10.2 A Stochastic Spatiotemporal Analysis of the Contribution of Primary versus Secondary Spread of HLB.

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The spatial and temporal dynamics of HLB in mature groves has been investigated in only very few cases, primarily from Reunion Island and People’s Republic of China (PRC) (Gottwald et al 1989; 1991a; 1991b; 2007) and more recently, preliminary studies are being conducted in Brazil and Florida. In all prior cases, only the Asian HLB pathogen, Ca. L. asiaticus, was known to be present. With the discovery of Ca. L. americanus in São Paulo, Brazil, some data on disease increase and spread of that organism is beginning to be collected as well (Bassanezi et al 2005). Prior studies presented an opportunity to investigate the disease in citrus plantings in two situations, where inoculum was introduced by infected planting stock, and where clean stock was utilized and the pathogen was introduced by the immigration of bacteria-contaminated psyllid vectors. The studies were conducted to establish preliminary rates of disease increase of citrus HLB under endemic conditions in the presence of vector populations, and develop preliminary temporal models to estimate the expected longevity of infected sweet orange and mandarin groves.

These previous studies of HLB increase and spread conducted in PRC and Reunion Island indicate a rate of disease increase leading to a multi-year epidemic similar to the CTV-Aphis gossypii pathosystem. In contrast, more recent studies in Brazil, Vietnam, and Florida suggest a much more rapid rate of disease increase and spread (Catling and Atkinson, 1974; Bassanezi et al 2005; Gatineau, 2006 (NOT CITED); Gottwald et al 2007). In the present study, an HLB epidemic was examined in a large 4,800+ ha plantation in South Florida where no new citrus had been introduced for 10 years and thus spread was entirely dependent on psyllid transmission. Objectives of this study were, 1) to characterize relationships of HLB-positive trees from a long-distance point of view to gain insights into psyllid transmission across a range of distances from immediately adjacent trees to regionally, and 2) to use stochastic modeling methods to parse disease spread over time into components using likelihood estimates of the ‘primary infection rate’ (introduction from outside the area under study) and ‘secondary infection’ (spread locally within the area of study).

Materials and Methods:
Based on the commercial survey of trees with visual symptoms of HLB, the spatial pattern of HLB was determined over a large contiguous planting of citrus (4856) ha in South Florida. The area was completely surveyed via a 100% census of trees five times over a 2-year period. The commercial planting was a mixture of sweet orange cultivars but predominately Valencia and Hamlin on various rootstocks. A subsection of the plantation composed of 180 blocks (~739 ha) was used for the study. Block size was of 14 rows of 110-115 plants per row or ~1,500 trees/block. Incidence of HLB was assessed by visual inspection. The GIS location of each
symptomatic tree and date when the symptoms were assessed were recorded on GIS referenced maps of the plantation.

For spatio-temporal analyses, data for each of 11 HLB–infected blocks were analyzed using the spatio-temporal stochastic model for disease spread which was fitted using Markov-Chain Monte Carlo (MCMC) stochastic integration methods. For a thorough description of the MCMC model, its application, and interpretation of results, refer to Gibson (Gibson 1997a; 1997b; 1997c; Gottwald et al 1999). The results of the spatio-temporal analysis can be viewed graphically in a two-dimensional parameter space representing a series of 'posterior density' contours of parameter densities, \( L(a) \). The two parameters represent local \((a_2)\) versus background \((b)\) interactions. The parameter \(b\) quantifies the rate at which a susceptible individual acquires the disease due to ‘primary infection’ independent of the infected trees in the plot and is therefore the simple interest or ‘primary infection rate’ in a spatio-temporal context. For many pathogens that are vector transmitted and dispersed, this usually means from sources of inoculum outside of the host population, i.e., plot. Whereas \(a_2\) represents the ‘secondary infection rate’ in a spatio-temporal context, \(b\) quantifies the manner in which the infective challenge presented to a susceptible individual by a diseased individual in the population decreases with the distance between them. As \(a_2\) increases, the secondary transmissions occur over shorter ranges and, so long as \(b\) is not so large that primary infections dominate, disease maps generated by the model exhibit aggregation. The MCMC analysis was accomplished by a simulation model using 500-1000 simulations. The citrus blocks studied were too large to allow this program to run due to memory restrictions. Thus we subdivided each plot into two smaller plots each with <750 trees.

**Results and Conclusions:**
The main objective of the study was to attempt to parse the spatial spread of HLB into distinct and identifiable components. This was done by the use of MCMC stochastic modeling methods to give likelihood estimates of the ‘primary infection rate’ (introduction from outside the area under study) and ‘secondary infection’ (spread locally within the area of study). We have employed this stochastic modeling technique previously to examine the spatio-temporal dynamics of citrus tristeza, blight, psorosis and other pathogens and the involvement and dynamics associated with various vector populations (Gottwald et al 1999; Castle and Gottwald 2005; Gottwald et al 2005 NOT CITED). Spatial disease spread between two assessment times was analyzed via the MCMC model when a minimum increase in disease incidence of 2.5% had occurred. Examples of posterior density contour graphs are shown in Figs. 1-3. These representative graphs are arranged into three groups which share common characteristics among their posterior density contours.

The overall interpretation was that there are two spatial processes that are ongoing during HLB epidemics, but not necessarily simultaneously. Figure 1 represents a smaller group of analyses, the largest probability category values (darkest contour color) of the posterior density \( L(a) \), extends exclusively from the vertical \(b\) axis. The highest probability category for \(b\) (the background parameter associated with secondary spread) varied from about 0.5 to 1.2 whereas the corresponding values of \(a_2\) (the local parameter relating to secondary spread) were of about 1.75 to 3.5 along the upper end of the parameter range of the \(a_2\) axis. Here the analyses indicate ‘background or primary spread of disease that originates from outside the plot areas’. This is the most hazardous kind of spread in that it indicates a spatial process of long distance or regional vector transmission. Background primary spread from outside the plot is the most devastating because no amount of spraying will stop psyllids from feeding on distant HLB-positive sources, migrating to uninfected trees at some distance, and transmitting the bacterial pathogen before
they die from insecticide applied to the new trees they settle on. Examples of purely background spread such as those shown in Figure 1 were the least common.

In all cases in the group shown in Figure 2, the largest probability category values of the posterior density \( L(a) \), corresponded to values of \( a_2 \) (the local parameter relating to secondary spread) of about 0.7 to 1.3 along the lower end of the parameter range, joined the \( a_2 \) axis, and the highest probability category for \( b \) (secondary spread) varied from about 0.05 to 1.1. For this collection of analyses, posterior density estimates provides evidence of ‘secondary spread via predominantly midrange local interactions for dispersal of inoculum within the boundaries of the plots through time’. This spatial process is characterized by vector transmissions that are not to nearest neighboring trees, but rather to trees that are nearby within a local area of influence. The prevalence of these two spatial processes is weighted much more heavily toward the secondary spatial process with only occasional evidence of background spread from external sources, i.e., random long distance transmissions of inoculum. Note that the graph is arranged along rows and from top to bottom of the graph with increasing posterior probability contour areas. Those at the top of the graph indicate little to no evidence of background spread whereas as we look further down and to the right of the individual graphs in Figure 2, we see increasing indications of some level of background or primary spread. The final graph to the lower right indicates a lower probability contour actually descending to the \( b \) axis indicating a definite but subordinate influence of background spread.

A third category of posterior density contour graphs are shown in Figure 3. These are perhaps the most common type of model result. In this case the posterior density contours generally do not always intersect either axis and indicate mixed spatial processes of primary and secondary spread occurring simultaneously. The top row indicates posterior density contours confined near the \( a_2 \) axis but still with the highest contour levels not extended to that axis indicating a prevalence of secondary spread but with significant influence of simultaneous background spread as well. For
the second row of graphs and the left portion of the third row, the contours rise above the $a_2$ axis and begin to increase in overall parameter space area, i.e., contours. This indicates a stronger but well mixed influence of ‘both primary and secondary spread without a clear prevalence of either’, however, the highest contour level is near the center of the $a_2$ range, indicating secondary spread that is mid range and neither nearest-neighbor nor short-range and local but somewhere in between. Lastly for the right two graphs on the third row, and all of the graphs on the fourth row, the lower probability density level contours begin to extend towards and generally intersect one or both axes, indicating a strong but mixed interaction of primary and secondary spread. The stochastic modeling analytical results characterized in Figure 3 are the

![Figure 2](image.png)

Figure. 2. Posterior density estimates of Markov-Chain Monte Carlo Simulation of the Spatio-temporal increase of huanglongbing in citrus plots in south Florida. MCMC posterior density likelihood estimations are for local and background influences on disease spread between two assessment dates for selected individual plots. Contour maps represent the posterior density estimations $L(a)$ between two assessment dates for each plot with a minimum of 2.5% increase in disease incidence between individual assessments. Plots in this figure demonstrate posterior density estimates that provide evidence of secondary spread via predominantly midrange local interactions (that are not to nearest neighboring trees, but rather to trees that are nearby within a local area of influence) for dispersal of inoculum within the boundaries of the plots through time. The graph is arranged along rows and from top to bottom of the graphic with increasing posterior probability areas. See text for interpretation and details.

most common type and indicate generally that HLB spread occurs as an incessant mixture of these two processes interpreted as a continuous introduction of inoculum from outside the plot and local spread from within the plot occurring simultaneously.
From the data presented we see that there are two spatial processes driving HLB epidemics, primary spread via psyllids carrying the HLB bacteria from sources of inoculum outside the plantings, and secondary spread via psyllids transporting HLB bacterial inoculum within the planting. It was rare to see instances when the predominate influence was primary spread from outside the planting (Figure 1), whereas, it was relatively common to find situations when secondary spread within the planting predominated with demonstration of some primary spread (Figure 2). However, the most common situation appeared to be a simultaneous and more balanced influence of both primary and secondary spread (Figures 2 and 3).

The data from this large commercial area in south Florida is unique in several ways. It represents the earliest and most heavily HLB-infected commercial area in Florida. The data are exclusive in that they provide the first regional examination of the spatial distribution and spread of HLB.
Even though the commercial industry in the area quickly and dramatically increased vector control and instituted roguing of HLB-infected trees, the age of the trees combined with the temporal latency of the disease provided a historical record of infection and spread that no amount of insect control or tree removal could mask within the two-year period of data collection. Finally, the level of psyllid population/infestation in this planting was unprecedented compared to other recorded psyllid infestations, probably because the insect was relatively newly introduced to Florida and this area in particular and out of balance with environmental constraints. Thus this HLB epidemic is undoubtedly one of the worst ever recorded. That being said, it demonstrates the true rapid and destructive nature of the HLB-pathosystem during a time period when virtually no mitigating measures were yet influencing the epidemic and serves as a warning to commercial citrus industries who would disregard the seriousness of HLB.

**Literature Cited:**