Proc. Fla. State Hort. Soc. 126:51–54. 2013.

Rehabilitation of HLB Infected Citrus Trees using Severe Pruning and Nutritional Sprays

Bob Rouse*

University of Florida, IFAS, Southwest Florida Research and Education Center, 2685 SR 29 N., Immokalee, FL 34142

Additional index words. HLB, Huanglongbing, citrus greening, PCR, nutrition, orange, 'Valencia'

Citrus trees infected with Huanglongbing (HLB) bacteria become weak and develop dieback, resulting in lost production. These trees eventually decline to a production level that is not economical to maintain in a citrus operation. Sixteen-year-old 'Valencia' (*Citrus sinensis* Macf.) orange trees on Swingle citrumelo rootstock [*C. paradisi* × *Poncirus trifoliata* (L.) Raf.], 100% infected with HLB, in decline and losing production, were severely pruned (buckhorned) to stimulate regrowth and the new flush treated with foliar nutritional sprays. Nutritional sprays included the "Boyd cocktail" and two other nutrient treatments that contained phosphites plus nickel and cobalt. Heavily pruned trees and unpruned standard control trees were compared for shoot growth and canopy development. Pruning was done in Feb. 2010 before the spring flush. Spring shoot growth on the pruned trees was twice the length of unpruned trees and had larger leaves. Summer shoot flush on the pruned trees was three times the length of the unpruned trees. Both pruned and unpruned trees bloomed and set fruit in the spring of 2011. The 2011–12 fruit crop on pruned trees was not significantly different from the unpruned trees. The 2012–13 crop on pruned trees was greater than on the unpruned trees and had reached economic production. There was no difference in fruit size between pruned and unpruned trees. Rejuvenation of HLB trees by severe pruning and foliar nutritional sprays may be an alternative to tree removal and replanting with new trees when existing citrus trees begin to lose production due to HLB infection.

Managing the spread and severity of Huanglongbing disease (HLB) in Florida has been challenging since the disease is vectored by a mobile insect. The disease is caused by the bacterium *Candidatus* Liberibacter asiaticus (CLas) and is vectored by the Asian citrus psyllid (ACP, *Diaphorina citri*). Symptoms of HLB include asymmetrical chlorosis of the leaves often referred to as "blotchy mottle." Eventually, dieback of the shoot and major limbs can occur and the entire tree can succumb to the disease (Bové, 2006). Affected fruit from affected trees often do not develop color properly, are of inferior quality, and prematurely abscise. The effects of HLB on Florida's industry is made more dire considering that the disease affects all known commercially available citrus genotypes and their relatives (Albrecht et al., 2012) and there is no known cure.

In Florida, many growers have turned to increasing their use of foliar sprays of micro- and macro-nutrients (Gottwald et al., 2012). Such foliar nutritional programs (hereafter referred to as "nutritional therapy") do not have bactericidal effects on CLas, but are employed to maintain adequate health and production of HLB-affected trees despite the colonization of CLas. Growers using nutritional therapy since 2006 to maintain tree health in HLB-affected groves have resulted in maintaining annual fruit yields that are similar to production levels of 'Hamlin' and 'Valencia' oranges prior to the appearance of the disease in Florida (Rouse et al., 2010, 2012).

After discovering HLB in their groves growers were reluctant to start new plantings and reset lost trees in existing groves. Planting and replanting citrus was at an all-time low from 2006 until 2010. The growers feared that newly planted nursery trees would become infected with HLB and not reach economic production. In 2010 growers realized that they would have to begin replanting or go out of business. Evidence was mounting that foliar applied nutrition was helping existing trees remain productive.

Severe pruning is a recognized practice to rehabilitate citrus damaged by freezing temperatures. If the damage is severe trees are pruned back to the scaffold limbs (Nesbitt, 2010; Rouse and Zekri, 2012). This severe pruning results in the maximum stimulation and the strongest regrowth.

A pruning experiment was established on 16-year-old HLB-infected 'Valencia' orange trees that had declined and were not economically productive. The objectives were to 1) salvage and rehabilitate the trees to avoid the cost and time required when replanting; 2) rebalance the shoot/root ratio of declining trees with pruning; and 3) feed the new foliage stimulated by pruning with foliar-applied nutrients. The rationale was that if existing trees could be rehabilitated, the costs of removing the old tree, purchasing a nursery tree and planting, plus the cost of tree care for 4 to 7 years until production was established, could be avoided. The objective of this experiment was to determine if HLB trees could be rehabilitated and resume economic production in less time than replanting a new tree.

Materials and Methods

The test site was at the University of Florida, Southwest Florida Research and Education Center located in Immokalee FL. The experimental block was 5 acres of mature 16-year-old 'Valencia'

*Corresponding author; phone: (239) 658-3400; email: rrouse@ufl.edu

oranges grafted to Swingle citrumelo rootstock. Tree density was 132/trees/acre planted at 15 ft in row by 22 ft between rows on Immokalee fine sand. This planting is a typical flatwoods citrus grove bedded with two tree rows per 44-ft-wide raised bed. Irrigation was by micro-sprayers. Ground-applied fertilizer was made twice per year using a slow release 14–0–18 + Mg, S, B and calcium nitrate 9–1–14 + Mg, Mn, Zn, Fe, Cu, B. The annually total amount of N was 160 lb/acre N and 205 lb/acre K_2O.

The experimental design was a factorial randomized complete block experiment with 10 tree plots, two tree treatments, four nutritional treatments, and seven replications. Leaf samples from all treatments were subjected to PCR analysis for determination of Huanglongbing disease (HLB) known as citrus greening. The 10-leaf sample from each tree was collected and tested with real time PCR for detection of HLB bacteria, *Candidatus Liberibacter Asiaticus* (Las), *Ca. Liberibacter Americanus* (Lam), and *Ca. Liberibacter Africanus* (Laf). Total DNA, was extracted from HLB symptomatic and non-symptomatic citrus leaf petioles, chopped and processed by a modified Tris-Base β-mercaptoethanol method of Li et al. (2006). Reactions were carried out in a 75000 Fast Real Time PCR System (Applied Biosystems, Foster City, CA). The Ct value is considered positive at 32 or below, negative at 36 or higher, and inconclusive between these two values. The field trial was monitored for psyllids and their management was by approved insecticides using a John Bean Redjet citrus speed sprayer (Durand-Wayland, Inc., Lagrange, GA) whenever psyllids were found. Sprays for psyllids were made four to five times per year including two dormant season applications.

The experiment began in Feb. 2010 with severe pruning (buckhorning) using a commercial mechanical hedger and topping machine. One tree row on each of the two-row beds were hedged to 4 ft wide in the row and topped 4 ft high to stimulate maximum forcing of shoot regrowth. The other row on the bed remained unpruned as the control. Large pruned limbs were removed from the site and smaller pruned limbs were mulched by mowing.

Four foliar nutrient treatments were applied to the regrowth foliage of the pruned trees and at the same time to the unpruned trees. The four foliar treatments were applied with a John Bean Redjet citrus speed sprayer. The products and amounts of the foliar applied nutrient treatments, applied in 125 gal/water/acre, are given in Table 1. Nutritional sprays were to the foliage four times/year when young vegetative flush was present. Nutritional applications were made in March when the spring flush emerged, two during summer flush in June and late July, and to fall flush in September.Growth measurements were taken of shoot length and tree canopy volumes. Tree canopy volume was determined by measuring the tree height and diameter in north–south and east–west direction and a mean diameter calculated. The canopy volume was calculated using the formula for an oblate spheroid $V = 0.5236 \times d^2h$ where d = diameter and h = height (Terrell, 1946). Fruit yield was determined by harvesting all fruit on each tree and weighed. All fruit yield is reported as boxes per acre (90 lb/box). Fruit size was determined on a commercial packinghouse line. Commercial fruit sizes categories were undersize (more than 125), 125, 100, 80, 64, 56, and 48 fruit per box. Juice quality was determined with an official commercial FMC juice extractor used by the Florida Department of Agriculture.

Results and Discussion

PCR leaf analysis results (not shown) confirmed that all trees were positive for HLB at the start of the trial in 2010 and each year thereafter in 2011 and 2012. The PCR values ranged from 20.8267 to 30.3987, all indicating positive for HLB. The control trees showed HLB symptomatic leaves throughout the year and became visibly yellow and chlorotic during the fall and winter. The Fortress treatments exhibited some chlorotic leaves during the growing season on select shoots and moderate visible symptomatic leaves during fall and winter. The Boyd-treated trees remained mostly free of HLB symptomatic leaves during the growing season with only minor symptomatic leaves during the winter.The growth measurement of shoot length from pruned and unpruned trees in each year is given in Table 2. It would be expected that pruning would stimulate good regrowth. In each year the pruned trees had significantly more and longer shoot growth than unpruned trees at each of the normal growth flush periods. Leaf size was noticeably larger on shoots from pruned trees. In the first year (2010) the pruned trees had two to three times the shoot length of unpruned trees. In the subsequent years (2011 and 2012) the pruned trees continued to have significantly more shoot growth than unpruned trees. Tree volumes are given

Table 1. Foliar nutrient treatments applied to pruned and unpruned trees. Products and amounts applied in 125 gal water/acre with a citrus speed sprayer.

Boyd mixture		Fortress[z] + KNO_3		Fortress[z] + urea		Control[y]	
Product	Amount	Product	Amount	Product	Amount	Product	Amount
3–18–20	8.0 gal	KNO_3	20.0 lb	Urea	2.0 gal	Mg	1.5%
K-phite	1.0 gal	P_2O_5	58.4%	P_2O_5	58.4%	S	4.0%
SAver	1.0 qt	Ca	4.0%	Ca	4.0%	Mo	0.003%
$MgSO_4$	7.5 lb	Mg	3.0%	Mg	3.0%	Fe	3.5%
$MnSO_4$	10 lb	B	0.6%	B	0.6%	B	0.16%
$ZnSO_4$	2.5 lb	Co	0.1%	Co	0.1%	Zn	0.75%
Na_2MoO_4	0.75 oz	Cu	0.6%	Cu	0.6%	Mn	0.75%
KNO_3	10 lb	Mn	3.5%	Mn	3.5%	Cu	0.06%
435 oil	5.0 gal	Zn	6.0%	Zn	6.0%		
		Ni	0.1%	Ni	0.1%		

[z]Sources: calcium phosphite, magnesium phosphite, boric acid, cobalt phosphite, copper phosphite, manganese phosphite, sodium molybdate, nickel EDTA, zinc phosphite.

[y]Control is a commercial liquid micronutrient product containing chelates.

Table 2. Shoot growth in 2010, 2011, and 2012 of pruned and unpruned HLB infected trees.

Treatment	Spring flush (inches)	Summer flush (inches)	Fall flush (inches)
	2010 shoot growth		
Pruned	7.7 a[z]	16.4 a	10.4 a
Unpruned	4.1 b	5.4 b	8.9 b
	2011 shoot growth		
Pruned	9.3 a	10.7 a	6.3 a
Unpruned	7.6 b	9.3 b	4.4 b
	2012 shoot growth		
Pruned	7.5 a	8.6 a	5.1 a
Unpruned	7.1 a	28.0 b	4.7 a

[z]Within a column, means followed by the same letter are not significantly different at $P > 0.05$ (Duncan's multiple range test).

Table 3. Pruned and unpruned annual tree volumes by treatment for seasons 2010–12.

Treatment		Tree volume (ft^3)		
Nutrient	Tree	2010	2011	2012
Control	Pruned	576.9 dc[z]	489.4 e	582.6 dc
Control	Unpruned	728.7 bc	815.6 b	803.2 b
Boyd	Pruned	647.9 cd	585.0 dc	563.9 dc
Boyd	Unpruned	718.1 bc	773.9 b	877.0 b
Fortress + KNO_3	Pruned	536.7 dc	578.3 dc	714.6 bc
Fortress + KNO_3	Unpruned	860.8 b	842.4 b	1015.4 a
Fortress + urea	Pruned	615.4 d	600.9 cd	694.8 bc
Fortress + urea	Unpruned	650.0 c	698.4 bc	956.8 a

[z]Within a column, means followed by the same letter are not significantly different at $P > 0.05$ (Duncan's multiple range test).

in Table 3. After the pruned trees grew in 2010, the trees increase in volume was reduced probably as a result of the heavy fruit load. Overall, there were no significant differences among the foliar nutrient treatments on shoot growth or canopy volume within pruned or unpruned trees. There was a difference in tree volume of pruned trees to unpruned trees.The crop production of the pruned and unpruned trees is given in Table 4. The 'Valencia' crop on the trees in Feb. 2010 at the start of experiment was harvested in Apr. 2011. The unpruned trees yielded about 75 lb of fruit and the control trees yielded 50 lb. This difference can be explained by the fact that the unpruned trees received the Boyd and Fortress nutrient applications from bloom in Mar. 2010 to the harvest in Apr. 2011. The pruned trees only had fruit in the row tree line since the canopy had been removed. The 2011 crop harvested in Apr. 2012 showed increased yield in the unpruned trees that had been receiving the foliar nutritional sprays. The pruned trees had reestablished a canopy and set a crop. The crop from the pruned trees equaled the unpruned trees. The 2012 crop harvested in spring of 2013 had equaled or exceeded the crop of the unpruned trees. The second production year after pruning the trees had returned to an economic production level. Fruit sizes were not significantly different among size categories for pruned and unpruned trees.The juice quality of citrus as measured by the legal maturity factors (Table 5) generally showed no significant

Table 4. Crop yield in 2010, 2011, and 2012 from pruned and unpruned HLB infected trees rehabilitated and receiving foliar nutrient sprays.

Treatment	Crop yield (lb/tree) 2010	2011	2012
Nutrient treatment to unpruned trees			
Boyd	77.4 a[z]	155.8 a	228.5 ab
Fortress + KNO_3	75.7 a	133.1 abc	195.2 b
Fortress + urea	75.7a	134.5 abc	197.3 b
Control	49.3 b	112.0 c	149.3 c
Nutrient treatment to pruned trees			
Boyd	22.0 c	144 ab	205.7 ab
Fortress + KNO_3	29.5 c	123.8 ac	264.7 a
Fortress + urea	29.5 c	133.0 abc	270.0 a
Control	27.4 c	116.8 bc	156.4 c

[z]Within a column, means followed by the same letter are not significantly different at $P > 0.05$ (Duncan's multiple range test).

differences in fruit quality. The pound solids per box, which is the basis for how growers are paid, were similar to fruit before HLB. Again, there were no significant differences among the foliar nutrient treatments on yield or fruit quality.Many citrus growers in Florida have adopted the practice of severe pruning to rejuvenate HLB trees. The grove managers have eliminated the hedging and refined the topping practice to only remove the top part of the canopy showing thinning of the foliage, dieback or decline. This procedure has been referred to as floating the tree tops when the topper is lowered or raised to only remove the thin canopy in the upper half of the tree. This practice has left the lower half of the tree intact and maintains cash flow for the grove. To date several thousand acres have been pruned in this manner.

Conclusions

Pruning of HLB infected citrus trees and foliar nutrition treatments appear to be a method to revitalize the tree and improve tree health and production. The objective is to stimulate new growth and reestablish the balance in the shoot/root ratio allowing the leaves and roots to support each other. In this trial, the new growth was supported with a foliar-applied nutritional treatment. Shoot regrowth the first year on pruned trees was twice the length of unpruned trees in the spring and three times the length during the summer flush. In years two and three, the shoot growth on pruned trees was still significantly greater than unpruned trees. Trees showing decline with dieback in the upper canopy were able to reestablish a healthy canopy and improve production. Yield from trees that had become economically non-productive reached economical production in the second crop year after pruning. Juice quality as measured by maturity factors percentage juice per fruit, acid, degree Brix, Brix/acid ratio, and pounds solids per 90-lb box was similar to juice from trees before HLB. Neither juice quality or fruit size was affected by HLB when treated with foliar nutritional therapy. Overall, the foliar nutrient treatments were all better than the control but no significant differences among the foliar nutrient treatments on canopy shoot growth, fruit yield or quality.

Table 5. 'Valencia' juice quality from pruned and unpruned trees receiving the Boyd nutrient cocktail, Fortress + KNO_3, Fortress + urea, and a control of a commercial liquid fertilizer with the summer applied citrus oil spray.

Treatment	Juice (%)	Acid	°Brix	Brix/acid ratio	Solids/box
		Crop year 2011–12			
Nutrient treatment to unpruned trees					
Control	54.4 a[z]	0.69 a	10.80 a	15.65 a	6.08 a
Boyd	55.4 a	0.52 a	11.99 a	23.06 a	7.00 a
Fortress + KNO_3	53.1 a	0.58 a	11.45 a	19.74 a	6.08 a
Fortress + urea	55.1 a	0.63 a	11.86 a	18.82 a	6.55 a
Nutrient treatment to pruned trees					
Control	50.7 a	0.66 a	10.59 a	16.05 a	6.24 a
Boyd	50.9 a	0.66 a	10.26 a	15.55 a	6.12 a
Fortress + KNO_3	50.9 a	0.63 a	10.28 a	16.32 a	6.11 a
Fortress + urea	50.9 a	0.64 a	10.34 a	16.16 a	6.09 a
		Crop year 2012–13			
Nutrient treatment to unpruned trees					
Control	55.6 a	0.59 ab	11.25 abc	19.02 a	6.25 a
Boyd	53.3 a	0.60 ab	11.26 abc	18.92 a	6.01 ab
Fortress + KNO_3	54.8 a	0.60 ab	11.41 ab	19.04 a	6.26 a
Fortress + urea	53.5 a	0.63 a	11.73 a	18.75 a	6.28 a
Nutrient treatment to pruned trees					
Control	53.8 a	0.58 ab	11.08 bc	19.27 a	5.96 ab
Boyd	52.7 a	0.55 b	10.71 c	19.79 a	5.63 b
Fortress + KNO_3	55.3 a	0.56 b	10.99 bc	19.85 a	6.07 ab
Fortress + urea	55.4 a	0.56 b	11.03 bc	19.84 a	6.12 ab

[z]Within a column, means followed by the same letter are not significantly different at $P > 0.05$ (Duncan's multiple range test).

Literature Cited

Albrecht, U., G. McCollum, and K.D. Bowman. 2012. Influence of rootstock variety on Huanglongbing disease development in field-grown sweet orange [*Citrus sinensis* (L.) Osbeck] trees. Scientia Hort. 138:210–220.

Bové, J.M. 2006. Huanglongbing: A destructive, newly-emerging century-old disease of citrus. J. Plant Pathol. 88:7–37.

Giles, F. 2009. An alternative approach. Florida Grower 102:6–8.

Gottwald, T.R., J.H. Graham, M.S. Irey, T.G. McCollum, and B.W. Wood. 2012. Inconsequential effect of nutritional treatments on huanglongbing control, fruit quality, bacterial titer, and disease progress. Crop Protection 36:73–82.

Li, W., J.S. Hartung, and L. Levy. 2006. Quantitative real-time PCR for detection and identification of *Candidatus* Liberibacter species associated with citrus huanglongbing. J. Microbiolog. Methods 66:104–115.

Nesbitt, M.L. 2010. Citrus freeze injury and care. Texas Agrilife Ext. Serv., Texas A&M Univ. <http://aggie-horticulture.tamu.edu/newsletter/hortupdate/2010/jan_feb/citrusfreeze.html>.

Rouse, B., M. Irey, T. Gast, M. Boyd, and T. Willis. 2012. Fruit production in a southwest Florida citrus grove using the Boyd nutrient/SAR foliar spray. Proc. Fla. State Hort. Soc. 125:61–64.

Rouse, B., P. Roberts, M. Irey, M. Boyd, and T. Willis. 2010. Monitoring trees infected with Huanglongbing in a commercial grove receiving nutritional/SAR foliar sprays in southwest Florida. Proc. Fla. State Hort. Soc. 123:118–120.

Rouse, R. and M. Zekri. 2012. Citrus culture in the home landscape. Univ. of Florida Hort. Sci. Dept. Ext. Publ. HS-867/HS132. <edis.ifas.ufl.edu/hs132>.

Terrell, F.M. 1946. Tables of surfaces and of prolate and oblate spheroids, and spheroidal coefficients. Univ. Calif. Press, Berkeley.