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SHORT COMMUNICATION

Ineffectiveness of pruning to control citrus huanglongbing caused by *Candidatus* Liberibacter americanus

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Abstract The huanglongbing (HLB) disease of citrus trees, caused by *Candidatus* Liberibacter asiaticus and *Ca.* Liberibacter americanus, was first reported in Brazil in March, 2004. The presence of the disease has caused serious concerns among growers. Pruning experiments were conducted to determine if removal of symptomatic branches or the entire canopy (decapitation) would eliminate infected tissues and save HLB-affected trees. Pruning was done in five blocks on a total of 592 3- to 16 year-old 'Valência', 'Hamlin' or 'Pêra' sweet orange trees showing no symptoms or with two levels of symptom severity. Ten decapitated trees per block were caged and all trees were treated with insecticides to control the psyllid vector, *Diaphorina citri*. Mottled leaves reappeared on most symptomatic (69.2%) as well on some asymptomatic (7.6%) pruned trees, regardless of age, variety, and pruning procedure. Presence of the pathogen (*Ca.* Liberibacter americanus) in all symptomatic trees was confirmed by PCR. In general, the greater the symptom severity before pruning the lower the percentage of trees that remained asymptomatic after pruning.

Keywords Greening · Inoculum source · *Diaphorina citri* · Disease management

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Huanglongbing (HLB), also known as citrus greening, is a disease caused by three species of *Candidatus* Liberibacter: *Ca.* Liberibacter asiaticus, *Ca.* Liberibacter africanus and *Ca.* Liberibacter americanus (Bové 2006). The pathogen is limited to the phloem of affected trees (Garnier et al. 2000) and is disseminated in the field by the psyllids *Diaphorina citri* and *Trioza erytreae* (McClean and Oberholzer 1965; Capoor et al. 1967). Affected trees show mottled leaves and deformed fruits that fall naturally. Usually, the symptoms appear first on mature leaves close to the top of the branches and then progress throughout the entire tree. In South Africa, trees affected by the less aggressive and heat sensitive *Ca.* Liberibacter africanus develop symptoms approximately 40 cm back from the site of vector feeding after 1 year (Van Vuuren 1993). Constant leaf drop, mainly during the cold months, leads to tree dieback and total loss of fruit production. The fast symptom development in affected trees and the rapid increase of diseased trees in affected orchards have made HLB the most serious citrus disease.

In Brazil, HLB is caused by the recently described *Ca.* Liberibacter americanus (Teixeira et al. 2005b) and *Ca.* Liberibacter asiaticus (Coletta-Filho et al.

2004; Teixeira et al. 2005a). Disease management involves the elimination of all symptomatic trees to reduce inoculum sources and the control of the insect vector *D. citri*. In order to verify whether removing HLB-affected branches of infected trees or removing the entire tree canopy by cutting off the trunks above the graft union (decapitation) would excise the infection and restore the trees to a healthy condition, five experiments were carried out from October, 2004 to July, 2005, in blocks of 3, 5 and 16 year-old 'Valencia', and one block each of 5 year-old 'Hamlin' and 9 year-old 'Pera' trees, all grafted in Rangpur lime rootstock (Table 1). The experiments were conducted in highly affected blocks in a region where the disease and the vector have been present and probably went unnoticed for several years.

All trees were inspected for the presence of HLB symptoms. Trees affected by both HLB and the citrus variegated chlorosis disease (blocks 14 and 19), caused by the xylem-limited bacterium *Xylella fastidiosa*, were not included in the experiment. When a symptomatic tree was identified, the total number of main branches (the branches that originated at the trunk) and those showing mottled leaves or mottled leaves plus dieback were counted. The extent of symptoms on the symptomatic branches were also recorded as to whether the mottled leaves were confined to the top 30–50 cm (level 1) or whether they were present also below 50 cm, close to the trunk (level 2). Because of an absence of leaves on the old internal branches of the 16 year-old 'Valencia' trees, level 2 consisted of trees with mottled leaves plus branch dieback. The incidence of trees showing HLB symptoms was recorded each month after pruning, ending in March (blocks 9 and 10) or July of 2005, when the experiments were terminated.

After recording symptom severity, the trees were pruned with a chain saw by removing the symptomatic branches entirely with the cuts done near the trunk (branch pruning), or by removing the entire tree canopy, with the cut done at the trunk, 15–20 cm above the graft union (decapitation). Asymptomatic trees used as controls were pruned in the same way. All 3 year-old trees of 'Valencia' in block 10, regardless of symptom severity, and all trees with more than two thirds of the main branches showing symptoms, in all blocks, regardless of symptom severity and tree age, were pruned at the trunk level only. In order to avoid sunburn, a 30% suspension of hydrated calcium was brushed onto the cut surface of the branch-pruned trees and to the entire trunk of the decapitated trees. Before growth of any suckers, iron cylinder cages (1.9 m long by 0.9 m diam) with a 50 mesh (0.78 by 0.26 mm) screen were placed on 10 individual trunks to protect the young suckers from access by psyllids. In addition, systemic insecticide (thiamethoxam, 0.75 g active ingredient per tree) was applied on the trunk at the soil level of all pruned trees, 1 week after pruning, and contact insecticide (deltamethrin, 1.5 ml active ingredient per 100 l water) sprayed twice on all pruned and non-pruned trees. Yellow sticky traps (12.5 by 10 cm, Biocontrole, São Paulo, SP) were distributed randomly throughout the orchard blocks (approximately one

Table 1 Incidence of HLB affected trees in the orchard blocks where the pruning experiments were conducted

Block	Sweet orange variety	Age (years)	Total trees	Cumulative number and % trees with HLB symptoms[a]	
				At the pruning date[b]	When the experiment ended[c]
10	Valencia	3	1668	41 (2.5)	50 (3.0)
15	Valencia	5	1252	146 (11.7)	238 (19.0)
19	Valencia	16	954	186 (19.5)	318 (33.3)
09	Hamlin	5	938	121 (12.9)	155 (16.5)
14	Pera	9	1935	195 (10.1)	267 (13.8)
		Total	6747	689 (10.2)	1028 (15.2)

[a] In blocks 14 and 19, 122 and 54 plants, respectively, found during the first inspection were not included in the experiment since they were also affected by the citrus variegated chlorosis disease, caused by the xylem-limited bacterium *X. fastidiosa*

[b] All plants were pruned during the month of October, 2004

[c] Includes pruned and unpruned trees. In blocks 9 and 10 the experiment ended in March, 2005, when all trees were removed. The experiment ended in July, 2005 for the remaining blocks

card per 100 trees), at the outer part of the tree canopy to monitor psylla populations.

Pruned trees were observed monthly for sucker development and symptom expression. Two months after pruning, the excess of sweet orange suckers and all suckers of Rangpur lime that developed on the trunks of the decapitated trees were removed, leaving the three to five most vigorous suckers. The top third of the remaining suckers were removed to force the development of secondary new branches. At each evaluation date, the number of suckers and branches showing mottled leaves or mottled leaves plus dieback was counted. In the branch-pruned trees, the number of old branches expressing leaf symptoms was also recorded. At the last evaluation date, 9 months after pruning, five mottled or chlorotic mature leaves were collected from each symptomatic or suspicious sucker or branch, processed and evaluated by PCR with primers specific for *Ca.* Liberibacter americanus and *Ca.* Liberibacter asiaticus, as described by Teixeira et al. (2005a). Pruned trees on blocks 9 and 10 were evaluated for only 5 months after pruning since all trees of these blocks were removed by the grower. The other blocks were evaluated until July, 2005, after which all symptomatic trees were destroyed. The Chi-square test was used for comparing the observed proportion of symptomatic and asymptomatic trees within and among pruning methods, sweet orange varieties, age and levels of symptom severity.

Regardless of the sweet orange variety, vigorous suckers grew on all trunks of the decapitated trees and on most branch-pruned trees close to the region that was pruned (Fig. 2). The number of suckers and their growth rate was highest for the trunks of the 3 year-old 'Valencia', where the suckers reached up to 1 m, 2 months after pruning. The growth of the young suckers on the larger trunks of the 16 year-old 'Valencia' was slow, requiring at least 3 months to reach 1 m in length. The rate of sucker growth on the branch-pruned trees was not uniform. Five months after pruning, suckers had developed in 29.5% and 19.4% of 5 and 16 year-old trees of 'Valencia', and in 32.6% and 40.3% of trees of 5 year-old 'Hamlin' and 9 year-old 'Pera', respectively.

Visual observation of the yellow sticky traps revealed the presence of *D. citri* in all blocks (Fig. 1), despite insecticide applications. Highest populations were observed on 7 December, 2004

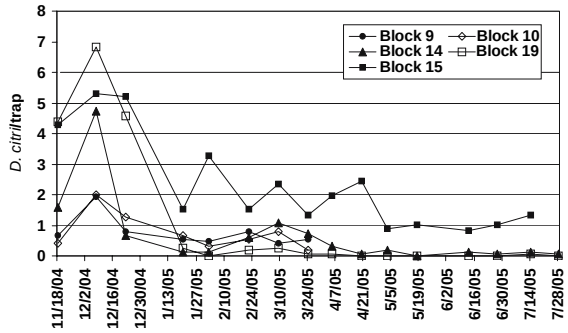

Fig. 1 Population dynamics of *D. citri*, shown as average numbers of adults per yellow sticky trap, in the blocks where the pruning experiment was conducted

when averages from 1.9 to 6.8 individual insects per catch were observed. After this date, the population decreased to less than one insect per trap on all except block 15, where the number of insects varied between 0.8 and 3.3 individuals per trap until July, 2005. The decrease in psylla population may be explained by insecticide applications, unfavourable climatic conditions, and absence of new plant growth flushes.

Four months after pruning, leaf mottling (Fig. 2B, C, D, E) was observed on suckers and/or old branches of most pruned HLB-affected trees (Table 2). Leaf mottling was also observed on some trees that had not expressed any visible symptoms at the pruning dates. The pattern of leaf mottling (Fig. 2E) was similar to that observed on mature leaves of old branches of branch-pruned trees, or on mature leaves of intact HLB-affected trees (Fig. 2F).

The presence of *Ca.* Liberibacter americanus in mottled leaves was confirmed by PCR but in no case was *Ca.* Liberibacter asiaticus detected. The proportion of symptomatic suckers increased over time in all blocks. Suckers with leaves showing mottling also had both young and mature leaves expressing variable degrees of yellowing, some symptoms being similar to those caused by zinc deficiency (Fig. 2). In a few trunks, the disease progressed to branch dieback.

Similar symptoms were observed on suckers of non-protected and cage-protected trunks, and on suckers of branch-pruned trees. The percentage of cage-protected trunks that developed symptomatic suckers was similar to that of non-protected ones. This result plus the fact that most symptoms appeared in a relatively short time interval (4 months)

Fig. 2 Patterns of HLB symptoms developed in Valencia trees 5 months after pruning down to the trunk (decapitation) non-affected (**A**) and affected trees (**B**, **C** and **D**) or after removing the symptomatic branch (**D**). **A**. Asymptomatic suckers on a non-caged trunk of an asymptomatic tree (control). **B**. Symptomatic suckers on a non-caged trunk of a symptomatic tree. **C**. Symptomatic suckers on a caged trunk of a symptomatic tree. **D**. Symptomatic suckers on a symptomatic non-caged tree. **E**. Mottled and asymptomatic leaves removed from symptomatic suckers. **F**. Mottled leaves on a branch of a non-pruned tree

regardless of the pruning procedure, variety, age and symptom severity levels indicates that pruning all visually diseased tissue from infected citrus trees was ineffective for eradicating the HLB disease from individual trees. Our observations suggest that reinfection of pruned trees did not occur as a result of new vector-transmitted infections. Even though adults psyllids were present (Fig. 1), pathogen transmission might have been limited by the preventive applications of systemic insecticide. No psyllids were found on the new young branches or suckers of the pruned trees during routine visits or experimental evaluation dates.

In general, the more severe the symptoms before pruning the higher the percentage of trees that showed symptoms after pruning, regardless of the pruning method, sweet orange variety or tree age (Tables 2 and 3). The decapitated 3 year-old 'Valencia' and 5 year-old 'Hamlin' trees were exceptions in that the most affected trees (level 2) before pruning resulted in a lower percentage of trunks with symptomatic suckers. The effect of symptom severity on pruning effectiveness is corroborated by statistical analysis of the data, except for the 16 year-old 'Valencia', and is a strong indication that the more severe the symptoms the greater the downward distribution of the pathogen in the tree.

This study demonstrates the inefficacy of pruning to control HLB caused by *Ca.* Liberibacter americanus in Brazil. The three sweet orange varieties showed similar responses to the pruning treatments (Table 3). On average, HLB symptoms reappeared in 63.8%, 55.3% and 60.9% of the 'Valencia', 'Hamlin' and 'Pera' trees, respectively. When disregarding the asymptomatic trees, the final infection level at the end of the experiment had increased to 71.7, 63.6 and 69.5 for the three varieties, respectively. These results confirm general field observations that these varieties

Table 2 Frequency and % pruned trees showing typical HLB leaf symptoms on new suckers or old branches at 5 or 9 months after pruning

Variety and age	Level of symptom severity before pruning[a]	After pruning at the trunk level (decapitation)		After removing the symptomatic or asymptomatic branch		Total	
		Symptomatic trees/ Total	%	Symptomatic trees/ Total	%	Symptomatic trees/ Total	%
Valencia 3 year	0	2/10	20.0	–	–	2/10	20.0
	1	9/10	90.0	–	–	9/10	90.0
	2	25/31	80.6	–	–	25/31	80.6
Chi-square		15.65 ($P = 0.0004$)		–		15.65 ($P = 0.0004$)	
Valencia 5 year	0	0/10	0	1/10 (1) [b]	10.0	1/20	5.0
	1	4/10	40.0	10/16 (8)	62.5	14/26	53.8
	2	65/86	75.6	27/34 (21)	79.4	92/120	76.7
Chi-square		25.58 ($P < 0.0001$)		16.04 ($P = 0.0003$)		39.95 ($P < 0.0001$)	
Valencia 16 year	0	1/5	20.0	1/4 (1)	25.0	2/9	22.2
	1	2/5	40.0	6/10 (5)	60.0	8/15	53.3
	2	14/27	51.9	18/22 (16)	81.8	32/49	65.3
Chi-square		1.81 ($P = 0.4054$)		5.73 ($P = 0.569$)		5.91 ($P = 0.0520$)	
Hamlin 5 year	0	1/10	10.0	0/10	0	1/20	5.0
	1	6/8	75.0	2/7 (2)	28.6	8/15	53.3
	2	50/76	65.8	19/30 (14)	63.3	69/106	65.1
Chi-square		12.28 ($P = 0.0022$)		13.04 ($P = 0.0015$)		24.61 ($P < 0.0001$)	
Pera 9 year	0	0/10	0	0/10 (0)	0	0/20	0
	1	10/17	58.8	15/26 (6)	57.7	25/43	58.1
	2	46/61	75.4	27/37 (12)	73.0	73/98	74.5
Chi-square		21.32 ($P < 0.0001$)		17.16 ($P = 0.0002$)		38.88 ($P < 0.0001$)	

[a] Pruned trees were asymptomatic (level 0), showing mottled leaves confined to the top 30 to 50 cm on usually one branch (level 1), or showing mottled leaves also below 50 cm or, more usually, distributed throughout one or more branches, associated with branch dieback. In 'Pera' sweet orange level 2 consisted of trees with mottled leaves plus branch dieback

[b] In parenthesis is the number of trees where symptoms developed on old branches or old branches plus suckers

are all very susceptible to HLB. The inefficacy of pruning for eradicating infections from infected trees was observed also in South Africa (Van Vuuren 1993), where HLB is caused by the apparently less aggressive *Ca.* Liberibacter africanus (Bové 2006). In a study in which single-infected branches of 'Valencia' trees were removed by cutting them at different levels towards the main trunk, typical HLB symptoms were observed in 29–86% of the suckers that developed on the pruned trees, 12 months later (Van Vuuren 1993).

In this study, no significant effect of tree age on recovery of trees from the disease by pruning (Chi square 2.41, $P = 0.4917$) was observed, contrary to observations made in South Africa (Buitendag and von Broembsen 1993). In that country, for the purpose of inoculum reduction, all affected trees up to 5 years of age must be removed. Trees older than 5 years must be pruned or removed depending on the extent of symptoms in the tree canopy (Buitendag and von Broembsen 1993). In Brazil, where HLB is apparently caused by more aggressive liberibacter species (*Ca.* Liberibacter americanus and *Ca.* Liberibacter asiaticus), all affected trees must be eliminated. Tree elimination is required by federal law but has spontaneously been done by most growers living in the HLB-affected areas.

Table 3 Overall effect of sweet orange variety, pruning method, HLB symptom severity, and age of the tree on success of eradicating HLB from infected trees

Parameter	Total Pruned trees	Trees that expressed symptoms after pruning	%
Variety Valencia	290	185	63.8
Variety Hamlin	141	78	55.3
Variety Pera	161	98	60.9
Chi-square 2.86 ($P = 0.2388$)			
Decapitation method	376	235	62.5
Branch removal method	216	126	58.3
Chi-square 1.00 ($P = 0.3171$)			
Asymptomatic	79	6	7.8
Symptoms at the end of the branch	109	64	58.7
Symptom on the entire branch	404	291	72.0
Chi-square 115.59 ($P < 0.0001$)			
3 year-old trees	51	36	70.6
5 year-old trees	307	185	60.3
9 year-old trees	161	98	60.9
16 year-old trees	73	42	57.5
Chi-square 2.41 ($P = 0.4917$)			

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