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Effects of huanglongbing on fruit quality of sweet orange cultivars in Brazil

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Abstract Citrus huanglongbing (HLB), associated with *Candidatus* Liberibacter asiaticus and *Ca.* L. americanus and transmitted by the Asian psyllid *Diaphorina citri*, is the most serious disease of citrus worldwide because of crop devastation and difficulty to control. Since 2004, approximately 3 million trees were eliminated in attempts to limit its spread in Brazil. Where HLB becomes endemic, the disease progression in the orchard and the increasing symptom severity throughout the tree canopy can be relatively fast, greatly reducing the economic life of affected orchards because of tree decline and yield reduction. Although the majority of the fruit from symptomatic branches drop before harvest, a significant amount of affected fruit remain attached, are available for harvest, and can affect juice quality. To quantify and compare the effects of HLB on fruit quality of the most important sweet orange cultivars grown in São Paulo State, 4–6 year-old sweet orange trees from 26 blocks (two of 'Valencia Americana', eight of 'Hamlin', four of 'Westin', seven of 'Pera', and five of 'Valencia') were selected prior to harvest. In each block, 14–21 HLB-symptomatic trees were chosen. In each tree, the quality of 20 fruit normal in appearance from asymptomatic branches and 20 symptomatic fruit from symptomatic branches were assessed. In general, compared to normal fruit, the symptomatic fruit were small, light, more acidic, and had lower juice percentage, Brix, total soluble solids per box, total soluble solids per fruit, and Brix/acidity ratio. These effects of fruit quality were less pronounced on early and mid season sweet orange cultivars than on late season cv. Valencia.

Keywords Epidemiology · Crop loss · Citrus greening · *Citrus sinensis*

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Introduction

In Brazil, citrus huanglongbing (HLB), also known as greening disease, is associated with the sieve tube restricted bacterium *Candidatus* Liberibacter asiaticus and *Ca.* Liberibacter americanus (Colleta-Filho et al. 2004; Teixeira et al. 2005). Both bacteria are transmitted by the Asian psyllid *Diaphorina citri* (Hemiptera: Psyllidae) (Cappor et al. 1967; Yamamoto et al. 2006). HLB has been described as the most important and destructive disease of citrus worldwide (Bové 2006; Gottwald et al. 2007). The reasons for its onerous reputation are the lack of known sources of HLB resistance and curative strategies for diseased trees (Bové 2006). In addition, it is very hard and expensive to manage HLB. Management is based on continuous and frequent reduction of bacterial inoculum by infected tree removal and chemical control of psyllid vectors to prevent trees from becoming infected (Gottwald et al. 2007). Also, in endemic situations or with no effective control of the bacterial inoculum and its insect vector, the disease progression in the orchard (Aubert et al. 1984; Bassanezi et al. 2006a; Catling and Atkinson 1974; Gatineau et al. 2006; Gottwald et al. 1991, 1989) and increased symptom severity evolution throughout tree canopy (Aubert 1990; Aubert et al. 1984; Regmi and Lama 1987; Roistacher 1996) are both relatively quick. These factors have greatly reduced the economic life of affected orchards (Aubert 1990; Aubert et al. 1984; Gottwald et al. 1991; Roistacher 1996), caused the destruction of tens of millions of trees, and the collapse of citrus industries in many countries of south and southeast Asia, Indonesia, Philippines, India, and the Arabian Peninsula (Halbert and Manjunath 2004; Toorawa 1998). Moreover, as disease severity increases, yield is severely reduced, mainly by early fruit drop from affected branches (Aubert et al. 1984; Bassanezi et al. 2006b; Catling and Atkinson 1974; Schwarz 1967). However, the percentage of affected fruit increases as disease severity increases and not all affected fruit drop before harvest. This affected fruit, could be harvested and sent to juice processing plants (Bassanezi et al. 2006b; Catling and Atkinson 1974). Symptomatic fruit have been described as small, light, lop-sided, and with mottled appearance and irregular maturation, with very acidic and bitter juice (McClean and Schwarz 1970). Quantitative data on HLB-affected fruit quality are scarce, but are fundamental to characterise the importance of the disease and to determine the effect of the disease on the quality of processed fruit for juice. In addition, there is no information comparing the effects of HLB on fruit quality of different sweet orange cultivars processed for juice. In 2004 and 2005, HLB was first reported in South and North America, respectively, and has threatened the sustainability of citrus production in São Paulo State — Brazil (Colleta-Filho et al. 2004; Teixeira et al. 2005) and in Florida State — USA (Halbert 2005), respectively, the first and second citrus and orange juice producing industries in the world. The goal of this study was to quantify and compare the effects of HLB on fruit quality of important sweet orange cultivars grown in São Paulo State.

Materials and methods

Citrus groves and data collection

This study was conducted on 4–6 year-old HLB-affected blocks of commercial sweet oranges grafted onto Rangpur lime, located around the municipality of Araraquara in the centre of São Paulo State, Brazil (Table 1). Because eradication of HLB-symptomatic trees is mandatory in São Paulo State, it is not easy to find groves with a sufficient number of diseased trees and symptomatic fruit at harvest for analysis. Therefore, the number of blocks and harvested trees of each cultivar were variable, as well as the harvest date.

The sweet orange cultivars assessed were Hamlin, Valencia Americana, Westin, Pera, and Valencia. Hamlin, Valencia Americana and Westin are early cultivars while Pera is mid-season and Valencia is late. For each block, 14–21 HLB-symptomatic trees were selected. At harvest, for each selected tree, 20 normal fruit from asymptomatic branches and 20 symptomatic fruit from symptomatic branches were arbitrarily selected for quality analysis (Redd et al. 1986). The quality variables assessed were fruit diameter, height (stem to stylar end orientation), weight, percentage of juice (grams of juice per 100 g of fruit), Brix (grams of total soluble solids per 100 ml of juice), kilograms of total soluble solids (TSS) per 40.8 kg box (TSS/box), grams of TSS per fruit (TSS/fruit), acidity (grams of titratable citric acid per 100 ml of juice), and Brix/acidity ratio.

Statistical analysis

For each cultivar, the mean values of each assessed quality variable for symptomatic fruit and normal fruit were compared by *t* test (STATISTICA, StatSoft, Tulsa, OK) considering each tree as one replication. To compare the HLB effect on fruit quality among cultivars, the means of absolute values of fruit quality variables were not used, because differences in fruit quality among healthy sweet orange cultivars have

Table 1 Description of commercial sweet orange blocks and harvest dates assessed for the effect of huanglongbing on fruit quality

Block	Cultivar	Fruit maturation characteristic	Age (years)	Harvest date	Number of harvested trees
SL08	Hamlin	Early season	5	16/07/2007	21
SL24	Hamlin	Early season	5	20/07/2007	15
SA53	Hamlin	Early season	5	13/06/2008	20
SA54	Hamlin	Early season	5	13/06/2008	20
SA55	Hamlin	Early season	5	13/06/2008	20
HE15	Hamlin	Early season	5	02/07/2008	20
HE16	Hamlin	Early season	5	02/07/2008	20
HE17	Hamlin	Early season	5	02/07/2008	20
SP17	Valencia Americana	Early season	6	18/07/2008	20
SP19	Valencia Americana	Early season	6	18/07/2008	20
CH01	Westin	Early season	5	11/08/2005	20
CU01	Westin	Early season	4	26/05/2006	20
SL11	Westin	Early season	6	08/08/2008	20
SL12	Westin	Early season	6	08/08/2008	20
SL21	Pera	Mid season	5	26/07/2007	16
SL22	Pera	Mid season	5	07/08/2007	14
LI01	Pera	Mid season	6	10/09/2007	20
LI02	Pera	Mid season	6	12/09/2007	20
SL26	Pera	Mid season	6	23/07/2008	20
SL39	Pera	Mid season	6	23/07/2008	20
SL41	Pera	Mid season	6	23/07/2008	20
CH02	Valencia	Late season	4	15/09/2004	20
SJ15	Valencia	Late season	5	15/07/2005	20
CH06	Valencia	Late season	6	18/10/2005	20
SL37	Valencia	Late season	5	13/08/2007	20
SL34	Valencia	Late season	5	15/08/2007	20

been well reported in the literature (Di Giorgi et al. 1993; Donadio et al. 1999; Figueiredo 1991). To eliminate the intrinsic cultivar differences in fruit quality, and to allow the comparison of the effects of HLB on assessed variables among cultivars, the reduction (−) or increase (+) in fruit quality variables of symptomatic fruit in relation to normal fruit were calculated for each selected tree according to Eq. (1), where SF=quality variable value for symptomatic fruit, and NF = quality variable for normal fruit.

$$D\% = (SF - NF)/NF * 100 \qquad (1)$$

The mean D% values of each assessed quality variable for each cultivar were then compared by one-way ANOVA with five treatments (five cultivars) and different numbers of replications per treatment (n=156 for Hamlin, n=40 for Valencia Americana, n=80 for Westin, n=130 for Pera, and n=100 for Valencia). When significant differences among variable means were detected by F test, the means were compared by Tukey's highly significant difference test (STATISTICA, StatSoft, Tulsa, OK).

Results

Symptomatic fruit were significantly lighter and smaller (reduction of diameter and height) than normal fruit for all tested sweet orange cultivars (Table 2). For the percentage of reduction of fruit weight, diameter and height in relation to normal fruit, there were significant differences among sweet orange cultivars (Table 3). Cultivars Valencia and Westin had a more pronounced reduction of fruit

Table 2 Fruit diameter (cm), height (cm), and weight (g) of symptomatic fruit from huanglongbing-symptomatic branches compared to normal fruit from asymptomatic branches for five sweet orange cultivars

Cultivar	Asymptomatic	Symptomatic	*P* [z]	*n*
	Diameter (cm) [y]			
Hamlin	6.91±0.55	6.05±0.89	0.000	156
Val. Americana	7.41±0.26	6.87±0.34	0.000	40
Westin	7.04±0.33	5.73±0.50	0.000	80
Pera	6.90±0.24	6.08±0.48	0.000	130
Valencia	7.31±0.34	5.92±0.38	0.000	100
	Height (cm) [y]			
Hamlin	7.22±0.76	6.33±1.14	0.000	156
Val. Americana	7.60±0.32	7.03±0.44	0.000	40
Westin	6.78±0.31	5.58±0.52	0.000	80
Pera	7.44±0.36	6.61±0.54	0.000	130
Valencia	7.37±0.45	6.11±0.44	0.000	100
	Weight (g) [y]			
Hamlin	173.11±35.48	128.59±54.47	0.000	156
Val. Americana	211.53±19.77	173.36±22.56	0.000	40
Westin	176.93±24.19	105.13±21.39	0.000	80
Pera	188.29±20.72	137.33±28.47	0.000	130
Valencia	208.10±29.55	118.91±20.52	0.000	100

[y] Mean ± standard deviation of *n* sampled trees. For each sampled tree 20 symptomatic fruit from huanglongbing-symptomatic branches and 20 normal fruit from asymptomatic branches were harvested.

[z] *P* represents the probability of error estimated by *t* test involved in accepting that the quality variable of symptomatic fruit from huanglonbing-symptomatic branches is different to normal fruit from asymptomatic branches.

weight, diameter and height than other cultivars. Symptomatic fruit of cv. Valencia Americana was less affected in weight and diameter than cvs Hamlin and Pera (not significantly different). Reduction of fruit height on symptomatic fruit of cv. Valencia Americana was not significantly different from cv. Pera.

For cv. Valencia Americana a significant increase in juice content of symptomatic fruit was observed in relation to normal fruit (Table 4). However, reduction of juice content of symptomatic fruit in relation to normal fruit was significant for all other cultivars (Table 4). Cultivar Valencia had a more pronounced reduction of juice content followed by cvs Westin, Hamlin, and Pera.

Fruit concentration of TSS (Brix) from symptomatic fruit was significantly less for all cultivars compared with normal asymptomatic fruit, except for cv. Valencia Americana (Table 4). The reduction of Brix from symptomatic fruit of cvs Hamlin and Pera in relation to Brix from normal fruit was less than the reduction of Brix for fruit of cvs Westin and Valencia (Table 3).

Both TSS/box and TSS/fruit were significantly reduced in symptomatic fruit for all cultivars tested compared with normal asymptomatic fruit, except TSS/box for cv. Valencia Americana (Table 4). However, differences were observed among sweet orange cultivars relative to decrease of TSS/box and TSS/fruit. The percent reductions of TSS/box and TSS/fruit of symptomatic fruit in relation to normal

Table 3 Reduction (−) or increase (+), in percentage, on fruit quality variables of symptomatic fruit from huanglonbing-symptomatic branches and normal fruit from asymptomatic branches for five sweet orange cultivars

Fruit quality variables	Reduction or increase (%)[y]				
	Val. Am. (*n*=40)	Hamlin (*n*=156)	Pera (*n*=130)	Westin (*n*=80)	Valencia (*n*=100)
Weight	−17.49 a	−27.53 b	−26.79 b	−39.62 c	−42.27 c
Diameter	−7.25 a	−12.51 b	−11.94 b	−18.32 c	−18.95 c
Height	−7.43 a	−12.73 b	−11.15 ab	−17.60 c	−16.96 c
Juice content	+4.78 a	−5.86 bc	−2.21 b	−6.97 cd	−10.40 d
Brix	−0.18 a	−6.39 a	−5.40 a	−16.14 b	−17.02 b
TSS/box	+4.32 a	−10.65 b	−7.09 b	−21.80 c	−25.15 c
TSS/fruit	−13.84 a	−32.66 b	−31.84 b	−51.82 c	−56.36 c
Acidity	+5.55 a	+20.35 b	+18.54 b	+17.83 b	+45.24 c
Ratio	−4.27 a	−18.91 b	−17.90 b	−27.40 c	−41.37 d

[y] Values with the same letter are not significantly different by Tukey test ($P>0.05$).

Table 4 Comparison of orange juice quality variables of symptomatic fruit from huanglongbing-symptomatic branches with normal fruit from asymptomatic branches for five sweet orange cultivars

Cultivar	Asymptomatic	Symptomatic	P^z	*n*
	Juice content [y] (grams of juice per grams of fruit)			
Hamlin	42.18±4.60	39.33±6.43	0.000	156
Val. Americana	51.63±4.00	53.97±4.93	0.023	40
Westin	46.83±4.34	43.55±5.83	0.000	80
Pera	51.97±3.53	50.67±4.05	0.006	130
Valencia	49.97±4.14	44.64±4.64	0.000	100
	Brix [y] (grams of total soluble solids per 100 ml)			
Hamlin	9.58±0.90	8.93±1.42	0.000	156
Val. Americana	9.31±0.82	9.26±1.31	0.843	40
Westin	10.71±1.23	8.93±1.63	0.000	80
Pera	9.89±1.04	9.32±1.43	0.000	130
Valencia	9.63±1.03	7.98±1.21	0.000	100
	TSS/box [y] (kilograms of total soluble solids per 40.8 kg box)			
Hamlin	1.65±0.24	1.44±0.37	0.000	156
Val. Americana	1.97±0.26	2.05±0.37	0.265	40
Westin	2.04±0.17	1.58±0.29	0.000	80
Pera	2.10±0.25	1.93±0.32	0.000	130
Valencia	1.96±0.26	1.46±0.28	0.000	100
	TSS/fruit [y] (grams of total soluble solids per fruit)			
Hamlin	6.89±1.24	4.80±2.58	0.000	156
Val. Americana	10.15±1.38	8.64±1.65	0.000	40
Westin	8.82±1.29	4.15±1.26	0.000	80
Pera	9.62±1.16	6.50±1.64	0.000	130
Valencia	9.92±1.38	4.29±1.25	0.000	100
	Acidity [y] (grams of titratable citric acid/100 ml)			
Hamlin	0.76±0.18	0.91±0.27	0.000	156
Val. Americana	0.75±0.10	0.79±0.12	0.141	40
Westin	0.95±0.25	1.11±0.35	0.001	80
Pera	0.96±0.22	1.14±0.32	0.000	130
Valencia	1.22±0.32	1.75±0.42	0.000	100
	Brix/acidity Ratio [y]			
Hamlin	13.12±2.63	10.72±3.67	0.000	156
Val. Americana	12.52±1.62	11.86±1.71	0.078	40
Westin	11.94±2.46	8.70±2.61	0.000	80
Pera	10.71±2.30	8.79±2.70	0.000	130
Valencia	8.34±2.11	4.80±1.33	0.000	100

[y] Mean ± standard deviation of *n* sampled trees. For each sampled tree 20 symptomatic fruit from huanglongbing-symptomatic branches and 20 normal fruit from asymptomatic branches were harvested.

[z] *P* represents the probability of error estimated by *t* test involved in accepting that the quality variable of symptomatic fruit from huanglonbing-symptomatic branches is different to normal fruit from asymptomatic branches.

fruit were significant and more pronounced for cvs Valencia and Westin (Table 3). For TSS/box and TSS/fruit, the percent reduction was significantly less for symptomatic fruit from cv. Valencia Americana, while the reduction for cvs Hamlin and Pera was intermidiate (Table 3).

Fruit acidity was the unique quality variable that was significantly increased for any cultivar, except for cv. Valencia Americana, compared with normal fruit (Table 4). The relative increase in fruit acidity was significantly more pronounced for cv. Valencia than for cvs Westin, Hamlin and Pera (Table 3).

Due to Brix decrease and acidity increase of symptomatic fruit, the Brix/acidity ratio of symptomatic fruit was significantly reduced for all tested sweet orange cultivars compared with normal fruit, except for cv. Valencia Americana (Table 4). Reduction of the Brix/acidity ratio was significantly lower for cvs Pera and Hamlin, intermediate for cv. Westin, and higher for cv. Valencia (Table 3).

Discussion

In this study we did not compared fruit from PCR-negative trees with fruit from asymptomatic branches of HLB-symptomatic trees. Liberibacters are unevenly distributed in HLB-affected trees. (Teixeira et al. 2008). Even though real-time PCR was able to detect liberibacters in symptomless leaves of affected trees, many other leaf samples from symptomless branches tested negative. Therefore, researchers can never be certain that a given asymptomatic tree or asymptomatic branch is actually uninfected. The confirmation of HLB pathogen presence in each harvested fruit or in leaves from branches using molecular tests would make this work unfeasible. Knowing that symptomless branches might still be infection-free or have very low liberibacter content, we would expect similar results on quality variables for fruit from asymptomatic branches of HLB-symptomatic trees and fruit from healthy trees. Additionally, fruit from asymptomatic trees and fruit from asymptomatic branches of symptomatic trees looked very similar in size, shape and peel colour.

For all tested sweet orange cultivars, the range of each of the fruit quality variables for normal fruits from asymptomatic branches were less than expected for healthy fruit of respective cultivars grown in the central region of São Paulo State and harvested in the same months as our samples (Nonino 1995). As the differences were similar for all sweet orange cultivars, the results could be compared directly. This is the first study to quantify the effects of HLB on symptomatic fruit compared to symptomless fruit on multiple sweet orange cultivars.

There were remarkable differences between HLB-symptomatic fruit from symptomatic branches and normal fruit from asymptomatic branches relative to the reduction of fruit size, weight, Brix, TSS/box, TSS/fruit, Brix/acidity ratio, and the increase of fruit acidity.

Fruit growth, final size, and weight result from accumulation of dry matter and water (Davis and Albrigo 1994; Guardiola 1992). For healthy trees under no water stress conditions, fruit size and weight have a positive relationship to Brix/acidity ratio and TSS/fruit, and a negative relationship to Brix, TSS/box, and acidity (Di Giorgi et al. 1993; Gazzola et al. 1991). Healthy trees under water stress usually produce smaller and lighter fruit with increased concentrations of total soluble solids (Brix and TSS/box) and citric acid (acidity), but with reduced percentages of juice and Brix/acidity ratio (Erickson and Richards 1955; Mostert and Van Zyl 2000). For two citrus diseases, citrus variegated chlorosis (CVC) and citrus sudden death (CSD), fruit from affected trees were smaller, lighter, had reduced Brix/acidity ratio, and reduced TSS/fruit, but with increased Brix, TSS/box, and acidity (Bassanezi et al. 2007; Laranjeira and Palazzo 1999). The authors suggested that these changes in affected fruit were the result of water transport blockage due to bacteria in the xylem, in the case of CVC (Laranjeira and Palazzo 1999), and a reduction of root system water uptake capacity, in the case of CSD (Bassanezi et al. 2007). In the case of HLB-symptomatic fruit, the impaired water transport and accumulation could explain partially the reduction in fruit size, weight, juice content, TSS/fruit, and Brix/acidity ratio and the increase in fruit acidity; however they could not explain the reduction of Brix and TSS/box. An additional effect is probably involved in the reduction of total soluble solid accumulation into the fruit from HLB-symptomatic branches.

Recent anatomical studies of sweet orange leaves infected by *Candidatus* Liberibacter asiaticus and *Ca.* L. americanus (Heredia et al. 2006; Kim et al. 2009) confirmed previous observations of Schneider (1968) with leaves affected with *Ca*. L. africanus. These studies indicated that HLB bacterium infection caused phloem disruption, plugged sieve pores, and starch accumulation. The phloem cell wall and cambium layer of infected leaves were thicker than uninfected control leaves. HLB bacteria did not form aggregates in citrus and phloem blockage resulted mostly from the up-regulation of the *pp2* gene related to callose deposition and plugging of the sieve pores in HLB-affected trees (Kim et al. 2009). Additionally, HLB pathogen infection up-regulated starch synthesis genes including AGPase, starch synthase, and granule-bound starch synthase in HLB-affected citrus leaves, but did not influence genes directly associated with photosynthesis (Kim et al. 2009). The authors concluded that up-regulation of key starch biosynthetic genes with photosynthesis was apparently unaltered, and together with restricted movement of photosynthates from leaves due to phloem plugging, likely led to the accumulation of starch in HLB-affected leaves. Sucrose, the major photoassimilate transported in sieve tubes from mature leaves to sink organs, is then impaired in moving out of HLB-diseased leaves and reaching the fruit of symptomatic branches, explaining the fruit Brix and TSS/box reductions observed in symptomatic fruit from symptomatic branches.

The observed difference among sweet orange cultivars in relation to relative loss of fruit quality could suggest that early and mid-season cultivars are slightly more tolerant to HLB or more efficient in the transport and accumulation of TSS into fruit affected by HLB. Flower anthesis occurs at the same time for both cultivars, but because the biochemical transformations in late cultivars are slower than in early and mid-season cultivars, more time or degree-days are required for late cultivars to achieve fruit maturity (Ortolani et al. 1991). Thus, the HLB effect would be less for cultivars with more efficient mechanisms of water and dry matter transport and accumulation such as early and mid-season cultivars. Also, as the duration of blockage increases, nutrient deficiency in sink organs and the hindrance of fruit maturation will be the likely consequences. The expression of foliar symptoms of HLB is seasonal, usually occurring from the beginning of autumn to the end of winter (from April to September in São Paulo, Brazil) when the concentration of bacteria into the tissues is higher (Gottwald et al. 2007). At the onset of foliar symptoms, fruit of early and mid-season cultivars are almost ready to be harvested at the end of autumn and winter (after June), while fruit of late cultivars harvested in spring and at the beginning of summer, become more affected by the disease.

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