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Recommendation for CDFA Regulatory Response to a Huanglongbing Detection Near Commercial Citrus

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As California regulatory bodies plan how to alter their management strategies towards the most effective response to huanglongbing (HLB) discoveries in commercial orchards, they require extensive knowledge about typical observed patterns of huanglongbing and Asian citrus psyllids (ACP) through time and space. This recommendation attempts to consolidate available research that describes both the plausible distances over which HLB may spread, and the strongly non-random distribution of HLB cases within newly invaded groves in order to provide the basis for a scientifically sound HLB sampling plan. Below, we describe briefly the relevant research on these two issues, as well as our recommendation.

Current CDFA Regulatory Response

1. A *C*Las qPCR positive tree triggers a 5-mile quarantine, a 400 meter insecticide treatment for both commercial and residential citrus, treatment of the infected tree, and tree removal.
2. A qPCR positive psyllid or tree triggers testing of all ACP and host plants within 400 meters for CLas in residential areas.
3. In commercial orchards, the current response protocol calls for surveying and testing 25% of orchard trees using a hierarchical sampling method (trees in groups of four, have 20 leaves per tree sampled).

Proposed replacement for the current sampling method

HLB and ACP both exhibit strong patterns of spatial distribution regardless of state or country (i.e. Texas, Florida, California, Brazil). These patterns should be used to inform regulatory plans, thereby maximizing efficiency of sampling and detection, and minimizing risk of HLB spread. Research has established that areas up to 400m from HLB or ACP detections are definitely at risk for ACP incursion and HLB spread. Additionally, a wealth of evidence shows that ACP and HLB are found in significantly higher prevalence in grove edges. Therefore, the following recommendations are suggested to replace number 3, above, for CA regulatory agencies. No changes are suggested at this time for other aspects of the Action Plan, such as abatement or trace-back activities.

- If any part of an orchard is within 400 meters of an HLB detection, survey and individually test all trees along the entire orchard perimeter.
- Sample the perimeter of the grove twice a year during periods of peak titer (late spring and late fall) with a direct testing method.

Supporting Evidence for Recommendation

1. Huanglongbing clustering and *Diaphorina citri* dispersal

Huanglongbing clustering

Huanglongbing is well known to be distributed non-randomly in citrus groves (Gottwald 2010). For example, research in Brazil has shown clustering of HLB (i.e. higher than expected frequency of HLB cases than due to chance) at distances up to approximately 500 m (around 0.3 mi) (Leal et al. 2010). In California, analyses of the spatial patterns of HLB cases in residential citrus suggests similar clustering. Of more than 650 confirmed CLas infected trees in Southern California dooryards, 95% were within 320m (<0.25 mi.) of another known infection (Fig. 1).

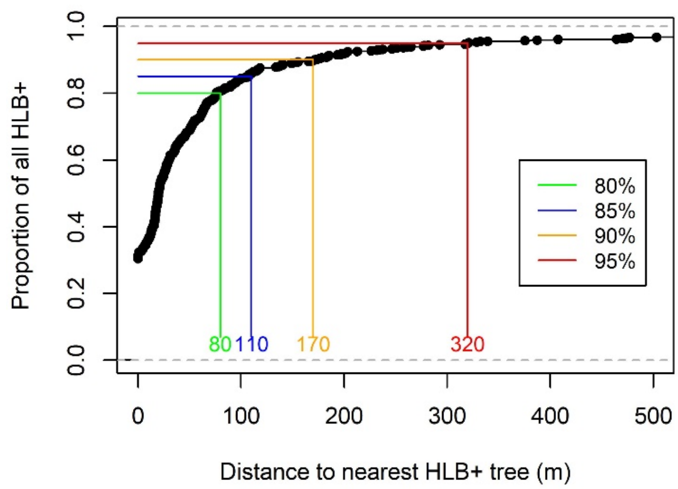

Figure 1. Cumulative distribution of CLas+ trees in residential areas of Southern California as a function of their distance to the nearest other CLas+ tree. Provided by T. Brenes-Arguedas.

These results suggest that areas of elevated HLB prevalence may occur on a similar spatial scale as the regulatory sampling plan for commercial groves, which calls for surveys and treatments within a 400 m radius (0.25 mile) surrounding an HLB detection. Yet, it is important to note that this radius is likely a conservative measure of the distance over which infected trees contribute to the risk of CLas spread via the natural dispersal of infective psyllids, as ACP are consistently shown to travel farther than 400 m. Researchers in Brazil, for example, have suggested that poorly managed citrus groves increase HLB incidence in groves upwards of 4 km (~2.5 mi) away due to the natural dispersal of infective psyllids (Belasque et al., 2010).

ACP Dispersal

Flight-mill studies have estimated that ACP are capable of flights up to 2.4 km (~1.5 mi), based on flight time (Martini et al. 2014). This is supported by the results of a mark-recapture study in Florida, in which psyllids were recaptured at 2 km (~1.25 mi), the farthest distance tested (Lewis-Rosenblum et al. 2015). The plausibility of ACP achieving these flight distances is also supported by studies of ACP invasion dynamics in Southern California. Records of psyllid occurrence in residential areas over the first several years of the invasion show significant spatial autocorrelation, which is often an indication of the scale of movement by insects (Thomas et al. 2017). **Autocorrelation was strongest up to approximately 200 m (~0.1 mi), suggesting frequent, relatively short-distance dispersal events by ACP, but was significant at distances upwards of 2 km (~1.25 mi), indicating longer dispersal events also occur (Fig. 2).**

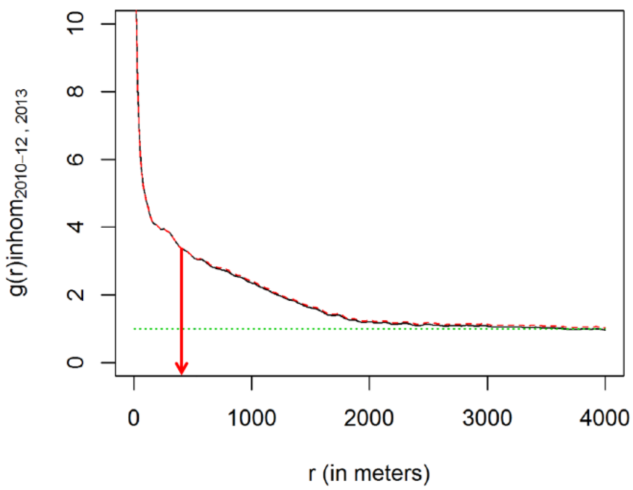

Figure 2. Paired correlation function analysis of autocorrelation in Southern California residential ACP occurrences in 2013 relative to years prior over a gradient in distance. Red arrow denotes 400 m, and the horizontal green line denotes no correlation (i.e. uniformity). From Thomas et al. 2017.

This conclusion is further bolstered by analyses of the invasion rate of commercial groves in Southern California following the first detections in 2011 and 2012. These analyses showed linkages between groves with ACP occurrences and the rate at which neighboring groves were invaded (MP Daugherty, unpublished results). Invasion rates were negatively related to distance to prior ACP detections, with an elevated risk of invasion up to approximately 2 km (~1.25 mi) from prior detections (Fig. 3; 7.6 on a log scale).

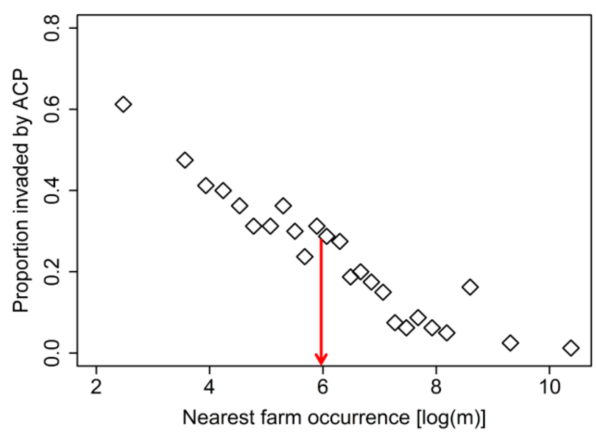

Figure 3. Proportion of Southern California commercial citrus groves with confirmed ACP detections in 2015 as a function of distance to farms with ACP detections in years prior (log transformed). Red arrow denotes 400 m. From M. Daugherty, unpublished results.

Collectively, research on ACP dispersal ability indicates substantial potential for *C*Las spread beyond 400 m. A 400m treatment and sampling area therefore represents a compromise between capturing a high proportion of probable dispersal events around an infected tree and the workload required to screen and treat other hosts within the zone (see Figures 2 and 3). The imposition of a larger surrounding quarantine zone accounts for movement beyond 400m.

2. Huanglongbing and *Diaphorina citri* edge effects within a grove

In addition to broad clustering of HLB cases, at finer spatial scales, HLB is well known to show strong gradients of prevalence within a grove (Gottwald 2010). Specifically, HLB exhibits edge effects, with high prevalence near features such as grove edges, roads, and ponds, and low prevalence toward the interior of blocks (Luo et al. 2010). These edge effects in disease likely stem from preferential recruitment to and elevated reproductive performance of ACP on grove edges (Gottwald 2010, Setamou and Bartels 2015), due to more favorable microclimates or enhanced flushing activity.

Edge effects in HLB distribution have been documented in Brazil, Florida, and Texas groves. For example, a multi-year study of a research grove in Texas showed HLB prevalence and distribution patterns that strongly depended on both proximity to the edge of the grove and time (i.e. survey #) (M. Setamou, unpublished data). In the first year of the study, no trees tested positive for *C*Las. Over the next two surveys the vast majority of HLB cases were found along the edge of the grove or one tree or row adjacent (i.e. within 10 m of edge) (Fig. 4). After the 3rd survey HLB became more prevalent throughout the grove, with 42% of trees infected by the last survey, though an edge effect was still apparent (Table 1).

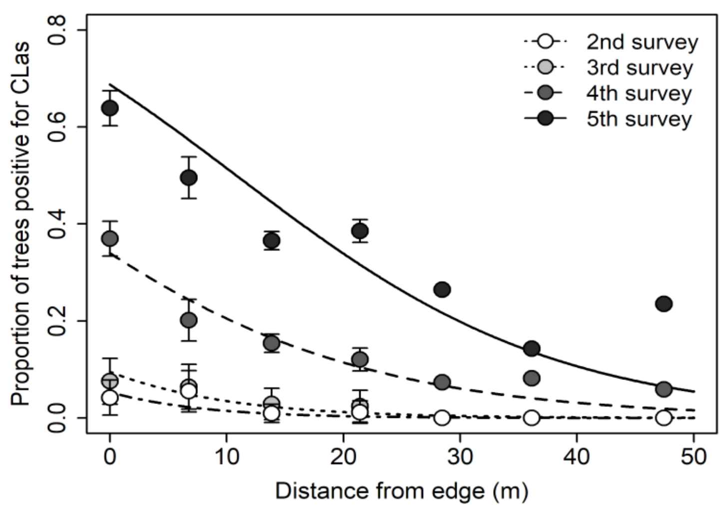

Figure 4. Mean (±SE) proportion of CLas+ trees detection rate in a 549 tree Texas grove among successive surveys as a function of distance from grove edge. No trees tested positive in the first survey. From M. Setamou, unpublished results.

Table 1. The extent of the edge effect seen in HLB detections in a Texas grove over time. From M. Setamou, unpublished results.

Survey Number	Edge effect (% of total infections detected in edge trees)	Grove infection (% of grove infected)
1	0	0
2	85%	2%
3	76%	4%
4	65%	19%
5	54%	42%

Strong evidence of edge effects, regardless of experiment location, implies that hierarchical sampling throughout the grove is not the most efficient method of detecting HLB or ACP. Rather, sampling plans should focus on edge trees to maximize efficiency with respect to determining whether HLB is present in a grove. This recommendation is supported by simulated surveys using actual HLB distribution data from Florida groves (T. Gottwald, unpublished results), which demonstrated that sampling inward from the perimeter only slightly improves HLB detection (Fig. 5).

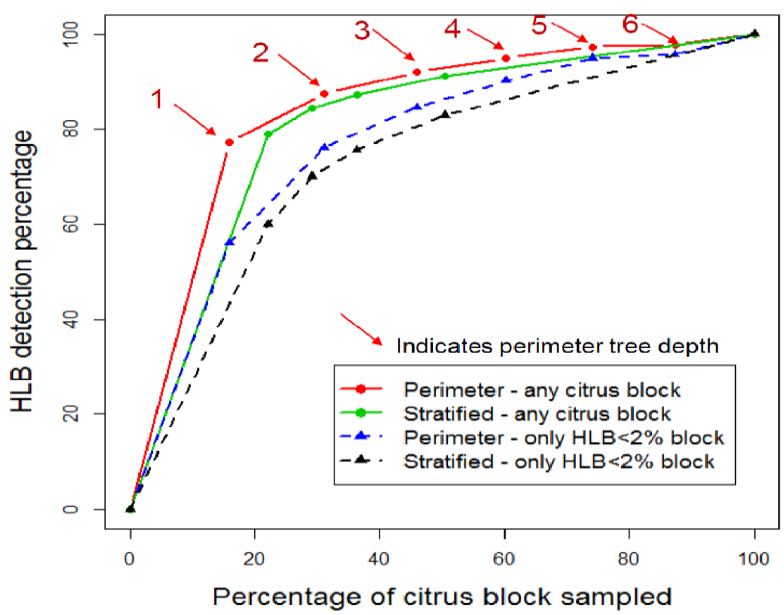

Figure 5. CLas+ detection probability as a function of distance from grove edge (tree depth) for simulated surveys using observed HLB data in Florida groves. From T. Gottwald, unpublished results.

The relationship shown in Figure 5 shows that increasing the number of sampled rows/trees included in the sampled "edge" has a declining marginal return. For example, sampling the outside row alone (1, in the figure) captures roughly 80% of infected trees, while adding a second row improves that figure by only another 5%. In situations where HLB incidence is low (<2% of trees infected) more sampling is required to achieve the same level of detection (to see this, compare the blue dashed line with the red line), but the same declining marginal return is apparent.

Although it is well-established elsewhere, there is currently no edge effect data for HLB in California, as HLB has not yet been found in commercial groves in the state. However, since disease edge effects stem from distribution patterns of ACP, such patterns could be used to predict likely disease distribution as well. Data from a pair of studies currently being conducted in California indicates ACP edge effects are similar to those in other regions. These studies have shown that, on average, ACP relative abundance on edge trees was 2 to 4-fold higher than on trees more than 1 row or tree space away from the edge (M. Daugherty, unpublished results; M. Rivera, unpublished results), and this was fairly consistent among ACP life stages (Fig. 6). Therefore, sampling for the purpose of determining whether HLB is *present* in commercial groves in CA, as in other areas, should focus on edge trees in groves near new HLB detections to maximize the probability of correctly detecting HLB when it is present. **It should be noted, however, that determining HLB prevalence within a block, or detecting every CLas+ tree, would require a different sampling plan.**

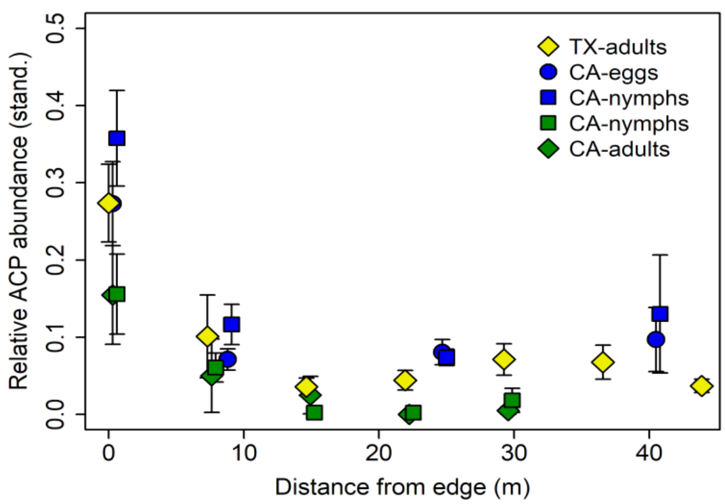

Figure 6. Mean (±SE) relative abundance of ACP at different distances from the grove edge in three studies (different colors) conducted in Texas and California. Counts or densities were standardized by dividing by the maximum value for that life stage (different shapes) in a given study. Data from Setamou and Bartels 2015, M. Daugherty, unpublished results, and M. Rivera, unpublished results.

Seasonal Testing and Grower HLB Response

To monitor the development of newly detectable infections, testing of orchard perimeters should be conducted twice a year, where risk exists based on proximity to previous HLB finds. This testing should be conducted in fall and late spring, when *C*Las concentrations are highest (Lopes et al., 2017; Sauer, 2015).

When HLB is found in a commercial orchard, the grower should follow the HLB Voluntary Grower Response Plan, which includes ACP control measures to minimize spread of the disease and additional HLB survey. Grower treatments should be conducted by the grower according to the best available evidence about efficacy and consistent with the orchard management strategy for the block in question. MAC-funded research in California has shown unequivocally that treatment programs based on broad spectrum insecticides with good residual properties are the most effective in reducing ACP numbers.

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