC, Vega J, Oliveira J, Gomes MMA (2005) *Sugarcane yellow leaf virus* infection leads to alterations in photosynthetic efficiency and carbohydrate accumulation in sugarcane leaves. *Fitopatologia Brasileira* **30**, 10-16

Herbers K, Tacke E, Hajirezaei M, Krause KP, Melzer M, Rohde W, Sonnewald U (1997) Expression of a luteoviral movement protein in transgenic plants leads to carbohydrate accumulation and reduced photosynthetic capacity in source leaves. *The Plant Journal* **12**, 1045-1056

Hofius D, Herbers K, Melzer M, Omid A, Tacke E, Wolf S, Sonnewald U (2001) Evidence for expression level-dependent modulation of carbohydrate status and viral resistance by the *Potato leafroll virus* movement protein in transgenic tobacco plants. *The Plant Journal* **28**, 529-543

Itaya A, Woo YM, Masuta C, Bao Y, Nelson RS, Ding B (1998) Developmental regulation of intercellular protein trafficking through plasmodesmata in tobacco leaf epidermis. *Plant Physiology* **118**, 373-385

Izaguirre-Mayoral ML, Carballo O, Alceste C, Romano M, Nass HA (2002) Physiological performance of asymptomatic and yellow leaf syndrome-affected sugarcanes in Venezuela. *Journal of Phytopathology* **150**, 13-19

Kim I, Cho E, Crawford K, Hempel FD, Zambryski PC (2005) Cell-to-cell movement of GFP during embryogenesis and early seedling development in *Arabidopsis*. *Proceedings National Academy of Science* **102**, 2227-2231

Kombrink E, Somssich IE (1995) Defense responses of plants to pathogens. In: Andrews JH, Tommerup IC (Eds) *Advances in Botanical Research* (incorporating Advances in Plant Pathology) Vol 21, Academic Press, London, pp 1-34

Ladygina ME, Babosha V (1996) Physiological and biochemical mechanisms governing viral pathogenesis and plant antiviral activity. *Russian Journal of Plant Physiology* **43**, 636-647

Lee BR, Kim K, Jung WJ, Avice JC, Ourry A, Kim TH (2007) Peroxidases and lignification in relation to the intensity of water-deficit stress in white clover (*Trifolium repens* L.). *Journal of Experimental Botany* **58**, 1271-1279

Lehrer A, Meinzer R, Moore P, Komor E (2001) Physiological consequences of *Sugarcane yellow leaf virus* infection on the sugarcane plant. *Proceedings of the International Society of Sugarcane Technologists* **24**, 657-659

Lehrer AT, Moore PH, Komor K (2007) Impact of *Sugarcane yellow leaf virus* (ScYLV) on the carbohydrate status of sugarcane: comparison of virus-free plants with symptomatic and asymptomatic virus-infected plants. *Physiological and Molecular Plant Pathology* **70**, 180-188

Lohaus G, Heldt HW, Osmond CB (2000) Infection with phloem limited *Abutilon mosaic virus* causes localized carbohydrate accumulation in leaves of *Abutilon striatum*: relationships to symptom development and effects on chlorophyll fluorescence quenching during photosynthetic induction. *Plant Biology* **2**, 161-167

Matthews REF (1991) *Plant Virology* (3rd Edn), Academic Press Inc., San Diego, pp 335-368

Mayo MA, Ziegler-Graff V (1996) Molecular biology of luteoviruses. *Advances of Virus Research* **46**, 416-468

Miteva E, Hristova D, Nenova V, Maneva S (2005) Arsenic as a factor affecting virus infection in tomato plants: changes in plant growth, peroxidase activity and chloroplast pigments. *Scientia Horticulturae* **105**, 343-358

Moonan F, Mirkov E (2002) Analyses of genotypic diversity among North, South, and Central American Isolates of *Sugarcane yellow leaf virus*: evidence for Colombian origins and for intraspecific spatial phylogenetic variation. *Journal of Virology* **76**, 1339-1348

Naderi M, Berger PH (1997) Effects of chloroplast targeted potato virus Y coat protein on transgenic plants. *Physiological and Molecular Plant Pathology* **50**, 67-83

Nelson RS, Van Bel AJE (1998) The mystery of virus trafficking into, through and out of the vascular tissue. *Progress in Botany* **59**, 476-533

Pastori GM, Foyer CH (2002) Common components, networks and pathways of cross-tolerance to stress. The central role of 'redox' and abscisic-acid-mediated controls. *Plant Physiology* **129**, 460-468

Peters D (1987) Spread of viruses in potato crops. In: Bokx JA, Van der Want JPH (Eds) *Viruses of Potatoes and Seed-Potato Production* (2nd Edn), Pudoc, Wageningen, pp 126-145

Peyrano G, Taleisnik E, Quiroga M, Forchetti S, Tigier H (1997) Salinity effects on hydraulic conductance, lignin content and peroxidase activity in tomato roots. *Plant Physiology and Biochemistry* **35**, 387-393

Rassaby L, Girard JC, Lemaire O, Costet L, Irey MS, Kodja H, Lockhart B, Rott P (2004) Spread of *Sugarcane yellow leaf virus* in sugarcane plants and fields on the islands of Réunion. *Plant Pathology* **53**, 117-125

Russin WA, Evert RF, Vanderveer PJ Sharkey TD, Briggs SP (1996) Modification of a specific class of plasmodesmata and loss of sucrose export ability in the *sucrose export defective1* maize mutant. *The Plant Cell* **8**, 645-658

Scagliusi SM, Lockhart BEL (2000) Transmission, characterization and serology of a luteovirus associated with yellow leaf syndrome of sugarcane. *Phytopathology* **90**, 120-124

Schenk S (1990) Yellow leaf syndrome – a new sugarcane disease. Hawaiian Sugar Planters Association: Annual Report

Souza IRP, Oliveira E, Purcino AC, Gama EG, Paiva E (1999) Atividade da peroxidase em linhagens de milho resistentes e susceptíveis ao mosaico causado por potyvirus. *Fitopatologia Brasileira* **24**, 355

Turgeon R (1989) The sink-source transition in leaves. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 119-138

Turgeon R (1995) The selection of raffinose oligosaccharides as translocates in higher plants. In: Madore MM, Lucas WJ (Eds) *Carbon Partitioning and Source-Sink Interactions in Plants*, American Society of Plant Physiologists, Rockville, USA, pp 195-203

Van Bel AJE (1993) Strategies of phloem loading. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**, 397-411

Van Loon LC (1976) Systemic acquired resistance, peroxidase activity and lesion size in tobacco reacting hypersensitively to *Tobacco mosaic virus*. *Physiology Plant Pathology* **8**, 231-242

Van Loon LC, Rep M, Pieterse CMJ (2006) Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology* **44**, 135-162

Vega J, Scagliusi SM, Ulian EC (1997) Sugarcane yellow leaf disease in Brazil: Evidence of association with a luteovirus. *Plant Disease* **81**, 21-26

Viswanathan R, Balamuralikrishnan M (2004) Detection of sugarcane yellow leaf virus, the causal agent of yellow leaf syndrome in sugarcane by DAS-ELISA. *Archives of Phytopathology and Plant Protection* **37**, 169-176

Zaitlin M, Hull R (1987) Plant virus-host interactions. *Annual Review of Plant Physiology* **38**, 291-315