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# Detection of Greening in Sprouts from Citrus Tree Stumps

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**A study was initiated to determine if stump sprouts from citrus trees that were removed because of visual citrus greening (huanglongbing, HLB) symptoms tested positive for *Candidatus* Liberibacter asiaticus (*Ca.* Las). The study was conducted in a commercial grove in DeSoto County, FL. All trees selected for the trial were visually determined to have HLB prior to removal. The 15 trees selected were removed using a standard tree shear mounted to a front-end loader. Remaining stumps were monitored on 30-day intervals to detect sprout formation from the stump or exposed lateral roots. Once sprouts appeared, the entire stump and exposed lateral roots were enclosed within a screen enclosure to exclude psyllids from feeding on new vegetative growth. At 160 days after clipping, leaf and stem tissues from sprouts were tested for *Ca.* Las by the DNA-based quantitative PCR (qPCR) method. During the study, 12 of the stumps sprouted and produced 2 to 26 sprouts per stump. Two of 12 stumps that sprouted had insufficient vegetative plant material to test. Eight of the 10 stumps (80%) had one or more sprouts that were greening positive. The percentage of greening positive sprouts recovered from a positive stump ranged from 33% to 100%. The broad range in percent greening positive sprouts from an individual stump reflects the uneven distribution of *Ca.* Las in the roots and trunk of infected trees. Controlling root and stump sprouts after removal of greening infected trees is essential in its control and spread.**

Citrus greening (huanglongbing, HLB) is a very serious disease resulting in tree decline and subsequent death. The disease causes phloem necrosis and is associated with the bacterium *Candidatus* Liberibacter asiaticus (*Ca.* Las). HLB affects all citrus cultivars. Early symptoms of decline include reduced fruit size, quality, yield, and eventually tree death after a number of years. The pathogen, *Ca.* Las, and its vector the Asian citrus psyllid (*Diaphorina citri* Kuwayama) are currently found throughout Florida (Gottwald et al., 2007).

Symptoms in the plant include an asymmetrical chlorosis or blotchy pattern in the leaves, yellow veins and/or lopsided fruit along with other symptoms. As the disease progresses, yellow shoots and defoliated twig dieback occurs. Visual diagnosis of the greening is often confirmed by quantitative PCR (qPCR) testing of symptomatic tissue in the laboratory (Southern Gardens Diagnostic Laboratory, Clewiston, FL; <http://www.flcitrusmutual.com/content/docs/issues/canker/sg_samplingform.pdf>). The bacterium can be distributed throughout the tree in the phloem including the roots (Tatineni et al., 2008). In studies by Lopes et al. (2007), pruning to remove symptomatic branches or the entire tree was ineffective for eliminating infected tissue and saving the affected tree.

Currently, a common practice is to treat the cut surface of the cut stump with an herbicide material to prevent sprouting from the stump or roots. Materials labeled for these treatments include various glyphosate formulations (Futch and Singh, 2009) and triclopyr (Remedy® Ultra, Dow AgroScience). The study reported here was conducted to determine if sprouts that grow from stumps or exposed roots of citrus trees that were visually diagnosed with greening symptoms are later positive for HLB.

## Materials and Methods

This study was initiated in a commercial citrus grove in DeSoto County, FL, on 14 Apr. 2008. The trees ranged in age from 7 to 15 years old and were grown primarily on Swingle citrumelo rootstock with a few trees on Carrizo citrange. All trees to be removed were visually surveyed for greening symptoms by a scouting crew trained to detect the disease (Yates et al., 2008). Suspect HLB trees were flagged and then reconfirmed by the senior scout to be visually positive for greening symptoms. Selected trees were not tested by laboratory methods to confirm the presence or absence of greening prior to removal; however, other trees in the grove were confirmed to be qPCR positive for HLB based on prior testing.

After marking and visual confirmation for HLB, 15 trees were removed using a standard tree shear mounted on a large front-end loader (Fig. 1). This tree-shearing process, commonly referred to as clipping, is used throughout the citrus industry in Florida to remove trees (Futch et al., 2008). Trees were sheared several inches above the soil surface leaving part of the stump and the entire root system intact. For this study, stumps were not treated with any herbicides to control sprouting. However, the recommendation to citrus growers is always to treat the stumps with registered herbicide material to control sprouting in commercial citrus operations.

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Fig.1. Front-end loader used in clipping citrus trees with tree shear attachment.

Removing trees by either clipping or pushing (removing the entire tree and the majority of the roots) has been shown not to have negative affects on tree health of subsequent replant trees (Futch et al., 2008; Noling and Futch, 1994). Thus, many citrus growers have adopted clipping as a standard tree removal method due to its lower cost than pushing as long as sprouts are aggressively controlled as recommended (Futch et al., 2008).

Stumps of presumptive positive greening trees were surveyed at approximately 30-d intervals, and sprout formation from the stump or lateral roots was recorded. When sprouts appeared, the entire stump and exposed lateral roots were enclosed within a screen enclosure to exclude psyllids from feeding on any new vegetative growth (Fig. 2). The enclosure was approximately 2 ft square at the base and 2.5 ft tall. The screen material selected for the enclosure was 80 mesh per square inch and of the type approved for psyllid exclusion in citrus greenhouses in Florida where certified nursery trees are produced.

At 160 d (17 Sept. 2008) after the beginning of the study, leaf and stem tissues from stump sprouts with sufficient vegetative growth were tested by DNA-based qPCR (Li et al., 2006) to determine if they were positive for the greening associated bacterium, *Ca*. Las by Southern Gardens Diagnostic Laboratory. Only individual sprouts large enough to obtain an adequate tissue sample were tested.

Fig. 2. Screen enclosure surrounding clipped stump to prohibit psyllid feeding on sprouts.

## Results

Observation of the stumps during the 160-d study period indicated 12 of the 15 stumps produced multiple sprouts. The time required for sprout development varied between stumps. There were 0, 5, 3, 1, and 3 additional stumps with sprouts observed at 29, 56, 85, 113, and 141 d after tree removal, respectively (Fig. 3). The number of sprouts per stump ranged from 0 to 26 and averaged 7.6 per stump at the end of the observation period (Fig. 4). At 160 d post-clipping, several stumps had sprouts exhibiting visual symptoms for HLB. The number of sprouts collected per stump ranged from 2 to 8 with an average of 6 per stump. Two of the 12 stumps did not have sufficient sprout tissue for sampling. Thus, sprouts collected from only 10 stumps were analyzed by qPCR. Based on qPCR testing, 8 of the 10 stumps (80%) had one or more sprouts that were positive for greening. The percentage of positive stumps could have increased with additional time and subsequent testing. The percentage of greening positive sprouts per stump ranged from 33% to 100%.

## Discussion

The fact that a majority (80%) of the sprouts originating from stumps of rogued trees tested positive for greening is important. Despite no initial testing the removed trees for greening by qPCR and coupled with issues regarding testing procedures, this study strongly supports the concept that sprouts from trees that were presumed positive by visual techniques are a rich source of inoculum. Sprout control is routinely practiced by citrus growers in the state of São Paulo, Brazil, due to the risk identified as previously discussed in a study evaluating the efficiency of pruning for controlling HLB in infected trees (Lopes et al., 2007). The broad range in percentage of greening positive sprouts within an individual stump reflects and further confirms the uneven distribution of *Ca*. Las in the root system of the tree or testing variability. The control of sprouts from citrus stumps has always been important for horticultural purposes (Futch et al., 2008). Sprout control from stumps of rogued HLB trees is now even more critical given the need to minimize the inoculum for spread of HLB within and between citrus groves where sprouts from clipped trees may contain the bacterium. This finding of HLB positive spouts is not surprising based on research on *Ca*. Las distribution in roots (Tatineni et al., 2008). Undoubtedly, the vigorous flushing condition of sprouts is highly attractive for psyllid feeding. Therefore, when clipping greening infected trees, the timely application of herbicide is essential for sprout control to suppress the spread of HLB in Florida citrus groves.

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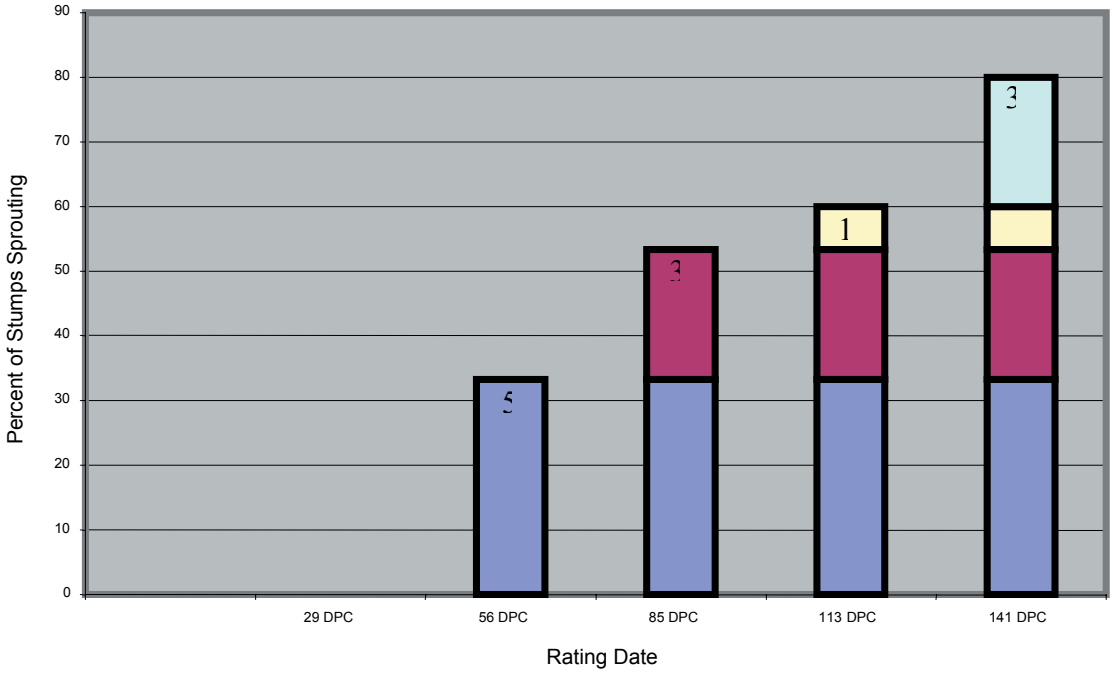


Fig. 3. Bars in the graph indicate the percentage of stumps that sprouted over time after clipping on 14 Apr. 2008. Color of bars represents the number of new stumps sprouting each time period with dark blue representing 5; red 3; yellow 1; and light blue 3. DPC = days post-clipping for each observation date.

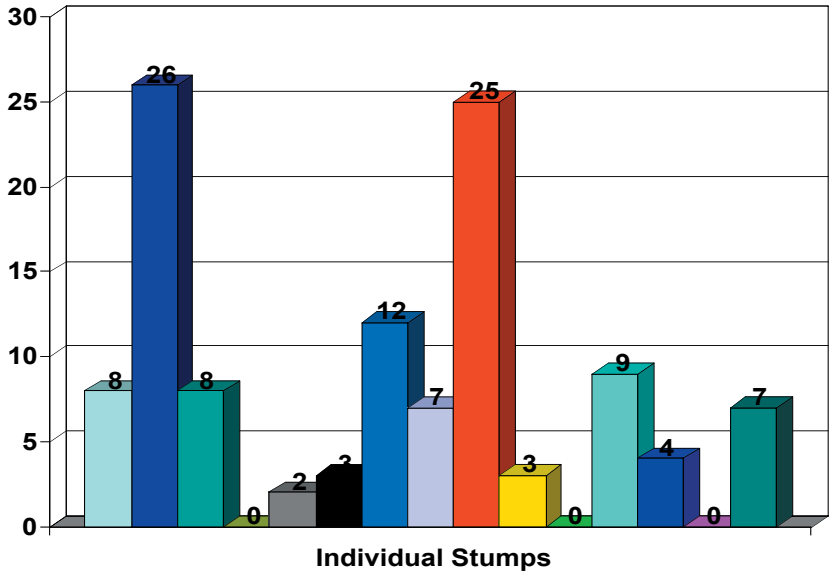


Fig. 4. Individual bars represent the number of sprouts per untreated stump and ranged from 0 to 26 with an average of 7.6 per stump.

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