



Development of HLB-positive trees at sites with a prior positive find

QBE Lab, October 2019

Overview

The goal of this analysis was to understand how likely it is for a site with 1 HLB-positive tree to develop another HLB-positive tree in the future. In Southern California, only 1 diseased tree per property is usually found. If additional trees on the property are detectably diseased, they are generally discovered during the same visit as the 1st tree. However, the only follow-up visit for most of these properties occurred within 2 months of the initial detection, so the dataset is not adequately longitudinal to make conclusions about the likelihood of HLB developing in a location over time.

The data

- Of all samples collected from 2012 to April 2019, 0.4% were positive (1,224 of 297,250)
- Positive samples were collected from 840 unique locations ("positive location")
 - Median = 1 positive per site
 - Mean = 1.46 positives per site (standard deviation = 0.973)
 - Few sites had more than 3 positive samples (Figure 1)
- 12% of all samples collected from positive locations tested positive (1,224 of 9,935)

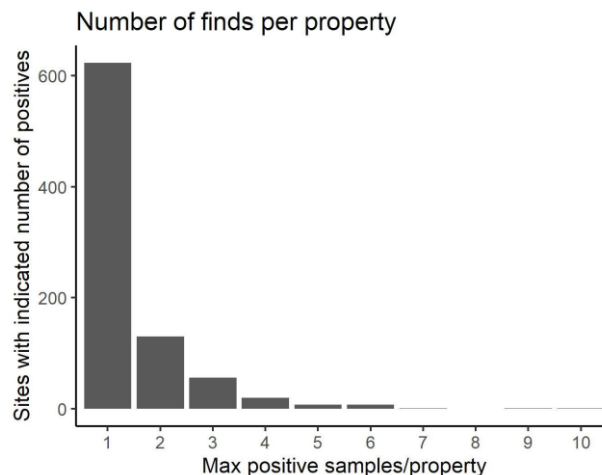


Figure 1. The distribution of the number of positive samples collected per site. The vast majority of sites have only one positive sample (74%). Few sites have more than 3 positive samples.

The descriptive statistics

- 26% of the positive locations had more than 1 positive tree (220 of 840). Of these,
 - 54% had all positive samples collected on the same date (118 of 220)
 - 46% had an additional detection on a different date (102 of 220). However, this accounts for only 12% of all positive locations (102 of 840), meaning that 88% of sites with any positive sample discover all possible positives on the same date (738 of 840).
- Of sites with multiple positive trees detected on different dates:
 - 94% had all positives found by the 2nd visit to the property (96 of 102)
 - 6% had all positives found by the 3rd visit (6 of 102) (Figure 2)
 - It typically takes 1 - 2 months to detect another diseased tree on the same property, but it can take more than 2 years (Figure 2)



- Only 22 sites with multiple positive samples were sampled 5 or more times (10% of all sites with multiple positives). Of these, the majority were sites with multiple positives found on the same day, implying that these sites are being sampled more frequently than sites with multiple positives found on different days.
- All sites with positives detected on the 1st, 2nd, and 3rd visits (6 sites total) are not indicated in the dataset as having been resampled for a 4th time. Of those 6 sites, 3 had their final sample collected in 2018, and 3 had their final sample collected in 2019.
- The only sites sampled more than 10 times were the original infections in Hacienda Heights (one of these sites was sampled 12 times; the other was sampled 21 times, including bimonthly in 2016 and up until May 2017.)

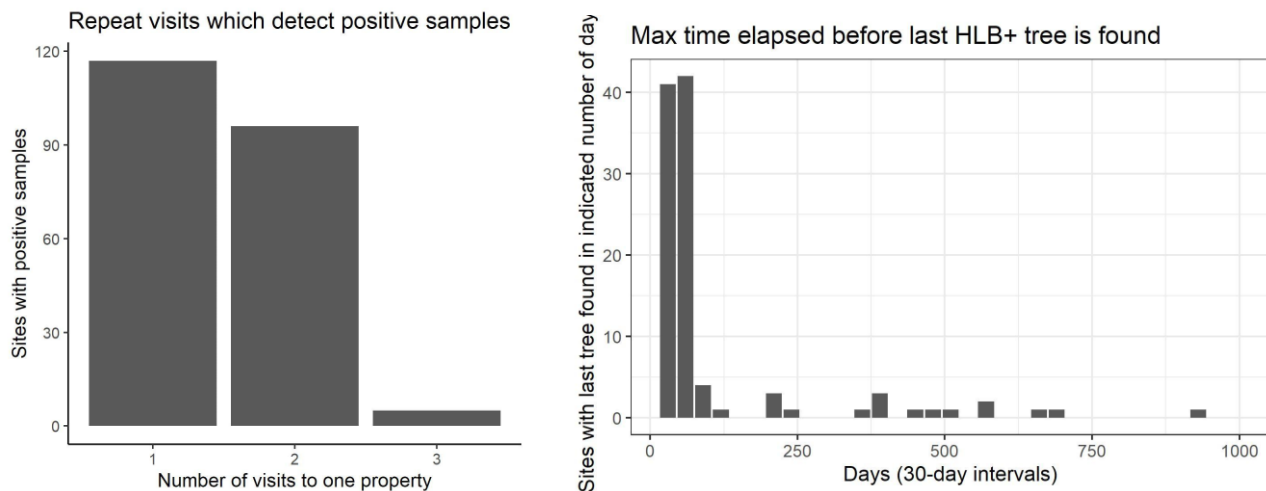


Figure 2. *Left:* On properties with multiple diseased trees, the majority of positive samples are collected after the 1st visit to a property. No additional positive trees have been detected after 3 visits to a site. *Right:* The maximum time interval between the first detection of an HLB+ tree at an address and the last HLB+ detection. Each column represents about 1 month (30 days).

Program trends

Length of time between repeat visits and additional detections

At locations with multiple positives detected on multiple days (MPMD), a follow-up visit typically occurs around 32 days after the 1st detection (standard deviation = 84, mean = 55 days) (Figure 3). The site is typically resampled for a 3rd time around 34 days after that (standard deviation = 156, mean = 136 days). MPMD sites were sampled, on average, 2.6 times (median = 2 visits).

Of the 6 sites with positives found at 3 separate sampling times, the following was found:

- Site 1 - a few months between 1st and 2nd positive, 11 months between 2nd and 3rd positive (last sample in 2019)
- Site 2 - a few weeks between 1st and 2nd positive, 12 months between 2nd and 3rd positive (last sample in 2019)
- Site 3 - One day between 1st and 2nd positive, 1 month between the 2nd and 3rd positive (last sample in 2018)
- Site 4 – Two months between 1st and 2nd positive, 12 months between 2nd and 3rd positive (last sample in 2019)
- Site 5 - One day between 1st and 2nd positive, 2 months between 2nd and 3rd positive (last sample in 2018)



- Site 6 - Six months between 1st and 2nd positive, 1 month between 2nd and 3rd positive (last sample in 2018)

Five MPMD sites were sampled 5 times, and one site was sampled more than 5 times. Only 1 site was sampled for the 6th, 7th, and 8th times. No diseased trees were detected during those visits.

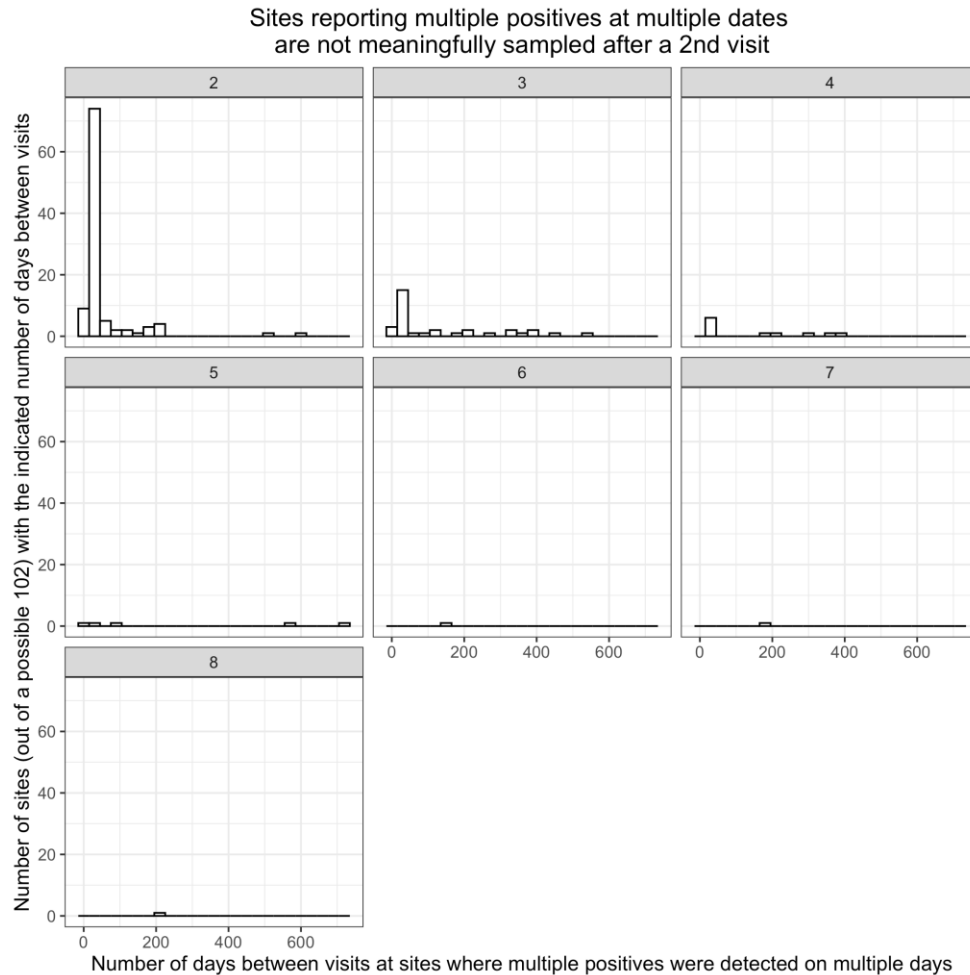


Figure 3. The number of days that pass between the previous visit and the next visit to a site, starting with the 2nd visit. Each facet represents a visit. The tallest bar represents 30-60 days.

Length of time between repeat visits without future detections

If a follow-up visit occurs for sites which ultimately yield only HLB-negative samples, it is typically over 200 days later (mean = 338 days). The maximum number of times a negative site has been sampled is 29, but they are typically resampled only 3 times, and are not significantly resampled after that (Figure 4).

If a site with multiple positive samples collected during a single visit is resampled, it is typically only revisited once (Figure 5). The median number of visits for these types of sites is 2; the maximum number is 21 (for reference, the maximum number of times an MPMD site is sampled is 8 times).

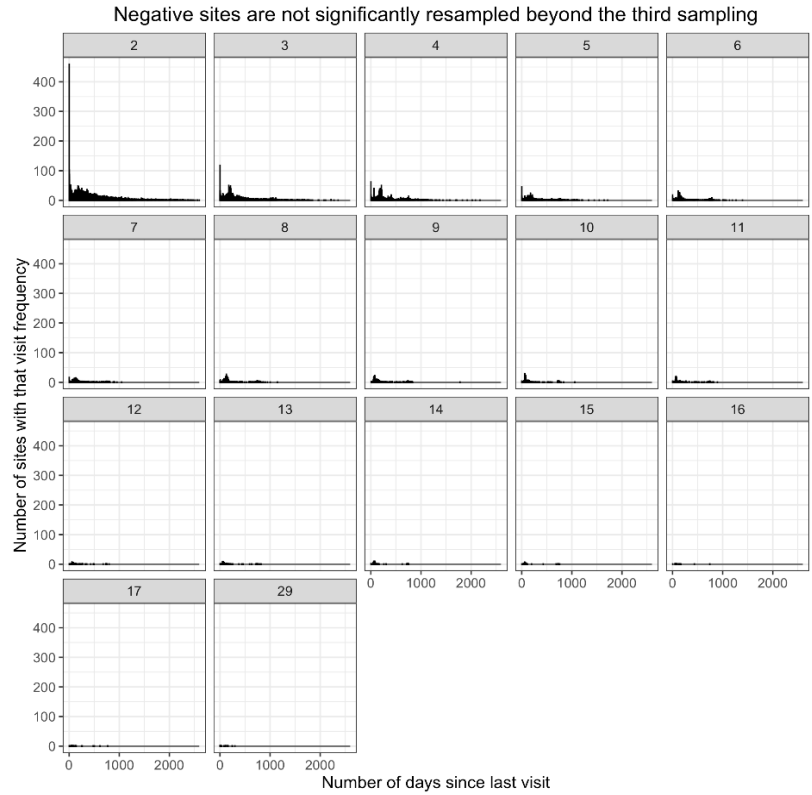


Figure 4. The number of days between visits to sites from which samples were always HLB-negative. Each facet represents a visit.

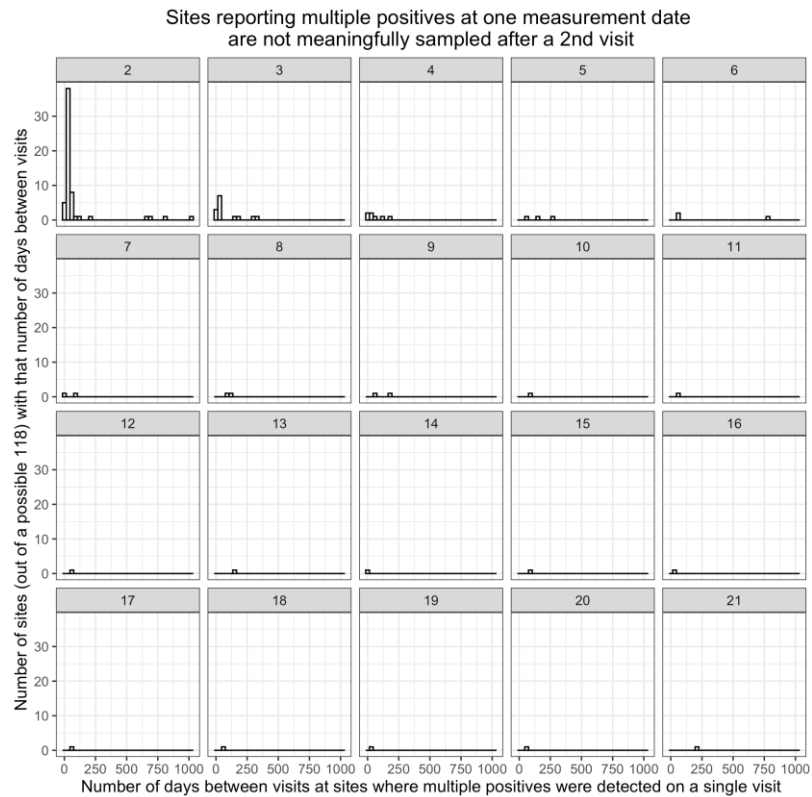


Figure 5. The number of days between visits for sites with multiple positives collected on the same day. Each facet represents a visit.

**In summary:**

- 26% of sites with 1 positive have another positive. Over 85% of these sites will find their 2nd positive on the same day as the 1st positive.
- 12.3% of all samples taken from all positive sites are positive.
 - The vast remainder are negative and not inconclusive. Because we do not know the number of trees per site, it is difficult to determine if the proportion of trees is becoming more positive at a given site over time, but the proportion of the whole dataset can be examined to determine how the positive proportion is changing over time.
- 12.1% of all positive sites record another HLB positive tree in the future.
 - This next positive find is typically found within 30 - 60 days of the initial positive find. This indicates that the disease was likely present but undetected at the time of the first sampling.
- CDFA samples negative sites at the same frequency, and in some cases more frequently, than positive sites.
 - MPMD sites were resampled up to 8 times (1 of 102 sites was sampled this many times); this 8th sampling occurred 1,209 days, or 3.3 years, after the first sampling at that site. MPSD sites were resampled up to 21 times. Negative sites were resampled up to 29 times.
 - The median MPMD site is sampled 2 times, with the 2nd sampling taken 3 months after the 1st sample. The median MPSD site is sampled 1 time, and the median negative site is sampled 1 time. However, among those that are followed up with, positive and negative locations are typically resampled twice.
- Most locations that have had multiple positives discovered over multiple dates are not sampled more than 5 times, encompassing a maximum duration of 1 - 2 years of sampling. The majority of sites are resampled no more than 4 months after the 1st positive detection, so the dataset is not adequately longitudinal to make conclusions about the likelihood of HLB developing in a location.