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Effects of *Sugarcane yellow leaf virus* on Sugarcane Yield and Root System Development

Antônio Carlos M. Vasconcelos[1†] • Marcos C. Gonçalves[2*] • Luciana R. Pinto[1] • Marcos G. A. Landell[1] • Dilermando Perecin[3]

[1] Instituto Agronômico, Centro de Cana - IAC, CP 206. 14001-970, Ribeirão Preto, SP, Brazil
[2] Instituto Biológico, Lab. Fitovirologia, Av. Cons. Rodrigues Alves 1252. 04014-002, São Paulo, SP, Brazil
[3] UNESP, Fac. Ciências Agrárias e Veterinárias/Dep. Ciências Exatas. 14884-900 Jaboticabal, SP, Brazil
[†] *In memorian*

Corresponding author: * **mcgon@biologico.sp.gov.br**

ABSTRACT

Sugarcane yellow leaf syndrome (YLS) causes significant yield losses in susceptible sugarcane varieties. In Brazil, YLS was not recognized as economical importance until early 1990s. However, with the drastic epidemics of the viral form of the disease in variety SP71-6163, breeders began to take into account its occurrence during the selection stages and its effects on vegetative development. The objective of the present work was to evaluate the effects of the *Sugarcane yellow leaf virus* (ScYLV), its main causal agent in Brazil, on sugarcane yield and root system development. The experiment was conducted in Ribeirão Preto, SP, Brazil, on Typic Hapludox soil, in variety IAC89-2135 during the plant cane cycle. ScYLV diagnosis was assayed by DAS-ELISA and RT-PCR for discrimination between infected and uninfected plants. Plants grown from ScYLV infected stalks showed typical infection symptoms as a consequence of virus perpetuation in the stalk. The infected plants showed significant reduction on roots dry weight and fresh weight of the above plant parts and an increase in Brix and sucrose content in the stalks. Although infected plants maintained regular root system vertical architecture, root dry weight was reduced and negatively correlated with fresh weight and stalk number, showing that alterations in root and vascular systems constitute important effects from ScYLV infection.

Keywords: *Luteoviridae*, *Polerovirus*, sugarcane breeding, sugarcane yellow leaf syndrome
Abbreviations: bp, base pair(s); **DAS-ELISA**, double antibody sandwich-enzyme linked immunosorbent assay; **RT-PCR**, reverse transcriptase-polymerase chain reaction; **ScYLV**, *Sugarcane yellow leaf virus*; **YLS**, yellow leaf syndrome

INTRODUCTION

Sugarcane diseases are amongst the main factors contributing to sugarcane yield losses worldwide. Sugarcane yellow leaf syndrome (YLS) has been associated with expressive yield losses in susceptible sugarcane varieties (Abu Ahmad *et al*. 2006). Symptoms of infection are characterized by intense yellowing of the midrib on the abaxial surface of mature leaves. Older leaves show a red coloration of the midrib on the adaxial surface. Afterwards the leaf blade becomes bleached, proceeding from the tip toward the base of the leaf, and tissue necrosis can eventually take place (Gonçalves *et al*. 2005). Frequently, there is also reduction in growth resembling that of drought stress. Symptoms of YLS have been attributed to many causes, both biotic and abiotic. The cause of the disease known as yellow leaf of sugarcane in Brazil is the infection by *Sugarcane yellow leaf virus* (ScYLV) (Vega *et al*. 1997; Maia *et al*. 2000; Moonan *et al.* 2000). ScYLV has been shown to cause significant yield losses in susceptible cultivars in several sugarcane growing countries (Lehrer and Komon 2008; Viswanathan *et al.* 2008; Xie *et al.* 2009). In Brazil, losses from 20 to 30% were reported in the variety SP71-6163 in the early 1990s (Vega *et al*. 1997). The losses caused by ScYLV in other Brazilian commercial varieties is poorly known, but the virus became endemic in the main cultivated areas making the development of resistant cultivars essential in sugarcane breeding programs. The Agronomic Institute of Campinas (IAC) sugarcane breeding program works applying a phenotypical clone selection strategy that takes 6 to 7 years for the regional phase assessments, and 10 to 12 years in total to release a genotype as a commercial variety (Landell *et al*. 2005). In IAC sugarcane breeding program, the main traits evaluated during selection of cultivars are productivity, sucrose content and disease resistance. The variety IAC89-2135 showed favorable characteristics and superiority compared to other commercial varieties until 1998, when the first symptoms of ScYLV infection were observed causing yield decline. Similarly, Comstock and Miller (2003) reported that in the CP-cultivar breeding program at Canal Point, USA, the incidence of samples with ScYLV infection generally increased from the first to the last stage of selection. Additionally, they emphasize that if marker assisted selection can be developed for ScYLV resistance, the process for the development of resistant cultivars would be greatly enhanced.

Several works have discussed the effects of viruses upon the above ground plant parts (Schenck *et al*. 2001; Izaguirre-Mayoral *et al*. 2002; Fontaniella *et al.* 2003; Rassaby *et al.* 2003; Gonçalves *et al.* 2005; Lehrer *et al*. 2007), however, their effects on the root system are poorly understood, partially because this type of study is extremely laborious and the roots are also affected by the soil physicochemical variability (Vasconcelos *et al*. 2003). For this reason, the present work was performed to investigate the effects of the ScYLV infection on variety IAC89-2135 root system development and its relationship with the yield.

MATERIALS AND METHODS

Field trials

A field study was conducted in Ribeirão Preto, São Paulo state, Brazil, on a Typic Hapludox soil, in variety IAC89-2135 planted

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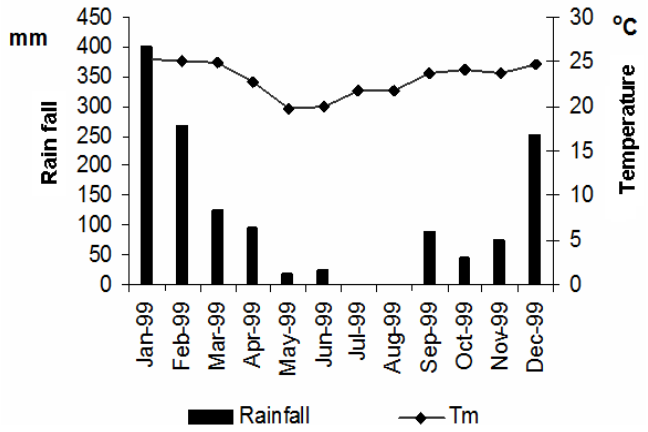

Fig. 1 Experimental field rainfall and air temperature averages conditions during 1999. Tm= air temperature.

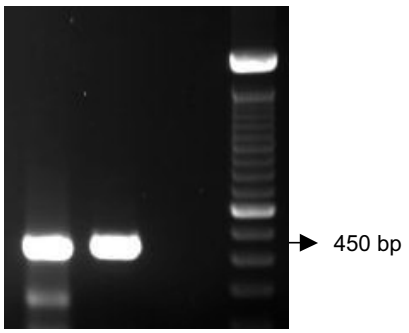

Fig. 2 Reverse transcription-PCR of total RNA extracted from sugarcane leaf samples. Lanes: 1, plant showing yellowing symptoms; 2, positive control (purified ScYLV); 3, negative control (healthy sugarcane plant); 4, 100 bp DNA molecular weight marker.

in February 1999. The rainfall and air temperature averages during vegetative period were typical of the regional climatic conditions, showing dry period between May and August (**Fig. 1**).

Field plots were 100 m wide by 50 m long, with 1.4 m between sugarcane rows. Nine months after planting, samples were randomly taken from 5 places where plants showed ScYLV symptoms, and from other 5 places with symptomless plants. Stalks were counted in 1 m of row, cut and fresh weighed. In each plot, stalks were counted in 1 m of row (stalk number per meter), cut and fresh weighed (fresh weight per meter) and 10 stalks were taken for the pooled analysis of Brix (%), fiber (%) and sucrose contents (SC). The samples obtained in each randomly location, were taken as replications for statistical analysis. In each sampling location, the roots were quantified from 3 depths: 0 to 20 cm, 20 to 40 cm and 40 to 60 cm. The trenches were dug under the cane row for extraction of soil monoliths with roots, measuring 150 cm, in the traverse sense to the line, and 20 cm longitudinal to the line. The roots were washed with pressurized water jet on 2 mm mesh sieves. Roots were collected and dried at 70°C, for 48 hours, until the weight was constant. After drying, the samples were sifted to separate vegetable impurity for subsequent weighing. Treatments were compared by the variance analysis (F statistics) and Tukey's test. Linear regression was done, separately between root dry weight and above ground plant parts attributes for each treatment (healthy or infected).

ScYLV diagnosis

Twenty sugarcane plants from both treatments were randomly collected and tested by DAS-ELISA using a ScYLV-specific polyclonal antiserum (Scagliusi and Lockhart 2000). Leaf tissue was diluted 1: 10 (w/v) in 100 mM sample buffer. Immunoglobulin-alkaline phosphatase conjugate, prepared with the specific antiserum, was diluted 1: 500 in sample buffer and the readings at 405 nm were performed after 30 min of incubation with the substrate *p*-nitrophenyl phosphate. As positive and negative controls were used 10 ng of purified virus and healthy plants grown in a greenhouse, respectively.

ScYLV infection in the same plants submitted to DAS-ELISA was also assayed by RT-PCR with the specific primer pair P1f/P2r (Gonçalves *et al*. 2002) based on the coat protein coding region of ScYLV (**Fig. 2**). The antisense primer P2r was used to reverse transcription reactions using total RNA extracted from leaf tissue. The reverse transcription was performed with 1 µl of antisense primer and 2 µg of RNA using the cMaster Rtplus kit (Eppendorf–Netheler, Hamburg, Germany) according manufacturer instructions. The PCR consisted of 1 µl of 10 µM sense primer, 1 µl of 10 µM antisense primer, 3 µl of cDNA samples and the reaction mix of cMaster Rtplus kit. PCR protocol followed as previously described (Gonçalves *et al*. 2002).

Perpetuation of ScYLV in stalks

In order to test virus presence and transmission in the vegetative material from a planting cycle to another, stalks from infected and healthy plants were planted separately in adjacent areas of 4 rows of 3 m. Symptoms were monitored and plants were photographed to illustrate the hypothesis of virus perpetuation. However, quantitative surveys of roots and above plant parts were not performed in these plots. At the end of the experiment, 20 symptomatic and 20 symptomless plants were randomly collected and their leaves tested for ScYLV presence by DAS-ELISA and RT-PCR.

RESULTS AND DISCUSSION

The experiments led us to draw some insights on the impact of ScYLV in sugarcane yield and root system development, confirming that symptoms caused by ScYLV were not limited to leaves and stalks of the infected plants.

Yield characteristics

Among the five features used to evaluate the effect of ScYLV on sugarcane yield, stalk number and fiber content showed no significant differences between infected and uninfected sugarcane plants. On the other hand, significant differences were observed for fresh weight, Brix and sucrose content (**Table 1**).

Fresh weight reduction of ScYLV infected plants was expected since yellowing and reduction in plant growth are characteristic symptoms of ScYLV infection, resembling that of drought stress (Schenck *et al*. 2001). In this research, the soil conditions and water supply were favorable during the period of large vegetative development (**Fig. 1**) and these factors were not sources of interference to the processes involved in absorption and transport of water and nutrients from soil to stalks and leaves. Therefore, the reduction in fresh weight was probably due to ScYLV infec-

Table 1 Sugarcane yield measurements of ScYLV infected and uninfected plants, at nine months after planting (immature plants). Means correspond to five samples per treatment.

Trials	Fresh weight (kg m^{-1})	Stalk number (per meter)	°Brix	Fiber (%)	Sucrose content (%)
Uninfected plants	8.00 a*	13.80 a	9.18 b	8.61 a	3.10 b
Infected plants	4.70 b	11.20 a	10.52 a	10.00 a	4.43 a
F (variance analysis)	8.76	2.62	10.39	1.02	8.43
Variation coefficient (%)	27.70	20.31	6.67	23.13	19.30

* Means within a column followed by the same letters do not differ by Tukey's test (P> 0.05)

tion interfering with the phloem sugar transport and consequently in the normal plant development. As already shown, infection by ScYLV in sugarcane causes alterations in photosynthetic metabolism and disorders in plant carbohydrate metabolism (Gonçalves *et al*. 2005; Lehrer *et al*. 2007).

Significant increase in Brix and consequently sucrose content, 14.6 and 42.9%, respectively, were observed in stalks of infected plants. It is well known that sugar is transported via phloem from leaves (blade and sheath) to stalks and then downward to roots. Thus, sucrose transport downward to roots was probably reduced as an influence of the virus in the phloem, leading to sucrose over accumulation in the stalks and probably in the leaves as previously shown (Gonçalves *et al*. 2005). It has been demonstrated that leaf midribs of ScYLV infected plants show Brix values two to three times higher than uninfected plants (Comstock *et al*. 1998). Sucrose contents in leaves are also increased in ScYLV infected plants and reduction in the content of sucrose in stalks could be expected as a secondary effect of low CO_2 exchange rates (Gonçalves *et al*. 2005). However, Schenck *et al*. (2001) did not find significant differences in sucrose content in stalk juice of ScYLV infected and uninfected H87-4094 variety plants. Similar results were observed in the variety Cuba 120-78 (Fontaniella *et al*. 2003). Corroborating our results, variation in response to ScYLV infection for yield characteristics such as stalk weight, height, diameter and sugar content were observed for three sugarcane cultivars (R570, R577 and R579) indicating that impact of ScYLV and tolerance of sugarcane to the virus may vary according to sugarcane variety (Rassaby *et al.* 2003).

Effect of ScYLV in the root system

ScYLV infected plants had an average root dry weight decrease of 43% compared to healthy plants, probably related to reductions in the metabolic transport via phloem from leaves to roots. However, the proportion of roots at each depth was not affected by ScYLV infection, and its vertical architecture distribution was preserved (**Fig. 3**). In ScYLV infected plants, besides the reduction in the quantity of roots and in the water absorption surface, a reduction in the aboveground plant parts was observed, probably as a result of alterations in photosynthetic metabolism and in the uptake of nutrients. According to Gonçalves *et al.* (2005), the potential quantum efficiency for photochemistry of photosystem (PSII) is reduced in ScYLV infected sugarcane plants. Alterations in the filling up of plastoquinone pool as well as reduction in photosynthetic leaf pigments contents, and chlorophyll a and chlorophyll b ratio are also observed in ScYLV infected sugarcane plants. Additionally, the accumulation of sugars in stalks and leaves of infected plants is a possible effect of the virus on non-photosynthetic processes, such as compartmentalization and metabolic transport via phloem. Luteoviruses are confined to the phloem tissue of its hosts, with sieve elements and companion cells frequently occupied. Sugar loading, mainly sucrose, and its distribution to the sinks require passage through these cells (Lalonde *et al.* 2003), which are somehow modified during virus movement through the phloem.

In healthy sugarcane plants, root dry weight was positively correlated with cane fresh weight, stalk number, Brix and SC contents (**Figs. 4**, **5**). These results show that the root system development is crucial for proper plant growth as it provides aboveground plant parts with sufficient water and nutrients. However, for such a scenario to be valid, plants must be healthy. On the other hand, both roots and aboveground plant parts showed smaller dry weight in infected plants than in healthy plants; but amongst ScYLV infected plants root dry weight was negatively correlated with cane fresh weight and number of stalks (**Figs. 4a, 4b**). Therefore, if ones evaluate separately the infected plants, those that suffered the largest reductions in development of above plant parts did not necessarily showed the largest reductions of the root system. Hence, nutrients like calcium that improves root growth (Ritchey *et al.* 1980) and is normally immobile in the phloem (Biddulph *et al.* 1958) might be allocated to the roots development. Our results show that a proper developed root system and greater water absorption surface do not necessarily implies in a good development of the above plant parts in ScYLV infected plants. This supports the hypothesis of 'spare capacity' in the root system of sugarcane. Smith *et al.* (2005) suggest that sugarcane plants usually have more roots than are needed for maximum growth, and in typical field conditions this spare capacity effectively buffers the plant against root loss or a sub-optimal soil environment.

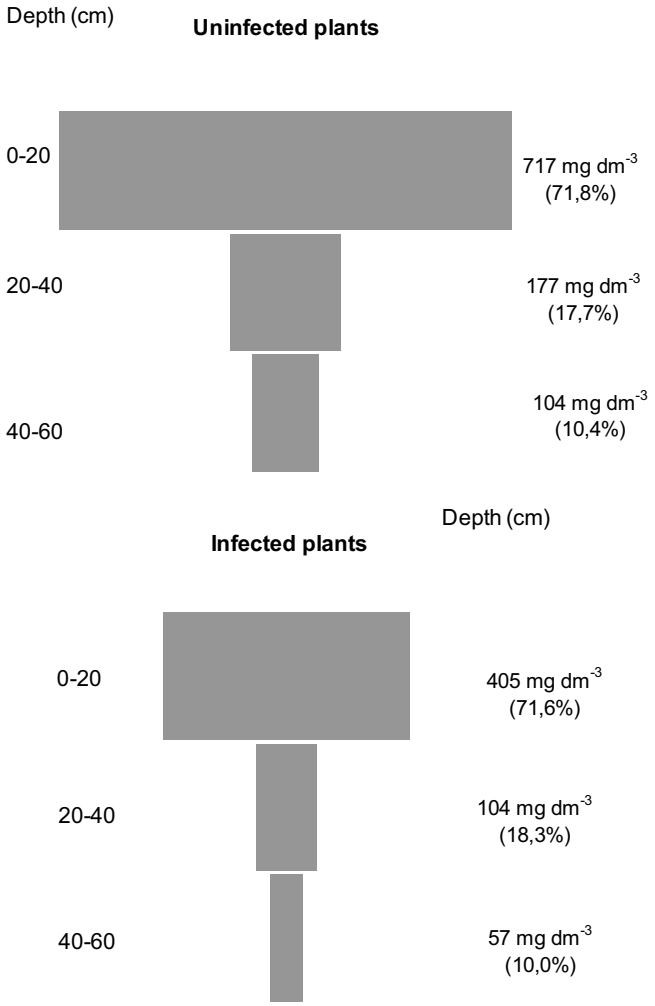

Fig. 3 Root system quantity (mg.dm^{-3}) and distribution in the soil profile (0 to 60 cm).

Interestingly, Brix and sucrose content were not significantly correlated with root dry weight in ScYLV infected plants (**Figs. 5a, 5b**), and negative correlation was not found as in the previous case, i.e., root versus fresh weight and number of stalks. This lack of correlation may be indicative of a rupture in the phloem transport of photo-assimilates and consequently xylem transport of nutrients between above plant parts and the root system.

ScYLV detection

The virus was not detected by DAS-ELISA in two of the tested symptomatic plants, probably due to the low virus titer in the leaf tissue. On the other hand, RT-PCR analysis revealed the presence of the virus in all twenty tested plants grown from stalks showing symptoms. Fragments of 450 bp, corresponding to the coat protein of ScYLV were amplified (**Fig. 2**). Plants grown from stalks without symptoms tested negative in RT-PCR. These results were confirmed at the

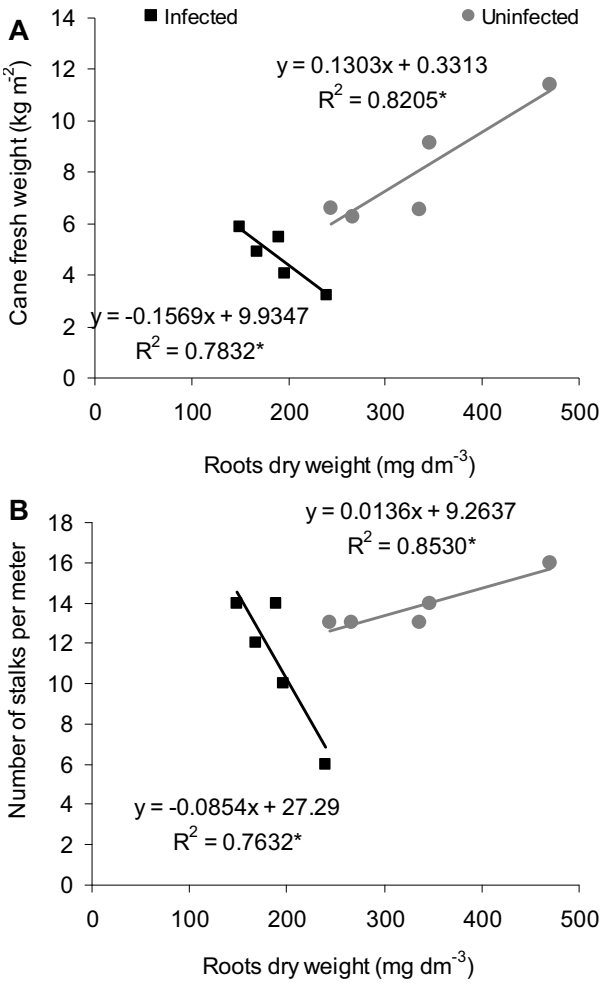

Fig. 4 Correlation between root dry weight (0-60 cm) and (A) leaves fresh weight, (B) number of stalks.

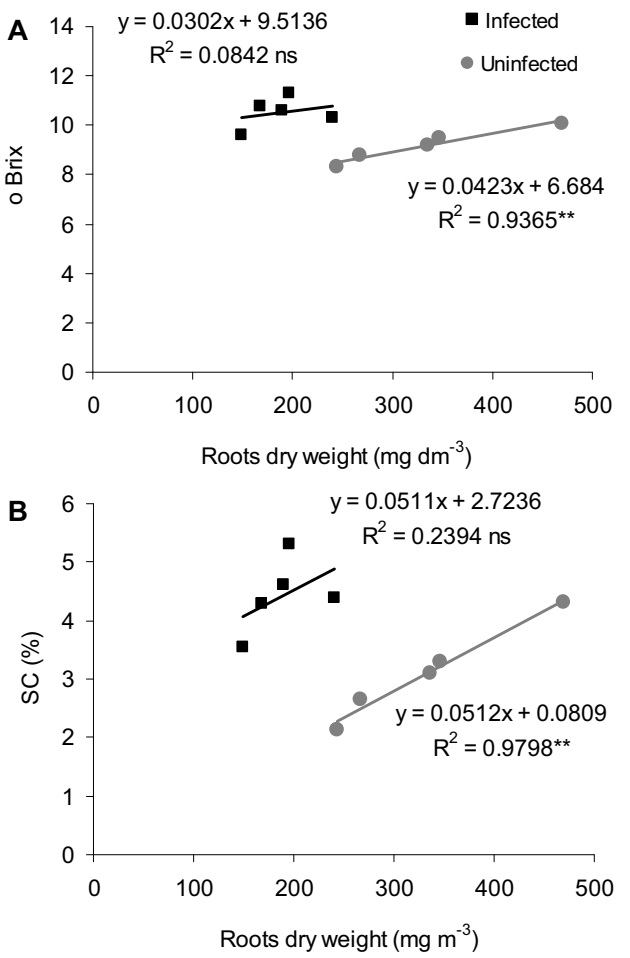

Fig. 5 Correlation between root dry weight (0-60 cm) and (A) Brix, (B) sucrose content (SC).

end of the cycle, detecting the virus only in symptomatic plants. RT-PCR has been successfully used as a diagnosis tool for ScYLV detection (Chatenet *et al.* 2001; Viswanathan *et al.* 2008; Xie *et al.* 2009). Although, in fewer cases, much contaminant RNA or PCR inhibitors can leads to false negatives, RT-PCR is more reliable than serological tests used for ScYLV detection, for instance DAS-ELISA and TBIA (Tissue-blot immunoassay) that depends on virus titer, which, in some cases, may be below the detection threshold of these serological techniques (Schenck and Lehrer 2000; Chatenet *et al.* 2001; Gonçalves *et al.* 2002). Our results corroborate these data, since DAS-ELISA did not show enough sensitivity to detect low virus titers in infected plants, as confirmed by RT-PCR analysis.

Perpetuation of ScYLV in stalks

Stalks of ScYLV infected plants whose virus infection was confirmed by RT-PCR analysis were planted in a plot field aside of stalks from virus free plants (uninfected). The plants grown from ScYLV infected stalks showed characteristic disease symptoms while plants from uninfected stalks did not show any symptoms (**Fig. 6**). These observations confirm the perpetuation of ScYLV in stalks from infected plants and its transmission by mean of infected shoots. Among the forms of ScYLV transmission, the use of infected stalk cuts from infected plants is the most important, as it constitutes the main propagation method for sugarcane planting (Rassaby *et al.* 2004). The virus is localized within plant phloem cells and chemical or heat treatment of stalks before planting is not effective enough to eliminate the virus from infected sugarcane (Schenck *et al.* 2001). Moreover, virus perpetuation has being reported in canes of second growth cycle even after long hot water treatment applied between two quarantine cycles (Chatenet *et al.* 2001). Our results confirm these observations and showed the effects of virus perpetuation in the sugarcane crop. Although infected plants maintained the root system vertical architecture, both roots and aboveground plant parts had lesser dry weight than healthy plants. On the other hand, root dry weight was negatively correlated with cane fresh weight and stalk number, and roots were less affected than aboveground plant parts. In general, changes in root and vascular systems were important effects of infection by ScYLV in sugarcane, leading to expressive reductions in yield.

Fig. 6 Stalk propagation of ScYLV infected and uninfected plants. GU = grown from uninfected stalks; GI = grown from infected stalks.

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REFERENCES

Abu Ahmad Y, Rassaby L, Royer M, Borg Z, Braithwaite KS, Mirkov TE, Irey MS, Perrier X, Smith GR, Rott P (2006) Yellow leaf of sugarcane is caused by at least three different genotypes of *Sugarcane yellow leaf virus*, one of which predominates on the Island of Reunion. *Archives of Virology* **151**, 1355-1371

Aiken RM, Smucker AJM (1996) Root system regulation of whole plant growth. *Annual Review of Phytopathology* **34**, 325-346

Biddulph O, Biddulph S, Cory R, Koontz H (1958) Circulation patterns for phosphorus, sulfur and calcium in the bean plant. *Plant Physiology* **33**, 293-300

Chatenet M, Delage C, Ripolles M, Irey MS, Lockhart BEL, Rott P (2001) Detection of *Sugarcane yellow leaf virus*, in quarantine and production of virus-free sugarcane by meristem culture. *Plant Disease* **85**, 1177-1180

Comstock JC, Irey MS, Lockhart BEL, Wang ZK (1998) Incidence of yellow leaf syndrome in CP cultivars based on polymerase chain reaction and serological techniques. *Sugar Cane* **4**, 121-124

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

Fontaniella B, Vicente C, Legaz ME, De Armas R, Rodriguez CW, Martinez M, Piñon D, Acevedo R, Solas MT (2003) Yellow leaf syndrome modifies the composition of sugarcane juices in polysaccharides, phenols and polyamines. *Plant Physiology and Biochemistry* **41**, 1027-1036

Gonçalves MC, Klerks MM, Verbeek M, Vega J, Van den Heuvel JFJM (2002) The use of molecular beacons combined with NASBA for the sensitive detection of *Sugarcane yellow leaf virus*. *European Journal of Plant Pathology* **108**, 401-407

Gonçalves MC, Vega J, Oliveira JG, 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

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

Lalonde S, Tegeder M, Throne-Holst M, Frommer WB, Patrick JW (2003) Phloem loading and unloading of sugars and amino acids. *Plant Cell Environment* **26**, 37-56

Landell MGA, Campana MP, Figueiredo P, Vasconcelos ACM, Xavier MA, Bidoia MAP, Prado H, Silva MA, Dinardo-Miranda LL, Santos AS, Perecin D, Rossetto R, Silva DN, Martins ALM, Gallo PB, Kanthack RAD, Cavichioli JC, Veiga Filho AA, Anjos IA, Azania CAM, Pinto LR, Souza SACD (2005) Variedades de cana-de-açúcar para o Centro-Sul do Brasil 15a liberação do programa cana IAC (1959-2005), IAC Technical Bulletin no. 197, Campinas, Brazil, 33 pp

Lehrer AT, Schenck S, Yan S-L, Komor E (2007) Movement of aphid-transmitted *Sugarcane yellow leaf virus* (ScYLV) within and between sugarcane plants. *Plant Pathology* **56**, 711-717

Lehrer AT, Komor E (2008) Symptom expression of yellow leaf disease in surgarcane cultivars with different degrees of infection by *Sugarcane yellow leaf virus*. *Plant Pathology* **57**, 178-189

Maia IG, Gonçalves MC, Arruda P, Vega J (2000) Molecular evidence that *Sugarcane yellow leaf virus* (ScYLV) is a member of the *Luteoviridae* family. *Archives of Virology* **145**, 1009-1019

Moonan F, Molina J, Mirkov TE (2000) *Sugarcane yellow leaf virus:* An emerging virus that has evolved by recombination between Luteoviral and Poleroviral ancestors. *Virology* **269**, 156-171

Rassaby L, Girard JC, Letourmy P, Chaume J, Irey MS, Lockhart BEL, Kodja H, Rott P (2003) Impact of *Sugarcane yellow leaf virus* on sugarcane yield and juice quality in Réunion Island. *European Journal of Plant Pathology* **109**, 459-466

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

Ritchey KD, Souza MG, Lobato E, Correa O (1980) Calcium leaching to increase rooting depth in a Brazilian Savannah Oxisol. *Agronomy Journal* **72**, 40-44

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

Schenck S, Lehrer T (2000) Factors affecting the transmission and spread of *Sugarcane yellow leaf virus*. *Plant Disease* **84**, 1085-1088

Schenck S, Lehrer AT, Wu WW (2001) Yellow leaf syndrome. Hawaii Agriculture Research Center. *Pathology Report* **68**, 1-5

Smith DM, Inman-Barber NG, Thorburn PJ (2005) Growth and function of the sugarcane root system. *Field Crop Research* **92**, 169-183

Vasconcelos ACM, Casagrande AA, Perecin D, Jorge LAC, Landell MGA (2003) Avaliação do sistema radicular da cana-de-açúcar por diferentes métodos. *Revista Brasileira de Ciência do Solo* **27**, 849-858

Vega J, Scagliusi SMM, Ulian EC (1997) Sugarcane Yellow Leaf Disease in Brazil: Evidence of association with a luteovirus. *Plant Disease* **81**, 21-26

Viswanathan R, Balamuraikrishnan M, Karuppaiah R (2008) Identification of three genotypes of *Sugarcane yellow leaf virus* causing yellow leaf disease from India and their molecular characterization. *Virus Genes* **37**, 368-379

Xie Y, Wang M, Xu D, Li R, Zhou G (2009) Simultaneous detection and identification of four sugarcane viruses by one-step RT-PCR. *Journal of Virological Methods*, in press