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Effect of enhanced nutritional programs and exogenous auxin spraying on huanglongbing severity, fruit drop, yield and economic profitability of orange orchards

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ABSTRACT

Citrus trees infected with '*Candidatus* Liberibacter asiaticus', the bacterium associated with huanglongbing (HLB), present asymmetric chlorosis and yellow veins on leaves, nutritional deficiencies due to impaired water and nutrient uptake by a reduced root system, and also premature fruit drop and progressive yield reduction. This motivates growers to adopt additional foliar nutritional and auxin plant hormone derivative 2,4-D sprays to try to mitigate HLB damage. However, long-term studies that compare the yield of both well-nourished asymptomatic (AT) and symptomatic trees (ST) under these programs are absent in the literature. This work presents a long-term field study that compared the effect of several enhanced nutritional programs (ENP) and exogenous auxin spray on the progress of HLB severity and on the fruit drop and yield of AT and ST. Moreover, the benefit–cost ratio of each program was estimated. Programs did not contribute to reduce HLB severity over time (mean final HLB severity > 50%). None of the programs contributed to reduce the percentage of fruit drop (remained higher than 40% in symptomatic branches) nor to increase fruit production of ST. Benefit/cost ratio was, on average, 1.49 times higher in AS than in ST. Grower standard nutritional program had the higher benefit-cost ratio (132.8 and 84.7 for AT and ST, respectively) than the others more costly programs. These observations suggest that ENP and 2,4-D sprays are not important in controlling HLB nor mitigating its damages, and their use, instead of removing diseased trees, can build up inoculum within a region. Diseased trees are inoculum source that psyllid vector *Diaphorina citri* can acquire the bacteria, transmit to a healthy tree, and, consequently, spread HLB. Rigorous vector control by insecticide sprays and eradication of diseased plants are still the best long-term HLB control.

1. Introduction

Huanglongbing (HLB), or greening, is the most severe and important disease affecting citrus in most citrus-growing regions, due to the difficulty of controlling it and the great crop loss (Bassanezi et al., 2020; Bové, 2006; Gottwald, 2010; Singerman et al., 2018). Typical symptoms of HLB on citrus trees consist of blotchy mottled leaves, severe asymmetric leaf chlorosis, yellow leaf veins or leaf vein corking, small lopsided fruit, color inversion of fruit, and fruit drop (Bové, 2006; da Graça and Korsten, 2004). Fruit production and fruit quality decrease drastically with the increase of HLB severity (Bassanezi, 2018; Bassanezi et al., 2009, 2011). Consequently, the economic longevity of the affected orchard is also reduced. Three bacteria species of '*Candidatus* Liberibacter sp.' are associated with HLB: '*Ca.* Liberibacter asiaticus' (CLas), in Asian, American, and African countries, '*Ca.* Liberibacter americanus' (CLam), in Brazil, and '*Ca.* Liberibacter africanus' (CLaf), in African countries (Bové, 2006; Teixeira et al., 2005). All '*Ca.* Liberibacter spp.' are mainly transmitted by plant propagation and insect vectors (Bové, 2006; Hall et al., 2013; Lopes et al., 2009). In Asia and the Americas, HLB can be spread by the Asian citrus psyllid vector *Diaphorina citri* and, in Africa, by *Trioza eritreae* (Aubert, 1987). Exclusion, eradication, and protective measures are the main HLB management practices, that should be carried out both within the farm and in the neighborhood around it for effective control (Bassanezi et al., 2013).

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Exclusion measures include the use of healthy vegetative material, for instance from a nursery with an insect-proof screen house; eradication measures include the removal and destruction of HLB-symptomatic plants, and protective measures include vector control by preventive insecticide sprays (Bové, 2006; Gottwald, 2010). Although eradication measures are strongly recommended, some growers are prone to maintain symptomatic plants in the orchards, due to the inherent cost of the plants, mainly economically productive bearing trees with early symptoms (Bassanezi et al., 2020). In this scenario, growers foresee measures to reduce crop losses and increase fruit quality in HLB-symptomatic trees. One of these measures is the combination of an enhanced nutritional program (ENP), that consists of supplementation with mineral nutrients, and a plant growth regulator, for instance the auxin plant hormone derivative 2,4-D, both mainly by foliar application, with a view to mitigating HLB symptoms in leaves and fruit and maintaining the production of diseased trees similar to that in healthy trees.

The colonization of '*Ca.* Liberibacter spp.' is associated with a decrease of some plant nutrients, such as phosphorous, calcium, magnesium, manganese, zinc, iron, and molybdenum (Masaoka et al., 2011; Mattos et al., 2020; Pustika et al., 2008; Zhao et al., 2013) and a decrease of plant biomass (da Silva et al., 2020). Colonization by these bacteria impairs water and nutrient uptake by roots, and also influences several aspects of citrus physiology, such as altering the levels of metabolites, altering the hormonal balance, and increasing the accumulation of carbohydrates in symptomatic leaves (Cevallos-Cevallos et al., 2012; Mattos-Jr et al., 2020; Sagaram and Burns, 2009; Schneider, 1968). The use of ENP and exogenous plant regulators aims to reduce these changes in plant nutrients and hormones caused by bacterial infection and colonization. Although several studies using such measures have been carried out (da Silva et al., 2020; Gottwald et al., 2012; Rouse, 2013; Shen et al., 2013; Stansly et al., 2014), few long-term field studies have assessed the yield of both diseased and healthy plants and discussed the benefit–cost ratio of using such programs (Gottwald et al., 2012; Stansly et al., 2014). Therefore, the present study was carried out with the goal to investigate the effect of different ENP and exogenous auxin spray programs on HLB severity progress, fruit drop, and yield in HLB-symptomatic orange trees compared with healthy or asymptomatic trees throughout several seasons, and also to estimate the benefit–cost ratio of the additional programs.

2. Material and methods

2.1. Experimental site

The experiment was conducted from March 2014 to December 2017 in a commercial citrus orchard with a drip irrigation system. The orchard was composed of 10-year-old 'Valencia' sweet orange trees grafted onto 'Swingle' citrumelo, planted in 2004 at 7.80 m by 3.80 m spacing (337 trees/ha). The orchard was located in the municipality of Cafelândia, in São Paulo state, Brazil (latitude 21°04′09″ S, longitude 49°36′16″ W and altitude 445 m). The experimental area was located within a plot near the farm border, in which there was natural infestation of the HLB vector *D. citri*. Insecticides to control the psyllid were sprayed over the orchard fortnightly all year round.

The soil of the experimental area had a sandy loam texture. Lime and gypsum were applied to obtain a soil pH between 5.5 and 6.0 and base saturation at pH 7 ($V = 100 \times [K + Ca + Mg]/[K + Ca + Mg + Al]$) above 70%. Chemical soil properties at 0–20 cm, before the experiment was installed were: pH ($CaCl_2$) = 6.0; O.M. = 14.0 g/dm^3; P = 82.0 mg/dm^3, Ca = 47.2 mmolc/dm^3; Mg = 22.2 mmolc/dm^3; K = 1.0 mmolc/dm^3; Al = 0 mmolc/dm^3, H+Al = 16 mmolc/dm^3; SB = 70.4 mmolc/dm^3; CEC = 86.4 mmolc/dm^3; and V = 81%, in which O.M. is organic matter, H+Al is the potential acidity, SB represents the sum of bases (SB = K + Ca + Mg), and CEC is the cation exchange capacity at pH 7 (CEC = K + Ca + Mg + H + Al). NPK fertilizers were applied to the soil between the end of spring and beginning of summer (October to December) by distributing 180–220 kg N/ha, 80–100 kg P_2O_5/ha, and 150–180 kg K_2O/ha, according to recommendations based on soil analysis. Boron was also applied to soil (2 kg B/ha).

2.2. Programs

Five programs (P1, P2, P3, P4, and P5) were sprayed in a row (4 rows per program); in the first season (2013–2014), applications were from March to June 2014, and from the second season onwards (2014–2015, 2015–2016, and 2016–2017), applications were from September, i.e. the beginning of flowering (BBCH scale 61; Agusti et al., 1995), to June of the next year, i.e. to fruit about 70% of final size (BBCH scale 77; Agusti et al., 1995). Detailed information on each program is given in Table 1. The application method for the programs P1, P2, P4, and P5 was monthly foliar spraying and for P3 was fortnightly fertigation. The foliar spraying was performed with a mounted air blast sprayer (Natali®, Limeira, Brazil), with 20 MGA lilac spray nozzles on each side, providing a 60° spray pattern at 4.8 bar and working speed of 5.0 km/h in 2013–2014, 2014–2015, and 2015–2016 (10-, 11-, and 12 year-old trees), and 10.3 bar and 3.2 km/h in 2016–2017 (13 year-old trees). The foliar spray volume was 1500 L/ha (75 mL/m^3 of tree canopy) in 2013–2014, 2014–2015, and 2015–2016; and 3500 L/ha (175 mL/m^3 of tree canopy) in 2016–2017. For fertigation, fertilizer was dissolved in a tank with a mixing system and applied by a direct system through pressure from a centrifugal pump in the experiment. The drip irrigation system had spacing among self-compensating drippers of 0.7 cm, flow rate of 2.3 L/h, and a water depth of 3 mm of water/day with replacement according to the estimated evapotranspiration. P1 was the standard program already performed at the citrus farm. P2 and P3, aimed to supply an enhanced dose of nutrients with a higher frequency of applications per season than P1 by foliar sprays (P2) or fertigation (P3). P4 and P5 are programs that have been commercialized in Brazil as "HLB control program". In spite of the nutrients present in these programs seems to be required by HLB-affected trees as there are a decrease of these nutrients in diseased trees, there are no previous studies to verify its efficacy in controlling HLB nor reduce yield losses. Exogenous auxin was sprayed, as 2,4-dichlorophenoxyacetic (2,4-D) acid (Aminol 806, Adama Agrociências, dose at 670 g/L), at P4 and P5 programs, in which this would mimic the action of the hormone auxin, aiming to reduce fruit drop caused by HLB.

2.3. Assessments

Twenty asymptomatic trees and 20 to 28 HLB-symptomatic citrus plants were selected within the two internal rows of each treated area. Each tree represented one experimental unit. Diseased plants were selected based on their initial percentage of HLB-symptomatic canopy (initial HLB severity at maximum of 10%). HLB severity was evaluated monthly in each selected symptomatic tree by visual inspection of the canopy using the methodology described by Gottwald et al. (1991). Yield (kg/tree) was evaluated in all selected plants from 2014 to 2017 in the harvest period.

For estimating fruit drop, the number of sweet orange fruits was assessed in 15 asymptomatic trees and 15 symptomatic trees selected within each program at the beginning of fruit formation (BBCH scale 72, April 4, 2015; March 1, 2016; and February 8, 2017) and at harvest (BBCH scale 83, September 22, 2015; September 26, 2016; and November 27, 2017). Three branches were evaluated from each asymptomatic tree (asymptomatic branch of asymptomatic tree – ABAT); from each diseased tree, three branches with no HLB symptoms (asymptomatic branch of symptomatic tree – ABST) and three branches with HLB symptoms (symptomatic branch of symptomatic tree – SBST) were evaluated. The sum of the number of fruits on the three branches ABAT, ABST, and SBST within the tree was considered as one replicate.

Table 1
List of five different programs (P1–P5) of the present experiment, products used in combination in each program, product rate (and unit) per application, and total number, frequency, and type of application within each season.

Prog.	Product	Product rate per application[h]					Applications per season[i]			
		2013–2014	2014–2015	2015–2016	2016–2017	Units	2013–2014	2014–2015	2015–2016	2016–2017
P1	Product A[a]	33.3	75	75	75	mL/100 L	1 foliar spray along with citrus black spot fungicide treatment	4 foliar sprays along with citrus black spot fungicide treatment	4 foliar sprays along with citrus black spot fungicide treatment	4 foliar sprays along with citrus black spot fungicide treatment
	Product B[b]	66.7	37.5	37.5	37.5	mL/100 L				
	Calcium nitrate	0	125	125	125	g/100 L				
	Magnesium chloride 8.5%	0	25	25	25	mL/100 L				
	Manganese chloride 14%	50	37.5	37.5	37.5	mL/100 L				
	Molybdenum 15%	18.3	13.8	13.8	13.8	mL/100 L				
	Phosphite 00-30-20	66.7	50	50	50	mL/100 L				
	Urea	166.7	125	125	125	g/100 L				
	Zinc chloride 21%	80	75	75	75	mL/100 L				
P2	Boric acid	56.3	60	60	60	g/100 L	4 monthly foliar sprays	10 monthly foliar sprays	10 monthly foliar sprays	9 monthly foliar sprays
	Calcium nitrate	125	150	150	150	g/100 L				
	Foliar copper 7%	25	25	25	25	mL/100 L				
	Magnesium chloride 8.5%	12.5	30	30	30	mL/100 L				
	Manganese chloride 14%	25	40	40	40	mL/100 L				
	Molybdenum 15%	5	5	5	5	mL/100 L				
	Phosphite 00-30-20	75	80	80	80	mL/100 L				
	Potassium carbonate 32%	12.5	20	20	20	mL/100 L				
	Purified MAP[c]	31.3	50	50	50	g/100 L				
	Urea	62.5	225	225	225	g/100 L				
	Zinc chloride 21%	56.3	82.5	82.5	82.5	mL/100 L				
P3	Ammonium nitrate	18.5	14.8	14.8	14.8	kg/ha	8 fortnightly fertigation	20 fortnightly fertigation	20 fortnightly fertigation	18 fortnightly fertigation
	Boric acid	844	585	585	585	g/ha				
	Calcium nitrate	12.1	9.7	9.7	9.7	kg/ha				
	Foliar copper 7%	813	470	470	470	mL/ha				
	Magnesium chloride 8.5%	2681	2275	2275	2275	mL/ha				
	Manganese chloride 14%	813	470	470	470	mL/ha				
	Molybdenum 15%	81	30	30	30	mL/ha				
	Phosphite 00-30-20	0.0	550	550	550	mL/ha				
	Potassium chloride white	22.9	15.5	15.5	15.5	kg/ha				
	Purified MAP[c]	14.7	11.7	11.7	11.7	kg/ha				
	Zinc chloride 21%	1094	620	620	620	mL/ha				
P4	Product C[d]	125	125	125	125	mL/100 L	4 monthly foliar sprays	10 monthly foliar sprays	10 monthly foliar sprays	9 monthly foliar sprays
	Calcium nitrate	250	250	250	250	g/100 L				
	Exogenous auxin[e]	0.4	0.4	0.4	0.4	mL/100 L				
P5	Product D[f]	250	250	250	250	mL/100 L	4 monthly foliar sprays	10 monthly foliar sprays	10 monthly foliar sprays	9 monthly foliar sprays
	Product E[g]	250	250	250	250	mL/100 L				
		250	250	250	250					

(continued on next page)

Table 1 (*continued*)

Prog.	Product	Product rate per application[h]					Applications per season[i]			
		2013–2014	2014–2015	2015–2016	2016–2017	Units	2013–2014	2014–2015	2015–2016	2016–2017
	Calcium nitrate					g/100 L				
	Exogenous auxin[e]	0.6	0.6	0.6	0.6	mL/100 L				

[a] Product A is a commercial mixture of 6% *Ascophyllum nodosum*, 3.6% nitrogen (N), and 9.6% amino acids.
[b] Product B is a commercial mixture of 44% calcium carbonate.
[c] Purified mono-ammonium phosphate with 12% N and 61% phosphorus pentoxide (P_2O_5).
[d] Product C is a commercial mixture of 4.04% N, 7.5% P_2O_5, 8.37% total phosphorus (P total), 0.03% potassium (K_2O), 0.03% calcium (Ca), 2.93% magnesium (Mg), 0.24% sulfur (S), 0.007% iron (Fe), 2.85% manganese (Mn), 0.07% cupper (Cu), 6.49% zinc (Zn), 0.006% boron (B), 0.11% sodium (Na), 0.0003% cobalt (Co), <0.0001% molybdenum (Mo), 0.01% aluminum (Al), and 3.83% organic matter.
[e] Aminol 806 (Adama Agrociências) with 670 g of 2,4-dichlorophenoxyacetic (2,4-D) acid equivalent per liter. This product had one less spray than the others in each season because it was not applied during flowering.
[f] Product D is a commercial mixture of 0.88% N, 13.60% P_2O_5, 13.87% P total, 13.30% K_2O, 0.01% Ca, 0.02% Mg, 0.01% S, 0.12% Fe, 0.03% Mn, 0.02% Cu, 0.03% Zn, 1.07% B, 0.41% Na, < 0.0001% Co, 0.01% Mo, 0.002% Al, and 8.87% organic matter.
[g] Product E is a commercial mixture of 10.89% N, 0.01% P_2O_5, 0.04% P total, 0.17% K_2O, 0.08% Ca, 3.47% Mg, 0.06% S, < 0.0001% Fe, 0.77% Mn, 0.001% Cu, 2.31% Zn, 0.005% B, 0.007% Na, < 0.0001% Co, 0.0005% Mo, < 0.0001% Al, and 1.04% organic matter.
[h] The foliar spray volume was 1500 L/ha in 2013–2014, 2014–2015, and 2015–2016; and 3500 L/ha in 2016–2017.
[i] Applications from March to June 2014 in 2013–2014, from September to June in 2014–2015 and 2015–2016, and from September to May in 2016–2017.

2.4. Data analysis

The average percentage of disease severity for each evaluated tree was plotted over time and the area under the disease severity progress curve (AUDSPC) was calculated by trapezoidal integration (Madden et al., 2007). Fruit drop (Fd) was calculated for each tree by $Fd = 100 \times (FNi - FN_H)/FNi$, where FNi corresponds to the number of fruit at the beginning of fruit formation and FN_H corresponds to the number of fruit at harvest. Relative yield corresponded to the ratio between the yield of symptomatic trees and that of asymptomatic trees.

Statistical analyses were performed using RStudio Version 1.2.5033 software (R Core Team, 2020). Initial disease severity (July 21, 2014), final disease severity (November 28, 2017), AUDSPC, yield of asymptomatic trees, yield of symptomatic trees, and relative yield and fruit drop of ABAT, ABST, and SBST were analyzed by generalized linear models (GLMs) using the GLM function from the "stats" package. Quasi-Poisson distribution was used to analyze the percentage of both initial and final disease severity, fruit drop from ABAT in 2016 and 2017, fruit drop from ABST in 2015 and 2016, and fruit drop from SBST in 2016 and 2017. Gaussian distribution was used to analyze AUDSPC, yield of asymptomatic trees, yield of symptomatic trees, the relative yield for each year and the accumulated yield, fruit drop from ABAT in 2015, fruit drop from ABST in 2017, and fruit drop from SBST in 2015. Family distribution was chosen based on the simulation envelopes with more points inside them using the "hnp" package (Moral et al., 2017). Least-squares means of programs were estimated by the "lsmeans" function from the "lsmeans" package (Lenth, 2016) and post-hoc comparisons among programs were conducted by Tukey's test at a significance level of alpha = 0.05.

2.5. Benefit–cost analysis

Benefit–cost analysis of each additional program was determined based on the total revenue in relation to production costs, considering the accumulated costs of all assessed seasons. The program cost was calculated based on February 2018 fertilizer product prices in São Paulo state. All costs in Brazilian currency (R$) were converted into U.S. dollars (US$) using the average exchange rate of R$ 3.24 = US$ 1 (Institute for Applied Economic Research – IPEA – database, https://www.ipea.gov.br/portal/). Both foliar spraying and fertigation were carried out associated with other operations and did not represent additional operational costs. Therefore, the cost of these operations was not accounted for in the benefit–cost analysis. In the case of foliar sprays, the products were mixed in a spray tank and applied with insecticides or fungicides, and for fertigation, the products were injected with the field irrigation.

The total revenue in United States dollars (US$) per hectare for each program was estimated based on the sum of the revenue per season, considering the yield (boxes/ha) and the average price of a sweet orange fruit box per season. The average price of a 40.8 kg sweet orange fruit box delivered to the juice industry in São Paulo state was converted into U.S. dollars (US$); it was US$ 4.16, US$ 3.58, US$ 5.25, and US$ 5.92 in the seasons 2013–2014, 2014–2015, 2015–2016, and 2016–2017, respectively (Center for Advanced Studies on Applied Economics – CEPEA – database, https://www.cepea.esalq.usp.br/en).

3. Results

The initial HLB severity was similar among the trees treated with the different programs and varied from 9.2% to 11.7% (Table 2). HLB severity increased rapidly over time in all assessed trees regardless of program (Fig. 1). Trees treated with P1 and P4 had higher final HLB

Table 2
Comparison of the percentage of initial and final severity of huanglongbing (HLB), and the area under the disease severity progress curve (AUDSPC) for trees treated with enhanced nutritional programs and exogenous auxin spray programs (P1, P2, P3, P4, and P5).

Program	Initial disease severity (%)[a]	Final disease severity (%)[b]	AUDSPC[c]
P1	9.2 (±1.4) a	63.2 (±1.6) a	36,798 (±2101) a
P2	11.7 (±1.6) a	53.3 (±3.2) b	24,980 (±2644) b
P3	8.4 (±1.1) a	60.8 (±2.7) ab	31,767 (±2663) ab
P4	11.2 (±1.3) a	63.9 (±1.5) a	40,274 (±1474) a
P5	9.2 (±1.6) a	58.9 (±3.2) ab	33,844 (±2938) ab
P value	0.40	0.02	<0.001

[a–c] Means followed by the same letter are not significantly different among programs, according Tukey's test at alpha set = 0.05.
[a] Mean value of the initial disease severity evaluated on July 21, 2014 per program ± standard error of the mean. Calculated P value obtained from the analysis of variance.
[b] Mean value of the final disease severity evaluated on November 28, 2017 per program ± standard error of the mean.
[c] Mean value of the AUDSPC per program ± standard error of the mean.

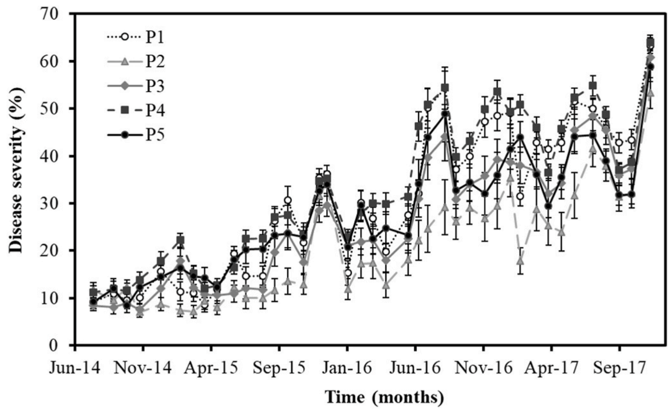

Fig. 1. Progress of severity of huanglongbing (HLB), as a percentage, from July 2014 to November 2017 in diseased trees treated with enhanced nutritional programs and exogenous auxin spray programs (P1, P2, P3, P4, and P5). Points represent the mean value and bars represent the standard error.

severity (P = 0.02) and higher AUDSPC (P < 0.001) than trees treated with P2 (Table 2). In spite of this difference, all treated trees had on average more than 50% of the canopy with HLB symptoms after 41 months (Table 2).

Nutritional and exogenous auxin program did not enhance fruit retention on the tree, either for ABAT or for ABST and SBST. However, the lowest average percentage of fruit drop was always observed for ABAT when compared to ABST and SBST (Fig. 2). For ABAT (Fig. 2A), estimated average fruit drop was 8.9% in 2015, 2.8% in 2016, and 7.5% in 2017, whereas for ABST (Fig. 2B), it was 3.1%, 7.7%, and 46.7% for the same respective years. Regardless of program, the average percentage of fruit drop for SBST (Fig. 2C) was mostly higher than 50%. In 2015 (P = 0.05) and 2016 (P = 0.05), fruit drop for ABAT was not significantly different among programs (Fig. 2A), whereas in 2017 (P = 0.003), trees treated with P4 had a higher percentage of fruit drop than trees treated with P1 (Fig. 2A). No program was consistently distinct from the others for fruit drop from symptomatic trees. For ABST, trees treated with P3 and P1 had the highest fruit drop in 2015 (P < 0.001) and 2016 (P < 0.001), respectively (Fig. 2B). For SBST, programs were significantly different only in 2016 (P = 0.04), when trees treated with P2 had the highest percentage of fruit drop, followed by P5, P1, P4, and P3 (Fig. 2C). No difference among the programs was observed for ABST (P = 0.21) and SBST (P = 0.14) in 2017 (Fig. 2B and C). As the severity of the disease increased over the years, the rate of premature fruit drop increased.

Generally, yield within the assessed years and the 4-year accumulated yield of asymptomatic (P = 0.24) and HLB-symptomatic (P = 0.52) trees were similar among the programs (Fig. 3A and B). Only in 2016, the yield of asymptomatic trees treated with P4 was higher than the other programs (P < 0.001, Fig. 3A). Also, in 2016, the yield of symptomatic trees treated with P5 and P4 was higher than P1 (P = 0.01, Fig. 3B). This difference is probably more related to the biennial yield fluctuation than to the program effect, as no difference was observed in the next year for either asymptomatic (P = 0.22) or HLB-symptomatic trees (P = 0.55) treated with different programs. The average yield of asymptomatic trees was 179.1, 455.5, 121.9, and 166.7 kg/tree in 2014, 2015, 2016, and 2017, respectively. It should be noted that the average yield of HLB-symptomatic trees was always lower than that of asymptomatic trees (on average 15.6 kg/tree lower in 2014, 132.9 kg/tree lower in 2015, 55.9 kg/tree lower in 2016, and 83.9 kg/tree lower in 2017) and the average relative yield decreased throughout the years, from 0.9 in 2014 to 0.5 in 2017 (Fig. 3C). The biennial yield fluctuation may also have been reflected in the relative yield, considering that there was a significant difference in relative yield among programs in 2014 (P < 0.001) and 2016 (P < 0.001), while in 2015 (P = 0.21) and 2017 (P = 0.14) no significant difference was observed (Fig. 3C). In spite of this difference, no treatment consistently stood out; for example, in 2014, P2 had the highest relative yield (0.99) and P1 had the lowest (0.83), whereas in 2016, P2 had the lowest relative yield (0.46) and P1 had the highest (0.70) (Fig. 3C).

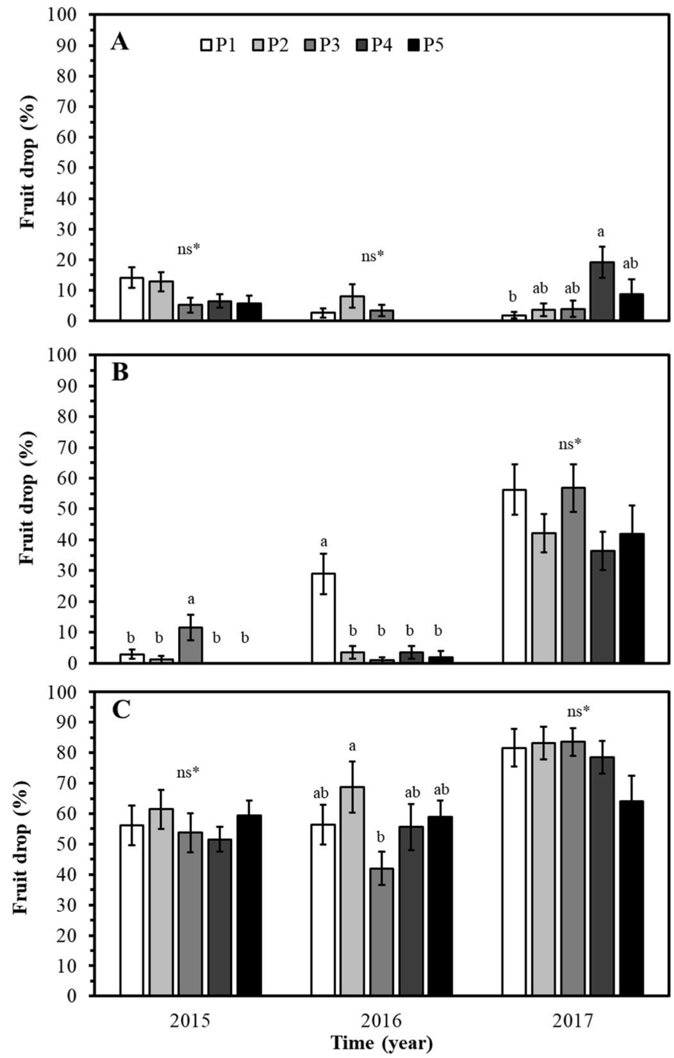

Fig. 2. Fruit drop, as a percentage, from asymptomatic branches of asymptomatic trees (A), asymptomatic branches of symptomatic trees (B), and symptomatic branches of symptomatic trees (C) treated with enhanced nutritional programs and exogenous auxin spray programs (P1, P2, P3, P4, and P5). Error bars represent the standard error. Means followed by the same letter are not significantly different among programs, according to Tukey's test at alpha set = 0.05. ns* = not significant.

Regardless of the program, the benefit–cost ratio was always greater for asymptomatic trees than for HLB-symptomatic trees (Table 3). A similar revenue was obtained for all nutritional programs; however, the revenue considering the yield of asymptomatic trees was, on average, US $ 11,000/ha higher than the yield of symptomatic trees (Table 3). The lowest program cost was obtained when trees were treated with the standard nutritional program already performed in the orchard (P1), with foliar sprays associated with black spot control (on average of three sprays per season). The 4-year accumulated cost of P1 was US$ 246.95/ha, with a benefit–cost ratio of 132.84 and 84.68 for asymptomatic and symptomatic trees, respectively (Table 3). The accumulated costs of P2 and P4 were on average 2.5 times higher than the cost of P1 (Table 3). P4 and P2 had a benefit–cost ratio of 55.07 and 56.98 for asymptomatic trees and 35.48 and 38.02 for symptomatic trees, respectively (Table 3).

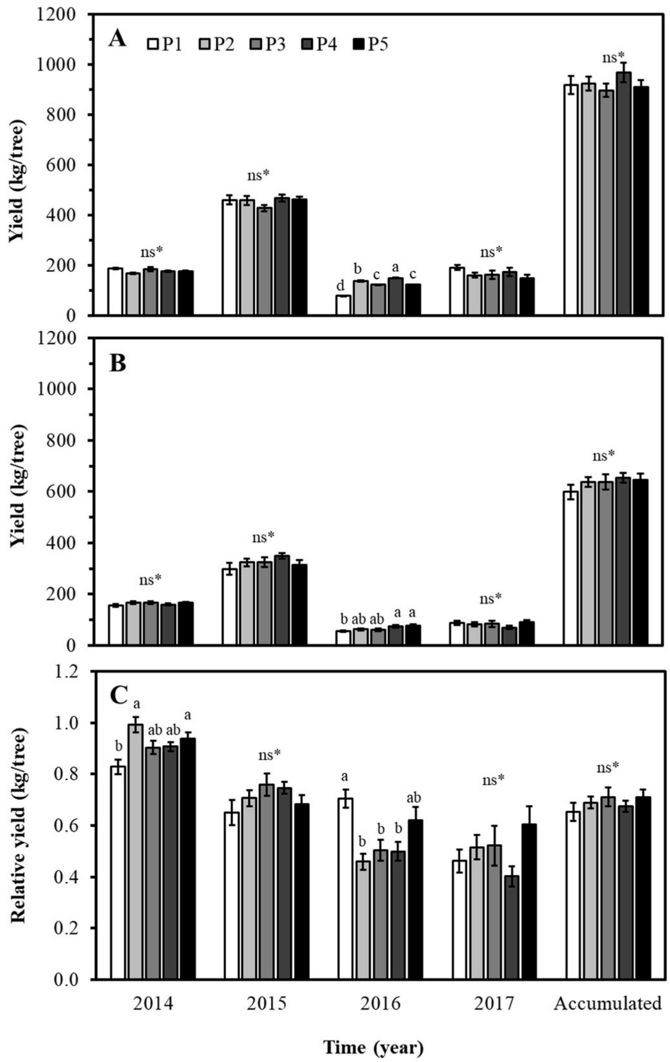

Fig. 3. Yield of asymptomatic trees (A) and of HLB-symptomatic trees (B). Relative yield (C) is the ratio between the yield of symptomatic trees and that of asymptomatic trees treated with enhanced nutritional programs and exogenous auxin spray programs (P1, P2, P3, P4, and P5). Error bars represent the standard error. Means followed by the same letter are not significantly different among programs, according to Tukey's test at alpha set = 0.05. ns* = not significant.

Table 3
Total revenue, program cost, and benefit–cost ratio of enhanced nutritional programs and exogenous auxin spray programs (P1, P2, P3, P4, and P5) applied to HLB-asymptomatic and symptomatic sweet orange trees.

	Program	Total revenue (US$/ha)[a]	Program cost (US$/ha)[a]	Benefit–Cost ratio
Asymptomatic trees	P1	32,805.12	246.95	132.84
	P2	33,110.27	581.12	56.98
	P3	32,229.95	2859.61	11.27
	P4	34,879.70	633.40	55.07
	P5	32,328.45	2453.31	13.18
Symptomatic trees	P1	20,912.42	246.95	84.68
	P2	22,093.71	581.12	38.02
	P3	22,149.24	2859.61	7.75
	P4	22,474.66	633.40	35.48
	P5	22,723.08	2453.31	9.26

[a] One hectare had 337 trees.

The accumulated costs of P3 and P5 were on average 10.8 times higher than the costs of P1 (Table 3). The benefit–cost ratios of P3 and P5 had the lowest values of, on average, 11.27 and 13.18 for asymptomatic and 7.75 and 9.26 symptomatic trees, respectively (Table 3).

4. Discussion

This paper reports the 4-year effect of different ENP and exogenous auxin spray programs on asymptomatic and HLB-symptomatic citrus trees. Specifically, our results show that ENP and exogenous auxin spray did not reduce the progress of HLB symptoms in the tree canopy or increase the yield of diseased trees in field conditions. Previous studies had already reported that enhanced nutritional programs do not reduce HLB progress over the years (Gottwald et al., 2012; Stansly et al., 2014). In contrast, existing studies have reported that application of foliar fertilizer has some influence on reduction or remission of HLB symptoms (da Silva et al., 2020; Li et al., 2019; Pustika et al., 2008; Rouse, 2013; Zhao et al., 2013). However, none reported that the treatment cured diseased trees, or compared results between HLB-symptomatic trees and well-nourished healthy citrus trees. Basically, citrus trees must receive all the necessary nutrients for their development to compare the effect of enhanced nutritional programs on the yield of asymptomatic and symptomatic trees. Otherwise growth and the increase in fruit production will probably be due to the application of the limiting nutrient (Mattos-Jr et al., 2020; Spann and Schumann, 2009). Unlike other studies, all programs received the recommended dose of nutrients for citrus via the soil. Afterwards, we evaluated the effect of additional nutrients via foliar spray or fertigation on the remission of HLB symptoms on leaves and fruit. HLB symptoms on leaves can be mistaken for symptoms of deficiency of nutrients such as nitrogen, iron, manganese, zinc, and magnesium (Pustika et al., 2008; Spann and Schumann, 2009). However, symptoms of HLB present an asymmetrical pattern across the leaf midvein, whereas nutrient deficiencies present a symmetrical pattern (Spann and Schumann, 2009; Vashisth et al., 2019). CLas colonization initially causes phloem disruption; induces callose accumulation into sieve elements pores, that can lead to cell collapse and inhibition of phloem transport; and also induces starch accumulation in cells of the phloem and vascular parenchyma in leaves and petioles (Etxeberria et al., 2009; Folimonova and Achor, 2010; Kim et al., 2009; Koh et al., 2012). The findings of the present work are probably connected to the degenerative and irreversible changes in the plant cells caused by CLas even at the beginning of the infection (Etxeberria et al., 2009; Folimonova and Achor, 2010; Kim et al., 2009; Koh et al., 2012). Nutritional treatments will hardly remedy these changes. An efficient program that may be capable of controlling HLB must not only focus on remission of symptoms but also kill the bacteria inside the phloem vessels, in order to break the intense colonization and damage caused to the tree.

The progress of HLB severity in adult trees was rapid (from 10 ± 0.6% to 60 ± 1.9% in 41 months) in all programs, and a seasonal pattern of symptom expression was observed throughout the years. HLB severity decreased during the growing season (spring and summer) regardless of program, due to the new asymptomatic branches and leaves and early fall of symptomatic leaves that mask the tree status. However, as the bacteria are within the phloem vessels, these new branches become mature and typical symptoms of HLB, such as blotchy mottled leaves, return to appear with greater disease severity in the subsequent season. A previous study has already reported that remission of HLB symptoms due to nutritional programs occurs only during the growing season (Rouse, 2013). Hence, the assessment of HLB severity must be done over the years to verify the effect of the program and not the seasonal effect that results in a natural decrease of HLB severity.

No program reduced fruit drop in both asymptomatic and symptomatic branches of symptomatic trees, as the disease severity increased. In this study, even the application of a chemical analogue of auxin (2,4-D) did not reduce the fruit drop due to HLB. Spraying only 2,4-D may reduce the natural fruit fall rate of healthy citrus trees (Almeida et al., 2004) but, in the same weather conditions, this spraying may be inefficient depending on the citrus variety, concentration sprayed, and/or phenological stage. Although the exogenous auxin spraying performed in this present work led to a cumulative 2,4-D rates of 28.8 mg/L (P4) and 43.2 mg/L (P5) in the entire season, these were applied out in nine monthly sprays of 2,4-D at 3.2 or 4.8 mg/L, respectively. Hence, the sprayed dose associated with several applications at different phenological stages had no significant effect in reducing fruit drop in HLB-affected trees. In another study, only one application of 2,4-D at 26 mg/L had a very low or null effect in reducing fruit drop in HLB-symptomatic trees (Albrigo and Stover, 2015). Synthetic auxins can promote a growth increase in peduncle vascular tissues and in the diameter of citrus fruit peduncles (Mesejo et al., 2003), but a high concentration of auxin in the peduncle could enhance premature fruit drop (Almeida et al., 2004). Besides that, several plant growth regulators may be involved in the regulation of fruit abscission (Chen et al., 2016; Rosales and Burns, 2011). Previous studies observed that a lower fruit detachment force was necessary to pull fruit from HLB-symptomatic trees when compared to fruit from asymptomatic trees (Chen et al., 2016), and levels of both abscisic acid (ABA) and indole-3-acetic acid (IAA), a plant hormone of auxin class, generally were higher in the flavedo of symptomatic fruit than in the flavedo of asymptomatic fruit (Rosales and Burns, 2011).

The benefit/cost ratio of ENP and exogenous auxin spraying was higher for healthy trees than for diseased trees in all programs, and the standard program (P1) was the most economical. The machinery and labor costs were not estimated; however, P1 had the lowest number of foliar applications throughout the years. The second and third programs had three, six, six, and six more foliar sprays than the standard program in 2013–2014, 2014–2015, 2015–2016, and 2016–2017, respectively, and this might not be cost-effective. Although the programs were carried out with other operational activities, for instance pesticide spraying, there is a tendency for citrus growers to reduce the number of sprays. In the case of the standard program (P1), which was applied with the fungicide sprays to control citrus black spot, there was already an improvement of adequate spray volume and the critical fungicide spraying period for control of this disease (Lanza et al., 2018). For the other programs (P2, P4, and P5), the application of nutrients and exogenous auxin was performed monthly with the insecticide spraying for psyllid control; if the grower chooses to spray insecticide based on an action threshold, it is feasible that some nutritional program spraying may be carried out separately from the insecticide application and this may lead to an addition to the programming of the orchard. Growers must keep a profitable orchard and, so far, foliar sprays can represent the largest cultural cost of production, as already occurs for processed oranges in Florida (Singerman, 2019). In summary, additional nutrients and exogenous auxin should be used with the aim of having a well-nourished orchard and not focusing on HLB control. The best long-term HLB control within orchards is still achieved by integrating the eradication of HLB-symptomatic trees with rigorous vector control by insecticide sprays, especially in area-wide management (Bassanezi et al., 2013).

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CRediT authorship contribution statement

Renato B. Bassanezi: Conceptualization, Methodology, Data curation, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Isabela V. Primiano:** Formal analysis, Data curation, Writing – original draft, Visualization. **Humberto V. Vescove:** Conceptualization, Methodology, Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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