

Effect of Citrus Sudden Death on Yield and Quality of Sweet Orange Cultivars in Brazil

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ABSTRACT

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Citrus sudden death (CSD) has greatly affected sweet orange cultivars grafted on Rangpur lime in São Paulo and Minas Gerais States, Brazil. To characterize and quantify CSD damage, fruit yield and quality were assessed in each combination of sweet orange cultivar (Hamlin, Pera, Natal, and Valencia), age class (3 to 5, 6 to 10, and 11 to 15 years old), and CSD severity class (0 = no symptom, 1 = initial symptoms, and 2 = severe symptoms). For each combination, 10 trees were harvested and 20 fruit were taken for quality analysis. Damage was characterized by reduction of: (i) total weight of fruit/tree (36 and 67% for severity class 1 and 2, respectively), (ii) number of fruit/tree (27 and 55%), (iii) fruit size (13 and 25% in diameter and height [stem to styler distance]), (iv) fruit weight (32 and 56%), (v) total soluble solids (TSS)/fruit (18 and 42%), and increase of (vi) Brix (14 and 34%), (vii) acidity (16 and 41%), and (viii) TSS/90-lb. box (21 and 33%). There was no alteration on Brix/acidity ratio and percentage of juice on fruit of affected trees. Sweet orange cultivars did not differ in percentage of reduction or increase of all yield and quality variables, with the exception of Pera, which expressed increases of Brix and acidity. For more severe affected trees, the youngest plants showed a higher reduction in fruit number/tree, whereas plants 6 to 10 years old showed a higher increase in fruit acidity and TSS/box. However, no differences in percentage of reduction or increase for other variables were observed among different age classes. The damage to the above probably was associated with reduced water absorption capacity of CSD-affected trees.

Additional keywords: *Citrus sinensis*, *C. limonia*

Citrus sudden death (CSD) was reported in southwestern Minas Gerais State and northern São Paulo State, Brazil, in the beginning of 2001 (18). This new and destructive disease affects groves of sweet orange (*Citrus sinensis* (L.) Osbeck) and some mandarin (*C. reticulata* Blanco) grafted on either Rangpur lime (*C. limonia* Osbeck) or Volkamerian lemon (*C. volkameriana* Pasq.). In spite of its unknown etiology, the causal agent is graft transmissible (35) and probably vectored by insects such as aphids, giving rise to spatial dynamics similar to those expressed *Citrus tristeza virus* (CTV) infection (1,2,26). The association of CTV variants and a new virus (possible *Tymoviridae*) with CSD-

affected trees has been studied (6,28), but Koch's postulate has not yet been completed. Like citrus tristeza, that causes quick decline of sweet orange grafted on sour orange (*C. aurantium* L.), CSD is a bud-union disease and inarching using tolerant rootstocks such as Swingle citrumelo (*Poncirus trifoliata* × *C. paradise* Macf.) and Cleopatra (*C. reticulata* Blanco) and Sunki (*C. sunki* Hort. ex Tanaka) mandarin can allow trees affected at the initial stage to recover (33).

Five years after its first report, CSD has been found restricted to 12 municipalities of southwestern Minas Gerais State and 19 of northern and northwestern São Paulo State (2,4). Although regionally localized, CSD has been responsible directly or indirectly for decline and eradication of almost 4 million trees, and continues to be a serious threat to the sustainable, commercial production of sweet orange grafted in Rangpur lime rootstock in the affected regions (4), which represents more than 25% of 170 million trees of São Paulo State. Inarching and new plantings with tolerant but drought-susceptible rootstocks increases the demand for irrigation and represents

additional costs to citrus growers of that region.

The generalized decline of affected trees caused by CSD is characterized by pale green coloration of leaves throughout the canopy, increased defoliation, reduction in new shoots, absence of shoots internal in the canopy, rot and death of a large portion of the root system, and the characteristic development of a yellow stain in the phloem of the rootstock (18). The rootstock bark near the bud union undergoes profound anatomical changes such as reduction size of phloem cells, collapse and necrosis of sieve tubes, overproduction and degradation of phloem, accumulation of nonfunctioning phloem, and invasion of the cortex by old nonfunctioning phloem (33). These symptoms intensify as the disease develops and can culminate with the sudden collapse and death of the tree, normally without fruit abscission (18). Sudden collapse and death of the tree often occurs when there are long periods of water deficit concurrent with high fruit set, which causes a severe unbalance between canopy water and nutrient demand and root system supply. However, the majority of affected trees display only initial decline symptoms during the first 2 years after a symptomatic tree appears in the grove, followed by the increased incidence of trees with more severe symptoms and increases in death (2). This gradual decline of trees usually occurs for a few years, during which periods of decline and recovery alternate. In this case, affected trees remain alive for some years and damage due to CSD is more related to fruit yield and quality reduction, or “economic tree death,” than “biological tree death.”

The economic death of the tree can be assessed by the affects on fruit yield and quality. There is little data available about CSD damage on fruit yield (3) and nothing concerning reduction of fruit quality. Because citrus fruit quality is related to photosynthesis (soluble solids content) and water potential (percentage of juice), it is possible to obtain an indirect indication of the effect of the disease on tree physiology. Also, CSD damage assessment is important to characterize the severity of the disease and to define the CSD incidence and severity threshold above which the affected grove becomes unprofitable. Thus, this

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*The e-Xtra logo stands for “electronic extra” and indicates that Figure 1 appears in color in the online edition.

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study was undertaken to assess the damage caused by CSD on fruit yield and quality as function of disease severity in plantings of different ages and cultivars of sweet orange commonly grown in Brazil.

MATERIALS AND METHODS

Citrus groves and data collection. This work was conducted in 48 plantings of sweet orange grafted on Rangpur lime expressing different CSD symptom severity during the 2004 season. The selected plantings were located in the municipalities of Colômbia (northern São Paulo State) and Planura, Frutal, Comendador Gomes, and Uberlândia (southwestern Minas Gerais State), Brazil.

The assessed sweet orange cultivars were Hamlin, Pera, Natal, and Valencia. For each cultivar, the age classes were 3 to 5 years old, 6 to 10 years old, and 11 to 15 years old. For each cultivar–age combination, four blocks were sampled (four replications).

For each block, all trees previously were classified according to the descriptive scale of CSD symptom severity (3): (a) class 0, asymptomatic tree; (b) class 1, tree with initial symptoms showing pale leaves and yellow stain in the inner tissue of rootstock bark below the bud union; and (c) class 2, tree with severe symptoms characterized by a combination of previous symptoms with partial defoliation and absence of internal shoots (Fig. 1).

For each class of CSD severity, 10 trees were chosen randomly and marked prior to harvest assessment. At harvest time (June to July for Hamlin, July to September for Pera, September to November for Valencia, and September to December for Natal), for each marked tree, the number of harvested fruit was counted and total fruit yields were weighed. Also, 20 fruit of each severity class in each block were arbitrarily sampled from marked trees for quality analysis (32). The quality variables assessed were fruit weight, diameter, height

(stem to stylar end orientation), Brix (grams of total soluble solids per 100 ml of juice), acidity (grams of titratable citric acid per 100 ml of juice), Brix/acidity ratio, percentage of juice (grams of juice per 100 g of fruit), kilograms of total soluble solids (TSS) per 40.8-kg box (TSS/box), and grams of TSS per fruit (TSS/fruit).

Statistics analysis. For each combination of cultivar–age, the mean values of each assessed variable of yield and quality for each severity classes (class 0, 1 and 2) were compared by main effects analysis of variance (ANOVA) with three treatments and four replications (blocks) per treatment according to a random block design. For each severity class, the value for yield variables was the mean of 10 trees, and the value for quality variables was a mean of 20 fruit.

The means of absolute values of fruit yield and quality variables were not compared among cultivars and age classes, because the differences in fruit yield and quality among sweet orange cultivars and age classes have been well reported in the literature (5,9–11,13,31) and that was not the goal of this study. To eliminate that intrinsic difference of cultivars and age classes on fruit yield and quality, and to allow the comparison of CSD effect on assessed variables among cultivars and among age classes, data for severity classes 1 and 2 were standardized as in the percentage of values of class 0 (asymptomatic) trees. Then, for each class of severity (class 1 and 2), one-way ANOVA was done with 12 treatments (factorial with four cultivars and three age classes) and four replications per treatment, according to a random block design. Because there was no significant interaction for cultivar–age class for yield and quality relative variables for the two classes of CSD severity, the effects of cultivar and class age are independent and, thus, they were analyzed separately.

When significant differences among variable's means were detected by *F* test, the means were compared by Tukey highly significant difference test.

RESULTS

CSD damage on sweet orange yield was characterized by reduction on yield per tree (total weight of harvested fruit) and number of fruit per tree. More severe symptoms of CSD resulted in higher damage estimates.

Reductions of yield and number of fruit per tree were significant for almost all combinations of cultivar–age class when severity class 1 and 2 were compared with class 0 (Table 1). There was no significant difference among sweet orange cultivars for the percentage of reduction of yield and fruit number per tree in relation to severity class 0. Mean reduction of yield per tree for severity class 1 and 2 was 36.0% (standard deviation [sd] = 16.4) and 67.0% (sd = 11.8), respectively. Mean reduction of fruit number per tree was 26.9% (sd = 20.0) and 54.8% (sd = 17.7) for severity class 1 and 2, respectively. Considering the age classes, there was no significant difference among age classes for percentage of reduction of yield per tree. For reduction on number of fruit per tree, no difference was observed among age classes for severity class 1; however, for severity class 2, the youngest trees showed a higher reduction of fruit number (65.7%, sd = 13.4) than trees older than 5 years (50.2%, sd = 15.1, for 6- to 10-year-old trees, and 48.7%, sd = 19.8, for 11- to 15-year-old trees).

Fruit quality damage caused by CSD was characterized mainly by reductions of fruit diameter, height, weight, and TSS/fruit, and increases of Brix, acidity, and TSS/box. Very light to no alteration of fruit ratio and percentage of juice were observed. As yield variables decreased, quality damage estimates increased in more severely diseased trees.



Fig. 1. Classes of citrus sudden death severity. From left to right: class 0, asymptomatic tree; class 1, tree with initial symptoms showing pale leaves and yellow stain in the inner tissue of rootstock bark below bud union zone; and class 2, tree with severe symptom characterized by previous symptoms and by partial defoliation and absence of internal shoots.

Reduction of fruit diameter had the same behavior as reduction of fruit height and both were significant for all combinations of cultivar–age class when severity classes were compared (Table 2). For the percentage of reduction of fruit diameter and height in relation to severity class 0, there was no significant difference among sweet orange cultivars or among age classes. For severity class 1 and 2, the mean reduction of fruit diameter was 12.8% (sd = 3.4) and 25.2% (sd = 4.9), respectively, whereas the mean reduction of fruit height was 13.1% (sd = 3.8) and 25.8% (sd = 5.0) for severity class 1 and 2, respectively.

Fruit weight also was significantly smaller for all combinations of cultivar–age class in more severely diseased trees (Table 3). There was no significant difference among sweet orange cultivars or among age classes when the mean percent reduction on fruit weight in relation to severity class 0 were compared. The mean reduction on fruit weight was 32.0% (sd = 6.8) and 55.9% (sd = 8.1) for severity class 1 and 2, respectively.

Fruit concentration of TSS (Brix) from severely affected trees was significantly higher for all cultivar–age class combinations compared with asymptomatic trees

(Table 4). Pera and Valencia trees with initial symptoms also had significantly elevated Brix compared with asymptomatic trees independent of age class

Table 3. Fruit weight (g) on different sweet orange cultivar–age class combinations as function of citrus sudden death (CSD) severity class

Combination		CSD severity class ^z		
Cultivar	Age (years)	0	1	2
Hamlin	3–5	195.6 ± 13.1 a	138.9 ± 10.5 b	90.5 ± 2.2 c
Hamlin	6–10	223.9 ± 38.6 a	148.8 ± 7.7 b	100.6 ± 9.1 b
Hamlin	11–15	193.6 ± 8.3 a	137.8 ± 10.6 b	85.0 ± 9.5 c
Pera	3–5	235.0 ± 17.8 a	142.0 ± 8.0 b	96.0 ± 9.1 c
Pera	6–10	249.7 ± 28.6 a	159.3 ± 12.1 b	92.8 ± 13.4 c
Pera	11–15	223.8 ± 17.1 a	148.2 ± 11.6 b	98.5 ± 11.2 c
Natal	3–5	217.1 ± 28.1 a	159.5 ± 10.9 b	96.4 ± 10.5 c
Natal	6–10	228.8 ± 17.7 a	156.6 ± 19.1 b	102.4 ± 13.0 c
Natal	11–15	219.7 ± 27.6 a	142.8 ± 8.7 b	80.5 ± 14.6 c
Valencia	3–5	250.1 ± 19.3 a	168.2 ± 6.6 b	126.5 ± 27.2 c
Valencia	6–10	221.6 ± 20.8 a	159.3 ± 5.0 b	106.2 ± 27.7 c
Valencia	11–15	220.8 ± 23.8 a	147.1 ± 17.7 b	97.9 ± 23.2 c

^z CSD symptom severity classes: 0 = asymptomatic tree, 1 = tree with initial symptoms showing pale leaves and yellow stain in the inner tissue of rootstock bark below the bud union, and 2 = tree with severe symptoms characterized by a combination of previous symptoms with partial defoliation and absence of internal shoots. Mean ± standard deviation of four blocks and 20 fruit per block. Values with the same letter in row were not different by Tukey highly significant difference test ($P > 0.05$).

Table 1. Fruit yield (kg) and number per tree for different sweet orange cultivar–age class combinations as function of citrus sudden death (CSD) severity class^y

Combination		Fruit yield per tree as function of CSD severity class ^z			Fruit number per tree as function of CSD severity class ^z		
Cultivar	Age (years)	0	1	2	0	1	2
Hamlin	3–5	119.7 ± 13.8 a	59.7 ± 4.0 b	37.1 ± 4.9 c	868.3 ± 113.2 a	480.2 ± 28.0 b	305.0 ± 68.6 c
Hamlin	6–10	180.0 ± 39.5 a	119.3 ± 25.4 b	72.2 ± 17.6 c	1,345.9 ± 265.1 a	1,049.6 ± 236.7 b	700.0 ± 164.6 c
Hamlin	11–15	212.5 ± 27.6 a	128.8 ± 26.7 b	62.5 ± 17.3 c	1,985.9 ± 383.0 a	1,196.0 ± 329.6 b	706.7 ± 217.7 b
Pera	3–5	34.0 ± 7.8 a	15.5 ± 11.6 b	7.8 ± 4.2 b	206.7 ± 45.2 a	105.4 ± 86.4 b	58.0 ± 30.8 b
Pera	6–10	90.1 ± 15.6 a	60.8 ± 20.2 b	32.7 ± 14.7 c	616.4 ± 125.6 a	482.5 ± 140.6 b	325.8 ± 122.7 c
Pera	11–15	120.0 ± 34.6 a	99.2 ± 20.5 a	59.6 ± 21.4 b	848.7 ± 246.4 a	784.3 ± 123.6 ab	531.1 ± 160.9 b
Natal	3–5	69.8 ± 40.0 a	39.3 ± 15.8 ab	16.3 ± 14.3 b	390.4 ± 161.9 a	262.2 ± 95.4 b	125.3 ± 106.0 c
Natal	6–10	120.8 ± 17.7 a	86.7 ± 17.7 b	37.5 ± 14.1 c	798.7 ± 166.5 a	670.1 ± 192.7 a	385.1 ± 131.9 b
Natal	11–15	115.7 ± 57.0 a	71.3 ± 32.4 ab	32.3 ± 11.0 b	808.9 ± 279.9 a	590.8 ± 174.6 ab	391.1 ± 137.1 b
Valencia	3–5	69.9 ± 37.9 a	49.8 ± 22.3 ab	31.0 ± 20.2 b	382.8 ± 202.8 a	304.5 ± 160.7 ab	191.4 ± 129.8 b
Valencia	6–10	153.4 ± 45.8 a	88.5 ± 37.6 b	43.3 ± 17.6 c	988.8 ± 306.6 a	729.1 ± 376.5 ab	421.8 ± 168.7 b
Valencia	11–15	102.7 ± 54.0 a	63.3 ± 32.1 ab	32.3 ± 11.3 b	718.8 ± 284.3 a	520.9 ± 185.1 ab	359.1 ± 131.5 b

^y CSD symptom severity classes: 0 = asymptomatic tree, 1 = tree with initial symptoms showing pale leaves and yellow stain in the inner tissue of rootstock bark below the bud union, and 2 = tree with severe symptoms characterized by a combination of previous symptoms with partial defoliation and absence of internal shoots.

^z Mean ± standard deviation of four blocks and 10 trees per block. Values with the same letter in row were not different by Tukey highly significant difference test ($P > 0.05$).

Table 2. Fruit diameter (cm) and height (cm) on different sweet orange cultivar–age class combinations as function of citrus sudden death (CSD) severity class^y

Combination		Fruit diameter as function of CSD severity class ^z			Fruit height as function of CSD severity class ^z		
Cultivar	Age (years)	0	1	2	0	1	2
Hamlin	3–5	7.4 ± 0.1 a	6.5 ± 0.2 b	5.6 ± 0.1 c	7.7 ± 0.2 a	6.7 ± 0.2 b	5.6 ± 0.1 c
Hamlin	6–10	7.6 ± 0.5 a	6.6 ± 0.2 b	5.8 ± 0.1 c	7.8 ± 0.6 a	6.6 ± 0.1 b	5.8 ± 0.2 c
Hamlin	11–15	7.2 ± 0.2 a	6.4 ± 0.2 b	5.4 ± 0.3 c	7.4 ± 0.2 a	6.5 ± 0.2 b	5.4 ± 0.2 c
Pera	3–5	7.6 ± 0.3 a	6.3 ± 0.2 b	5.5 ± 0.2 c	8.0 ± 0.1 a	6.7 ± 0.1 b	5.8 ± 0.3 c
Pera	6–10	7.7 ± 0.3 a	6.6 ± 0.2 b	5.4 ± 0.3 c	8.2 ± 0.5 a	6.9 ± 0.2 b	5.7 ± 0.2 c
Pera	11–15	7.4 ± 0.3 a	6.4 ± 0.2 b	5.5 ± 0.2 c	7.8 ± 0.2 a	6.8 ± 0.2 b	5.9 ± 0.3 c
Natal	3–5	7.4 ± 0.3 a	6.6 ± 0.2 b	5.5 ± 0.2 c	7.7 ± 0.4 a	6.9 ± 0.1 b	5.7 ± 0.2 c
Natal	6–10	7.5 ± 0.3 a	6.5 ± 0.3 b	5.6 ± 0.3 c	7.8 ± 0.2 a	6.8 ± 0.3 b	5.9 ± 0.3 c
Natal	11–15	7.3 ± 0.3 a	6.3 ± 0.1 b	5.2 ± 0.3 c	7.7 ± 0.4 a	6.6 ± 0.2 b	5.4 ± 0.4 c
Valencia	3–5	7.7 ± 0.1 a	6.7 ± 0.1 b	6.0 ± 0.5 c	8.0 ± 0.2 a	7.0 ± 0.1 b	6.3 ± 0.5 c
Valencia	6–10	7.4 ± 0.3 a	6.7 ± 0.2 b	5.8 ± 0.5 c	7.7 ± 0.2 a	6.8 ± 0.1 b	5.8 ± 0.4 c
Valencia	11–15	7.4 ± 0.3 a	6.4 ± 0.3 b	5.6 ± 0.5 c	7.6 ± 0.3 a	6.6 ± 0.2 b	5.7 ± 0.5 c

^y CSD symptom severity classes: 0 = asymptomatic tree, 1 = tree with initial symptoms showing pale leaves and yellow stain in the inner tissue of rootstock bark below the bud union, and 2 = tree with severe symptoms characterized by a combination of previous symptoms with partial defoliation and absence of internal shoots.

^z Mean ± standard deviation of four blocks and 20 fruit per block. Values with the same letter in row were not different by Tukey highly significant difference test ($P > 0.05$).

whereas, for Hamlin and Natal, there was no difference. CSD-affected Hamlin, Natal, and Valencia had similar Brix increases compared with severity class 0 (average of 10.6%, sd = 7.8, for severity class 1 and 27.4%, sd = 12.1, for severity class 2); however, these increases were smaller than those for affected Pera (24.0%, sd = 13.7, and 52.3%, sd = 23.6, for severity class 1 and 2, respectively). There was no difference among age class relative to increase of fruit Brix. Mean Brix increase was 13.9% (sd = 11.3) and 33.6% (sd = 19.2) for severity class 1 and 2, respectively.

The changes in TSS/box were higher for more severely affected trees (Table 5). No differences were observed among sweet orange cultivars relative to increase of TSS/box, or among age classes for initially affected trees. For severely affected trees, the age class 6 to 10 years old had a higher increase (40.9%, sd = 22.7) of TSS/box, followed by 3- to 5-year-old (33.1%, sd = 19.3) and 11- to 15-year-old classes (23.8%, sd = 17.0). The mean of percentage of increase on TSS/box was 20.8% (sd

= 14.9) for initially symptomatic trees and 32.8% (sd = 20.7) for severely symptomatic trees.

Although the soluble solids concentration increased in fruit taken from more affected trees, conversely, as severity of CSD increased, TSS/fruit decreased (Table 5). No difference was observed among sweet orange cultivars or among age classes relative to reduction of TSS/fruit. The mean of percent loss in TSS/fruit was 18.1% (sd = 11.1) for initially symptomatic trees and 41.7% (sd = 41.4) for severely symptomatic trees.

Except for cv. Pera, fruit acidity of initially affected trees did not differ from asymptomatic trees for any cultivar-age class combination compared (Table 4). For more severely affected trees, for almost all combinations, there was an increase of fruit citric acid concentration (Table 4). Initially and severely CSD-symptomatic Pera trees had higher increases of fruit acidity compared with other cultivars. For Pera, the mean increase of fruit acidity was 34.9% (sd = 21.1) and 92.6% (sd = 40.8)

for severity class 1 and 2, respectively, whereas the mean for other cultivars ranged from 7.2% (sd = 10.6) to 12.6%, (sd = 14.4) for severity class 1 and from 13.9% (sd = 18.8) to 34.0% (sd = 26.4) for severity class 2. For the age class 6 to 10 years old, the increase of fruit acidity was highest, followed by 3 to 5 years old and 11 to 15 years old, and was independent of severity class. Increases ranged from 7.4% (sd = 15.0) to 24.0% (sd = 21.4) for severity class 1 and from 21.7% (sd = 28.7) to 59.0% (sd = 48.6) for severity class 2.

Whereas fruit Brix and acidity increased in similar proportion, the Brix/acidity ratio did not change for all cultivar-age class combinations tested that showed initial symptoms of CSD (Table 6). An increase of fruit ratio in relation to severity class 0 trees was detected only for severity class 2 trees of Hamlin-3- to 5-years-old, Pera-3- to 5-year-old, Pera-6- to 10-year-old, and Valencia-11- to 15-year-old combinations.

Fruit juice content also was not significantly reduced in any cultivar-age class combination compared with asymptomatic

Table 4. Fruit Brix (grams of total soluble solids/100 ml) and acidity (grams of titratable citric acid/100 ml) on different sweet orange cultivar-age class combinations as function of citrus sudden death (CSD) severity class^y

Combination		Fruit Brix as function of CSD severity class ^z			Fruit acidity as function of CSD severity class ^z		
Cultivar	Age (years)	0	1	2	0	1	2
Hamlin	3-5	9.3 ± 0.5 a	10.0 ± 0.8 ab	11.2 ± 0.4 b	0.61 ± 0.04 a	0.64 ± 0.04 a	0.62 ± 0.07 a
Hamlin	6-10	9.4 ± 0.8 a	10.4 ± 0.6 a	12.2 ± 1.2 b	0.62 ± 0.07 a	0.70 ± 0.09 ab	0.80 ± 0.15 b
Hamlin	11-15	10.1 ± 0.8 a	10.6 ± 0.5 a	12.1 ± 1.2 b	0.67 ± 0.08 a	0.69 ± 0.13 a	0.74 ± 0.13 a
Pera	3-5	9.4 ± 0.2 a	11.2 ± 0.2 b	13.3 ± 0.7 c	0.61 ± 0.08 a	0.79 ± 0.02 a	1.13 ± 0.11 b
Pera	6-10	9.6 ± 1.1 a	12.9 ± 1.0 b	16.5 ± 0.9 c	0.54 ± 0.12 a	0.81 ± 0.11 b	1.26 ± 0.25 c
Pera	11-15	9.8 ± 1.2 a	11.4 ± 0.8 b	13.6 ± 0.5 c	0.61 ± 0.04 a	0.74 ± 0.13 b	0.95 ± 0.10 c
Natal	3-5	9.9 ± 1.3 a	11.2 ± 0.2 ab	12.8 ± 0.8 b	0.78 ± 0.13 a	0.87 ± 0.09 a	1.05 ± 0.12 b
Natal	6-10	10.8 ± 1.1 a	12.1 ± 1.1 a	14.6 ± 1.5 b	0.66 ± 0.04 a	0.76 ± 0.07 ab	0.90 ± 0.15 b
Natal	11-15	11.6 ± 0.9 a	12.4 ± 0.6 a	14.9 ± 1.5 b	0.66 ± 0.21 a	0.67 ± 0.25 a	0.85 ± 0.44 a
Valencia	3-5	9.0 ± 0.3 a	10.4 ± 0.4 b	11.5 ± 0.6 c	0.66 ± 0.17 a	0.75 ± 0.16 a	0.91 ± 0.21 b
Valencia	6-10	10.5 ± 0.5 a	12.1 ± 1.1 b	13.8 ± 1.5 c	0.80 ± 0.22 a	0.95 ± 0.35 ab	1.13 ± 0.37 b
Valencia	11-15	12.1 ± 0.9 a	13.0 ± 0.5 a	14.8 ± 1.3 b	0.66 ± 0.08 a	0.72 ± 0.21 a	0.64 ± 0.14 a

^y CSD symptom severity classes: 0 = asymptomatic tree, 1 = tree with initial symptoms showing pale leaves and yellow stain in the inner tissue of rootstock bark below the bud union, and 2 = tree with severe symptoms characterized by a combination of previous symptoms with partial defoliation and absence of internal shoots.

^z Mean ± standard deviation of four blocks and 20 fruit per block. Values with the same letter in row were not different by Tukey highly significant difference test ($P > 0.05$).

Table 5. Total soluble solids (TSS) per 90-lb. box (kg) and per fruit (g) on different sweet orange cultivar-age class combinations as function of citrus sudden death (CSD) severity class^y

Combination		TSS per box as function of CSD severity class ^z			TSS per fruit as function of CSD severity class ^z		
Cultivar	Age (yrs)	0	1	2	0	1	2
Hamlin	3-5	1.38 ± 0.18 a	1.64 ± 0.25 ab	1.88 ± 0.12 b	6.6 ± 1.3 a	5.6 ± 1.0 ab	4.2 ± 0.2 b
Hamlin	6-10	1.61 ± 0.30 a	1.93 ± 0.20 b	2.16 ± 0.21 c	8.9 ± 2.9 a	7.1 ± 1.0 ab	5.3 ± 0.8 b
Hamlin	11-15	1.75 ± 0.25 a	1.92 ± 0.05 a	2.09 ± 0.21 a	8.3 ± 1.0 a	6.5 ± 0.5 a	4.4 ± 0.8 b
Pera	3-5	1.99 ± 0.13 a	2.44 ± 0.05 b	2.69 ± 0.05 c	11.4 ± 1.0 a	8.5 ± 0.6 b	6.3 ± 0.6 c
Pera	6-10	1.90 ± 0.20 a	2.74 ± 0.33 b	2.89 ± 0.33 b	11.5 ± 1.3 a	10.7 ± 1.7 a	6.6 ± 1.5 b
Pera	11-15	1.94 ± 0.15 a	2.33 ± 0.10 b	2.57 ± 0.10 c	10.6 ± 0.5 a	8.4 ± 0.4 b	6.2 ± 0.8 c
Natal	3-5	1.91 ± 0.27 a	2.19 ± 0.09 a	2.32 ± 0.18 a	10.0 ± 0.8 a	8.6 ± 0.9 a	5.5 ± 0.5 b
Natal	6-10	2.05 ± 0.17 a	2.48 ± 0.26 b	2.86 ± 0.21 b	11.5 ± 1.3 a	9.5 ± 1.0 b	7.2 ± 0.9 c
Natal	11-15	2.26 ± 0.31 a	2.55 ± 0.12 a	2.82 ± 0.11 a	12.0 ± 0.6 a	8.9 ± 0.5 b	5.2 ± 1.0 c
Valencia	3-5	1.59 ± 0.18 a	1.96 ± 0.16 b	2.09 ± 0.08 b	9.7 ± 1.0 a	8.1 ± 0.6 a	6.5 ± 1.4 b
Valencia	6-10	1.95 ± 0.27 a	2.30 ± 0.43 ab	2.53 ± 0.28 b	10.5 ± 1.2 a	9.0 ± 1.4 a	6.5 ± 1.2 b
Valencia	11-15	2.28 ± 0.26 a	2.55 ± 0.17 ab	2.79 ± 0.39 b	12.3 ± 1.8 a	9.1 ± 0.6 b	6.6 ± 1.1 b

^y CSD symptom severity classes: 0 = asymptomatic tree, 1 = tree with initial symptoms showing pale leaves and yellow stain in the inner tissue of rootstock bark below the bud union, and 2 = tree with severe symptoms characterized by a combination of previous symptoms with partial defoliation and absence of internal shoots.

^z Mean ± standard deviation of four blocks and 20 fruit per block. Values with the same letter in row were not different by Tukey highly significant difference test ($P > 0.05$).

trees, except for fruit of Pera, which was severely affected in all age classes (Table 6).

DISCUSSION

The CSD damages on fruit yield and quality increased with severity of disease symptoms. Trees in the initial stages of CSD infection had, on average, 36.0% less yield (kilograms) per tree than asymptomatic trees, whereas severely affected trees had, on average, 67.0% less yield per tree. In general, for all cultivars of healthy citrus trees, there is a positive linear relationship between yield per tree and number of fruit per tree (21,22,25), and a negative relationship between fruit number per tree and fruit size and weight (20,25). In the case of CSD-affected trees, the reduction on fruit yield was related to the reduction of number of fruit per tree, and the reduction of fruit size and, fruit weight. Compared with asymptomatic trees, the number of fruit per tree with initial and severe CSD symptoms was 26.9 and 54.8% smaller, respectively. The average of fruit diameter, height, and weight were reduced 12.8, 13.1, and 32.0%, respectively, for initially affected trees, and 25.2, 25.8, and 55.9%, respectively, for severely affected trees.

These results corroborate those observed 1 year earlier by Bassanezi et al. (3); however, the damage was greater in 2004 compared with the 2003 season despite similar yield and fruit number per tree for asymptomatic trees in both seasons. The differences in duration of water stress and rain distribution observed in 2003 compared with 2004 probably were the reason for the difference in damage estimates. In 2003, the rain deficit (<30 mm/month) occurred over 3 months (5 mm in June, 4 mm in July, and 20 mm in August) whereas, in 2004, it occurred over 4 months, with higher stress during the last 2 months when the majority of harvest was accomplished (17 mm in June, 25 mm in July, 0 mm in August, and 3 mm in September).

The significant reduction of yield, number of fruit per tree, and fruit size and weight for CSD-affected trees was related to symptom development post tree infection. The first observed alterations in Rangpur lime tissues below the bud-union zone of CSD-affected trees were the reduction of phloem cell size, collapse and necrosis of sieve tubes, non-functioning phloem accumulation, and cortex invasion by old non-functioning phloem leading to a poor function and degradation of rootstock phloem cells (33). In response to phloem cell degradation, vascular cambium promotes overproduction of new phloem cells, which are further degenerated (33). This continual cyclic process of synthesis and degradation impairs the transport of photosynthesis products from canopy to root system. The restricted flux of nutrients in the sap to roots and feeding roots over a long time exhausts their carbohydrate reserves, causing general root starvation, and favors invasion by secondary pathogens. Due to the resultant debilitated root system, water and mineral salt uptake also is impaired. The unbalanced water demand by the canopy versus water supply by the roots is responsible for general decline symptoms that develop in the canopy, such as wilted and pale leaves, absence of vigorous new shoots, and defoliation (18).

Foliage prevalence and health is positively related to citrus fruit size and quality (15). The greater number of leaves per fruit, the larger the fruit produced. The quantity of leaves also is related to yield and number of fruit. Citrus trees under water and nutritional stresses and with small leaf area index have less photosynthetic efficiency and, consequently, reduced capacity for carbohydrate accumulation (34). Once environmental conditions necessary for flowering induction and fruit set have been satisfied, the availability of carbohydrate reservoir in the tree is one of the most important limitations to citrus fruit production (7,16,19). The higher the

carbohydrate availability, the greater the number of flowers and the less the fruit initiated will drop. Consequently, a greater set number of fruit will be harvested per tree (24).

Water composes a major portion of the fruit mass (85 to 90% by weight) and carbohydrates contribute to 75 to 80% of total soluble solids (8). Fruit growth and its final size and weight result from dry matter and water accumulation, which depend on fruit competition for metabolites, availability of these metabolites in the tree, and root system water uptake capacity (8,14,23). Fruit size and weight from healthy trees have a positive relation to Brix/acidity ratio and TSS/fruit, and are negatively related to Brix, TSS/box, and acidity (10,17). Healthy trees under water stress usually produced smaller fruit with increased concentration of total soluble solids (Brix) and citric acid (acidity), but with reduced juice percentage and Brix/acidity ratio (12,30). Citrus variegated chlorosis (CVC), a disease caused by *Xylella fastidiosa* bacteria that live in the xylem vessels, block water transport, and decrease the nutrient availability to the tree, reduced the fruit size, weight, Brix/acidity ratio, and TSS/fruit, but increased Brix, TSS/box, and acidity (27,29). The positive relationship found between fruit size and TSS/fruit and the negative relationship between fruit size and Brix (TSS concentration) suggested to the authors that the internal alterations observed in CVC-affected fruit occurred mainly by blockage of water transport due to bacteria in xylem. Except for Brix/acidity ratio and juice percentage, two factors that were not significantly affected, fruit from CSD-affected trees had the same behavior as fruit from water-stressed healthy trees; that is, reduced size, weight, and TSS/fruit, and increased Brix, TSS/box, and acidity.

The absence of difference among sweet orange cultivars and age classes in relation

Table 6. Fruit ratio (Brix/acidity) and juice content (%) on different sweet orange cultivar–age class combinations as function of citrus sudden death (CSD) severity class^y

Combination		Fruit ratio as function of CSD severity class ^z			Fruit juice content as function of CSD severity class ^z		
Cultivar	Age (years)	0	1	2	0	1	2
Hamlin	3–5	15.2 ± 1.3 a	15.6 ± 1.0 a	18.1 ± 2.0 b	36.2 ± 3.2 a	40.2 ± 3.0 a	41.4 ± 2.8 a
Hamlin	6–10	15.3 ± 1.6 a	15.0 ± 1.3 a	15.6 ± 2.3 a	41.6 ± 4.4 a	45.4 ± 2.6 a	43.4 ± 0.6 a
Hamlin	11–15	15.3 ± 1.7 a	15.7 ± 3.3 a	16.8 ± 4.1 a	42.5 ± 5.7 a	44.8 ± 3.2 a	42.4 ± 2.1 a
Pera	3–5	15.5 ± 1.8 a	14.3 ± 0.3 ab	11.8 ± 0.6 b	51.7 ± 2.2 a	53.1 ± 1.7 a	49.6 ± 2.1 b
Pera	6–10	18.8 ± 6.0 a	16.1 ± 3.0 ab	13.5 ± 2.2 b	48.5 ± 0.9 a	52.1 ± 2.6 a	42.8 ± 4.0 b
Pera	11–15	15.9 ± 0.9 a	15.6 ± 2.0 a	14.5 ± 1.3 a	48.9 ± 3.4 ab	50.4 ± 1.9 a	46.3 ± 2.0 b
Natal	3–5	12.9 ± 0.5 a	13.0 ± 1.1 a	12.3 ± 1.5 a	47.1 ± 1.3 a	47.8 ± 1.9 a	44.6 ± 3.8 a
Natal	6–10	16.5 ± 1.3 a	16.1 ± 1.9 a	16.6 ± 3.2 a	46.4 ± 2.8 a	50.4 ± 2.0 a	48.0 ± 3.0 a
Natal	11–15	18.7 ± 4.7 a	20.8 ± 7.8 a	20.3 ± 7.6 a	47.4 ± 3.1 a	50.5 ± 1.1 a	43.5 ± 5.3 a
Valencia	3–5	14.2 ± 3.4 a	14.2 ± 2.5 a	13.0 ± 2.3 a	43.0 ± 3.6 a	46.0 ± 2.5 a	44.7 ± 2.3 a
Valencia	6–10	14.1 ± 5.3 a	14.3 ± 6.1 a	13.4 ± 5.3 a	45.6 ± 6.3 a	46.5 ± 6.6 a	45.0 ± 1.6 a
Valencia	11–15	18.5 ± 3.1 a	19.4 ± 5.9 a	24.2 ± 5.8 b	46.4 ± 3.4 a	48.0 ± 1.9 a	46.1 ± 3.1 a

^y CSD symptom severity classes: 0 = asymptomatic tree, 1 = tree with initial symptoms showing pale leaves and yellow stain in the inner tissue of rootstock bark below the bud union, and 2 = tree with severe symptoms characterized by a combination of previous symptoms with partial defoliation and absence of internal shoots.

^z Mean ± standard deviation of four blocks and 20 fruit per block. Values with the same letter in row were not different by Tukey highly significant difference test ($P > 0.05$).

to relative yield reductions and loss of fruit quality (except for a few fruit quality variables for Pera and for 6-to 10-year-old trees) suggest that most sweet orange cultivars have similar tolerance to CSD, or that those reductions depend mainly on alterations caused by the CSD agent on the rootstock and the capacity of the rootstock's vascular cambium to regenerate functional phloem and permit the normal flux of nutrients to feed the roots responsible for water uptake to supply the canopy demand.

After the appearance of first CSD-symptomatic trees in the grove, disease symptoms could show up in 60 to 100% of trees in the next 2 or 3 years. However, during the same time, the majority of symptomatic trees will show less severe symptoms (2). At the beginning of CSD epidemics, inarching affected trees with tolerant rootstocks can stop CSD severity progress in the tree or even reverse symptoms from severity class 2 to 1 and from 1 to 0 (33), extending the life of affected trees. Based on the results of this work, it may be possible to estimate the economic viability of an affected grove prior to eradication or inarching measures being undertaken, considering the expected average yield and fruit quality of each severity class of CSD-symptom, the box production costs, the box price, and the incidence of affected tree in each severity class.

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LITERATURE CITED

- Bassanezi, R. B., Bergamin Filho, A., Amorim, L., Gimenes-Fernandes, N., Gottwald, T. R., and Bové, J. M. 2003. Spatial and temporal analyses of citrus sudden death as a tool to generate hypotheses concerning its etiology. *Phytopathology* 93:502-512.
- Bassanezi, R. B., Bergamin Filho, A., Amorim, L., and Gottwald, T. R. 2005. Spatial and temporal analyses of citrus sudden death in Brazil. Pages 217-229 in: Proc. 16th Conf. IOCV, Monterrey, Mexico.
- Bassanezi, R. B., Busato, L. A., Sanches, A. L., and Barbosa, J. C. 2005. Danos da Morte Súbita dos Citros sobre a produção de laranja. *Fitopatol. Bras.* 30:497-503.
- Bassanezi, R. B., Gimenes-Fernandes, N., and Massari, C. A. 2006. Progresso da morte súbita dos citros no Estado de São Paulo no período 2002-2005. *Laranja* 27:13-29.
- Blumer, S., Pompeu Junior, J., and Garcia, V. X. P. 2003. Características de qualidade dos frutos de laranjas de maturação tardia. *Laranja* 24:423-431.
- Coletta Filho, H. D., Targon, M. L. P. N., Takita, M. A., Muller, G. W., Santos, F. A., Dorta, S. O., Souza, A. A., Astua-Monge, G., Freitas-Astua, J., and Machado, M. A. 2005. Citrus tristeza vírus variant associated with citrus sudden death and its specific detection by RT-PCR. Page 147 in: Proc. 16th Conf. IOCV, Monterrey, Mexico.
- Davenport, T. L. 1990. Citrus flowering. *Hortic. Rev.* 12:349-408.
- Davis, F. S., and Albrigo, L. G. 1994. Citrus. CAB International, Wallingford, UK.
- Di Giorgi, F., Ide, B. Y., Dib, K., Marchi, R. J., Triboni, H. R., and Wagner, R. L. 1990. Contribuição ao estudo do comportamento de algumas variedades de citros e suas implicações agroindustriais. *Laranja* 11:567-612.
- Di Giorgi, F., Ide, B. Y., Dib, K., Triboni, H. R., Marchi, R. J., and Wagner, R. L. 1993. Qualidade de laranja para industrialização. *Laranja* 14:97-118.
- Donadio, L. C., Stuchi, E. S., Pozzan, M., and Sempionato, O. R. 1999. Novas variedades e clones de laranja doce para a indústria. *Boletim Citricola* 8. FUNEP, Jaboticabal.
- Erickson, L. C., and Richards, S. J. 1955. Influence of 2,4-D and soil moisture on size and quality of Valencia oranges. *Am. Soc. Hortic. Sci. Proc.* 65:109-112.
- Figueiredo, J. O. 1991. Variedades copas. Pages 228-257 in: *Citricultura Brasileira* v. 1. O. Rodriguez, J. Pompeu, Jr., and V. Pinto Viégas, eds. Fundação Cargill, Campinas.
- Fishler, M., Goldschmidt, E. E., and Monselise, S. P. 1983. Leaf area and fruit size in girdled grapefruit branches. *J. Am. Soc. Hortic. Sci.* 108:218-221.
- Fudge, B. R. 1936. The relation of foliage to tree maintenance and fruit production. *Proc. Fla. State Hortic. Soc.* 49:14-19.
- Garcia-Luis, A., Fernes, F., Sanz, A., and Guardiola, J. L. 1988. The regulation of flowering and fruit set in *Citrus*: relationship with carbohydrate level. *Israel J. Bot.* 37:189-201.
- Gazzola, R., Oliveira Junior, J. P., Correa, G. C., Fonseca, E. B. A., Paula, C. M. P., Almeida, N. A., and Rocha, M. R. 1991. Correlação entre características químicas e físicas nos frutos de laranja (*Citrus sinensis* (L.) Osbeck cv. Natal). *Ciê. Prát.* 15:154-158.
- Gimenes-Fernandes, N., and Bassanezi, R. B. 2001. Doença de causa desconhecida afeta pomares cítricos no norte de São Paulo e sul do Triângulo Mineiro. *Summa Phytopathol.* 27:93.
- Goldschmidt, E. E., Ashkmezai, M., Herzano, Y., Shaffer, A. A., and Monselise, S. P. 1985. A role for carbohydrate levels in the control of flowering in citrus. *Sci. Hortic.* 26:159-166.
- Goldschmidt, E. E., and Monselise, S. P. 1977. Physiological assumptions toward the development of a citrus fruiting model. *Proc. Int. Soc. Citric.* 2:668-672.
- Guardiola, J. L. 1987. Factores internos que determinan el tamaño del fruto en los agrios. *Levante Agríc.* 279/280:247-250.
- Guardiola, J. L. 1988. Factors limiting productivity in citrus: a physiological approach. *Proc. 6th Int. Citrus Congr.* 1:381-394.
- Guardiola, J. L. 1992. Frutificação e crescimento. Pages 1-24 in: *Anais do Segundo Seminário Internacional de Citros: Fisiologia*. L. C. Donadio, coord. Fundação Cargill, Bebedouro.
- Guardiola, J. L. 2000. Regulation of flowering and fruit development: endogenous factors and exogenous manipulation. Pages 342-346 in: *Proc. 9th Int. Citrus Congr. Orlando, FL.*
- Guardiola, J. L., Agustí, M., Almela, V., and Garcia-Marí, F. 1982. The regulation of fruit size in *Citrus* by tree factors. (Abstr.) 21st Int. Hortic. Cong. 1:1363.
- Jesus Junior, W. C., and Bassanezi, R. B. 2004. Análise da dinâmica e estrutura de focos da morte súbita dos citros. *Fitopatol. Bras.* 29:399-405.
- Laranjeira, F. F., and Palazzo, D. A. 1999. Danos qualitativos à produção de laranja 'Natal' causados pela clorose variegada dos citros. *Laranja* 20:77-91.
- Maccheroni Junior, W., Alegria, M. C., Greggio, C. C., Piazza, J. P., Kamila R. F., Zacharias, P. R. A., Bar-Joseph, M., Kitajima, E. W., Assumpção, L. C., Camarotte, G., Cardozo, J., Casagrande, E. C., Ferrari, F., Franco, S. F., Giachetto, P. E., Girasol, A., Jordão Junior, H., Silva, V. H. A., Souza, L. C. A., Aguilard-Vildoso, C. I., Zanca, A. S., Arruda, P., Ferro, J. A., and Da Silva, A. C. R. 2005. Identification and genomic characterization of a new virus (*Tymoviridae* Family) associated with citrus sudden death disease. *J. Virol.* 79:3028-3037.
- Menegucci, J. L. P., Paiva, L. V., Souto, R. F., Carvalho, S. A., Marinho, C. S., Amaral, A. M., and Souza, M. 1995. Alterações físico-químicas de frutos de laranja 'Valência' com sintomas de clorose variegada dos citros. *Rev. Bras. Frutic.* 17:153-155.
- Mostert, P. G., and Van Zyl, J. L. 2000. Gains in citrus fruit quality through regulated irrigation. *Acta Hortic.* 516:123-130.
- Paulino, S. E. P. 2005. Pages 38-42 in: *Modelos agrometeorológicos para estimativa do número de frutos por planta em cultívers de laranja doce na região de Limeira-SP*. Ph.D. thesis, University of São Paulo, Brazil.
- Redd, J. B., Hendrix Junior, C. M., and Hendrix, D. L. 1986. Quality control manual for citrus processing plants. Book I: regulations, citrus methodology, microbiology, conversion charts, tables, other... Intercit Inc., Safety Harbour.
- Román, M. P., Cambra, M., Juárez, J., Moreno, P., Duran-Vila, N., Tanaka, F. O. A., Alves, E., Kitajima, E. W., Yamamoto, P. T., Bassanezi, R. B., Teixeira, D. C., Jesus, W. C., Jr., Ayres, A. J., Gimenes-Fernandes, N., Rabenstein, F., Giroto, L. F., and Bové, J. M. 2004. Sudden death of citrus in Brazil: a graft-transmissible bud union disease. *Plant Dis.* 88:453-467.
- Syvrtsen, J. P., and Lloyd, J. J. 1994. Citrus. v. 2. Pages 65-99 in: *Handbook of Environmental Physiology of Fruit Crops*. B. Schaffer and P. C. Anderson, eds. CRC Press, Boca Raton, FL.
- Yamamoto, P. T., Jesus, W. C., Jr., Bassanezi, R. B., Sanches, A. L., Ayres, A. J., Gimenes-Fernandes, N., and Bové, J. M. 2003. Transmission of the agent inducing symptoms of citrus sudden death by graft-inoculation under insect-proof conditions. (Abstr.) *Fitopatol. Bras. (Suppl.)* 28:S265.