

Yield loss caused by huanglongbing in different sweet orange cultivars in São Paulo, Brazil

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Accepted: 15 November 2010 / Published online: 9 April 2011
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Abstract Citrus huanglongbing (HLB) reduces an affected orchard's economic life. This work aimed to characterize yield loss due to HLB for different sweet orange cultivars and determine the relationship between disease severity and yield. Disease severity and yield were assessed on 949 individual trees distributed in 11 different blocks from sweet orange cultivars Hamlin, Westin, Pera and Valencia. In each block, plants showing a range of HLB severity levels and asymptomatic plants were selected. Total yield (weight of harvested fruit), mean weight of asymptomatic and symptomatic fruit, relative yield (symptomatic tree yield/mean yield of asymptomatic trees from the same block) and relative number of fruits (fruit number from symptomatic tree/mean number of fruits from asymptomatic trees from the same block) were determined. The weight of symptomatic fruit was lower than the weight of asymptomatic fruit, but the weights of asymptomatic and symptomatic fruit were not correlated with disease severity, indicating

that the effects of HLB were restricted to symptomatic branches. The relationship of the relative yield with HLB severity can be satisfactorily described by a negative exponential model. The rates of yield decrease as a function of disease severity were similar for all assessed cultivars. A relative yield (up to 19%) was observed even for trees where disease severity was 100%. The strong linear relationship between relative number of fruits per tree and the relative yield per tree suggested that the yield reduction was due primarily to early fruit drop or lack of fruit set on affected branches.

Keywords Epidemiology · Yield-disease severity relationship · Citrus greening · *Citrus sinensis* · *Candidatus Liberibacter asiaticus*

Introduction

Citrus huanglongbing (HLB) is the most serious citrus disease worldwide (Bové 2006; da Graça 1991; Gottwald et al. 2007a). In Brazil, where the disease was first detected in 2004, it is associated with the bacterial species *Candidatus Liberibacter asiaticus* and *Ca. Liberibacter americanus* (Coletta-Filho et al. 2004; Teixeira et al. 2005). Both species are spread in the field by the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) (Capoor et al. 1967; Yamamoto et al. 2006) and can also be transmitted from plant to plant by grafting (Lopes et

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al. 2009). All major commercial sweet orange cultivars grown in Brazil are susceptible to this disease and the concentrations of these pathogens in leaf tissues do not vary among cultivars. However, *Ca. Liberibacter asiaticus* is found in higher concentrations than *Ca. Liberibacter americanus* within the same cultivar (Lopes et al. 2009).

Infected trees usually develop one or more conspicuous yellow shoots, which appear in contrast to the other healthy or symptomless parts of the tree and give a sectored appearance to the disease. As a phloem inhabitant, the bacteria spread systemically throughout the plant (Tatineni et al. 2008) and the symptoms can be observed on leaves and fruits. The most characteristic foliar symptom is a blotchy mottled pattern, which is asymmetrical on the two halves of a leaf. In later stages, zinc-like deficiency symptoms can develop, with small upright leaves becoming leathery and with a prominent vein, followed by leaf drop and twig dieback (Gottwald et al. 2007a; McClean and Schwarz 1970). Fruit produced on infected branches are not marketable because they are small and lopsided with yellow-stained vascular bundles (Bové 2006; da Graça 1991; McClean and Schwarz 1970) and of very poor quality (Bassanezi et al. 2009a). Since there is no curative method or available resistant sweet orange cultivar, HLB management has been conducted through the continuous removal of symptomatic trees in order to reduce the amount of inoculum, and frequent insecticide applications to reduce vector population and disease spread (Bové 2006; da Graça 1991; Gottwald et al. 2007a, b).

The destructive impact of HLB on the citrus industry in many countries due to crop devastation and limited citrus production is well documented (Aubert 1992; Bové 2006; da Graça 1991; Gottwald et al. 2007a). When HLB becomes established and there is no effective control by reduction of the bacteria inoculum and its vector, the disease incidence increases quickly. The evolution of disease severity throughout the tree canopy, especially in young trees, is considered to be very fast, greatly reducing the production life span of the affected orchards (Aubert et al. 1984; Gottwald et al. 1991; Roistacher 1996). There has been only one attempt to model the impact of HLB on citrus yield over time in non-controlled groves, which was based on hypothetical rates of disease incidence and severity increase according to the tree age at infection (Bassanezi and Bassanezi

2008). To improve this model, it is necessary to assess the development of disease incidence and severity over time accurately and to determine the relationships between yield and the variables of the epidemic (Madden et al. 2007). The progress rates of HLB incidence are well documented for many places in Asia, Africa, South and North America (Aubert et al. 1984; Bassanezi et al. 2006, 2009b; Catling and Atkinson 1974; Gatineau et al. 2006; Gottwald et al. 1989, 1991, 2007b). Trees with severe symptoms have been observed within 5–8 years after planting (Aubert et al. 1984; Regmi and Lama 1988; Roistacher 1996) or 1–5 years after symptom onset in the tree (Aubert 1992; Schwarz et al. 1973). However, only one study, which was conducted in China, estimated the HLB severity progress rate of an affected grove (Gottwald et al. 1989) but not the disease severity progress rate for individual trees. It was also reported that as the disease severity increases, the fruit yield and quality are seriously reduced (Aubert et al. 1984; Catling and Atkinson 1974; Schwarz 1967). However, these studies were not done with the same sweet orange cultivars grown in Brazil and none of them was able to establish relationships between disease severity and yield loss.

Although some reports are available in the literature, a great deal of quantitative information needs to be gained through field experiments and then validated for local conditions. The yield loss caused by a disease can be determined in experimental plots as the difference in the yield of any given crop unit and the yield of a disease-free crop unit (Madden and Nutter 1995). The data collected to assess and model losses must be representative, reliable, and realistic with regards to the range of disease incidence or severity encountered. To achieve this objective, data are usually collected either from fields in which natural epidemics are occurring or from fields in which attempts are made to create disease intensity differentials (Campbell and Madden 1990). In Brazil, where HLB control is regulated by the government through eradication of HLB-symptomatic trees, it is not possible to create disease intensity differentials under experimental conditions. Disease and yield loss can only be estimated in commercial fields under natural infection. Because of the difficulties in artificially establishing a HLB severity gradient, the single plant method, where the experimental unit is a single plant, can be used to estimate the yield loss

caused by a disease (Madden et al. 2007). This method is utilized in most cropping areas because large variation in disease intensity is often encountered from plant to plant (Campbell and Madden 1990). Furthermore, single plants are frequently used for the purpose of assessing disease-yield loss responses, as it is more important to have more treatments with different disease intensities than to have fewer treatments with replications (Teng and Oshima 1983).

Despite the importance of HLB, only a few studies have attempted to quantify the yield reduction caused by HLB in Reunion Island and South Africa (Aubert et al. 1984; Catling and Atkinson 1974). This information is fundamental for characterizing the impact of the disease and for defining economic models to predict the economic life of a given HLB-affected grove. Thus, the aim of this work was to assess the yield loss caused by HLB for different Brazilian sweet orange cultivars and to determine the relationship between disease severity and yield.

Materials and methods

Disease severity and yield assessments

The terminology used in this paper follows Savary et al. (2006) and Madden et al. (2007). The single plant approach was used to estimate yield loss

caused by HLB. Disease and yield were assessed on 949 trees (116 symptomless, and 833 with varying levels of disease severity), distributed in 11 different commercial blocks of 4–6 year-old sweet orange (*Citrus sinensis* Osbeck) grafted on Rangpur lime (*Citrus limonia* Osbeck), where the presence of *Candidatus* Liberibacter species was confirmed by PCR according to Teixeira et al. (2005). The citrus blocks are described in Table 1: four were from the early cultivars Hamlin and Westin, three were from the mid-season cultivar Pera and four were from the late cultivar Valencia. All blocks were located in commercial groves which follow the recommended management practices for citrus production in Araraquara County, the most HLB-affected region of São Paulo State, Brazil. In each block, 37 to 100 trees showing a wide range of severity levels, including 6 to 15 symptomless plants, were selected. The assessment of disease severity was performed according to Aubert et al. (1984) and Gottwald et al. (1991) on the same day of fruit harvest. Each tree was divided into an upper and lower hemisphere by an imaginary horizontal plane at mid-canopy height. Each hemisphere was subdivided into four equal quadrants by two imaginary perpendicular planes passing through the axis of the tree trunk. The resulting 8 sections were scored individually on a linear category scale with levels 0, 1, 2, 3, 4, and 5, corresponding to 0, 20, 40, 60, 80, and 100% of the canopy section area with HLB symptoms. Disease

Table 1 Citrus block number, sweet orange cultivar, harvest and assessment date, tree age on the date of harvest and disease assessment and number of asymptomatic/symptomatic trees in the areas where data were collected

Block	Sweet orange cultivar	Fruit maturation	Harvest and assessment date	Tree age (years)	Asymptomatic/Symptomatic trees per block
1	Hamlin	Early	16 Jul 2007	5	10/51
2	Hamlin	Early	20 Jul 2007	5	6/31
3	Westin	Early	11 Aug 2005	5	11/90
4	Westin	Early	26 May 2006	4	9/91
5	Pera	Mid	5 Sep 2007	6	10/51
6	Pera	Mid	26 Jul 2007	5	10/78
7	Pera	Mid	7 Aug 2007	5	10/90
8	Valencia	Late	15 Sep 2004	4	15/85
9	Valencia	Late	18 Oct 2005	5	15/85
10	Valencia	Late	13 Aug 2007	5	10/90
11	Valencia	Late	15 Aug 2007	5	10/90

severity per tree was the mean of the 8 section severity scores. The fruit harvest date varied for each block according to fruit maturation of each cultivar (Table 1), from May to October. The disease symptoms were conspicuous at harvest date allowing good disease severity assessment. Each assessed tree was individually harvested at one time, and the normal (asymptomatic) and symptomatic fruit were counted and weighted separately. Lopsided fruit with mottled appearance and irregular maturation were considered symptomatic for HLB (McClean and Schwarz 1970). For each tree, the following variables were calculated: total yield (weight of all harvested fruit), mean weight of asymptomatic fruit (weight of asymptomatic fruit/number of asymptomatic fruit), mean weight of symptomatic fruit (weight of symptomatic fruit/number of symptomatic fruit), relative yield (yield of symptomatic tree/mean yield of asymptomatic trees from the same block), relative number of fruits (number of fruits from symptomatic tree/mean number of fruits from asymptomatic trees from the same block), and percentage of symptomatic fruit per tree.

Data analysis

Spearman's rank correlations were performed to test the dependence between the number of symptomatic fruit per tree and disease severity, between the proportion of harvested fruit with HLB symptoms and disease severity, and between the weight of asymptomatic or symptomatic fruit and disease severity. Relationships between relative yield (dependent variable) and disease severity (independent variable) were tested by linear and non-linear regression analyses for each group of cultivars: early, mid-season, and late. Three models were fitted to describe the relationship between relative yield (proportion) and disease severity: the negative exponential model [$y = \exp(-b \cdot x)$], a deterministic version of the half-Cauchy distribution [$y = b_1 / (1 + (b_2 \cdot x)^2)$], and a generalized gradient model [$y = b_1 \cdot \exp(-b_2 \cdot x^{b_3})$], with the last two models being recommended for gradient analysis (Madden et al. 2007). Coefficient of determination and residual pattern were used to choose the best model describing the relationship between relative yield and disease severity. The parameters of the chosen model obtained for each group of cultivars were compared by a *t* test. The linear model was fitted

to describe the relationship between relative yield (dependent variable) and relative number of fruits per tree (independent variable). The parameters of the linear equations obtained for each group of cultivars were also compared by *t* test. The mean weights of asymptomatic fruit from asymptomatic trees and symptomatic and asymptomatic fruit from symptomatic trees were compared by analysis of variance. Mean comparisons were done by Tukey's highly significant difference test. All analyses were performed using the software STATISTICA 7.1 (Stat Soft Inc., Tulsa, OK).

Results

Within each citrus block, high variation in yield and number of fruits was observed among symptomless trees as well as among trees with the same disease severity, even within trees with the same scion/rootstock variety, age, management, and similar canopy size. In addition, the yield and number of fruits in symptomless trees were quite different among blocks for the same cultivar and among blocks of different cultivars (Fig. 1a, d, g). The relative yield and relative number of fruits were calculated to normalize the data and allow for comparison among cultivars (Fig. 2).

In general, the number of symptomatic fruit harvested per tree was small. The mean number of symptomatic fruit harvested per tree (\pm standard error) was 38.3 (± 2.6), 15.0 (± 1.3), and 29.3 (± 1.4), respectively, for early, mid-season and late cultivars (Fig. 1b, e, h). A poor correlation was observed between the number of symptomatic fruit per tree and disease severity for all tested cultivars (Table 2, Fig. 1b, e, h). However, the proportion of harvested fruit with HLB symptoms significantly increased with disease severity (Table 2, Fig. 1c, f, i).

The weights of symptomatic and asymptomatic fruit were not correlated with disease severity, except for the late cultivar Valencia, in which the weight of asymptomatic fruit was negatively correlated with disease severity (Table 2, Fig. 3). For all cultivars, the mean weight of asymptomatic fruit from symptomatic trees was not different from the mean weight of asymptomatic fruit from asymptomatic trees, but both were significantly greater than the mean weight of symptomatic fruit (Table 3). On average, symptomatic fruit were 22.6, 30.4 and 37.6% lighter than asymp-

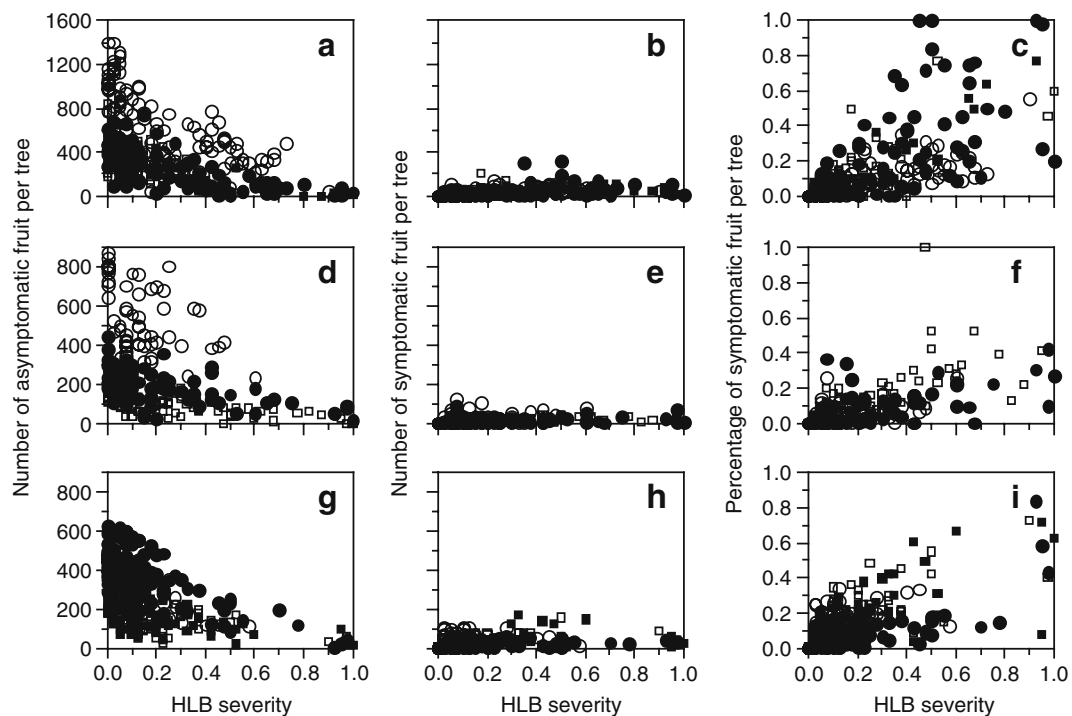


Fig. 1 Relationship between HLB severity (proportion of symptomatic canopy area) and the number of asymptomatic (a, d, g) and symptomatic fruit (b, e, h) per tree and the

percentage of symptomatic fruit per tree (c, f, i), in early (a–c), mid-season (d–f) and late (g–i) sweet orange cultivars in São Paulo State. Different symbols refer to different citrus blocks

tomato fruit from early, mid-season and late cultivars, respectively.

All models fitted to describe the relationship between relative yield and HLB severity presented similar coefficients of determination and none of them showed any apparent residual pattern (*data not shown*). The negative exponential model was chosen to describe the relationship between relative yield and HLB severity (Fig. 2, Table 4) because it is the simplest with only one parameter. The rates of yield reduction as a function of disease severity were similar for all cultivars ($b=1.66$ to 1.95 , Table 4). The relative yield (y)-HLB severity (x) relationship for the sweet orange cultivars was described by a single equation $y = \exp(-1.85x)$. In all cases, a relative yield in the range of 14% to 19% was expected in trees where 100% of the canopy showed symptoms (Fig. 2). The relative number of fruits per tree was positively related linearly to the relative yield per tree, with 93, 94 and 78% of the total variation in the relative yield explained by the relative number of fruits for early, mid-season and late cultivars, respectively (Fig. 4).

Discussion

A significant relationship between HLB severity and citrus yield was established for the main citrus cultivars in Brazil. Despite of the low coefficient of determination for the severity-yield model which reduces its predictive value for individual trees, this equation has a predictive value for an orchard as whole and shows that HLB reduces yield even at low levels of disease severity. The difficulty of establishing a quantitative relationship between disease severity and yield in perennial fruit crops is mainly due to the high variability of yield among individual trees at the same disease severity level. The tendency of fruit trees to bear heavy crops in a year and very little or no crop at all in the next year is termed alternate or biennial bearing. Citrus is a major fruit crop that suffers from alternate bearing, leading to temporal variation in fruit yield and quality among individual trees (Ye et al. 2008a, b). In contrast to coffee where alternate bearing is homogeneous within a season for each block of plants, in citrus, neighbour plants show different behavior in the same year, with high yielding

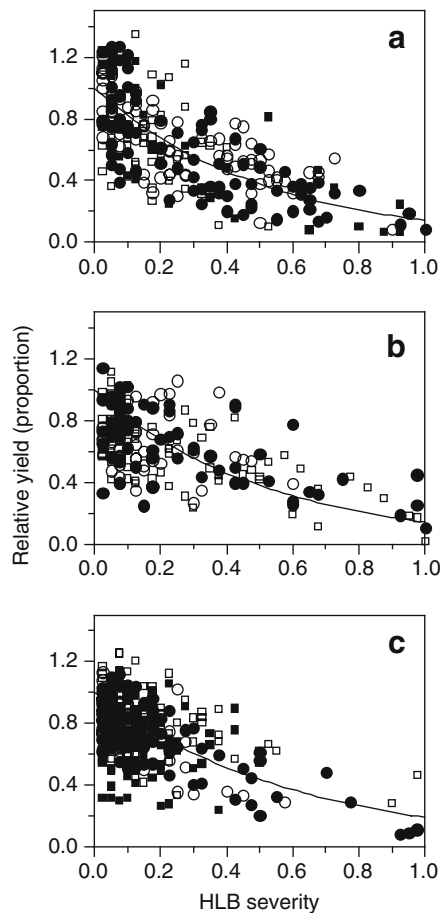


Fig. 2 Relationship between HLB severity (proportion of symptomatic canopy area) and relative yield (yield from symptomatic tree/mean yield from asymptomatic trees) in early (a), mid-season (b) and late (c) sweet orange cultivars in São Paulo State. Different symbols refer to different citrus blocks. The lines represent the fitted curves by the negative exponential model (Parameters of the negative exponential model in Table 4)

and low yielding plants, which makes it impossible to forecast the yield based on the previous year's data (Ye et al. 2008a, b). Large variation in fruit yield

and number of fruits per tree for the same healthy sweet orange cultivars has also been observed in other studies (Bassanezi et al. 2007; Palazzo 1993). Such variation contributes to the relatively low coefficients of determination (R^2 from 0.10 to 0.44) of the negative exponential model for relative yield and HLB severity even though these relationships were statistically highly significant ($P < 0.01$) for all cultivars (Table 4).

The negative exponential model satisfactorily described the relationship between yield and HLB severity for all sweet orange varieties surveyed in this study. This model represents a type I curve as described Mumford and Norton (1987), where the greatest rate of change in yield occurs at low disease severity (Madden and Nutter 1995), suggesting that even when the initial symptoms appear, the yield will be reduced. Type I curves also indicate that the main effect of the injury is reduction of the radiation use efficiency in photosynthetically active tissues (Johnson 1987). HLB is a phloem disease that does not reduce leaf area by necrosis, but instead, alters the host metabolism. However, the yield loss observed at low disease severity does not agree with preliminary results obtained by Catling and Atkinson (1974) in South Africa and by Aubert et al. (1984) in Reunion Island, who were not able to observe a significant yield reduction at low incidences of foliar symptoms. Catling and Atkinson (1974) only observed a yield reduction for trees with foliar symptoms with ratings of 3 and 4 (where 1 = slight and 4 = severe), although symptomatic fruit accounted for 6.6 to 18.5% of the fruit at harvest for trees with ratings of 1 and 2. Aubert et al. (1984) observed normal yield for trees showing only small symptomatic areas in the canopy, with severity ranging from 0 to 12.5%. However, the previous work grouped trees with different ranges of

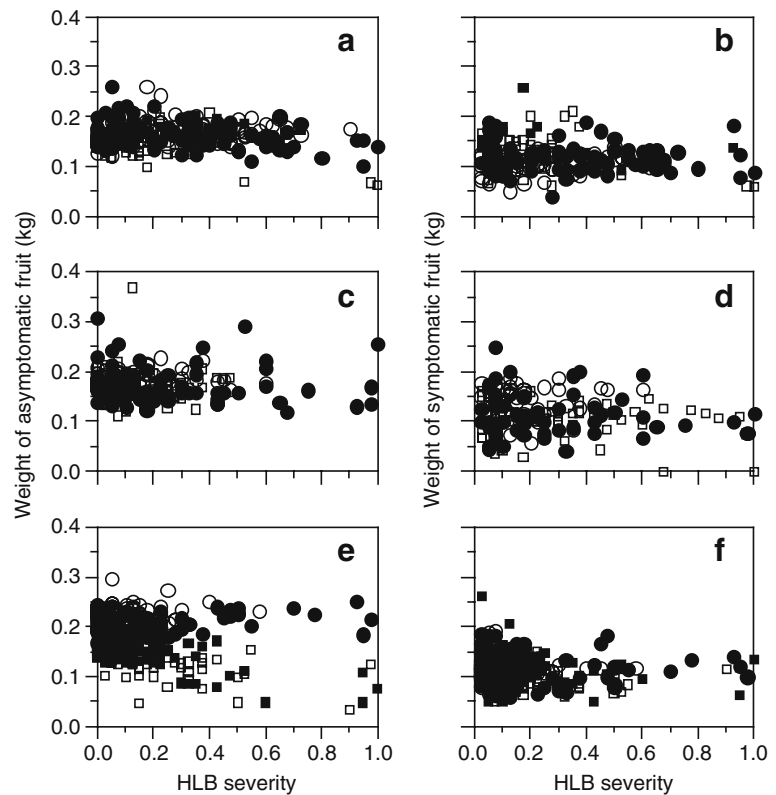
Table 2 Spearman's rank correlation coefficients between huanglongbing (HLB) severity and other variables: number of fruits with HLB symptoms per tree, proportion of harvested

fruit with HLB symptoms per tree, weight of asymptomatic fruit, and weight of symptomatic fruit for early, mid-season, and late sweet orange cultivars

Cultivars	Number of symptomatic fruit per tree	Proportion of harvested symptomatic fruit per tree	Weight of asymptomatic fruit	Weight of symptomatic fruit
Early	0.53*	0.71*	0.11	−0.08
Mid-season	0.41*	0.54*	−0.11	−0.02
Late	0.31*	0.51*	−0.26*	−0.11

* = Significant correlation ($P \leq 0.01$)

Fig. 3 Relationship between HLB severity (proportion of symptomatic canopy area) and the mean weight (kg) of asymptomatic (a,c,e) and symptomatic fruit (b,d,f) in early (a,b), mid-season (c,d) and late (e,f) sweet orange cultivars in São Paulo State. Different symbols refer to different citrus blocks



disease severity into few classes. In our work, we generated a continuum of disease severity and thus considered that the difference between our results and the previous ones may result from the variation in the fruit yield per tree for the same level of disease severity, as discussed previously.

Yield per tree is composed of the number of fruits per tree and the individual weight of those fruit. With constant fruit weight, yield per tree will be totally dependent on the number of fruits per tree. However, as the variation in fruit weight among individual fruit from a single tree increases, the dependence of relative yield on the number of fruits per tree

decreases. The high correlation between relative yield and number of fruits indicates that most of the reduction in yield caused by HLB could be due to the reduction of the number of fruit on symptomatic trees due to early fruit drop or lack of new fruit in affected branches rather than only reduction in fruit weight, which is observed with citrus variegated chlorosis (CVC, caused by *Xylella fastidiosa*). Although CVC significantly reduces the weight of the affected fruit in symptomatic branches, these fruit remain on the canopy until harvest and do not reduce the number of fruits; therefore, trees with different disease severity still have similar numbers

Table 3 Mean weight (g) of asymptomatic fruit from asymptomatic trees and mean weight of asymptomatic and symptomatic fruit from huanglongbing symptomatic trees of early, mid-season, and late sweet orange cultivars

Cultivars ^y	Mean weight of asymptomatic fruit from asymptomatic trees	Mean weight of asymptomatic fruit from symptomatic trees	Mean weight of symptomatic fruit from symptomatic trees
Early	157.8 a	160.6 a	123.3 b
Mid-season	185.3 a	176.2 a	125.8 b
Late	193.7 a	176.0 a	115.3 b

^y Values with the same letter in the rows are not significantly different by Tukey highly significant difference test ($P > 0.01$)

Table 4 Equation parameters ($b \pm$ standard error) and coefficients of determination (R^2) of the negative exponential model [$y = \exp(-b \cdot x)$, where y is the relative yield (proportion) and x is the huanglongbing severity (proportion of symptomatic canopy area)] fitted to observed data of relative yield over different disease severities from citrus trees with symptoms of huanglongbing from early, mid-season, and late sweet orange cultivars, and to combined cultivars data as well (Graphical representation of data in Fig. 2)

Cultivars	$b \pm$ std error	d.f.	R^2	P
Early	1.95 ± 0.12	263	0.44	<0.01
Mid-season	1.94 ± 0.13	203	0.10	<0.01
Late	1.66 ± 0.09	326	0.20	<0.01
Combined data	1.85 ± 0.06	794	0.33	<0.01

d.f., degrees of freedom; R^2 , coefficient of determination; P , F probability

of fruit and the relationship between relative yield and number of fruits is not as strong as for HLB. Using data from Palazzo (1993) for the yield and number of fruits from healthy and CVC-affected Natal sweet oranges grafted on Cleopatra mandarins in two consecutive years, only 65% of the variation in the relative yield was explained by the relative number of fruits. The poor correlation observed in our study between the number of HLB-symptomatic fruit per tree and disease severity for all tested cultivars is also an indication that, in the case of HLB, fewer fruit were set on symptomatic branches or more symptomatic fruit had dropped early with increasing disease severity, leading to only a small proportion of fruit (probably the most recently affected ones) remaining attached on symptomatic branches until harvest. Catling and Atkinson (1974) also found a weak relationship between the number of symptomatic fruit per tree and the severity of HLB. Similar to our results, they observed that the proportion of harvested symptomatic fruit significantly increased with increasing disease severity. Independent of HLB severity, there was always a low number of symptomatic fruit with poor juice quality that could be harvested and brought to market or juice processing plants.

No significant difference between asymptomatic fruit weight from asymptomatic trees and asymptomatic branches of symptomatic trees was observed, but symptomatic fruit from symptomatic branches were significantly lighter than asymptomatic fruit from asymptomatic branches. These results supported

the findings by Bassanezi et al. (2009a) of the remarkable differences between HLB-symptomatic fruit from symptomatic branches and normal fruit from asymptomatic branches in terms of fruit size, weight, and other fruit quality variables, such as Brix, acidity, Brix/acidity ratio and total soluble solids (TSS) content. These data indicate that the effects of HLB on yield, as well as on fruit quality, are restricted to symptomatic branches even if the pathogen is present in symptomless branches of affected trees (Tatineni et al. 2008).

Corroborating the results of Bassanezi et al. (2009a), we found more pronounced effects of HLB on fruit weight on the late season cultivar Valencia than on the early and mid-season sweet orange cultivars. A weakly significant relationship was

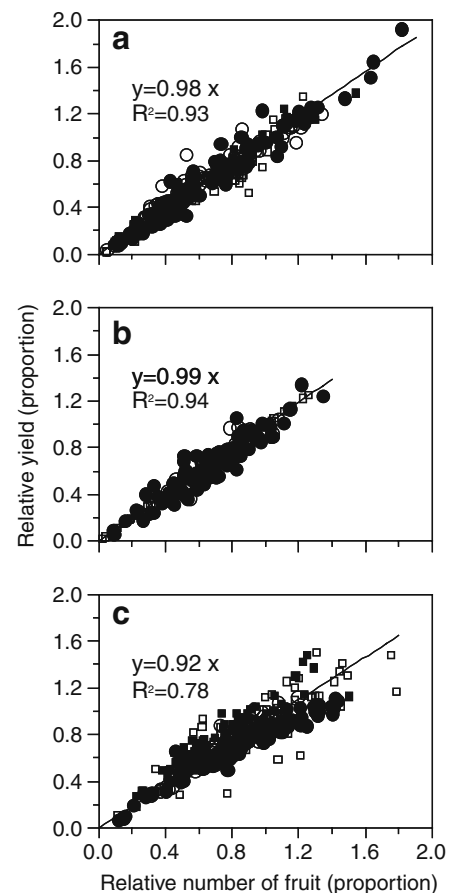


Fig. 4 Relationship between the relative number of fruits (number of fruits from symptomatic tree/mean number of fruits from asymptomatic trees) and relative yield (yield from symptomatic tree/mean yield from asymptomatic trees) in early (a), mid-season (b) and late (c) sweet orange cultivars in São Paulo State. Different symbols refer to different citrus blocks

observed between fruit weight and disease severity only for cv. Valencia. Also, the difference between the weight of symptomatic fruit and normal fruit was larger for the late cultivar than for early and mid-season cultivars. Bassanezi et al. (2009a) suggested that early and mid-season cultivars could be slightly more tolerant to HLB or more efficient in the transport and accumulation of TSS into fruit affected by HLB. However, the absence of differences in concentrations of *Ca. Liberibacter* spp. in the leaf tissues among tested cultivars (Lopes et al. 2009) and the similar rates of yield decrease as a function of disease severity for all tested cultivars observed in this study are indications that all cultivars have a similar tolerance to HLB and an explanation based on a difference in efficiency of transport and accumulation of TSS might be more appropriate. A single equation can be used to describe the relative yield-HLB severity relationship for the most common sweet orange cultivars in Brazil: $y = \exp(-1.85x)$. Such a general equation could be incorporated in economic models to predict the economic life of HLB-affected groves as proposed by Bassanezi and Bassanezi (2008). These models can address for long-term strategic decisions, as pointed out by Savary et al. (2006).

Acknowledgements We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the fellowships of first, fourth and fifth authors.

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