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Physicochemical characteristics of 'Pera' sweet orange *Citrus sinensis* affected by citrus leprosis

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Abstract

Citrus leprosis is among the most serious viral diseases of citrus in South and Central America. Our objective was to study the physicochemical characteristics of the *Citrus sinensis* 'Pera' sweet orange grafted on *C. reshni* 'Cleopatra' mandarin with different severity of citrus leprosis. The experiment was conducted from 2003 to 2010 in Reginopólis county, São Paulo, Brazil. We used different acaricides against vector mite *Brevipalpus yothersi* to obtain plants with different levels of disease. For crop years 2009 and 2010 were determined average weight, average diameter, juice yield, soluble solids, total titratable acidity, ratio and disease severity determined using a visual scale. The highest severity levels were observed in plants without acaricide application and plants treated with spirodiclofen and cyhexatin in rotation showed the lowest levels of citrus leprosis severity. Plants treated with lime sulfur presented intermediate levels of severity this disease. The quality of fruits that remained on the plant was not affected by citrus leprosis measured by physicochemical characteristics, however the productivity was reduced substantially. This work proved in the field that citrus leprosis does not affect juice quality even at high level of severity. However, causes high fruit drop reducing substantially the productivity and affects negatively the fruits appearance.

Keywords: CiLV, *Brevipalpus yothersi*, flat mite, Total Soluble Solids.
Abbreviations: EECB_Experimental Citrus Station of Bebedouro; FAPESP_Fundação de Amparo à Pesquisa do Estado de São Paulo; JY_Juice Yield; TI_Technological Index; TSS_Total Soluble Solids; TTA_Total Titratable Acidity.

Introduction

Citrus leprosis is one of the most serious viral diseases of citrus in South and Central America (Bastianel et al., 2010; Cruz-Jaramillo et al., 2014). Two forms of *Citrus leprosis virus* (CiLV) are known, CiLV cytoplasmic type (CiLV-C) and CiLV nuclear type (CiLV-N). CILV-C prevails in the majority of cases (Nunes et al., 2012; Rodrigues et al., 2003).
CiLV is considered endemic to citrus orchards of Brazil and is transmitted by *Brevipalpus* spp. mites (Navia et al., 2013). CiLV has been found in Argentina, Bolivia, Paraguay, Venezuela, and Colombia, and is spreading north ward through Panama, Costa Rica, Nicaragua, Guatemala, Honduras, El Salvador, and Mexico (Bastianel et al., 2010; Cáceres et al., 2013; Gómez et al., 2005; Izquierdo-Castillo et al., 2011; Leon et al., 2006). Brazil is the world's leading citrus producer. The cost control of citrus leprosis is estimated between US$75 and 80 million each year (Cáceres et al., 2013). Most of the costs are associated with the purchase and application of acaricides, since the only known method to prevent the occurrence of the disease is by controlling the mite vector. Plants affected by citrus leprosis show a reduction in photosynthetic capacounty, branch dieback, and pronounced defoliation. During years of severe incidence of disease, orchards may become economically unfeasible due to high levels of fruit drop and consequent reduction in productivity. Further, plant death can occur without adequate disease control (Garita et al., 2013).

Citrus leprosis is part of a complex pathosystem due to multiple factors involved, including complex interactions among the vector, virus, host plant, and environmental factors (Bassanezi and Laranjeira, 2007; Garita et al., 2014) and management difficulties that require special expertise (Bassanezi and Laranjeira 2007; Garita et al., 2014).

Several physiological and biochemical changes can occur in plants affected by citrus leprosis, including increased respiration, and changes in protein synthesis and amino acid sequences (Andrade et al., 2008). Furthermore, infections caused by viruses, because of greater specificity with the plant in general, have significant effects on plant metabolism (Freitas-Astúa et al., 2007; Hull, 2002). Viral infections can accelerate or delay fruit ripening, as well as alter the physical and chemical features.Quantitative and qualitative losses caused by diseased fruits of different citrus varieties were investigated by Rodrigues (2000). For the *Citrus sinensis* (L.) Osbeck 'Lima Verde', an increase in the number of fruit injuries corresponded to reduction in fruit weight. Variables related to the chemical characteristics of *C. sinensis*, however, were not affected by lesions of citrus leprosis.
In spite of these observations, there is a lack of studies showing the effects of citrus leprosis on the whole plants after years of infection. For citrus industry it is very important to know if fruits from plants with citrus leprosis has modified juice when compared to fruits of plants without citrus leprosis. Additionally, is required to determine the

specific type of virus that affects the plant (Garita et al., 2013; Locali-Fabris et al., 2006). Therefore, the present study aimed to assess the physicochemical characteristics of 'Pera' sweet orange fruit in plants with different severity levels of citrus leprosis caused by CiLV-C.

Results

Physicochemical characteristics

No differences due to treatments were noted in the JY and transverse and longitudinal diameters (Tables 1, 2 and 3). Fruit weight differed between treatments only in the first collection, conducted in August 2009 (Table 1). The lowest fruit weights in August 2009 were observed in the spirodiclofen and cyhexatin treatment. TTA, TSS, and fruit ratio showed no significant differences between treatments (Tables 1, 2 and 3). TI, also known as industrial yield, represented the amount of soluble solids per 40.8 kg box of oranges and showed a significant difference between treatments in the first fruit collection. Fruit treated with spirodiclofen and cyhexatin ranked highest on the TI (Table 1). The *ratio* parameter differed between the three harvests with the lowest level observed in December 2010. By contrast, the highest *ratio* was observed in the harvest of August 2009, with an approximate value of 14. Variation in TTA was higher in all treatments in the December 2010 collection (Fig. 1A). In general, TSS content was lower in the August 2010 harvest. The treatment with lime sulfur in this harvest had the lowest TSS which differed from the other treatments (besides the without acaricide treatment harvest) in August 2010 (Fig. 1B).

Citrus leprosis and physicochemical characteristics correlations

Despite these results, severity levels of citrus leprosis differed among treatments with and without acaricide in all harvests. The highest severity levels were observed in plants without acaricide application. Of the three harvests, plants treated with spirodiclofen and cyhexatin showed the lowest levels of citrus leprosis severity. By contrast, plants treated with lime sulfur presented intermediate levels of severity (Tables 1, 2, and 3 and Fig. 2). In the severity assessment performed in July 2010, there was a considerable increase of citrus leprosis in all three treatments; however, the assessment performed in October 2010 found a reduction of the levels of severity in the treatments without acaricide and with lime sulfur (Fig. 2). Correlations performed between the physicochemical parameters of fruits (transverse and longitudinal diameters, fruit weight, JY, TTA, TSS, *ratio* and TI) and citrus leprosis severity were not significant in any of the harvested crops.

Productivity of plants

In 2009, the largest crop production was observed in the spirodiclofen and cyhexatin treatment. Crop production was lowest in the treatment without acaricide. Treatment with spirodiclofen and cyhexatin yielded approximately 650 boxes of oranges (40.8 kg per box) per hectare. Treatment with lime sulfur did not differ from the other two treatments in this crop, yielding an approximate production of 350 kg per hectare (Fig. 4). On the other hand, in 2010, the average harvest volume was lower and there were no differences between treatments (Fig. 4).

Discussion

CiLV found in experiment plants

Based on symptomatology, the predominant CiLV in the experiment strongly suggested CiLV-C (Bastianel et al., 2010). CiLV-C is associated with baciliformes particles in the endoplasmic reticulum and the presence of dense cytoplasm viroplasma (Kitajima et al., 2010). The two forms of CiLV not share genomic sequences is probably genetically distinct viruses (Bastianel et al., 2010). Locali-Fabris et al. (2006) and Pascon et al. (2006) completed the sequencing of the genome CiLV-C and found that CiLV, typically regarded as a member of the family Rhabdoviridae, should be grouped within a new genus of viruses called Cilevirus.

Citrus leprosis severity after five years

Fruit maintained in 'Pera' orange plants were not affected in quality by citrus leprosis, even after five years without control. These results corroborate Rodrigues (2000), who observed that disease does not affect the main features of Lima Verde juice as compared to juice from disease free fruit. However, this study only considered the severity of citrus leprosis in fruit by counting the number of lesions present and not in the plant as a whole. Plants treated with lime sulfur demonstrated moderate disease severity compared to other treatments. Although these plants provided the lowest number of fruit lesions, the lesions were larger in diameter, which resulted in severe fruit drop though smaller in proportion when compared to treatment without acaricide. The lime sulfur treatment maintained the population of *Brevipalpus yothersi* Baker below the level of control, but did not prevent the onset and progression of citrus leprosis injuries in branches, leaves, and fruit.

Physicochemical characteristics and CiLV-C

Furthermore, characteristics related to the quality of the juice have seasonal and annual variation, being part of complex physiological processes. Acidity decreases gradually, while the amount of soluble solids content and *ratio* increases. These parameters are therefore best evaluated by studies conducted over longer periods of time (Domingues et al., 1996). Several authors consider disease as a factor that can directly influence the maturation of fruit. Serious infection can be caused by diseases, especially those caused by viruses due to the higher specificounty with plants. These diseases have significant effects on plant metabolism, affecting many essential cellular processes such as synthesis of nucleic acids and proteins, lipids and carbohydrate metabolism and hormones, and enzyme function (Hull, 2002). In contrast, the incidence of *Coffee ringspot virus* (CoRSV), also transmitted by *Brevipalpus* spp. to coffee plants directly, affects the drink quality. Boari et al. (2006) showed that fruits infected with CoRSV produced lower quantities of reducing sugars and a higher electrical conductivity as compared to healthy fruits. Marques et al. (2010) observed that the CiLV affects chloroplasts, leading to hypertrophy of the lamellar system and destroyed stomata that are responsible for plant transpiration. The authors demonstrated that the central region of injury caused by citrus leprosis was the palisade parenchyma, as it was devoid of chloroplasts. Furthermore, they observed that CiLV-C causes hypertrophy of parenchymal cells and lipid accumulation. The presence of CiLV and other injuries affect the physiological processes of plants, including increasing transpiration and cellular

Table 1. Average values of physico-chemical parameters of fruits collected in August 2009 and August 2010 severity citrus leprosis according to different acaricides used to control *Brevipalpus yothersi* mite.

Treatments	Fruit diameter (cm)		Fruits weight (g)	Juice yield (%)	AT[1] (g/100 mL)	SS[2] (°Brix)	*Ratio* (SS/AT)	Technological index	Leprosis severity
	transversal	longitudinal							
No acaricide	6.5 a	7.2 a	181.1 ab	53.2 a	0.8 a	10.3 a	12.9 a	2.2 ab	3.8 a
Lime sulfur	6.8 a	7.2 a	194.8 a	53.2 a	0.9 a	10.0 a	11.1 a	2.1 b	2.5 b
spirodiclofen/cyhexatin	6.8 a	7.1 a	176.7 b	55.0 a	0.9 a	10.5 a	11.6 a	2.4 a	1.2 c
C.V.(%)[3]	0.7	6.1	9.5	4.5	3.4	2.4	3.5	1.7	4.5

Original data were converted to ln (x + 5). Same letters in column do not differ by Tukey's test at a 5% significance level. [1]total titratable acidity; [2]soluble solids; [3]Variation coefficient.

Table 2. Average values of physico-chemical parameters of fruits collected in August 2010 and severity citrus leprosis according to different acaricides used to control *Brevipalpus yothersi* mite.

Treatments	Fruit diameter (cm)		Fruits weight (g)	Juice yield (%)	AT[1] (g/100 mL)	SS[2] (°Brix)	*Ratio* (SS/AT)	Technological index	Leprosis severity
	transversal	longitudinal							
No acaricide	6.8 a	7.0 a	169.0 a	54.7 a	0.7 a	9.5 a	13.6 a	2.1 a	4.4 a
Lime sulfur	6.7 a	7.0 a	168.4 a	56.0 a	0.7 a	9.3 a	13.3 a	2.1 a	3.5 b
Spirodiclofen/cyhexatin	6.9 a	7.2 a	177.9 a	54.1 a	0.7 a	10.1 a	14.4 a	2.2 a	1.7 c
C.V.(%)[2]	1.1	1.3	2.4	5.0	1.6	2.5	3.1	3.2	9.2

Original data were converted to ln (x + 5). Same letters in column do not differ by Tukey's test at a 5% significance level. [1]total titratable acidity; [2]soluble solids; [3]Variation coefficient.

Table 3. Average values of physico-chemical parameters of fruits collected on December 2010 a severity citrus leprosis according to different acaricides used to control *Brevipalpus yothersi* mite.

Treatments	Fruit diameter (cm)		Fruits weight (g)	Juice yield (%)	AT[1] (g/100 mL)	SS[2] (°Brix)	*Ratio* (SS/AT)	Technological index	Leprosis severity
	transversal	longitudinal							
No acaricide	6.2 a	6.4 a	132.6 a	50.0 a	1.3 a	10.1 a	7.8 a	2.1 a	3.7 a
Lime sulfur	6.4 a	6.7 a	150.3 a	51.0 a	1.4 a	10.1 a	7.2 a	2.1 a	2.6 b
Spirodiclofen/cyhexatin	6.4 a	6.5 a	147.6 a	53.7 a	1.2 a	10.4 a	8.7 a	2.3 a	1.2 c
C.V.(%)[3]	1.4	1.2	3.2	2.1	3.9	2.6	8.2	2.4	9.2

Original data were converted to ln (x + 5). Same letters in column do not differ by Tukey's test at a 5% significance level. [1]total titratable acidity; [2]soluble solids; [3]Variation coefficient.

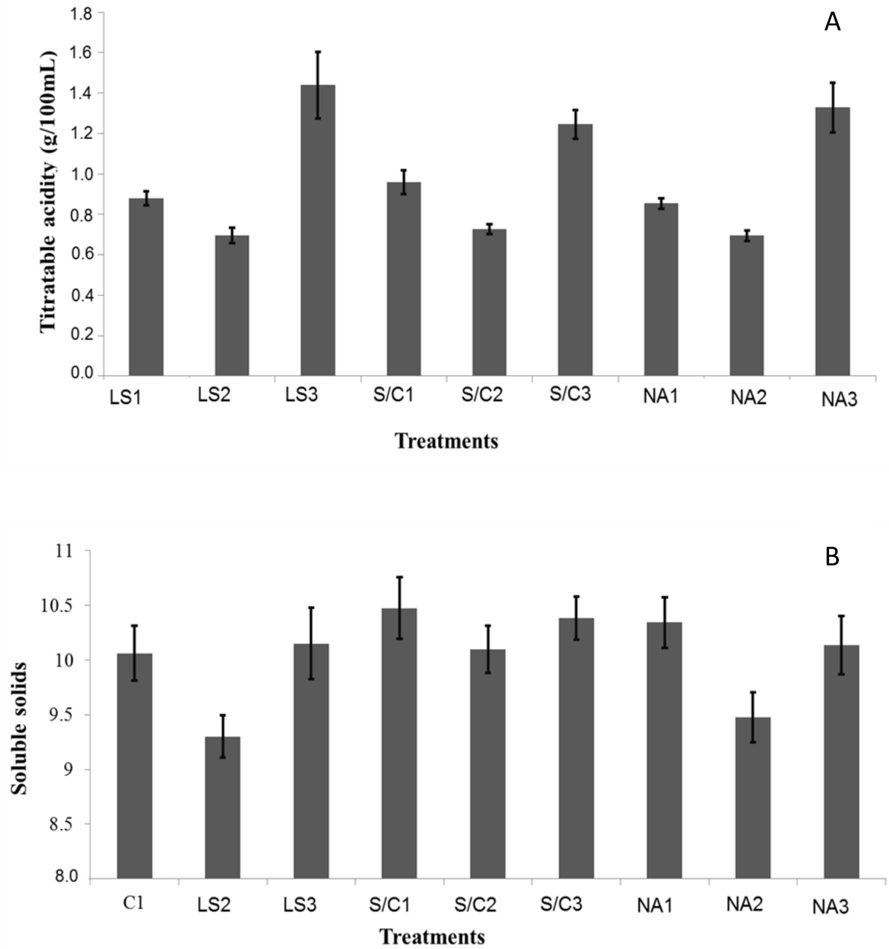

Fig 1. Average values of total titratable acidity (A) and total soluble solids or Brix (B) of citrus fruit from three harvests for the assessment of physical-chemical characteristics: on August 2009 (LS1, S/C1 and NA1); on August 2010 (LS2, S/C2 and NA2) and December 2010 (LS3 S/C3 and NA3). LS = lime sulfur, S/C = spirodiclofen/cyhexatin and NA = no acaricide.

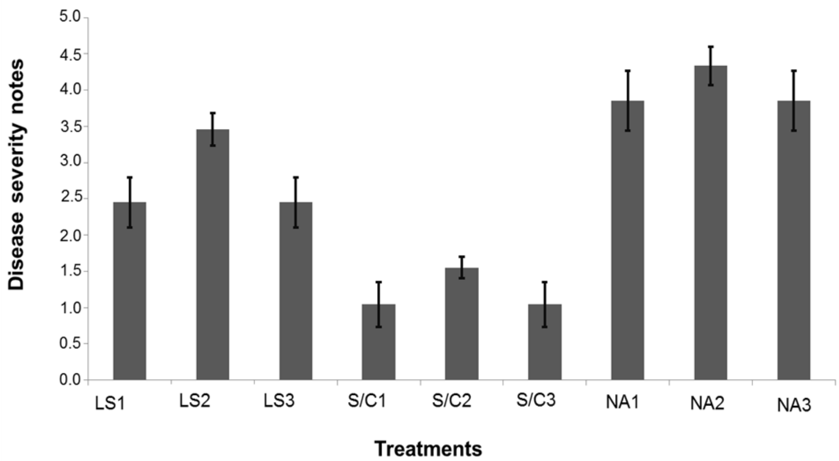

Fig 2. Average values of disease severity notes of citrus leprosis in plants in three ratings: on July 2009 (LS1, S/C1 and NA1); on July 2010 (LS2, S/C2 and NA2) and October 2010 (LS3 S/C3 and NA3). LS = lime sulfur, S/C = spirodiclofen/cyhexatin and NA = no acaricide.

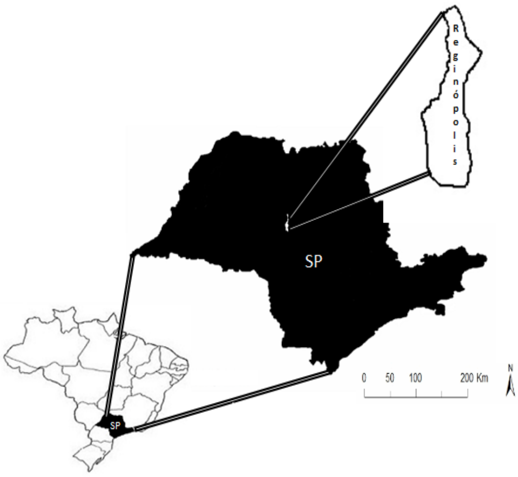

Fig 3. Illustrative map to location of the Reginópolis county, São Paulo State - Brasil, where the experiment was conducted.

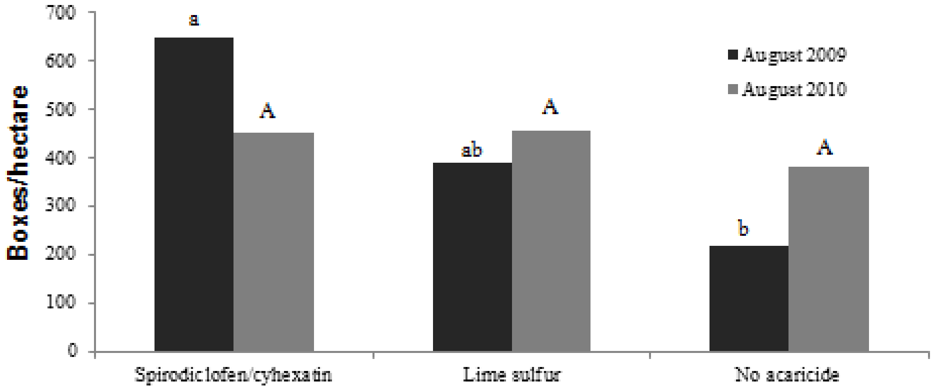

Fig 4. Average productivity of citrus plants in boxes of 40.8 kg/hectare, according to different acaricides used to control *Brevipalpus yothersi*, referring to harvest carried out on August 2009 and August 2010. Same letters lowercase concerning harvest August 2009 and same letters uppercase concerning harvest August 2010 do not differ by Tukey's test at a 5% significance level.

respiration rates (Andrade et al., 2008; Freitas - Astúa et al., 2007). In plants infected with disease causing viruses, the rate of cellular respiration increases, often before symptoms appear, and respirationincreases as the disease develops (Hull, 2002). Freitas-Astúa et al. (2007) studied CiLV infected and non-infected plants of *C. sinensis* and demonstrated that at the molecular level, repression of gene expression related to photorespiration occurs in the beginning stages of CiLV infection even before the appearance of visible lesions.

On the other hand, Pascholati (1995) reported that plants attacked by viruses typically do not change the rate of transpiration; rather, that conditions leading to a disruption of the cuticle surface leads to an increase the rate of transpiration. Studies related to the effect of citrus leprosis on host-plant water balance should be performed separately. Citrus leprosis affects plants non-systemically, which is uncommon among viral plant diseases since most affect their hosts systemically.

The weight of fruits

Fruit weight differed among treatments for the August 2009 harvest. The lowest fruit weight was observed for treatment with spirodiclofen and cyhexatin, in which there was minimal fruit and leaf drop due the absence of citrus leprosis. Efficient control of *B. yothersi* due to the effectiveness of these products resulted in retaining a greater number of fruit on the plants at harvest. Evidence supports the hypothesis that treatment with spirodiclofen and cyhexatin created a higher division of water and photosynthates, resulting in lower fruit weight than that in the other treatments, which yielded less number of fruits at harvest. The transverse and longitudinal diameters of fruit differed between harvests, ranking the lowest in December 2010. The fruit from this harvest refers to the second flowering of the year, thus, competing for photosynthates with the fruit of the first flowering. 'Pera' sweet orange, typically, has three or four annual flowerings resulting in fruit production several times throughout the year. Therefore, competition between these fruits results in a negative correlation between the number of fruit per plant and the fruit size at harvest.

Technological index (TI)

In 2009, a record-breaking annual precipitation of 2,017.33 mm was recorded. In 2010, 1,396.18 mm was recorded. Fruit collected in 2009 treated with spirodiclofen and cyhexatin demonstrated the highest TI. The industrial yield is given by the TI and represented by the physical and chemical characteristics of the fruit. The method used to determine the maturity of fruit at harvest is the relationship between the percentages of TTS and TTA, known as maturity index or *ratio*. Higher values of TI are an indicator of maturity and can also be used as indicator of fruit quality (Souza and Góes, 2010). According Pozzan and Triboni (2005), citrus juice with a *ratio* between 14 and 16 are the most appreciated by consumers worldwide, due to an ideal balance of sugar and acid content.

Materials and methods

Location of the experimental field and plant materials

The experiment was conducted from 2003 to 2010 and located in the county of Reginopólis, state of São Paulo, Brazil. The geographical coordinates of the orchard are latitude 49º 13' 24" W and longitude 21º 56' 26" S at an altitude of 468 m (Fig. 3). The experimental plots were of the soil order Oxisol, classified as a dystrophic Red-Yellow Latosol with a claylike texture, as defined by Santos et al. (2006). 'Pera' sweet orange (*C. sinensis)* was grafted onto 'Cleopatra' mandarin (*C. reshni* hort ex Tanaka) and drip irrigated. The experiment began in October 2003 and this moment the plants were 12 years old.

Experimental design and conduct of the study

Approximately 500 plants were examined for the presence of citrus leprosis following the methodology of Rodrigues (2002). Eighty-six percent of the plants had disease in early stages of infection. The presence of *B. yothersi* was also evaluated using sampling methodology (Rodrigues et al., 2003). *Brevipalpus yothersi* mites were observed in less than 5% of the inspected plants.The experiment was designed in randomized blocks with three treatments and 16 replications. Each replication consisted of three plants arranged in line.

Applications of miticides and citrus leprosis levels

In order to obtain plants with different levels of citrus leprosis, different acaricides were applied depending on their effectiveness in controlling of *Brevipalpus* spp. The first treatment used was lime sulfur (Super S20®), a concentration of 20% sulfur and 8% calcium per liter of product with a volume of 4 liters added to 100 liters of water. The second treatment consisted of spirodiclofen (Envidor 240 SC®) and cyhexatin (Sipcatin 500 SC®) in rotation, applied in doses of 20 and 50 mL in 100 liters of water, respectively. In the third treatment any acaricide was not applied. The products were applied using a trailed sprayer coupled with Jacto hand guns with a 2.000 L capacounty. The applications contained sufficient spray volume to provide adequate coverage of the plants.

Sampling of Brevipalpus spp. and citrus leprosis severity

In each plot, the central plant was sampled. Three fruits located inside the plant canopy were examined with the aid of a pocket magnifier of 10× magnification. In the absence of fruit, three branches were evaluated at the beginning of suberization. The average length of the three branches was 25 cm. An infested fruit was defined as having at least one mite present at any stage of development, except at the egg stage. Treatment applications were performed based on the extent of *B. yothersi* infestation. Control levels of infested fruit were set at 8.3%. The *Brevipalpus* species was identified by morphology of the dorsal propodosoma, opisthosoma, and leg chaetotaxy using a scanning electron microscopy (SEM) based on the characteristics proposed by Beard et al. (2015).
Severity assessments of citrus leprosis in all plants was performed in July 2009 and July and October 2010, based on a visual scale ranging from 0 to 5, where (0) represented no injury; (1) a few lesions in any organ, restricted to one sector of the plant; (2) lesions in more than one organ and/or distributed in more than one sector; (3) numerous lesions in all organs and distributed throughout the plant; (4) numerous lesions throughout the whole plant and leaf and/or fruit drop; and (5) numerous lesions throughout the whole plant, leaf and/or fruit drop, and branch dieback (Rodrigues, 2002).

Physicochemical characteristics determination

Ten fruits were randomly collected off the central plant of each plot just before a typical harvest season. Collections were performed in August 2009 and August and December of 2010. The harvested fruit samples were sent to the Chemical Analysis Laboratory at the Experimental Citrus Station of Bebedouro (EECB), located in Bebedouro county, state of São Paulo, Brazil in order to determine key physicochemical characteristics. The characteristics determined were as follows: (i) average fruit weight: determined gravimetrically using a digital balance model Filizola MF-1 with accuracy of 0,2 g. The average weight was obtained by dividing the weight of all fruit in each treatment by the number of fruit. (ii) average fruit diameter: the longitudinal and transverse diameters of each fruit were measured with a pachymeter graduated in cm with accuracy de 0,05mm. (iii) juice yield (JY): fruit juice was extracted with the aid of a Centenário Model 1800 OTTO juice extractor. The juice extractor belonged to the EECB. The extracted juice was filtered with a measuring cylinder to determine volume, expressed in mL per 100g of fruit. JY was expressed as a percentage, calculated by the relationship between juice weight and fruit weight. (iv) total soluble solids (TSS): determined by refractometry at 20° C and expressed in Brix (g per 100 mL) according to methods established by Redd et al. (1986). (v) total titratable acidity (TTA): determined by titration with NaOH 0.1N, with phenolphthalein as an indicator and Tecnal digital counter top pHmeter. TTA was determined by the quantity of NaOH consumed. The results were expressed in grams of citric acid per 100 mL of juice. (vi) maturation index or ratio: calculated by difference between TSS and TTA (vii) technological index (TI): the amount of TSS of citrus per 40.8 kg box was calculated using the equation proposed by Di Giorgi et al. (1990).

$$TI = TSS * JY * 40.8 / 10000$$

Where: TI = technological index, TSS = total soluble solids (° Brix), JY = juice yield, and 40.8 as the industry standard of weight in kg for a box of oranges.

Statistical analysis

Using a computer program, data was subjected to an analysis of variance (ANOVA), the F-test, and the averages compared by the Tukey test at 5% probability. Before being subjected to ANOVA, data was transformed to $\ln(x + 5)$ in order to normalize the results. The physicochemical characteristics of fruit were correlated to the average values of average severity of disease obtained in each experimental plot. Variation of physicochemical parameters of fruit among harvests was also analyzed.

Conclusion

The citrus leprosis does not affect juice quality of the 'Pera' sweet orange grafted on 'Cleopatra' mandarin even at high level of severity disease in the field. However, causes high fruit drop reducing substantially the productivity and affects negatively the fruits appearance.

Besides the acaricides application are recommended other control measures to avoid the citrus leprosis dissemination, for example, pruning affected parts by disease.

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References

Andrade DJ, Oliveira CAL, Falconi RS, Pattaro FC, Fernandes EJ (2008) Effect of water stress and *Citrus leprosis virus* on sweet orange leaf temperature and its relationship with *Brevipalpus phoenicis* population and leprosis severity on citrus. Laranja. 29(1): 1-15.

Bassanezi RB, Laranjeira FF (2007) Spatial patterns of leprosis and its mite vector in commercial citrus groves in Brazil. Plant Pathol. 56(1): 97-106.

Bastianel M, Novelli VM, Kitajima EW, Kubo KS, Bassanezi RB, Machado MA, Freitas-Astúa J (2010) Citrus leprosis: centennial of an unusual mite virus pathosystem. Plant Dis. 94(3): 284-292.

Beard JJ, Ochoa R, Braswell E, Bauchan GR (2015) *Brevipalpus phoenicis* (Geijskes) species complex (Acari: Tenuipalpidae) - a closer look. Zootoxa. 3944(1): 1–67.

Boari AJ, Figueira AR, Neder DG, Santos R de C, Goussain MM, Nogueira NL, Rossi ML (2006) *Coffee ringspot virus* (CoRSV): influence on the beverage quality and yield of coffee beans. Summa Phytopathol. 32(2): 192-194.

Cáceres S, Aguirre A, Costa N, Coll O, Segnana LG, Fariña N, Tassi AD, Calegario RF, Moraes GJ, Freitas-Astúa J, Pereira JA, Salaroli RB, Kitajima EW (2013) Present status of citrus leprosis in Argentina and Paraguay. Trop Plant Pathol. 38(4): 282-294.

Cruz-Jaramillo JL, Ruiz-Medrano R, Rojas-Morales L, López-Buenfil JA, Morales-Galván O, Chavarín-Palacio C, Abrahán Ramírez-Pool JA, Xoconostle-Cázares B (2014) Characterization of a proposed *Dichorhavirus* associated with the citrus leprosis disease and analysis of the host response. Viruses. 6(7): 2602-2622.

Di Giorgi F, Yde BY, Dib K, Marchi RJ, Trioni HR, Wagner RL (1990) Contribuição ao estudo do comportamento de algumas variedades de citros e suas implicações agroindustriais. Laranja. 11(2): 567-612.

Domingues ET, Teófilo Sobrinho J, Mattos Júnior D, Pompeu Júnior J, Oliveira LA (1996) Qualidade e maturação das laranjas Ovale, Ovale de Siracusa e Ovale San Lio. Laranja. 17(1): 143-158.

Freitas-Astúa J, Bastianel M, Locali-Frabris EC, Novelli VM, Silva-Pinhati AC, Palmieri AC, Targon ML, Machado MA (2007) Differentially expressed stress-related genes in the compatible citrus-*Citrus leprosis virus* interaction. Genet Mol Biol. 30(3): 980-990.

Garita LC, Tassi AD, Calegario RF, Freitas-Astúa J, Salaroli RB, Romão GO, Kitajima EW (2013) Common bean (*Phaseolus vulgaris* L.): experimetal local lesion host for *Citrus leprosis virus* C (CiLV-C) and some other cytoplasmatic-type *Brevipalpus* transmited viruses. Plant Dis. 97(10): 1346-1351.

Garita LC, Tassi AD, Calegario RF, Freitas-Astúa J, Salaroli RB, Romão GO, Kitajima EW (2014) Experimental host range of *Citrus leprosis virus* C (CiLV-C). Trop Plant Pathol. 39(1):43-55.

Gómez EC, Vargas MR, Rivadameira C, Locali EC, Freitas-Astúa J, Astúa-Monge G, Rodrigues JCV, Mesa Cobo NC, Kitajima, EW (2005) First report of *Citrus leprosis virus* on citrus in Santa Cruz, Bolivia. Plant Dis. 89(6): 686.

Hull R (2002) Matthews' Plant Virology. 4th edition. Academic Press, Orlando, 1,001 p.

Izquierdo-Castillo II, Diaz LFZ, Mendez W, Otero-Colina G, Freitas-Astua J, Locali-Fabris EC, Moraes GJ, Calegario RF, Tassi AD, Kitajima EW (2011) Confirmation of the presence of the *Citrus leprosis virus* C (CiLV-C) in Southern Mexico. Trop Plant Pathol. 36(6): 400-403.

Kitajima EW, Rodrigues JCV, Freitas-Astúa J (2010) An annotated list of ornamentals naturally found infected by *Brevipalpus* mite transmitted viruses. Sci Agric. 67(3): 348-371.

Leon GA, Realpe CE, Garzon PA, Rodriguez JA, Moreno MG, Childers CC, Achor D, Freitas-Astúa J, Antonioli-Luizon R, Salaroli SB, Cobo NCM, Kitajima EW (2006) Occurrence of *Citrus leprosis virus* in Llanos Orientales, Colombia. Plant Dis. 90(5): 682-682.

Locali-Fabris EC, Freitas-Astúa J, Souza AA, Takita MA, Astúa-Monge G, Antonioli-Luizon R, Rodrigues V, Targon MLPN, Machado MA (2006) Complete nucleotide sequence, genomic organization and phylogenetic analysis of *Citrus leprosis virus* cytoplasmic type. J Gen Virol. 87(1): 2721-2729.

Marques JPR, Kitajima EW, Freitas-Astua J, Appezato-da-Glória B (2010) Comparative morpho-anatomical studies of the lesions caused by *Citrus leprosis virus* on sweet orange. An Acad Bras Ciência. 82(2): 501-511.

Navia D, Mendonça RS, Ferragut F, Miranda LC, Trincado RC, Michaux J, Navajas M (2013) Cryptic diversity in *Brevipalpus* mites (Tenuipalpidae). Zool Scripta. 42(1): 406-426.

Nunes MA, Oliveira CAL, Oliveira ML, Kitajima EW, Hilf ME, Gottwald T, Freitas-Astúa J (2012) Transmission of *Citrus leprosis virus* C by *Brevipalpus phoenicis* (Geijskes) to alternative host plants found in citrus orchards. Plant Dis. 96(5): 968-972.

Pascholati SF (1995) Fitopatógenos: fitotoxinas e hormônios. In: Bergamin Filho A, Kimati H, Amorin L. (Ed). Manual de fitopatologia, 3.ed., São Paulo: Agronômica 1: 365-392.

Pascon RC, Kitajima JP, Breton MC, Assumpção L, Greggio C, Zanca AS, Okura VK, Alegria MC, Camargo ME, Silva GGC, Cardozo JC, Vallim MA, Franco SF, Silva VH, Jordão Júnior H, Oliveira F, Giachetto PF, Ferrari F, Aguilar-Vildoso CI, Franchiscini FJB, Silva JMF, Arruda P, Ferro JA, Reinach F, Silva ACR (2006) The complete nucleotide sequence and genomic organization of citrus leprosis associated Virus, Cytoplasmatic type (CiLV-C). Virus Genes. 32(3): 289-298.

Pozzan M, Triboni HR (2005) Colheita e qualidade do fruto. In: Mattos Junior D, De Negri JD, Pio RM, Pompeu Junior J (Ed). Citros. 1: 801-822

Redd JB, Hendrix Junior CM, Hendrix DL (1986) Quality control manual for citrus processing plants book. I – Regulation, citrus methodology, microbiology

Rodrigues JCV (2000) Relações patógeno-vetor-planta no sistema leprose dos citros. (2000).168p. Tese. (Doutorado em Ciências) USP - Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba.

Rodrigues JCV (2002) Programa de redução do inóculo da leprose dos citros. Laranja. 23(2): 307-332.

Rodrigues JCV, Kitajima EW, Childers CC, Chagas CM (2003) *Citrus leprosis virus* vectored by *Brevipalpus phoenicis* (Acari: Tenuipalpidae) on citrus in Brazil. Exp Appl Acarol. 30(1-3):161-179.

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Oliveira JB, Coelho MR, Lumbreras JF, Cunha TJF (Ed.) (2006) Sistema brasileiro de classificação de solos. 2.ed. Rio de Janeiro: Embrapa Solos, 306p.

Souza PFC, Goes A (2010) The reaction of sweet oranges to the resistance of *Guignardia citricarpa.* Rev Bras Frutic. 32(3): 718-725.