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#### Variability of direction of tree-to-tree spread of HLB over time

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Candidatus Liberibacter americanus and Candidatus Liberibacter asiaticus are two bacterial species that cause huanglongbing (HLB) disease in citrus-growing regions of Brazil. A concentrated sampling plan of a grove in Matao, Brazil was initiated to evaluate the spatial and temporal spread of these bacteria. The exact location of each of 8697 trees was recorded, and each symptomatic tree was assessed by PCR for the presence of Ca. L. americanus and Ca. L. asiaticus during 17 different months from April 2006 to May 2008 (Fig. 1). In the first month, only five trees were confirmed to have Ca. L. asiaticus. The first trees with confirmed cases of Ca. L. americanus were not found until February 2007. By the end of the study, 43 trees were confirmed to have Ca. L. americanus, 1164 trees were confirmed to have Ca. L. asiaticus, and three trees were coinfected. For the new trees each month that were confirmed to have Ca. L. asiaticus, their distance (m) from previously infected trees was calculated. This process continued for each of the 17 months and for all lag times from 3 to 24 months (lag is defined here as the time from visual symptoms on one tree to visual symptoms on a newly infected tree). For the example shown in Figure 2 with a lag of 6 months, 95% of the new infections were within 50 m of a previously infected tree. Therefore, it was assumed that a new infection could be from psyllids and bacteria of any previously infected tree within a 50 m radius. Directionality of disease spread was then examined. The absolute difference between the direction (North =  $0^{\circ}$ ) from an infected tree to other trees it was determined to possibly infect and the direction from that infected tree to a newly infected tree (Fig. 2A) were found. Results indicate that predictions of tree-to-tree spread should take the direction of previously found infections into account versus assuming that all trees around an infected tree (i.e., a 360° circle around an infected tree with some radius) have equal probability of being infected by psyllids from a symptomatic tree. The example in Figure 2A indicates that about 80% of new infections (i') appeared to emanate from a prior infected tree (i) that was already found to infect other trees within  $\pm 45^{\circ}$  (or 90° total) cone extending from the older infection (i). Therefore, in future development of spatio-temporal models for HLB, it is suggested to attribute higher probabilities of infection for trees that are in the same direction as previous suspected travel routes of psyllid populations. One cause for the results found may be that new infections from a prior infected tree all occurred during one or a few spread events where the psyllids moved in similar directions (possibly due to similar environmental influences), but the variability in lag period among the trees resulted in symptoms identified during different scouting times. Similar results as shown for the lag period of 6 months were also found for most of the other lag times, but the shortest periods of 3 and 4 months had more uniform results than is shown in Figure 2A. Other possible reasons for the findings were investigated but were found to not be the cause. First, the direction of the infections has no pattern (i.e., uniform distribution) in this data (Fig. 2B), which indicates there is variability in psyllid movement over the 2 years of the study. Second, it was thought the results might be

dependent on the angle of the rows (Fig. 2C). However, only 11% of new infections were indicated to be caused by a tree within the same row. That is 244 out of the 270 observations in the first bin of Figure 2C (i.e., there were 270 cases where the angle between the row and the new infected tree was between 0 and 5° and 244 of those were within the same row), resulting in an overall uniform distribution. Third, the results found were the same when trees near the edges were excluded (since the evaluations could only be in a single direction) (data not shown).

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Figure 1. Citrus orchard in Matao, Brazil with 8697 trees. Those confirmed to have the bacterial species *Candidatus* Liberibacter americanus and *Candidatus* Liberibacter asiaticus are colored red and blue, respectively. Note variable and complex planting patterns, i.e., row directions along elevation contours.



A Difference between direction from each previously infected tree within 50 m of a new infection and previously infected trees by the same source

Figure 2. A) Directional (North =  $0^{\circ}$ ) differences from an infected tree to newly infected trees that are within 50 m. B) Direction from an infected tree to newly infected trees that are within 50 m. C) Directional difference between the angle of the row the infected tree is in and the angle to the newly infected tree.