

### 10.3 HLB Survival Analysis – A Spatiotemporal Assessment of the Threat of an HLB-positive Tree to its Neighbors

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The spread of HLB is complex and can be thought of as occurring over various spatial scales, i.e., from an infected cell to surrounding cells, within an individual tree, among immediately adjacent trees within a local area, among trees within a block or plantation, among plantations within an industry, and among industries nationally and internationally. Various spatial mechanisms affect spread at each spatial hierarchy, including human movement of plant material and psyllid transmission. Bacteriferous psyllids also move and transmit the HLB bacteria within multiple spatial hierarchies. One concern voiced by citrus producers is the threat of an HLB-infected tree to surrounding trees within a planting block or the entire plantation.

Survival analysis is a class of statistical methods for studying the occurrence and timing of events and is often applied to the study of deaths. Survival analysis has been used for many years in medical studies and by the insurance industry for generation of actuarial tables. However this analytical method has served many disciplines and has been referred to by other names in other disciplines, for example event history analysis (sociology), duration analysis or transition (economics), reliability analysis or failure time analysis (engineering), etc. (5,6). Survival analysis has been used only recently in botanical epidemiology to examine plant disease epidemics and the factors affecting these epidemics through time, such as the effect of roguing of diseased plants (2,3,4,6). This paper explores the contribution of short distance transmissions of HLB by psyllids and the influence they have on the overall spatial pattern of disease that develops through time. The goal is to examine the threat of HLB-infected trees to other trees within the planting.

**Materials and Methods:** For this study we selected 11 blocks in a large commercial plantation in south Florida, each 4 ha (10 acre) in size, with 14 row of ca. 100-110 trees per row. We intensively mapped the spatial position of all trees within each of the blocks and their HLB status through 6 assessments over a 2.5 year period. HLB status was determined visually.

**Survival analysis** methods were applied first to determine the general survival characteristics of HLB in each plot using the Kaplan-Meier Survival model,

$$S(t) = \prod_{t_i \leq t} \left[ \frac{r_i - d_i}{r_i} \right]$$

where  $t$  = time in months,  $\Pi$  denotes the geometric mean,  $r$  = the hazard ratio, and  $d$  = disease status (0 or 1) for the  $i^{\text{th}}$  = individual tree. Next, the data were examined to gauge if trees with existing HLB infection from previous assessment periods could be used to explain the occurrence of new infections that occurred within different distances from the potential infection source (defining the area of influence). Radii of 7.6, 15.2, 22.9, 30.5, and 38.1 m (25, 50, 75, 100, and 125 ft) surrounding infected trees were queried. The number of HLB-positive trees found within those areas that existed during the previous assessment period, were numerated. For individual

trees occurring near the edge of the plot, the radii often extended beyond the plot boundaries. To adjust for edge effects in the data, an edge correction calculation was performed to adjust the number of HLB-positive trees within each area defined by the radii. Using these parameters, the covariate that was tested via survival analysis was the number of prior infections within areas described by these radii.

The semi-parametric Cox proportional hazards model was fitted to the data, which specifies the hazard for an individual tree  $i$  at time  $t$  as,

$$h_i(t) = h_0(t) \exp(\beta' \mathbf{X}_i)$$

where  $h_0(t)$  is an unspecified baseline hazard function,  $\mathbf{X}_i$ , a vector of time-constant covariates values and  $\beta'$  the vector of covariate coefficients that are estimated by partial likelihood (2,4,5). The potential effect of a covariate is quantified using the hazard ratio (HR), expressed in terms of an exponential of the corresponding estimated  $\beta$  coefficient for one unit change in the value of the given variable. An HR value of 1 ( $\beta^{\wedge} = 0$ ) indicates no significant effect of the covariate tested. The explanatory covariate tested was the number of infected trees within an area of influence in a prior assessment (time period). The hazard function modified for this purpose was,

$$h_i(t) = h_0(t) \exp(\beta' \mathbf{X}_i(t))$$

with  $\mathbf{X}_i(t)$ , the vector of values at time  $t$  of the time-dependent covariates as well as the values of the time-independent covariates; and  $\beta'$  is the vector of associated coefficients (6). Analysis was performed using the Survival library of S-PLUS (Data Analysis Products Division, Mathsoft Inc.).

### Results and Conclusions:

Variability of disease increase among the 11 commercial citrus blocks studied is shown in Figure 1. Note that all of the blocks increase rapidly in HLB incidence then begin to plateau after about 400 days.

Spatial patterns maps are shown in Figure 2 for two commercial citrus blocks each 2.5ha in size.

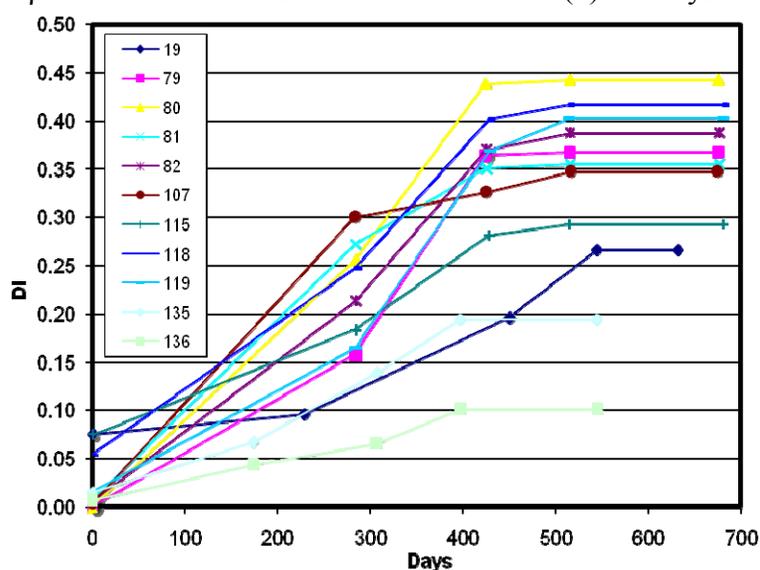


Figure 1. Disease increase of HLB in 11 commercial citrus blocks each 2.5 ha in area.

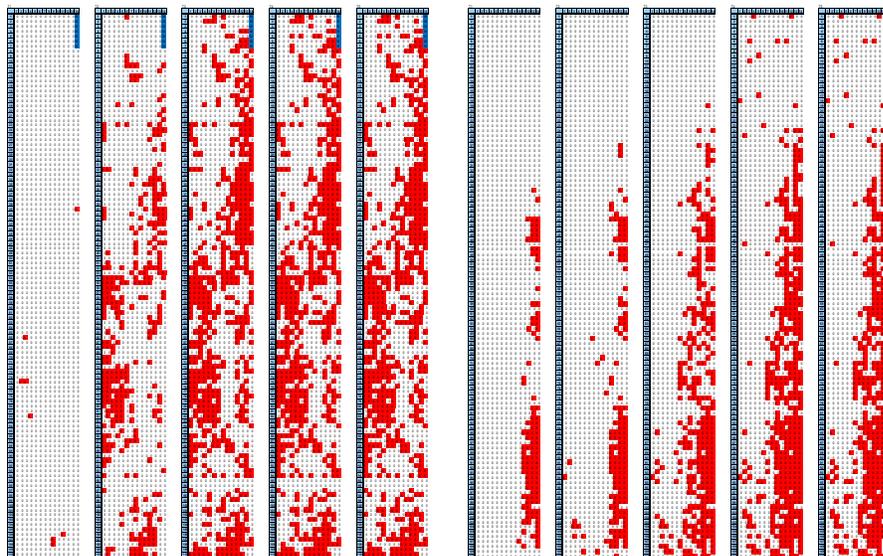


Figure 2. Spatial pattern maps for two commercial citrus blocks showing the first 5 assessments over a 2-yr period. Trees were assessed visually. Red dots indicate the spatial position of HLB + trees.

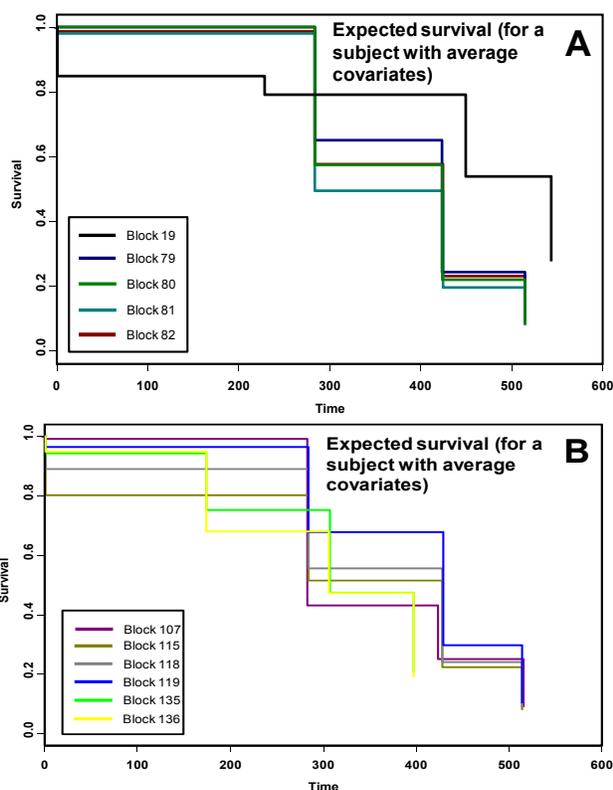


Figure 3. Survival graphs describing plants remaining in a non-symptomatic state within individual 2.5 ha blocks of commercial citrus. Time is in days.

Survival analyses of the blocks individually agree with the characteristics of disease progress presented above. Survival of trees in the HLB-free condition as estimated by the Kaplan-Meier model with average covariates, decreased with time as expected (Figure 3). This model takes into account distance as well as time on survival. The range of survival within individual blocks varied as expected and fell below 50% within 310 to 550 da ( $x=420$  da). The temporal difference in survival

corresponds well to the difference in initial onset of HLB infection within each block.

The second objective of this study was to examine the effect of a prior infection of HLB within a tree or trees within the surrounding area on subsequent infection of a HLB-symptomless tree. This was done by first looking at the effect of all HLB-symptomatic trees from previous assessment periods within a given distance on the survival (remaining in a HLB-asymptomatic state) of each remaining asymptomatic tree in the block. Secondly, survival was further examined as the effect of all HLB-symptomatic trees from previous assessment periods within a given annulus of distance on the survival of each remaining asymptomatic tree in the block. This second approach allowed us to examine the influence or

“hazard” of prior HLB-symptomatic trees over a range of distances from each asymptomatic tree on their probability of each asymptomatic tree remaining asymptomatic. Examination of the survival characteristics of trees due to surrounding trees within various radii of asymptomatic trees, show that there is very little influence of increasing the radii, that is, all of the graphs are very similar (Figure 4, A and B). These graphs are also very similar to the individual nonparametric Kaplan-Meyer curves for each block (Figure 3). Thus it appears that there is very little additional influence in survival that can be attributed to nearby trees and there is no difference among radii tested. Similarly, the same trend was seen for the influence of HLB-positive trees located within various concentric annuli from asymptomatic trees on survival (Figure 4, C and D). These annuli survival graphs are also very similar to the individual nonparametric Kaplan-Meyer curves for each block (Figure 3). Therefore, no additional influence in survival could be attributed to nearby trees within concentric annuli and there was no difference among annuli tested.

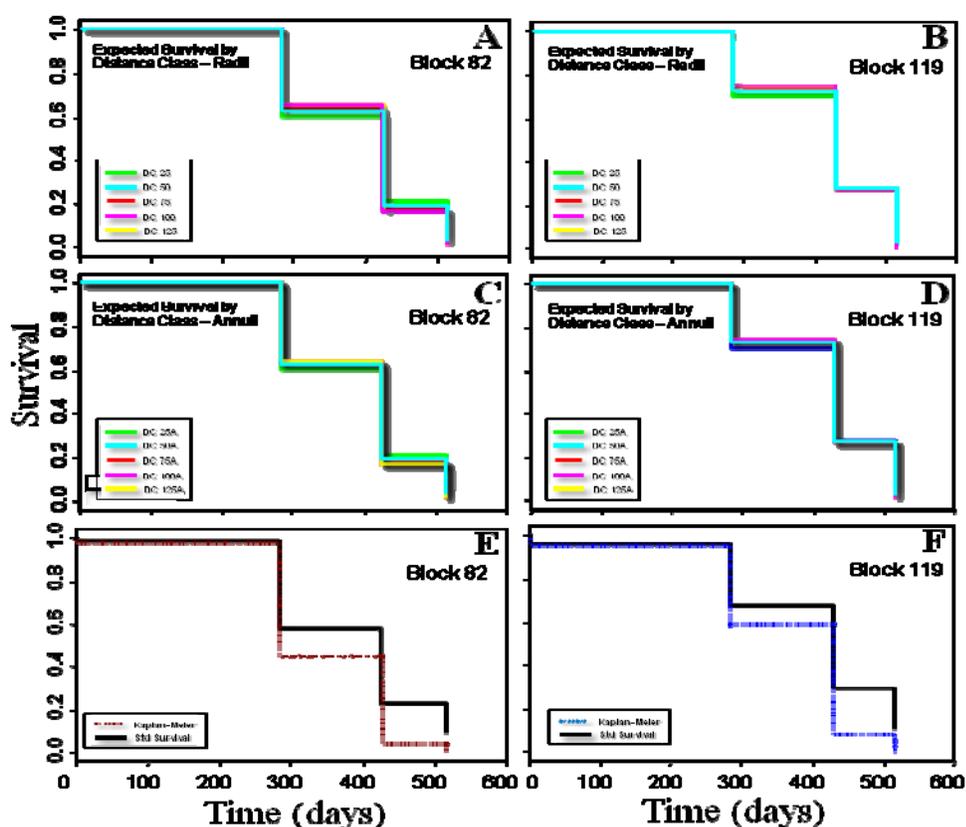


Figure 4. Survival graphs for two commercial citrus blocks of 2.4 ha, showing the probability of asymptomatic trees remaining asymptomatic (i.e., survival) based on the influence or ‘hazard’ imposed by HLB-positive trees within defined A, B) radii or C, D) annuli from each asymptomatic tree. E, F) Standard survival model with covariates of *time* and *distance* considered, and Kaplan-Meier nonparametric model with *time* only as a variable considered.

lack of such influences seen for HLB suggests quite different spatial processes at work. HLB is known to spread regionally. In another paper in this proceedings, the spatiotemporal influence of

These findings differ sharply from those found for other citrus and prunus pathosystems (2,4). For Citrus Tristeza Virus (CTV) and Plum Pox virus (PPV), both vectored by aphid species, there was a marked influence, i.e., “hazard”, of diseased trees within the immediate vicinity on the continued health “survival” of asymptomatic trees. For CTV, this influence was pronounced within 32 m of non-infected trees and incremental radii and concentric annuli could be seen to contribute significantly and differently. The

psyllid transmissions were examined by stochastic Markov-Chain Monte Carlo modeling (3). In that study, for HLB, the influence of ‘primary’ infections from outside of the blocks was the overwhelming influence on the spatiotemporal increase and spread of the disease. Only on rare occasions was it possible to distinguish the influence of ‘secondary’ spread from within the block. This demonstrates the regional aspects to HLB increase and spread and the difficulty of establishing or seeing appreciable influence of nearby Las-infected trees. This same characteristic of HLB epidemics is demonstrated in Figure 4. The survival graphs for the range of various radii and annuli tested are not appreciably different (Figure 4 A-C). The radii and annuli survival graphs are also not appreciably different from the Kaplan-Meier expected survival curves, demonstrating a lack of influence of HLB-symptomatic plants within the immediate area. The radii and annuli survival graphs also do not appreciably differ from the Kaplan-Meier nonparametric survival curves. The Kaplan-Meier nonparametric survival analysis does not consider distance as a variable. Thus the influence of ‘distance’ from prior symptomatic trees in the near vicinity or even within the block in general does not contribute greatly to ‘survival’, i.e., the probability of remaining disease free. Thus this study confirms that the overarching influence in HLB epidemics is the migration and transmission of Las via psyllids from outside the block, i.e., the influence of primary spread. It also indicates that attempting to control HLB locally is probably futile. Significant control will likely only be achieved from regional disease management strategies.

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