APPENDIX B

DISTANCE NECESSARY TO CIRCUMSCRIBE PROCEDURE

"Everything is possible but nothing is real" Song by Living Colors

1. INTRODUCTION

In Appendix A, some very basic questions were asked:

- Where was the survey data collected?
- When did data collected begin and end?
- How often were inspections conducted?
- Was citrus canker prevalent outside of these sites?
- What happened if repeated surveys conflicted, such as the number of trees surveyed, health status of trees, type of tree?

Appendix A identified numerous conflicting statements made by the 2002 article by Gottwald et al and the FDACS concerning this very basic information. The Department tried their best to bridge over these problems, by declaring the study was approximately 12 to 18 month long, when some documents stated the study lasted for 12 months and others 18 months. The size of sites was also problematic, as the size estimates varied from presentation to presentation. These conflicting pieces of data could not be easily be dismissed.

Appendix A also summarized the inspection protocols which were used by the eradication program. The canker eradication program inspections were ongoing prior to and during the field study. The routine inspection for citrus canker involved repeated visits to residents' yards. If citrus canker was discovered, the discovery date would be noted on the inspection form.

According to the Department, in year 2000, a second visit was generally done to confirm citrus canker. The second confirming visit is described by Schubert et al. (3) and was in effect during year 2000. It is unknown if second visits were employed in 1998- 1999 during the field study.

Amazingly, the Department claims that they retained none of the field study data. This seemed very odd. It was collected by FDACS inspectors at the Department's expense. The entire dataset could be scanned and likely contained on a few CD's. The Department claimed all the data were with the USDA. All attempts to obtain release of the data from the USDA failed, as they claimed this was a continuing research study.

The Department maintained a database of all healthy and diseased citrus trees and the dates of inspections for each yard. (3) Since there were discoveries of canker in the Miami-Dade sites prior to announced start of the study on February 26, 1998, these would also be included in the list of diseased trees. There were many problems with inspections, which is discussed in Appendix A.

In many respects, the surveys were the same as the normal surveys for the CCEP. However, there were two important exceptions. The inspectors would note the age of the oldest lesion on the tree. Second, using GPS meters, the coordinates of every citrus tree on the property (healthy/ diseased) would be identified. Despite requests, the USDA has never made this collected data public.

This appendix takes the next step. It is a review of the most essential set of calculations in the field study as a result of the distance necessary to circumscribe (DNC) procedure. As part of the procedure, a new time scale of disease incidents is calculated based on oldest lesion ages on each tree. The distance necessary to circumscribe (DNC) procedure is described by Dr. Gottwald et al. in the 2002 article (1) and in other documents provided by the FDACS (2). The procedure was used to calculate the distance values and other results as shown in Tables 1 to 5 in the 2002 article. The "2002 article" in this appendix refers to reference 1. All articles and results as referred to in this appendix are posted on the supporting documents website, under DNC support documents.

It is, unfortunately, not only necessary to describe how these distance values were calculated, but also what was not done. It is likely there has been considerable study description has been misunderstood by the public based on the wording in Commissioner Crawford's press release on February 26, 1999, the postings by the FDACS ("Summary of the Justification for Removing Canker-Exposed Trees within 1900 feet of an Infected Trees") and within the 2002 article. The press release refers to experimental sites, and the article refers to experimental design (page 363, used as a heading). The study was not an experiment, but an observational study. This difference is critical, as with an experiment, a source tree or plant would be used so there is no ambiguity as to the initial source of the disease. The definitions of experimental and observational studies is well defined in the literature.

Further, the study is not based on perspective study or one that is done in real time, although the FDACS website justification statement of June 2000 certainly implies an active tracking of the disease, as follow::

> The study was done in an area where canker was only recently established, where citrus leafminer was present (a new factor in the epidemiology equation for the Western Hemisphere) and where thousands of trees in four separate sites could be monitored to provide the data for the study.
>
> Four study sites were selected based on their relative isolation from each other, the recent appearance of only a few infected trees in each area and the absence of disease in the surrounding citrus. At the beginning, all citrus (ca. 19,000) in the vicinity were identified and their location plotted using satellite-based global positioning technology. The disease status of each tree in the study area was then determined on a 30-day basis by a field plant pathologist.

The impression might be that in the first few days of the study, the whole area of the sites and periphery area was surveyed and only a few infected trees were found. For a four square mile area, as the 2002 article describes Site D1, there are 8 sections bounding the site on all sides, for a total of 12 square miles.

FDACS does not routinely inspect residential areas which they believe are absent of disease. There is no evidence in the 2002 published article that the study sites were any different. In fact, citrus canker may have been quite prevalent in the sites prior to the start of the study. It was just a matter of time until it had been discovered. As stated in Dr. Schubert's presentation at the 2000 International Citrus Canker Research Technical Workshop:

> We're looking at history when we find the disease and the pathogen has gone ahead of us as we can tell from the epidemiology studies that Tim Gottwald and others have done.

The study's data is relevant to infection events which occurred prior to their discovery. Thus the study is a look back in time or a retrospective study. The different types of studies was reviewed in Chapter 6.

The key concept of the DNC procedure is to transform real time scale of discovered infected trees to a synthetic time sequence, based on the initial infection dates (IID). The initial infection date is the day the tree became infected with the citrus canker.

The nomenclature used in this appendix is not the same as in the 2002 article. The 2002 article makes no specific reference to either a DNC procedure, synthetic time line or a synthetic time sequence. These terms are used to add clarity to the discussion. The DNC procedure is clearly described in this appendix and an example provided, prior to any critical review of the procedure.

Following the detail discussion in this appendix B, there is one final twist to this discussion. It is discussed in Appendix B1, titled "Unusual Field Results", which further explores the distance procedure in review of other presentations of the field study.

Two related short notes, posted on the website are:

SN 3.3 Misidentification and Detection Errors and Eradication Policy
SN 6.2 The1900-ft Policy and the Published Articles by Dr. Gottwald et al.

2. SELECTED EXCERPTS FROM THE PUBLISHED ARTICLE

Selected Excepts from Gottwald, T.R., X. Sun, Riley, T. Graham, J.H., Ferrandino, F. and Taylor, E., 2002, Geo-Referenced Spatiotemporal Analysis of the Urban Citrus Canker Epidemic in Florida, Phytopathology, Vol 92, No. 4. (Reference 1)

Every effort has been taken to transcribe the excerpted passages exactly as published. Figures and table numbers used in this section are based on the published article. These selected sections may exclude important details, so it is recommended that the full article be reviewed. The full article may be downloaded free of charge from a number of websites, including www.citruscankerdocs.com. Selected discussion of surveys, methodology, results and conclusions were excerpted. Only a partial listing of the DNC results as presented in Tables 1-4 are provided

-- Survey page 366:

Experimental design. During 1998, three study sites were selected within the urban Dade County area with a few widely spaced focal trees from which to measure disease spread. Two additional sites north of the ACC quarantine boundary as it existed at that time were subsequently selected in Broward County. Within the Dade County sites, the CCEP did not undertake eradication efforts during the study but rather continued to address the large backlog of ACC-affected trees elsewhere in the Dade County area. Miami, Dade County sites D1 and D2, consisted of ≈10.3 and 5.2 km^2 (4 and 2 mi^2) of urban area, respectively, as defined by section-township-range (STR) boundaries. Site D3 consisted of three infected trees all within 15.2 m (50 ft) of each other at the beginning of the study, and spread was measured from this small focus of infection to the surrounding area circumscribed by a 1.6-km (1.0-mi) radius. The Broward County sites B1 and B2 consisted of 2.6 and 15.5 km^2 (1.0 and 6.0 mi^2) of urban areas, respectively. Because the Broward County sites were north of the quarantine boundary and were considered a potential imminent source for continued northward spread of the disease, they were treated differently. In Broward County, ACC-affected trees were removed as they were identified. The majority of tree removals occurred within 2 to 4 weeks of detection.

Within each site, surveys were conducted to identify all diseased and asymptomatic citrus trees. Over 16 inspectors, experienced at recognizing ACC symptoms, conducted inspections in the experimental sites. The diseased trees were visually examined, evaluated, and rated by a single inspector, X. Sun, to maintain consistency. A total of 18,769 trees among the five study sites were assayed. Using differential global positioning system (GPS), latitudinal and longitudinal coordinates were recorded for each tree. Other data recorded relative to each tree included (i) species/ cultivar, (ii) tree age, (iii) age of oldest lesion, (iv) severity of infection, and (v) section of tree infected (i.e., directional quadrant—N, E, S, and W, and portion of tree infected—top third, middle third, and bottom third). Surveys were conducted at least three times in each study area approximately 60 days apart. The age of lesions during a given survey can be based on phenotypic characteristics of infected host tissue. From the above data, the infection initiation date (IID), i.e., the date that the oldest lesion found in a tree, was estimated to have been established, relative to the date discovered and taking into account latency of infection of approximately 14 days. The oldest infection among the five study sites was used to establish the temporal origin of the study period, and all IIDs were referenced to that date. IID for each tree was estimated as survey date (expressed as the number of days post- temporal origin) minus age of oldest lesion expressed in days for each tree. This date was used in all future calculations.

-- Methodology:

Page 363, Second Paragraph, left hand side.

Nearest neighbor distance calculations. The distance between any two trees was calculated as the length of the hypotenuse of a right triangle whose perpendicular sides represent the north-south and east-west distances between the two points determined by DGPS. Each tree was assigned an IID value, based on an estimate of the date the tree became infected. For the purposes of the study, the IID values were separated into contiguous 30-day categories. This was done because some error in visual estimation of the infection date was assumed. For each site, the nearest neighbor distance measurements between focal trees and newly infected trees were calculated for various temporal periods. These temporal periods were 30, 60, 90, and 120 days in duration and were chosen for their relevance to regulatory procedures. That is, the ability of the CCEP to return to resurvey an area every 30, 60, 90, or 120 days. Resurveys were dependent upon the expanding quarantine area and available manpower. For example, to accommodate a 30-day survey period, the infected trees that existed in the area during the first 30 days were considered focal or "alpha trees" that gave rise to the subsequent diseased trees had IIDs in the second 30-day period. For the next 30-day temporal period, the diseased trees, which occurred in the first 60 days of the study, were considered focal trees that gave rise to the subsequent diseased trees, which had IIDs in the third 30-day period, etc. The entire data set was parsed into 25 time periods (designated TI to T25), each 30 days in duration, encompassing the period from 25 October 1997 through 15 November 1999. A visual basic application (VBA) was used to calculate the distances between each newly diseased tree and all prior focal trees. The shortest distance was stored and the process was repeated for each new diseased tree in the study area. This "nearest infected neighbor" concept was used throughout the study. Although it is very possible that pathogen spread occurred from source trees to secondary infected trees that were not nearest neighbors, this nearest distance was used because it was considered to be the most conservative estimate of spread.

-- Methodology: Discussion of the holdover/ overlap procedure is provided in the footnotes of the tables as follows:

Page 365, below Table 1 (Same in all tables):

The 30-day and 60-day time periods are consecutive and thus the number of existing focal trees plus the number of new infected trees is equivalent to the number of focal trees at the beginning of the next temporal period. However, the 90-day and 120 -day time periods overlap with previous periods by 30 and 60 days, respectively, and thus the number of focal trees is not additive from one period to the next.

-- Results:

Abstract, Page 361:

For each area studied, distance measurements between focal trees and newly infected trees were calculated for various temporal periods of 30, 60, 90, and 120 days in duration, corresponding to intervals of inspection survey. A visual basic application was used to calculate the distances between each newly diseased tree and all prior focal trees. The nearest distance was used because it was considered the most conservative estimate possible. It is therefore likely to be an underestimate of spread but is a good estimate of the minimum possible distances of spread. For the first four 30-day periods among the five study areas, calculated maximum distances of spread ranged from 12 to 3,474 m, indicating a broad continuum of distance for bacterial spread was possible.

Page 372, beginning right hand column.

Results from the spatiotemporal studies of disease spread are presented in Tables 1 to 5 associated with Dade and Broward counties. Each table presents the proportion of newly diseased trees that was estimated to occur within 38.1 m (125 ft) of nearest focal trees. Subsequent table columns represent the distance category ±15.2 m (50 ft) that was necessary to circumscribe 90, 95, 99, and 100% (maximum distance) of all newly infected trees during the indicated time period. Example histograms of the frequency distribution for selected temporal periods are provided as examples (Fig. 2). Table 1 demonstrates that as the density of infected trees increases through time, the distance calculation between temporal periods becomes more conservative. This results because more infected trees lie closer to each other and thus distance measurements to newly infected trees decreased through time.

Therefore, the most important estimates of spread resulted from consideration of the first few temporal periods, during which longer distance estimates were less obscured. For site D1, the ranges of the maximum distances of spread measured for the first four 30-, 60-, 90-, and 120-day periods were 494 to 1,159, 552 to 1,739, 552 to 1,768, and 441 to 1,829 m (1,622 to 3,803, 1,814 to 5,705, 1,814 to 5,801, and 1,450 to 6,000 ft), respectively. In this site, during the first 30-day period, four ACC-affected trees gave rise to 10 newly infected trees, the farthest was 1,159 m distant and none were within the first 38 m. For site D2, the ranges of distance measurements were considerably less at first but longer distance measurements up to 618 m (2,030 ft) were obtained for 30-day periods after 23 February 1998. Site D3 is of interest because there were only two diseased trees in the area at the onset of the epidemic and all surrounding areas for several miles were free of disease. When the 30-day period measurements were examined, they represented the emergence of only a few new diseased trees from very few previously infected trees, and thus provided relatively unobscured measurements of spread. The longest distance measurement for D3 occurred during the 30-day period following 21 December 1998, when eight previously infected trees gave rise to six newly infected trees with a maximum distance of 588 m (1,929 ft). Site B1 had the longest calculated distance of spread measurement, 3,474 m (11,398 ft), which occurred during the first 30-day period when two previously infected trees gave rise to 28 newly infected trees. Site B2 resulted in maximum calculated distances of spread during 30-day intervals that ranged from 55 to 475 m (181 to 1,557 ft).

-- Results, Page 376, Discussion provides some connection to 1900-ft policy:

In January 2000, a "1,900-ft regulation" was adopted by CCEP to define exposed trees for removal within that radius of an infected tree (10). If we consider the first four 30-day temporal periods over all of the study sites during which spread occurred, 3 of 12, 4 of 12, 7 of 12, and 7 of 12 of these 30-day periods had calculated distances of spread that required >579 m (1,900 ft) to circumscribe 90, 95, 99, and 100% of the newly infected trees, respectively. Thus, 579 m is a distance that is neither the longest nor the shortest distance calculated but rather a compromise that represents a common distance of disease spread during a 30-day period. It should be considered that spread of the disease over some of the larger distances measured could have been the result of movement of inoculum or infected plant materials by human or mechanical means. Thus, the distance estimates needed to circumscribe 95 or 99% of the newly infected trees, rather than 100%, would be a conservative estimate of maximum possible spread. Even so, it would appear from examination of results of the calculations presented that radii of ≥579 m would be necessary to define exposed trees for removal to contain spread in many cases.

--- Partial table of results are presented on page 365. Please consult the article for all results.

TABLE 1. Nearest neighbor distance calculations from citrus trees previously infected with Asiatic citrus canker caused by *Xanthomonas axonopodis* pv. *citri* to newly infected citrus trees in Miami. Dade County, site D1[a]

Temporal period	Dates	Alpha tree age (30-day period)	Secondary tree age (30-day period)	No. of focal (alpha) trees	No. of secondary infected trees	% Captured at 38.1 m	Distance (m) needed to circumscribe			Max. distance (m)	Disease incidence (DI)	Delta disease incidence (DDI)
							90%	95%	99%			
30-day												
1st	10/26/97–11/24/97	>25	>24	4	10	0.00	1,127.76	1,159.32	1,159.32	1,159.32	0.0007	0.0007
2nd	11/25/97–12/24/97	>24	>23	14	9	22.22	769.35	769.35	769.35	769.35	0.0023	0.0017
3rd	12/25/97–1/23/98	>23	>22	23	17	0.00	548.64	599.16	599.16	599.16	0.0038	0.0015
4th	1/24/98–2/22/98	>22	>21	40	31	19.35	441.96	441.96	494.59	494.59	0.0066	0.0028
5th	2/23/98–3/24/98	>21	>20	71	51	50.98	137.16	228.60	360.40	360.40	0.0117	0.0051
6th	3/25/98–4/23/98	>20	>19	122	142	35.21	228.60	289.56	457.20	496.65	0.0201	0.0084
7th	4/24/98–5/23/98	>19	>18	264	216	44.44	152.40	228.60	441.96	552.98	0.0436	0.0234
8th	5/24/98–6/22/98	>18	>17	480	55	50.91	167.64	182.88	205.35	205.35	0.0793	0.0357
9th	6/23/98–7/22/98	>17	>16	535	68	63.24	106.68	106.68	135.10	135.10	0.0883	0.0091

3. REVIEW OF DNC PROCEDURES

The term “observation time lag” was coined in Chapter 3 to cover the numerous factors delaying discovery of canker. The DNC procedure is a method introduced by Dr. Gottwald (1) to overcome the problem of observational time lag in developing parent-offspring relationships between infected trees. If plant “A” becomes infected with a disease, and some time later a second plant “B” becomes infected, the “tree pairs” form a possible parent/ offspring relationship. Certainly, if no other plant in vicinity of the plants has the disease, then the potential parent/ offspring relationship becomes more likely.

The difficulty is that infection events resulting in dissemination of bacteria are not observable, only the display of symptoms, which may be discovered months or even years after the initial infection events. Every time it rains or the citrus tree is watered, there can be some dissemination of bacteria, either within the foliage of the tree or to other trees. In the case of multiple potential parents in the vicinity, there is ambiguity as to which parent was responsible for the offspring infected tree. If the most likely distance between parent (or prior infected “PI” tree) and offspring (or newly infected “NI” tree) is desired, then using the closest PI tree to the NI tree would be appropriate.

TERMINOLOGY

In all respects, the description carefully adheres to the 2002 article. However, some liberties have been taken with the terms used for purposes of clarity. Specifically:

1. No name was given to the procedure, hence the label “distance necessary to circumscribe” or DNC procedure was selected based on the results it produced.

2. The terms “prior infected (PI) trees” and “newly infected (NI) trees” were not in the 2002 article. They are the equivalent to alpha or focus trees and secondary trees.

Table 1: Terminology

In this Appendix	2002 Published Article
Newly Infected Tree (NI)	Secondary Infected Tree
Prior Infected Tree (PI)	Alpha or focal tree
Distance necessary to circumscribe method	Near neighbor calculations
Initial Infection Date (IID)	Same
Synthetic time line	Not in article
Synthetic time periods, time periods or bins	Temporal periods
Hypothetical infection event	Not in article
Real time line - time from first survey to last one that were included in the study	Not in article

Inspectors surveyed the designated sites and discovered many infected trees. The dates of discovered infected trees are events on the real time line.

But, there is another time line or sequence composed of hypothetical initial infection events. The initial infection event itself is not observed, so its date of occurrence is hypothetical.   It is based on the discovery date minus the age of the oldest lesion on the infected tree. This forms a different sequence of events, which is denoted as the "synthetic" time sequence or time line. The synthetic time line procedure is described in the next section, under DNC method, step 2.

There are many references to infection events which occurred on certain dates, or prior to or after certain dates. All are based on the synthetic time line. For example,   "For the next 30-day temporal period, the diseased trees, which occurred in the first 60 days of the study, were considered focal trees that gave rise to the subsequent diseased trees, which had IIDs in the third 30-day period, etc."   is a direct reference to the synthetic time line.

DNC METHOD PER THE 2002 ARTICLE

1.   Identification Step: Locations of canker infected citrus trees are identified from surveys within the specified study sites. The time of discovery of trees and the age of the oldest lesion on the tree is noted on survey sheets. The initial incident date (IID) is calculated for each infected tree as: IID = Discovery date minus the age of the oldest lesion. This age is expressed in days (Ref 1, page 363).

2.   Parsing of Data and Creation of Tables Step: The infected trees are parsed into "temporal periods" based on their IID's.   These periods will also referred to synthetic time periods. This establishes a "synthetic time line" with hypothetical sequence of infection events in accordance to the terminology in Table 1.

This terminology is necessary to distinguished between events which occur in real time (discovery of infected tree and their location) verses those which are contingent on an assumption (ability to accurately identify the oldest lesion) and other undisclosed data. As discussed in later sections, the ability to accurately identify the oldest lesion has been questioned by a highly regarded scientist.

The real time line would span the interval from the first survey of an area to the last survey. The pseudo time line begins with the minimum IID. In each of the five sites, the pseudo time line begins sometime between 10/26/97 to 11/24/97, the first time bin.

3. Association Step: Potential sources of inoculum for the trees (NI trees) in one time bin are limited to only the trees in the prior time bins. The PI tree closest to to the NI tree is considered the source of inoculum. If the duration of a time bin is 30 days, at the end of the 30 days, all NI trees are considered PI trees. This 30 day period is also called the hold over period, because within the 30 days, all the NI trees in this period, can not infect other trees.

4. Distance and Percentile Calculation Step: Distances are calculated from all NI trees to the associated source trees (nearest PI tree) and these are used to calculate estimates of distance or DNC probabilities using percentiles.   This would be calculated by ranking each distance, k = 1, 2 ... n, from lowest to highest. From examination of results, it appears that the probability levels (90, 95 and 99%) are calculated as $p = \lceil k/n \rceil \cdot 100$ where $\lceil \cdot \rceil$ means the values are rounded to the next lower integer. Thus, in each of the time periods with 10 or fewer NI trees, the 90, 95 and 99% distances would be the same number and equal to the maximum distance.

Table 2: DNC Procedure Summary

Step	Data Required	Result	Secondary Result
1. Identification	Age of oldest lesion. discovery date	Initial incident date (IID) calculated.	
2. Parsing of Data and creation of tables	IID per step 1. Assumed overlap/ hold over period	Newly infected trees assigned to a specific time period, creating a synthetic time line in discrete time periods. Results presented in a table format.	Prior infected trees assigned to multiple time periods based on hold over/ overlap procedure and added to table.
3. Association	Infected tree locations, PI and NI tree designations for each time period per step 2.	Selection of the closest PI tree as the responsible tree for infecting the NI tree for each time period.	
4. Distance and Percentile Calculations	Pairing of NI and PI trees as determined in Step 3.	Distances necessary to circumscribe for each infected tree. This set of distances equals the number of infected trees in the site.	Percentiles are calculated from the set of distance to determine distances at various levels (90%, 95% and 99%). Other statistics are calculated.

DISCRETIZATION IN TIME

Continuous processes are frequently discretized in time and space for purposes of numerical analysis. However, the release and later entry of citrus canker depends on wet conditions, thus in this case, discrete events (rain storms) are being parsed into discrete time periods. This leads to several complications. It was shown in the second Broward Court case that there were no rainstorms in some of the 30-day time periods, yet the results showed an increase in disease incidences.   Thus, all distances in all sites calculated for the no rain periods, would be in error, as the disease incidences could not be related to a prior existing source tree.

The 2002 article states that the 30 day period was chosen to compensate for visual examination of the error in determination of oldest lesion age. As stated, NI trees are unable to transmit the disease during this time. Extending the duration of the time period also leads to complications, as it assumes that an NI tree unable to transmit the disease for longer periods, i.e. 60, 90 or 120 days. These topics are discussed in the “Overlap/ Hold Back Procedure” and Lesion age estimation sections.

DISEASE ORIGIN, TEMPORAL ORIGIN AND TIME PERIOD NOMENCLATURE

Each time period has a duration of 30 days.   The end date is 30 days after the start date of the period. The published article states the following on page 366 :

> The oldest infection among the five study sites was used to establish the temporal origin of the study period, and all IIDs were referenced to that date. IID for each tree was estimated as survey date (expressed as the number of days post- temporal origin) minus age of oldest lesion expressed in days for each tree. This date was used in all future calculations.

A more direct definition would be the IID equals the survey date minus oldest lesion age. Since the survey date is a specific date, the lesion age must be given in days. Referencing dates to the earliest IID (temporal origin) seems to be superfluous, since any date can be restated in terms of a reference date and an offset, i.e. 5 days after December 25. Why state a date is 5 days after December 25, instead of December 30?

For purposes of this discussion, a period will be defined as any 30 day time interval in the synthetic time line.   The T1 is the time period between 10/26/97 to 11/24/97. The 2002 article states calculations were performed for some of the analyses for   time periods T1 to T25.   The T25 time period is from 10/16/99 to 11/14/99.

To better understand the procedure, it is helpful to define a zero time period, T0, as containing any infected tree with an IID less than 10/26/97. This period contains the presumed original source trees, according to the DNC procedure.   All trees in T0 would be the source trees (PI trees) for all subsequent time periods. The duration of this T0 period is unknown.   Since these trees have no associated pre-existing source tree, it is unknown how these trees became infected.

From Tables 1- 5, the number of trees in T0 period are: 4, 0, 2, 2, 1 for Sites D1, D2, D3, B1 and B2, respectively.   In D2, there are 10 original source trees given in the T1 period. No inter-tree distances are calculated for these source trees because there can be no associated parents.   The end date of the T0 period is 10/25/97 seems arbitrary.   If the end date of the T0 period is arbitrary, then the number of original source trees is subjective. For example, in Site B1, if the end of the T0 period was 11/24/97, instead of 10/25/97, there would be 30 trees “originally” infected at the start of the survey.

The "temporal origin" is a hypothetical time, because it is impossible to observe the first arrival of citrus canker into any area. Unlike a grove, there are no routine inspections in residential areas, so discovery of canker may occur long after observable lesions first appear. The term "observation time lag" was coined in Chapter 3 to cover the numerous factors delaying discovery of canker. As discussed in Chapter 6, a particular advantage of experimental studies is there can be no question of the initial source of a disease, because the disease plants are introduced in the experiment.

CALCULATION EXAMPLE

The following example is used only to explain the procedures. For this reason, our fictional study takes place in arbitrary time period in year 2005. It is assumed at the beginning of the study, one tree is known to be diseased identified as A, and four more infected trees, identified as B, C, D, and E, as a result of the study

Table 3: Example of IID Calculations (DNC Step 1)

Survey Data and IID Calculations

Trees ID	Discovery Date	Oldest Lesion Date (Days)	Initial Infection Date
A	1-Apr-05	210	3-Sep-04
B	1-Jul-05	60	2-May-05
C	22-Jul-05	140	4-Mar-05
D	1-Sep-05	50	13-Jul-05
E	29-Sep-05	170	12-Apr-05
Study start	1-Apr-05		3-Sep-04
Study end	30-Sep-05		13-Jul-05
Study time (days)	183		221
Survey starts	Jul-05		
Survey ends	Sep-15		
Survey time	3 months		

How long did the study last in this example? In real time, surveying lasted 3 months. Because there had been one tree identified in the site in April 2005, one could state the studies duration to be 6 months. But, if one examines the synthetic time line, the study time is 221 days, more than twice time devoted to inspections.

The sequence or order by which the trees became infected is determined by the discovery date and oldest lesion age. The variability in lesion ages (~ 2 to 9 months) in this example is in the same range as shown by Dr. Gottwald in his presentation in November 2000 in Broward Court.

Presumably, to calculate IID's of infected trees which had already been discovered prior to the start of the study, inspectors would have to go back and create a new discovery date corresponding to the oldest lesion age, if the tree still existed. Homeowners could cut down, kick over or pull out infected trees. A small tree can be pulled out of the ground.

The next step is to parse the data into time periods, as shown below:

Table 4: Example, (DNC Step 2)

Creation of Synthetic Time Line

		# PI trees	# NI trees	PI Tree	NI Tree
Period 1	Mar-05	1	1	A	C
Period 2	Apr-05	2	1	A,C	E
Period 3	May-05	3	1	A,C,E	B
Period 4	Jun-05	4	0	A,B,C,E	
Period 5	Jul-05	4	1	A,B,C,E	D

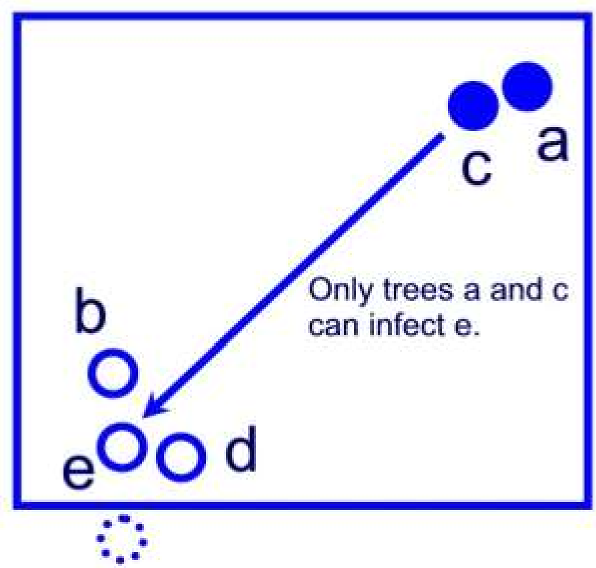
Synthetic time line	March to July-2005
Study real time line	April to Sept-2005
Survey real time line	July to September-2005

The real time sequence was tree a, b, c, d, e. The re-sequenced time line is a, c, e, b, d. Five months of results are generated from three months of survey data by the back dating of discovery dates to create the IIDs. We have 5 infected trees by the end of July 2005, when actually only 3 infected trees had been discovered by this time.

There was considerable confusion in the Florida field study, as to the beginning and ending dates. The Department has never revealed publically when surveys dedicated to the Florida field study began and ended in each study site.

The final steps are to calculate the distances between tree pairs based on their locations. In Figure 2, the filled circles are the prior infected trees, and the open circles are the newly infected trees. Infected trees b and c were discovered within 3 weeks of each other, so it is known that tree b had citrus canker for three months before tree E

Figure 1: Example Distance calculation for Period 2 (Steps 3 and 4)



As shown in Figure 1, it is possible there was another tree just outside of the site, which caused trees E, D and B to be infected. If this is the case, the calculated long inter-tree distance between A to C, would be incorrect.

After the distances are calculated, the 90 - 99% distance levels are calculated by percentages. In accordance with the percentiles, and the distance from points C to E would appear as the distance at 90, 95, 99% probability levels and maximum distance.

Based on this example, it can be seen that two clusters of infected trees at opposite sides of a site, which are back dated in a certain sequence, can result in a series of long distances.

OVERLAP/ HOLD-BACK PROCEDURE

An NI tree can not infect another NI tree during the 30-day period. At the end of the 30-days, it is reclassified as a PI tree and may be a potential parent of any NI tree, If the time period is extended, the tree stays longer as an NI tree for a longer period. The NI tree is held back for a month or more from reclassification. Discussion of the overlap or hold-back procedure is given as a footnote in Tables 1 to 5 of the published article, where it is stated:

> Time periods are 30 days in duration. The 30-day and 60-day time periods are consecutive and thus the number of existing focal trees plus the number of new infected (focal) trees is equivalent to the number of focal trees at the beginning of the next temporal period. However, the 90-day and 120-day time periods overlap with previous periods by 30 and 60 days.

In the table below, a0 is the number of infected trees with IIDs less than the start of the first period (10/27/97) and a1, a2 and a3 are the number of infected trees parsed as NI trees into the periods 1, 2 and 3, based on the calculated IID's.

Table 5: Calculation Template for NI and PI Tree Counts

30 day Scenario		
30 day duration	PI Trees	NI Trees
Period 1	a0	a1
Period 2	a0 + a1	a2
Period 3	a0 +a1 +a2	a3
60 day Scenario		
60-day duration		
Period 1	a0	a1 + a2
Period 2	a0 + a1 + a2	a3 + a4
Period 3	a0 + a1 + a2 + a3 + a4	a4 + a5
90 day Scenario		
90-day duration		
Period 1	a0	a1 + a2 + a3
Period 2	a0 + a1 + a2	a3 + a4 + a5
Period 3	a0 + a1 + a2 + a3 + a4	a5 + a6 + a7
120 day Scenario		
120-day duration		
Period 1	a0	a1 + a2 + a3 + a4
Period 2	a0 + a1 + a2	a3 + a4 + a5 + a6
Period 3	a0 + a1 + a2 + a3 + a4	a5 + a6 + a7 + a8

The authors state on page 363 their reasoning for the different durations, as follows (page 363, right hand column of paper):

> These temporal periods were 30, 60, 90 and 120 days in duration and were chosen for their relevance to regulatory procedures. That is, the ability of the CCEP to return and resurvey an area every 30, 60, 90 or 120 days. Resurveys were dependent upon the expanding quarantine area and the available manpower.

Following the rules set forward on the DNC procedure, any tree which is parsed into a particular time bin is newly infected and therefore remains incapable of infecting other trees for up to 30, 60, 90 or 120-days, depending on the particular scenario.

The rationale for this procedure is it is relevant to survey intervals. It defies logic to state that in the 120-day scenarios for about 4 months after a tree is assumed infected, it can not infect another tree and in the same published article, state that a tree may create a lesion within 14 days of being infected.

The rules governing these hypothetical scenarios do not resemble any disease process. A set of trees labeled as NI trees is incapable of being the source of infection for a discrete time period, then are reclassified as sources of the disease for the rest of the study. The authors do not provide supporting evidence for the procedure.

REMOVAL OF TREES - EFFECT ON DNC PROCEDURE

The one year moratorium, as announced by Commissioner Crawford February 26, 1998 banned the destruction of healthy trees. It was officially lifted on June 17, 1999, when the Commissioner stated the study was done. The Commissioner did not at any time ban the destruction of infected trees, either in Miami-Dade or Broward County.

Infected trees within the study sites in Miami-Dade were allowed to remain for duration of the study according to the 2002 article. (1) Exactly when the surveys ended and cutting of infected trees began within the study sites in Miami-Dade County is unknown.

In the Broward site, trees were cut as soon after they were discovered. Once cut, they could no longer be considered the sources of citrus canker. But, a destroyed tree could be considered a source tree (PI tree) prior to its eradication. For example, a tree is discovered in June 1999 with 8 months lesions would be placed in the November 1998 time slot, as a newly infected tree (NI tree). So, it can be in the study for 8 months, although its real existence from discovery to eradication is only a few weeks.

So, continuing with the example, let us suppose that the tree is cut down the next month, in July 1999. The procedures, as stated by Dr. Gottwald, led to the conclusion that the tree would remain as a source tree. So, it is a phantom source tree. Distance calculations continue to be made between actual infected trees and those which no longer exist.

All infected trees in Broward County ultimately become phantom trees. If a tree has 8 month old lesions, and the IID is not less than the start date of the first period, it is likely to be a real infected tree (PI tree) for about 8 time periods, and a phantom source tree for the rest of the periods. It is likely nearly all infected trees within the last 6 periods (T18- T25) do not actually exist.

The effect of the phantom trees is impossible to determine. There is a chance the phantom trees would decrease the distance statistics in later periods. However, it is also possible the phantom trees could be the PI trees matched up with the more distant NI trees. In all cases, the distances would be invalid.

To clean up the field study data set, these phantom trees could have been removed from the post-eradication periods. Why was this not done? To remove phantoms would likely required a link between information within the CCEP database and the field study data set and likely lead to further disclosure.

The mechanics of removing a "phantom free" would be fairly difficult. Given the poor precision of the GPS meters with an location error of 50 ft, a citrus tree in the backyard could be in the homeowner's lot, the adjacent back lots, the two side lots or the two corner lots. The Department relied on hand drawn sketches of back yards to mark the location of the citrus trees. If these hand drawn sketches also had the GPS readings (longitude/ latitude) of each citrus tree, then a fairly reliable re-location of a tree would be possible. This is exactly the same as the problems of re-inspection as discussed in Appendix A.

This discussion likely extends beyond just the Broward sites. Certainly, with repeated inspections of yards, homeowners would likely know if their trees had canker. It would be logical to assume some homeowners would remove their infected trees. Trees are removed regularly in back yards for landscaping purposes particularly as homes are sold.

RESULTS PROVIDED IN TABLES 1 TO 5 IN THE 2002 ARTICLE

Distance necessary to circumscribe values are shown in Tables 1 to 5 of the 2002 published article. Only the first 9 synthetic time periods of the total of 25 periods are shown. The upper portion of Table 1 from page 368 is shown below. A period is 30 days.

Referring to Table 6, column 1 is labeled alpha tree age. As shown below, for time period 1, the alpha tree (PI) lesion age of all trees is greater than 25 periods, as calculated from the last time period (T25) in table. It has no real significance. If the study had been limited to 5 periods, then this number would have been 5 instead of 25.

Column 2 is the lesion age for secondary trees (NI) but the inequality sign is incorrectly. The first line in each table should read, $\geq 24\ and\ < 25$ instead of > 24. Similarly, the 60-day period, first line in each table should read, $\geq 23\ and\ < 25$ instead of > 23.

Columns 3 and 4 are the number of focal (PI) and secondary infected (NI) trees as per step 2 of the DNC procedure. Columns 5- 9 are calculated as per the DNC procedure, steps 3- 4. Columns 5 (% captured) is likely obtained by linear interpolation between two percentile value.

Columns 10 and 11 provide the disease incidence (DI) and delta disease incidences (DDI), respectively. The disease incidences are the fraction of trees diseased at the start of the pseudo time period, which is the number of PI trees divided by the total number of trees. The delta disease incidences are the incremental changes in incidences.

Table 6: Site D1, DNC Results

TABLE 1. Nearest neighbor distance calculations from citrus trees previously infected with Asiatic citrus canker caused by *Xanthomonas axonopodis* pv. *citri* to newly infected citrus trees in Miami, Dade County, site D1[a]

		(1)	(2)	(3)	(4)	(5) - (9)					(10)	(11)
Temporal period	Dates	Alpha tree age (30-day period)	Secondary tree age (30-day period)	No. of focal (alpha) trees	No. of secondary infected trees	% Captured at 38.1 m	Distance (m) needed to circumscribe			Max. distance (m)	Disease incidence (DI)	Delta disease incidence (DDI)
							90%	95%	99%			
30-day												
1st	10/26/97–11/24/97	>25	>24	4	10	0.00	1,127.76	1,159.32	1,159.32	1,159.32	0.0007	0.0007
2nd	11/25/97–12/24/97	>24	>23	14	9	22.22	769.35	769.35	769.35	769.35	0.0023	0.0017
3rd	12/25/97–1/23/98	>23	>22	23	17	0.00	548.64	599.16	599.16	599.16	0.0038	0.0015
4th	1/24/98–2/22/98	>22	>21	40	31	19.35	441.96	441.96	494.59	494.59	0.0066	0.0028
5th	2/23/98–3/24/98	>21	>20	71	51	50.98	137.16	228.60	360.40	360.40	0.0117	0.0051
6th	3/25/98–4/23/98	>20	>19	122	142	35.21	228.60	289.56	457.20	496.65	0.0201	0.0084
7th	4/24/98–5/23/98	>19	>18	264	216	44.44	152.40	228.60	441.96	552.98	0.0436	0.0234
8th	5/24/98–6/22/98	>18	>17	480	55	50.91	167.64	182.88	205.35	205.35	0.0793	0.0357
9th	6/23/98–7/22/98	>17	>16	535	68	63.24	106.68	106.68	135.10	135.10	0.0883	0.0091

-- Disease Progression

Disease incidences is shown in Figure 2 of the published article. Based on the results in Tables 1- 5, 18.3% of the trees became infected by the end of the study as shown below:

Table 7: Disease Progression in Study Sites

Site	Infected Trees	All Citrus	Percent Infected
D1	1758	6056	29.0
D2	971	6072	16.0
D3	26	798	3.3
B1	450	4730	9.5
B2	229	1113	20.6
Total	3434	18769	18.3

It is unknown the time span covering the discovery of the 3,434 infected trees, because the authors do not state when the earliest date which infected trees were discovered, nor when the last infected tree was discovered. This lack of information is discussed in Appendix A.   The best we can determine in the surveys continued at least until November 14, 1999, the end of the T25 period. The percentage of infected trees ranges from 3.3 to 29%.   There is no indication within the article of why there is such wide variations among the sites.

-- Inter-Tree Distances

The maximum inter-tree distance based on the 30-day scenarios is 3474 m (11,395 ft), from Site B1, period 1.   The minimum inter-tree distance based on the 30-day scenarios is 3.72 M (12.2 ft) from Site D3.

The authors state that the earlier time periods are more meaningful, as the long distances are less obscured.   This is based on the observation that as the density of infected trees increases, the inter-tree distances, generally declines. The basic observation is that the inter-tree distances in the first four periods, would bracket the 579 m (1900-ft) distance, if Site D3 is excluded. However, if Site D3 is included, the inter-tree distances would also bracket the 38.1 (125-ft) distance.

The complete table of inter-tree distances from T1 to T25 are not provided in the published article or in any other presentations.

--- Results used in other analysis

The results developed by the DNC procedure, in terms of the infection scenarios with NI and PI trees are used in most of the other analysis. So, the problems in obtaining reliable results with the DNC procedures, are not isolated to the results found in Tables 1- 5, but would permeate through the other analyses including the contour mapping.

4. Why Preliminary Results are Impossible

Drs. Gottwald and Graham published an article indicating that the origins of citrus canker in Miami-Dade County dated back to 1992 - 1993, based on an infected tree with 2- 3 year old lesions. The topic of lesion age estimation is discussed in the next section.

Of importance in this discussion is the effect of a newly discovered infected tree on the results of the field study. In accordance to the procedure, a newly discovered tree will be assigned a time period as an NI tree based on the calculated IID. However, this tree will also be assigned as a PI tree for every subsequent period.

Imagine if on the last day of the survey, an infected tree is discovered with lesions more than 2 years old. Because this tree would pre-date all NI trees, it would have to be assigned as a PI tree in every single one of the 25 time periods. Every single distance would have to be re-calculated.

There are five statistics presented for each time period (given 30-day scenarios), the percentage captured at 125-ft, the distances necessary circumscribe at 90, 95 and 99% levels, and the maximum distance. It is virtually impossible to have the same identical statistics with the addition of an infected tree which appears in every time period following its assigned time period as an NI tree.

If the lesion ages were small and relatively consistent, this would not be the case. However, in the Broward Court presentation by Dr. Gottwald, lesion ages were in the range of 2 to 9 months.

5. Lesion Age Estimation

The appearance of lesions are described in Chapter 3 of the book. Many articles as referenced in this chapter provide excellent discussions of the appearance of lesions.

Figure 2: Citrus Canker Lesions

For reference, the sections on lesion age estimation per the published article (1) follows:

> The diseased trees were visually examined by a single inspector, X. Sun, to maintain consistency... Other data recorded relative to each tree included ... (iii) age of the oldest lesion ... The age of lesions during a given survey can be based on phenotypic characteristics of infected host tissue.
>
> Surveys were conducted at least three times in each study area approximately 60 days apart. The age of lesions during a given survey can be based on phenotypic characteristics of infected host tissue. From the above data, the infection initiation date (IID), i.e., the date that the oldest lesion found in a tree, was estimated to have been established, relative to the date discovered and taking into account latency of infection of approximately 14 days.
>
> Each tree was assigned an IID value, based on an estimate of the date the tree became infected. For the purposes of the study, the IID values were separated into contiguous 30-day categories. This was done because some error in visual estimation of the infection date was assumed.

It is mentioned in the above paragraph that latency is about 14 days. The discussion is vague on how this was taken into account, but presumably, 14 days were added on to estimates of the oldest lesion.

THE 30-DAY PERIOD- A REMEDY FOR LESION AGE ERRORS?

In the excerpt sections, it is claimed the 30-day time period was chosen to reduce the effect of lesion age error. Interestingly, the authors previously claimed the other time periods, 60, 90, and 120 days were selected to correspond to the time to resurvey an area. Further, the DNC procedures do not permit NI trees to infect other NI trees for 30 or 60 days, depending on the time period used.

However does the 30-day time period help correct for lesion age error? Let us suppose that lesion ages can be determined to within 5 days, so the error could be in the range [-5, 5], with each value having equal probability. What is the probability that a single infected tree is misclassified and put in the wrong time period? The table below shows the percentages of errors. Listing of the program used in these calculations is provided at the end of the appendix.

Table 7: Lesion Error Effect on Misclassification (30 day periods)

Error	Range*	% error
5 days	-5 to 5 days	9.1
10 days	-10 to 10 days	18.0
15 days	-15 to 15 days	26.0
30 days	-30 to 30 days	51.0
60 days	-60 to 60 days	75.0

* Occurrence of errors assumed uniform. discrete distribution.

Actually, the problem is worse than shown above, because every error creates an infected tree in a time period where it should not be, and leaves out an infected tree in the correct time period. If there are 100 trees that should be in a certain time period, it is expected that 36 trees will be misclassified and placed in the prior time period and 36 trees will be placed in the subsequent time period, leaving 28 trees correctly classified.

The consequence of misclassification leads to incorrect inter-tree distance values. In a memorandum from Mr. Richard Gaskalla, it was suggested that the errors in lesion age would cancel out as the number of infected trees increased. Just the opposite occurs, as the number of infected trees increases, the chances of the number of NI trees in any period being correct decreases, as does the probability of any of the percentile measures being correct.

CANKER IDENTIFICATION AND LESION AGE ASSESSMENTS

The information needed to calculate the initial infection date is the age of the oldest lesion. An error of one day can cause the tree to be assigned to the incorrect time period.

There are serious identification problems with citrus canker. This information is also discussed in Short Note 3.3 Misidentification and Detection Errors and Eradication Policy. If canker can not be reliably identified in every lesion, then one can not make a reliable determination of the oldest lesion age.

Identification errors occur on leaves and fruit of citrus trees. On leaves, citrus canker can be confused with citrus bacterial spot (CBS), greasy spot, citrus scab, leprosis. On fruit, citrus canker may be confused with alternaria, citrus scab, damage, and leprosis. In the presence of damage, the lesion may follow the contours of the damage therefore not being circular. This is true if citrus canker is in the serpentine mines left by the citrus leafminer larvae. In older lesions a saprophytic white fungus may grow over the center of the lesion. (11)

Problems with Older Lesions

Dr. Canteros has been studying citrus canker for over 30 years in Argentina. She states in a 2004 article that most misidentification errors occur when only old lesions are present. (9) One problem is fungal diseases growing in the center of the lesion. A second problem is the chlorotic yellow and brown halos begin to fade. The images are from Dr. Chaloux with the USDA/ APHIS. The complete presentation is posted to this website. (10) The oldest lesion on a tree, is likely to be the smallest one and likely mistaken for citrus scab.

Figure 3: Chlorotic yellow and brown halos fade with age on leaves

The final problem relates to the care given to citrus trees by residents. As canker symptoms be came evident, residents who did not want their tree to be discovered by canker inspectors or felt they could treat the tree themselves, it is likely they would prune the lower branches. The less evident older lesions on the top branches would be missed.

Lesions in Wounds or Citrus Leafminer

Canker bacteria can enter injuries to leaves and insect wounds. As stated in reference 11:

> In the presence of damage, the lesion may follow the contours of the damage therefore not being circular. This is true if citrus canker is in the serpentine mines left by the citrus leafminer larvae.

Examples are non-circular lesions are provided by Gottwald et al (reference 12) and provided in the online supporting documents website.

Figure 4: Citrus canker in Serpentine Mine of CLM (Ref 12)

Figure 5: Citrus canker in leaf wounds (Ref 12)

Symptoms of canker were apparent in only a portion of the mines in Figure 4. The symptom may be only a small irregular brown spot. Leaf wounds may be irregular, or slight pin pricks. Damage on leaf edges way be caused by the larvae of giant swallowtail. Pruning activities such as topping off of trees can cause many wounds. It may be difficult early on to distinguish between the damage caused by the injuries and citrus canker, particularly if inspectors are expecting the more common circular patterns.

Inspection Difficulties

The inspectors did not bring ladders. Citrus trees in the backyards of residents can be 15-ft or taller Unless a citrus tree is small, a complete inspection is impossible. Citrus cankers can appear on the stems, fruit and leaves of the plant. Lesions are likely to be occur first on the most susceptible parts of the plant- the new flushes. Many of these may be located at the very top of the tree. Homeowners can prune or topped off by their owner. Citrus canker encourages early leaf drop (3), although leaf drop particularly during the colder months is normal. One can see immediately why a homeowner might want to simply prune off fruit with so many cankers in hopes of getting rid of the disease.

Lesion Age Estimation

Lesions are obviously in a continuous process of change. The plant has mechanisms to resist infection, but is being slowly overwhelmed by the bacteria. Dr. Gabriel of the University of Florida described the process similar to a volcano which has erupted. The bacteria are never in the state of dormancy, but the multiplication of cells is reduced during the winter, resulting in a breakthrough to the surface. Similarly, if it appears the expansion rate has stopped, in reality the changes are visually imperceptible.

According to Schubert et al. (3) symptoms may take 60 days or more to appear under non-optimal incubation and infection conditions. This is likely an observation from commercial groves as reported by Loucks (1934). The period may be considerably longer, perhaps 5 to 6 months if the infection occurs late in the summer. The symptoms may not appear until the following spring or summer. New flushes are particularly susceptible.

According to Schubert's article, lesions on the leaves of citrus plants expand at a rate of approximately 1 mm/month (0.04"/month) for the first 6 to 8 months and their expansion rate slows or stops after this period. Schubert concludes it is possible to determine approximate age of lesions <u>within a few months</u>. This range of accuracy, I believe, may be possible in some cases, and impossible in others. As discussed, in other cases, the error needs to be expressed in a few years.

The prevalence of lesions on a citrus tree is likely a poor indicator determinant of the time to first infection. The concentration of bacteria which oozes from a lesion when in contact with water is dependent on many factors. Pruvost identified temperature as the most critical factor. Winter temperatures are highly variable during December in the southern area of Florida, and would influence the spread of canker on citrus trees.

It is further suggested, from the scientific literature, that it is impossible to reliably estimate the initial infection event as <u>a specific calendar date</u> as required by the DNC procedure based on lesion size or the characteristics of individual lesions. The rate of expansion is far to small and irregular, as easily observed from many photographs, to possibly back date each lesion to the day it became infected. No one can observe a 1 mm change in diameter, looking up at a tree, with lesions 10 to 20 ft away in some cases. At least, to date, there are no articles within the scientific literature which show estimation of lesion ages in terms of days, can be determined through visual observations.

It is also noted that the lesion ages of infected trees during the data collection of the field study and an essential part of distance calculations has never been made public.

LESION AGE DETERMINATION- DR. SUN COMMENTS

On June 17, 2002, the Miami Herald published a discussion of the Florida field study, prepared by Georgia Tasker, a long time feature writer at the Miami Herald (4). The article included my comments, along with Dr. Sun's response:

> Lord also believes it's hard to precisely identify the age of a canker lesion when looking for canker spread patterns. "There's no real scale [such as] 'if the lesion is one millimeter, it has to be 15 days old.' There's no guide."
>
> Sun said he begins with the most heavily infected area within the tree. "So I looked at the site, then the branches and leaves. Then if the lesion is on a new flush of leaves of 1 - to 2- year old leaves. When I look at the leaves, I look at the lesion size, the texture, whether it is raised and ruptured, if it has a secondary fungal infection.
>
> It takes time. But I am able to determine within <u>two weeks</u> whether a lesion is 2, 3, 4, 5 or 6 months old."

I have underlined two weeks because it is critical. This two weeks accuracy is, of course, contradicted by Schubert's article of stating lesion ages could be identified approximately <u>within several months.</u> How long it takes any lesion to appear, could be 60 days or longer, according to Schubert.

But Dr. Sun is a co-author of Schubert's 2001 article. So, in essence, he is contradicting himself. The article was published in April 2001, and in June 2002, he is saying something different. Even if the lesion age could be determined +/- 15 days, it would still create 26% classification errors.

Dr. Sun implies in the Miami Herald article (4) that he personally inspected all the infected tree as the follows, "Survey people found trees with signs of canker, they reported to me and I went to the field," Sun said. A total of 3444 infected trees was tabulated from the published article on the field study. Dr. Sun would normally work at FDACS/ DPI headquarters, located in Gainesville, Florida.

At the Rule Development Workshop, Public Hearing on November 14, 2001, the question and answers between Mr. Gaskalla and I were as follows:

> Mr. Lord: Doctor Sun participated in this data collection, so he was responsible for the estimation of the oldest lesions. Is that correct?
> Mr. Gaskalla: Doctor Sun and others.
> Mr. Lord: The wording in this document says he was solely responsible for the estimation of the oldest lesion.

Mr. Gaskalla: For the estimation.
Mr. Lord: Did he actually go to each property to find the oldest lesion?
Mr. Gaskalla: To the best of my knowledge, yes.

The 2002 published article states that diseased trees were inspected by a single inspector, Dr. Sun. Mr. Gaskalla contradicts the 2002 article by saying that the inspections were done by Dr. Sun and others. Yet his response lacks clarity. According to Jack Haire, the Chief Plaintiff against the Department in the Broward cases, Dr. Sun stated he gave permission for a helper to do some of the inspections during a deposition.

Lesion Ages- Qualitative Approach

Precise estimation of lesion age in terms of days seems impossible, given all the factors involved including the frequency of inspections The overwintering, or a period of dormancy lasting several months, was first recognized in an article published in 1918. (13) Even in this study, the leaves were inoculated with the bacteria, so scientists knew what to look for and where lesions would likely appear.

The manifestation of symptoms is a continuous process, but for descriptive purposes, it may be perfectly rational to group symptoms, into an early, middle and late stage. Each stage is fraught with problems in lesion age identification. At the early stage, misidentification is likely. Few lesions are present, which may disappear with pruning or leaf drop. The period of dormancy varies depending on the cultivar and temperature. Likely, other factors play a role. The presence of citrus leaf miner, is known to accelerate the development of lesions.

Late stage development, where raised lesions are prevalent, would minimize misidentification problems, but other problems become more probable, such as an owner pruning off infected leaves and fruit. An owner can easily remove the infected plant.

Schubert et al states that the 1997 campaign met with only limited success, mainly due to a lack of resources and being understaffed. If this were the case, would the Department in 1998 allocated inspectors to conduct repeated surveys on an area of 7 to 13 square miles for a period of 18 months?

6. Concluding Remarks

In this review, it was necessary to explain clearly how the DNC procedure worked, before examining the necessary data. An example of the DNC procedure was used to clarify the procedure. The procedure requires estimation of the initial infection date (IID) based on the age of the oldest lesion.

If the date of initial infection could be determined, this would seem to be of great benefit to grove owners. Let us suppose that a seedling shows the oldest lesion that predates the purchase of a tree. Then the grove owner could go back to the nursery with proof that the tree was already infected with canker prior to purchase.

Unfortunately, the oldest lesion age can not be determined exactly, so the DNC procedure is not a reliable method to calculate distance of travel of citrus canker between two infected trees. To avoid errors, the age of the oldest lesion must be estimated within one day.

Since citrus trees regularly defoliate, the oldest lesion may be long gone when an inspector discovers an infected tree. Pruning activities is another way the oldest lesion can vanish.

Further, incorrect associations may include source trees at locations “just over the fence” from the infected trees which are excluded because they are not within the site. So the problems with the procedure occur in both time and space.

As a practical matter, the fruit with the most lesions is likely the first the homeowner would discard as it is unappealing. Similarly, the homeowner would likely prune off stems and branches showing lesions. If inspections were occurring regularly on the property, this would provide incentives to homeowners to remove infected limbs to save the tree from the eradication crews.

I was unable to find any example of the DNC procedure used for citrus canker or any other plant disease after the published article in 2002. I can not say that I have searched all the journals on plant diseases and epidemiology, so if I receive any article, it will be posted on the website.

Short notes 3.3 and 6.2 provided related information on identification problems and the relationship between the published articles and the 1900-ft policy. It is important to remember that Dr. Gottwald, the principal investigator of the field study, stated that the 1900-ft policy was not decided upon by any report, but by the consensus of regulators, researchers and others at an ad hoc meeting in December 1999.

References

1. Gottwald, T.R., X. Sun, Riley, T. Graham, J.H., Ferrandino, F. and Taylor, E., 2002, Geo-Referenced Spatiotemporal Analysis of the Urban Citrus Canker Epidemic in Florida, Phytopathology, Vol 92, No. 4.

2. Gottwald, T.R. , 1999, Citrus Canker Spread Study in Urban Miami, internal document submitted to the Broward Court, in Case 00-18394 (08) CACE. The report is undated, but a transmittal cover letter from Dr. Gottwald to Dr. Dixon of FDACS/ DPI is dated October 13, 1999.

3. Schubert, T.J. , Gottwald, T.R., Rizvi, S.A., Graham, J.H., Sun, X., Dixon, W.N., 2001, Meeting the Challenge of Eradicating Citrus Canker in Florida- Again, Plant Disease, Vol. 85-4.

4. Tasker, Georgia, 2002, The 1900-ft War, Miami Herald article, June 17, 2002.

5. Pruvost, O., Bober, C., Brocherieux, C., Nicole, M., Chiroleu, F., 2002, Survival of *Xanthomonas axonopodis* pv *citri* in Leaf Lesions Under Tropical Environmental Conditions and Simulated Splash Dispersal of Inoculum, Phytopathology, Vol 92.

6. Public Hearing Rule Development Workshop Transcript, November 14, 2001, page 203. beginning line 16, link available on website.

7. November 5, 2000 Canker Hotline shows breadth of residents; ire 22,609 complaints logged in 13 months, Miami Herald

8. Gottwald, T.R., Hughes, G., Graham, J.H, Sun, X., Riley, T., 2001, The Scientific Basis of Regulatory Eradication Policy for an Invasive Species, Phytopathology, 91:30-34.

9. Canteros, B.I., 2004, Citrus Canker in Argentina— A Review, Proc. Int. Soc. Citriculture, Paper No. 9, 2004.

10. Citrus Canker Pathology Training Presentation, Citrus Health Response Program, Texas A+M, (provided on website).

11. Website: www.idtools.org, based on collaborative effort of the USDA/APHIS and FDACS/DPI. Citrus canker factsheet:

http://idtools.org/id/citrus/diseases/factsheet.php?name=Citrus%20canker

12. Gottwald, T. R., Graham, J. H., and Schubert, T. S. 2002. Citrus canker: The pathogen and its impact. Online. Plant Health Progress doi:10.1094/PHP-2002-0812-01-RV.

13. Peltier, G.L., Neal, D.C., 1918. Overwintering of the citrus-canker organism in the bark tissue of hardy citrus hybrids. Journal of Agricultural Research, September 9, 1918.

MATLAB Program

```
%
%  ---  Simulation of Misclassification error
%
niter = 100000;
x = randi(30,niter,1);
r = 5;
a = 2*r+1;
b = r+1;
err = randi(a,niter,1)-b;
d = x + err;
c = d > 30 | d < 1;
tally = sum(c)/niter*100
```

In the above program, there are 100,000 iterations. The matrix x contains random numbers corresponding to the iterations, given that the actual IID of the oldest lesion is equiprobable from 1 to 30 days.   A 5 day error results in a range from -5 to 5 days, so “err” generates error values. The matrix d contains all iterations of the estimated values given the error and “tally” provides the estimated occurrence of error.