



**Figure 1 (left).** Front-end loader used in clipping citrus trees with tree shear attachment.

**Figure 2 (above).** Screen enclosure surrounding clipped stump to prohibit psyllid feeding on sprouts.

# Detection of greening in sprouts from citrus tree stumps

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A study was conducted to determine if sprouts that grow from stumps of citrus trees that were removed because they exhibited visual greening (HLB) symptoms will later produce sprouts that will be positive for greening in subsequent tests. The study was initiated in a commercial citrus grove in DeSoto County, Florida on April 14, 2008. All trees in the selected block were visually surveyed for greening symptoms by a scouting crew trained to detect greening positive trees. Suspected greening trees were flagged and then reconfirmed by the senior scout to be greening positive. Marked trees were not tested by laboratory methods to confirm the presence or absence of greening prior to removal.

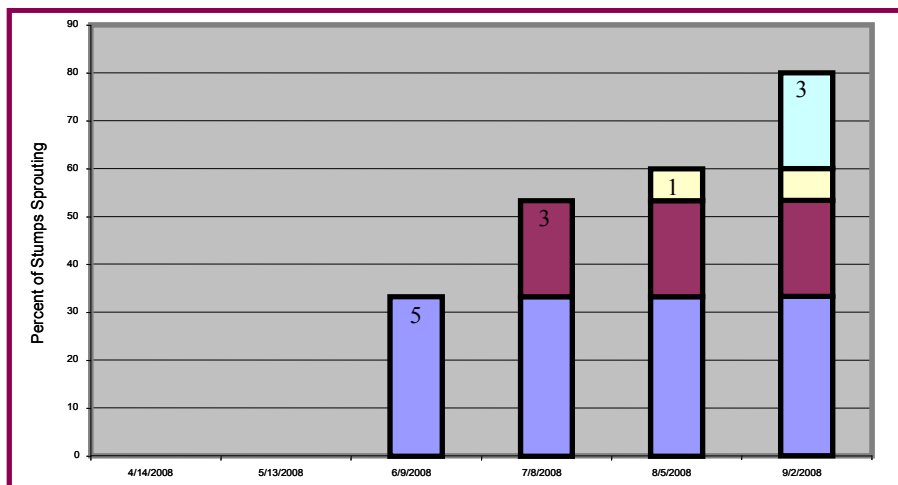
After marking and visual confirmation for greening, the 15 selected trees were removed using a standard tree shear that is mounted to a large front-end loader (Figure 1). This tree-shearing process, commonly referred to as clipping, is often used in the citrus and timber industries throughout Florida to remove trees of various sizes. Clipped trees were sheared off several inches above the soil surface leaving part of the stump and the entire root system intact. A common grower practice is to treat the surface of the cut stump with herbicide material to prohibit sprouting from

the stump or roots. However, for this study, stumps were not treated with any herbicidal products which would prohibit sprouting.

Stumps from visual greening positive trees were surveyed at approximately 30-day intervals to detect any sprout formation from the stump or lateral roots. Once a stump had any sprout formation, the entire stump and exposed lateral roots were enclosed within a screen enclosure to prohibit psyllid feeding on new vegetative growth that arose (Figure 2). The en-

closure is approximately 2 feet square and 3 feet tall. The screen material selected for the enclosure was 80 mesh and of the type approved for citrus greenhouses where nursery trees are produced. This woven mesh screen is designed to prohibit psyllid movement through the material.

At 160 days (September 17, 2008) after the beginning of the study, leaf and stem tissue from sprouts on stumps with sufficient vegetative growth were tested by a DNA-based laboratory method (PCR) to determine if they were positive for greening. Only individual sprouts large enough



**Figure 3.** Bars in the graph indicate the percentage of stumps that sprouted over time after clipping on April 14, 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. Days post clipping (DPC) for each observation date: May 13, 29 DPC; June 9, 56 DPC; July 8, 85 DPC; August 5, 113 DPC; and September 2, 141 DPC.

to obtain an adequate tissue sample were selected, marked and tested using the PCR method.

During the study period, 12 of the 15 untreated stumps sprouted. Sprouts were noted over the study period with 0, 5, 3, 1 and 3 of the 15 stumps sprouting at 29, 56, 85, 113 and 141 days after tree removal, respectively (Figure 3). The number of sprouts per stump ranged from 2 to 26 and averaged 8 per stump at the end of the study. Two of the 12 stumps that sprouted did not have sufficient vegetative growth to properly analyze the plant material for the greening bacterium. Thus only sprouts from 10 stumps were tested. All sprouts of sufficient size were collected from stumps and individually analyzed using real time PCR. The number of collected sprouts per stump ranged from 2 to 8 with an average of 6 per stump.

Prior to testing the sprouts at 160 days post clipping, several stumps had sprouts exhibiting visual symptoms for greening (Figure 4). Based on PCR testing, eight of the 10 stumps (80 percent) had one or more sprouts that



**Figure 4.** HLB symptoms on sprout from one stump in the study which was confirmed as positive for the bacterium.

were greening positive. The percentage of greening positive sprouts recovered from a given stump ranged from 33 percent to 100 percent. The broad range in percentage of greening positive sprouts within individual stumps reflects the uneven distribution in the roots and further confirms the uneven distribution of greening within the tree.

The fact that a majority of the

sprouts originating from stumps of rogued trees tested positive for greening is important because the infected sprouts can serve as a source of inoculum and further the spread of greening in groves where growers are attempting to control the disease.

While the control of sprouts from citrus stumps has always been important, it is even more essential today, given current needs to minimize the spread of greening within and between citrus groves. Sprouts from stumps of clipped trees can harbor the greening bacterium and the vigorous young flushing condition of sprouts also is highly attractive for psyllid feeding. Therefore, with current production practices involving clipping and tree removal, it is imperative to keep all stumps sprout free. Timely herbicide application after clipping and periodically checking for sprouting is essential in controlling the spread of HLB.

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