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Agricultural Research Service

South Atlantic Area

U. S. Horticultural Research Laboratory
2120 Camden Road
Orlando, FL 32803-1419
Voice: (407) 897-7347
Fax: (407) 897-7309
Email: t.r.gott@worldnet.att.net

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Dr. Wayne Dixon
Bureau Chief
Florida Dept. of Agriculture and Consumer Services
Division of Plant Industry
P.O. Box 147100
1911 SW 34 St.
Gainesville, FL 32614-7100

Dear Wayne,

Here is the draft report on the Miami Citrus Canker Epidemiology study that I was in hopes of getting to you about a week ago. Sorry for the delay and again sorry for missing the teleconference last week. However, it is probably better that I did miss it or I would have been hacking and wheezing in everyone's ears.

Please note that the report is marked *Not For Circulation* and is really just a comprehensive update on some of the data and analyses to date for your information. It is not in any way intended to be a publishable paper and there are a number of additional analyses I want to run prior to publishing the data. The paper that will likely result will be much more comprehensive in Materials and Method, Results and Conclusions. However, the data will not change, so for regulatory purposes this report may be useful for planning eradication/disease suppression activities.

I have made several copies of the report for the RAG people and of course Gaskella. You may need to make more copies if I have miscounted. We may want to share some form of this report with the Canker SIWG in the future also. See you next week at the Canker TAC meeting.

Sincerely,

Tim

Tim R. Gottwald
Research Plant Pathologist

Agricultural Research Service

FDAS000289

Citrus Canker Spread Study in Urban Miami
[Preliminary Draft Report, Not for Circulation!!]

Tim R. Gottwald, Xiaon Sun, Tim Riley, Jim Graham, and Gareth Hughes

Background: Questions continue to arise concerning the use of 125 foot radius around diseased trees to identify "Exposed" trees for eradication in an urban setting. Mr. Ken Bailey and Mr. John Thomas requested assistance to determine the validity of 125-foot radius as previously suggested by Stall and Miller, from experimental observations in Argentina. The concerns are: 1) Are the experimental and meteorological conditions under which the data were collected in Argentina applicable to Florida? and 2) Since the Argentine data were collected under grove conditions, how do these relate to the urban situation where eradication is ongoing in Florida?

In August 1998 a cooperative CCEP, ARS, and UF research effort was established to address these issues. At that time the Florida Commissioner of Agriculture placed a moratorium on further destruction of exposed trees by the CCEP until the research was completed.

Objectives:

- Determine the distance of spread from point sources (foci of infection) in an urban setting.
- Examine spread resulting from normal rainstorm events.
- Examine spread resulting from catastrophic meteorological events, i.e., tropical storms, hurricanes, tornadoes, if they occurred during the study period.
- Evaluate current use of the 125-foot radius for defining exposed trees in relation to research results.
- If necessary, provide demonstrable evidence for any adjustment of radius distance.

Materials and Methods:

Experimental Design: Three study sites were selected within the urban Miami area with discrete focal trees from which to measure disease spread. A fourth site was subsequently selected in Broward Co., just north of the quarantine boundary at that time. Within the Miami sites, the CCEP agreed to curtail all eradication efforts until the experiment was concluded. This was not an imposition because at that time the CCEP had a large backlog of canker-infected trees to remove over the entire Miami, Dade Co. area. Eradication efforts were simply shifted to other areas. Miami, Dade Co. sites 1 and 2 consisted of ca. 4 and 2 square miles of urban area, respectively, as defined by STR's. Site 3 consisted of 3 infected trees all within 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 1.0-mile radius. Because the Broward County site was north of the quarantine boundary and was considered a potential imminent source for continued northward spread of the disease, it was treated differently. In Broward County, canker-infected trees were removed as they were identified. Most removals occurred within 2-4 weeks of detection.

Within each site, surveys were conducted to identify all diseased and healthy citrus trees. Using differential GPS, the exact position (+/- 2 to 7.5 m) was recorded for each healthy and diseased tree. Other data recorded relative to each tree was: 1) Cultivar, 2) Age, 3) Age of oldest lesion (estimated by visual examination by Dr. Xiaoan Sun), 4) Severity of infection, and 5) section of tree Infected, i.e., directional quadrant – N, E, S, W and portion of tree infected – Top third, Middle third, Bottom third). Surveys were conducted at least three times in each study area approximately 60 days apart. From the above data the "infection initiation date" (IID), i.e., the date of the oldest lesion found in a trees was calculated to have been established. This date was used in all future calculations.

Differential GPS Measurements: When measuring distances between two points, GPS accuracy must be taken into account. For this project Garmin hand held GPS units were modified by the addition of differential GPS (DGPS) modules. These modules used a US Coast Guard transponder in Biscayne Bay, Miami as a known reference point to correct the signal from individual satellites for the U.S. Military imposed inaccuracy algorithm imposed on civilian GPS units for national security reasons. The use of DGPS provided routine readings in the Miami area with an accuracy of ±7.5 m meters or less. Therefore when calculation distances between two points determined by DGPS, each position is considered to be accurate within a radius of ±15 meters (±49 feet) considering the worst case measurement error between two points (Fig. 1). In practice the majority of position readings were more accurate than ±7.5 m and thus this error is an over estimate in most cases.

Latitude and longitude position for each tree was taken in decimal degree format. These were converted to Universal Transverse Mercator (UTM) via a series of spreadsheet calculations provided by Dr. Donald Stierman, Associate Professor, Department of Geology, University of Toledo. This series of transformations changes Latitude/longitude measurements made on the surface of the earth, i.e., a sphere, to a flat Cartesian coordinate plane for more accurate calculation of distances between points in meters. This was done for all trees in all four study sites.

Distance calculations: The distance between any two points in the Cartesian coordinate plane was calculated as the length of the hypotenuse of a right triangle whose perpendicular sides represent the north and east distances between the two points.

Each tree was associated with an IID, which was 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 it was believed that some error in visual estimation of the IID was possible and estimates to the nearest 30-day period were both valid and sufficient for the purposes of the study. For each site, distance measurements between focal trees and newly infected trees were calculated for various temporal windows. These temporal windows were 1, 2, 3, and 4 months in duration and were chosen for their relative regulatory significance. That is, the ability of the CCEP to return to an area every 30, 60, 90, or 120 days to resurvey it. Resurveys are dependent upon the ever-expanding quarantine area and available manpower. Therefore, for example for 30-day windows, the infected trees which existed in the area during the first 30 days were considered focal or 'alpha trees' that gave rise to the subsequent diseased trees which had IIDs in the second 30 day period. For the next 30-day temporal window, 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.

A Visual Basic routine was used to calculate the distances between each newly diseased tree and all prior focal trees. The shortest distance was stored and then the process repeated for each newly diseased tree in the study area. This 'nearest neighbor' concept was used throughout the study. It is of course very possible that pathogen spread occurred from source trees to secondary infected trees that were not nearest neighbors. However, this nearest distance was used because it was considered to be the most conservative estimate possible. Data generated in this way is therefore likely to be an ***underestimate*** of spread but is a good estimate of the minimum possible distances of spread. Because of the possible error associated with the DGPS estimates explained above, distances of spread were parsed into consecutive 15 meter (~50 ft) distance categories and plotted as frequency distributions. This was done by second Visual Basic routine.

A Third Visual Basic routine was used to calculate the distance from focal trees to all possible subsequent infected trees. For ease of calculation, these distances were parsed into contiguous 100ft distance categories. This calculation represents an ***overestimate*** of spread but calculates the longest or maximum distance of spread that could have occurred. The ***actual*** distance of spread can never be determined from this data set, but intuitively would be between these two extremes.

Additional data caveats: As time progresses, the distance calculation between temporal windows becomes more and more conservative. This is because as the number of focal trees increases over time and the possible distance of spread from a newly-infected tree to it's nearest focal tree decreases simply because the number of focal trees and their distribution within the study site increases. Therefore the most accurate estimates of spread result from consideration of the first or second temporal windows. Another factor is of course weather. Meteorological conditions continually change and thus no two time periods have the same number of storms, and each storm has different amounts of rainfall, rain intensity, wind speed, and wind direction. Finally, host tree susceptibility is continually changing. Unlike a commercial citrus plantation, urban trees are not of uniform cultivar or care. Therefore, the number and duration of new flushes of foliage continually changes over time and is dependent on cultivar, age, fertilization and general health of a tree. These factors varied widely and were continually in flux within the urban areas studied.

Temporal disease progression: Disease progress curves for each of the four study sites and an overall disease incidence considering all study sites were plotted versus time. In addition ***rain*** and ***rain*wind*** indices was also plotted versus time. Cross correlation analyses were conducted to determine the temporal offset of disease progress for each stud site in relation to the weather indices parameters.

Results:

Preliminary results from the spatio-temporal studies are presented in Tables 1 to 4 associated with Miami study sites 1, 2, 3 and Broward Co., respectively. Each table presents the proportion of newly diseased trees that were estimated to occur within 125 ft of nearest focal trees. Subsequent table columns represent the distance category (±15 meters ~49 feet) that was necessary to circumscribe 90, 95, 99 and 100 % of all newly infected trees, during the indicated time period (temporal window) of the test.

Histograms of frequency distributions were produced for each of the temporal windows tested for each of the test sites. These were too numerous to present here. Therefore, Figure 2 is presented as an example and represents a single frequency distribution of citrus canker spread relative to one of the temporal windows for one of the test sites.

Figures 3 to 6 represent frequency distributions for each study site. These frequency distributions represent all possible distances of spread from minimum to maximum with the study area. Peaks in this distribution represent the most common distance categories calculated.

Both the normalized [cumulative precipitation (cm per day)*100] index and the normalized [wind gust (meters per sec)*precipitation (cm per day)*100] index were found to correlate well with normalized disease increase [number of diseased trees over time in days] (Table 5 and Fig. 7). However, from a biological perspective, the index that accounted for both wind and rain makes more since and had more reasonable temporal offsets. When considering both wind and rain in the index, the cross correlation analysis indicated a temporal offset of 13 to 198 days with the highest correlation between disease incidence and the precipitation*wind-gust index of $r^2 > 0.96$. Perhaps study sites 1 and 2 are the most interesting because they have the largest number of diseased trees to use for the estimate. For these two study sites, the temporal offset of 101 to 111 days was indicated. Study site three was not as informative because it only had 31 diseased trees at the end of the study. The Total disease category (includes all sites) is also of interest because it takes into account all diseased trees in the study from all study sites. When considering all diseased trees within the study, the temporal offset was determined to be 79 days. These results indicate that disease was visually detected with the highest accuracy by survey teams ca. 79 to 111 days after infection caused by wind-blown rain takes place. The Broward Co. Study site does not correlate well with either weather index (best correlation is minus 8 days). This may be because the weather information used for the indices was collected by the National Weather Service at the Miami International Airport which is 13-16 miles south of the Broward study site. Thus the weather in the Broward site may have been somewhat different and not well represented by the Miami Airport weather data.

Conclusions:

The tables and figures presented are preliminary results to date. More exhaustive analyses are continuing in collaboration with Dr. Gareth Hughes, University of Edinburgh, Scotland, UK. Future analyses will perhaps include exploration of the use of Ripley's *k* statistic and stochastic modeling of spatial point patterns over time as suggested by Peter Dingle in '*Statistical Analysis of Spatial Point Patterns*', Academic Press, London, 1983. In addition the temporal aspects of spread are being examined in relation to meteorological data from the National Weather Service to examine the wind and rain patterns that occurred in Miami during the duration of this study and to more closely correlate specific meteorological events with spread. To accomplish this, Doppler radar scans of the Miami area will be used to examine rainfall and wind vectors

[Note: this page has been transcribed from a copy of the original, as the copy in parts was not legible after scanning]

Doppler radar scans of the Miami area will be used to examine rainfall and wind vectors associated with significant storm events and attempts will be made to correlate these data with the patterns of disease spread.

Although these further analyses will likely provide greater insight into the spatial distribution and dynamics of citrus canker in an urban environment, they not change the preliminary results, which may be the most important for regulatory purposes, For regulatory purposes probably the most important results obtained were the measurement of minimum distances of spread from focal trees to newly infected trees in the study areas. **As** indicated above, these measurements are highly conservative and based solely on nearest neighbor distance estimates. In many cases, spread of citrus canker was undoubtedly due to dissemination of inoculum from focal trees at greater distance than simply the nearest previously diseased tree. Unfortunately, this cannot be determined from the present data sets. However, it is the belief of the authors, that the data presented here conclusively demonstrate that spread of citrus canker in urban Miami occurs over distances considerably greater than 125 and that 125 ft would be inadequate to contain the disease and or curtail further spread.

We should consider that spread of the disease over some of the larger distances measured could have been the result of movement of inoculum by human or some other mechanical means Thus we might want to use the 95 or 99% levels to estimate maximal spread. Even so, it would appear from examination of results of the calculations presented that radii of 2000 ft or greater would be necessary to define exposed trees for removal to contain spread in most cases.

If we consider the temporal results mentioned above, the best visual detection of disease was a little over three months after a dissemination event. There has always been considerable discussion that increasing survey efforts to decrease the time period between repeat surveys of an area would offset the need to increase the 125 ft radius. This would not appear to be upheld by the results of this study for several reasons: 1) Single storm events seem to be capable of moving the disease far in excess of the 125 ft distance, 2) the ever-expanding canker quarantine area makes it less and less possible to resurvey all infested sections in **a** timely manner even with increases in manpower 3) survey crews never have 100% access to all properties and 4) surveys are less sensitive than we would like and numerous small infestations of the disease are not picked up until subsequent surveys. Thus the conclusion that the 125 ft radius could still be used could well lead to a false sense of security that the disease can be managed simply by increasing the frequency of resurvey. In our estimation this is unlikely to hold true.

Consistency of findings: The findings of the current study of spread in urban Dade and Broward Counties are not inconsistent with those previously described under South Florida Grove conditions (Smoak Grove) discovered in October 1990. See Plant Dis. 76:389-396. 1992. In the case of the Smoak Grove outbreak, inoculum was determined to have originated from lemon trees in adjacent property and disseminated to new foci of infection in the neighboring Smoak grove to the West. Meteorological data from the National Weather service were examined. The age of the oldest lesions in the Smoak grove was temporally consistent with a mid-August 1989 storm that passed over the area. No other storms of significance occurred around this time. Source –to-secondary-foci distances for the Smoak Grove outbreak were measured to be:

- Focus 1 - 230 m (754 ft.)
- Focus 2 - 410 m (1345 ft.)
- Focus 3 - 810 m (2657 ft.)

These measurements are within the same ranges found in the urban Miami study discussed above.

[Note: Tables were transcribed from originals as shaded areas (first row and column) were illegible after scanning]

Table 1: Citrus Canker Urban Miami Epidemiology Spread Study Site 1

Temporal Window	No Focal (Alpha) Trees	No 2ndary Infected Trees	% Captured at 125 ft	90%	95%	99%	Max Distance
1st 1-Mo. Window	38	15	13.3	800	4150	4150	4150
2nd 1-Mo. Window	52	39	41.0	1450	1450	1650	1650
3rd 1-Mo. Window	90	73	43.8	1200	1600	1900	1900
4th 1-Mo. Window	162	235	35.7	700	800	1450	1850
5th 1-Mo. Window	396	124	44.4	350	500	700	750
6th 1-Mo. Window	519	32	71.9	250	950	950	950
1st 2-Mo. Window	38	53	28.3	1450	1450	4150	4150
2nd 2-Mo. Window	90	307	25.7	1050	1400	1650	2100
3rd 2-Mo. Window	396	155	45.8	350	600	950	950
4th 2-Mo. Window	530	490	64.1	300	350	700	850
1st 3-Mo. Window	38	125	25.6	1400	1450	3200	4150
2nd 3-Mo. Window	90	430	25.1	950	1250	1600	2100
3rd 3-Mo. Window	396	420	54.3	350	450	700	950
1st 4-Mo. Window	38	359	15.6	1400	1650	2150	4150
2nd 4-Mo. Window	90	461	24.7	950	1300	1800	2250
3rd 4-Mo. Window	396	644	54.0	350	650	850	950

Table 2: Citrus Canker Urban Miami Epidemiology Spread Study Site 2

Temporal Window	No Focal (Alpha) Trees	No 2ndary Infected Trees	% Captured at 125 ft	90%	95%	99%	Max Distance
1st 1-Mo. Window	21	17	17.5	2050	3400	3400	3400
2nd 1-Mo. Window	28	7	14.3	950	950	950	950
3rd 1-Mo. Window	30	1	0.0	450	450	450	450
4th 1-Mo. Window	53	23	43.5	450	500	700	700
5th 1-Mo. Window	130	31	51.6	450	1050	2050	2050
6th 1-Mo. Window	253	48	75.0	400	450	550	550
1st 2-Mo. Window	21	24	12.5	2700	3050	3400	3400
2nd 2-Mo. Window	30	23	43.5	450	500	700	700
3rd 2-Mo. Window	82	80	55.0	400	500	2050	2050
4th 2-Mo. Window	253	205	42.9	800	800	1900	1950
1st 3-Mo. Window	21	24	12.5	2750	3050	3400	3400
2nd 3-Mo. Window	30	54	40.7	450	700	2050	2050
3rd 3-Mo. Window	82	179	42.5	550	1050	3050	3050
4th 3-Mo. Window	253	210	42.9	800	1000	1900	1950
1st 4-Mo. Window	21	47	12.8	2150	2750	3400	3400
2nd 4-Mo. Window	30	102	38.2	450	600	1600	2050
3rd 4-Mo. Window	82	283	35.0	1500	2250	3050	3100
4th 4-Mo. Window	253	278	40.6	750	1000	1950	2200

Table 3: Citrus Canker Urban Miami Epidemiology Spread Study Site 3

Temporal Window	No Focal (Alpha) Trees	No 2ndary Infected Trees	% Captured at 125 ft	90%	95%	99%	Max Distance
1st 1-Mo. Window	2	2	50.0	200	200	200	200
2nd 1-Mo. Window	8	6	33.0	1950	1950	1950	1950
3rd 1-Mo. Window	15	10	10.0	900	900	900	900
4th 1-Mo. Window	18	5	20.0	850	850	850	850
1st 2-Mo. Window	2	4	0	650	650	650	650
2nd 2-Mo. Window	4	3	33.3	200	200	200	200
3rd 2-Mo. Window	7	7	28.6	1950	1950	1950	1950
4th 2-Mo. Window	15	15	13.3	900	900	900	900
1st 3-Mo. Window	2	2	0	650	650	650	650
2nd 3-Mo. Window	3	3	33.3	200	200	200	200
3rd 3-Mo. Window	7	7	0.0	1950	1950	1950	1950
4th 3-Mo. Window	15	16	12.5	2000	2200	2200	2200
1st 4-Mo. Window	3	4	0.0	650	650	650	650
2nd 4-Mo. Window	7	3	33.3	200	200	200	200
3rd 4-Mo. Window	7	7	0.0	1950	1950	1950	1950
4th 4-Mo. Window	9	16	12.5	2200	2200	2200	2200

Data for the above table were not obtained from consecutive windows as were the data for sites 1 and 2. This is because the limited number of infected plants in this very small study site. Rather, temporal windows were chosen which represented time periods when disease changes were noted.

Table 4: Citrus Canker Urban Miami Epidemiology Spread Study Site Broward Site

Temporal Window	No Focal (Alpha) Trees	No 2ndary Infected Trees	% Captured at 125 ft	90%	95%	99%	Max Distance
1st 1-Mo. Window	3	42	7.1	1140	19450	19700	19700
2nd 1-Mo. Window	44	50	40.0	1100	10750	20800	20800
3rd 1-Mo. Window	93	15	13.3	1350	2700	2700	2700
4th 1-Mo. Window	107	15	40.0	2950	58850	58850	58850
5th 1-Mo. Window	121	109	50.5	900	1400	3150	3200
6th 1-Mo. Window	229	63	61.9	700	2050	2350	2350
1st 2-Mo. Window	3	91	3.3	19400	19650	40300	40300
2nd 2-Mo. Window	93	29	20.7	2850	2950	58850	58850
3rd 2-Mo. Window	121	170	49.1	900	1600	3150	3200
4th 2-Mo. Window	291	173	33.5	850	1750	4450	10100
1st 3-Mo. Window	3	105	2.9	19400	19650	19800	19800
2nd 3-Mo. Window	93	137	40.9	1700	2800	3200	58850
3rd 3-Mo. Window	121	216	45.4	1150	2100	2400	3200
4th 3-Mo. Window	291	306	24.3	1250	1850	3500	10100
1st 4-Mo. Window	3	118	2.5	19400	19650	19800	19800
2nd 4-Mo. Window	93	199	40.7	2250	2850	4300	58850
3rd 4-Mo. Window	121	340	31.5	1400	2050	2400	3200
4th 4-Mo. Window	291	327	22.1	1250	1850	4450	10100

This study site differs from the other three in that infected trees were removed as rapidly as possible after detection. This was not the case for sites 1, 2, 3 in Miami, Dade County.

Table 5. Cross-Correlation of Cumulative Rain and Wind with Disease Incidence.

Study Site	Disease vs. Precipitation X 100		Disease vs. Precip. X 100 X Wind Gust	
	Offset (days)	Corr. r^2	Offset (days)	Corr. r^2
1	59	0.988	101	0.987
2	55	0.983	111	0.982
3	198	0.959	198	0.962
Broward	-8	0.995	13	0.986
Total (All Sites)	39	0.991	79	0.986

Number of offset days indicated refers to the number of days following the indicated weather parameter(s) that best correlated with disease incidence.

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Figure 1. Calculation of distances between two points via DGPS. Radius of each circle represents worst possible error from each GPS reading.

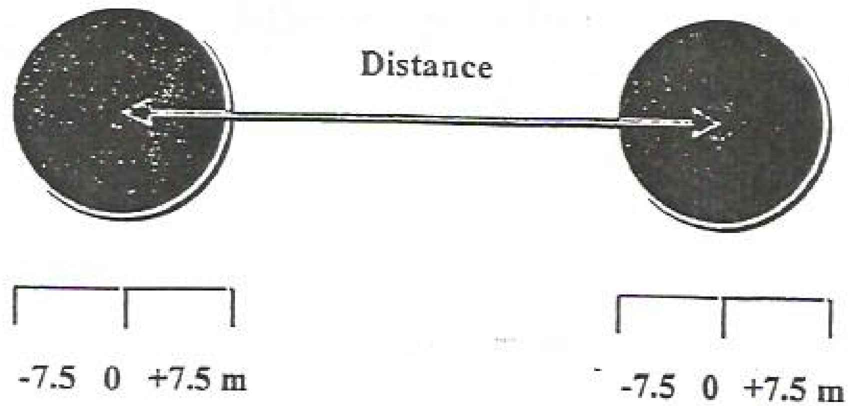

Fig. 2. Site1 Second 2 Month Window

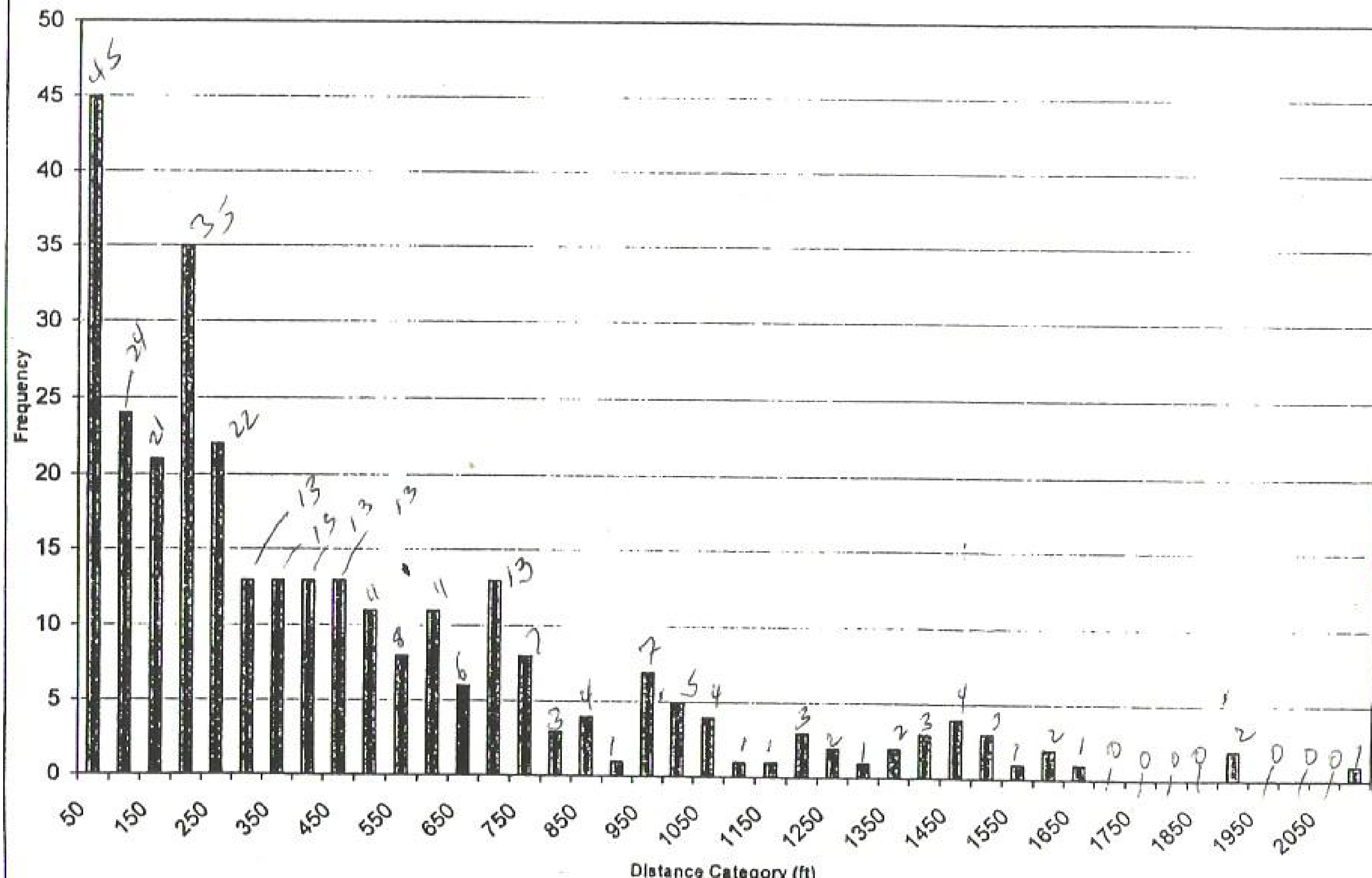

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Fig. 3. Citrus Canker Epidemiology Spread Study in Urban Miami
Site 1: Distances from Focal Tree to Secondary-Infected Trees

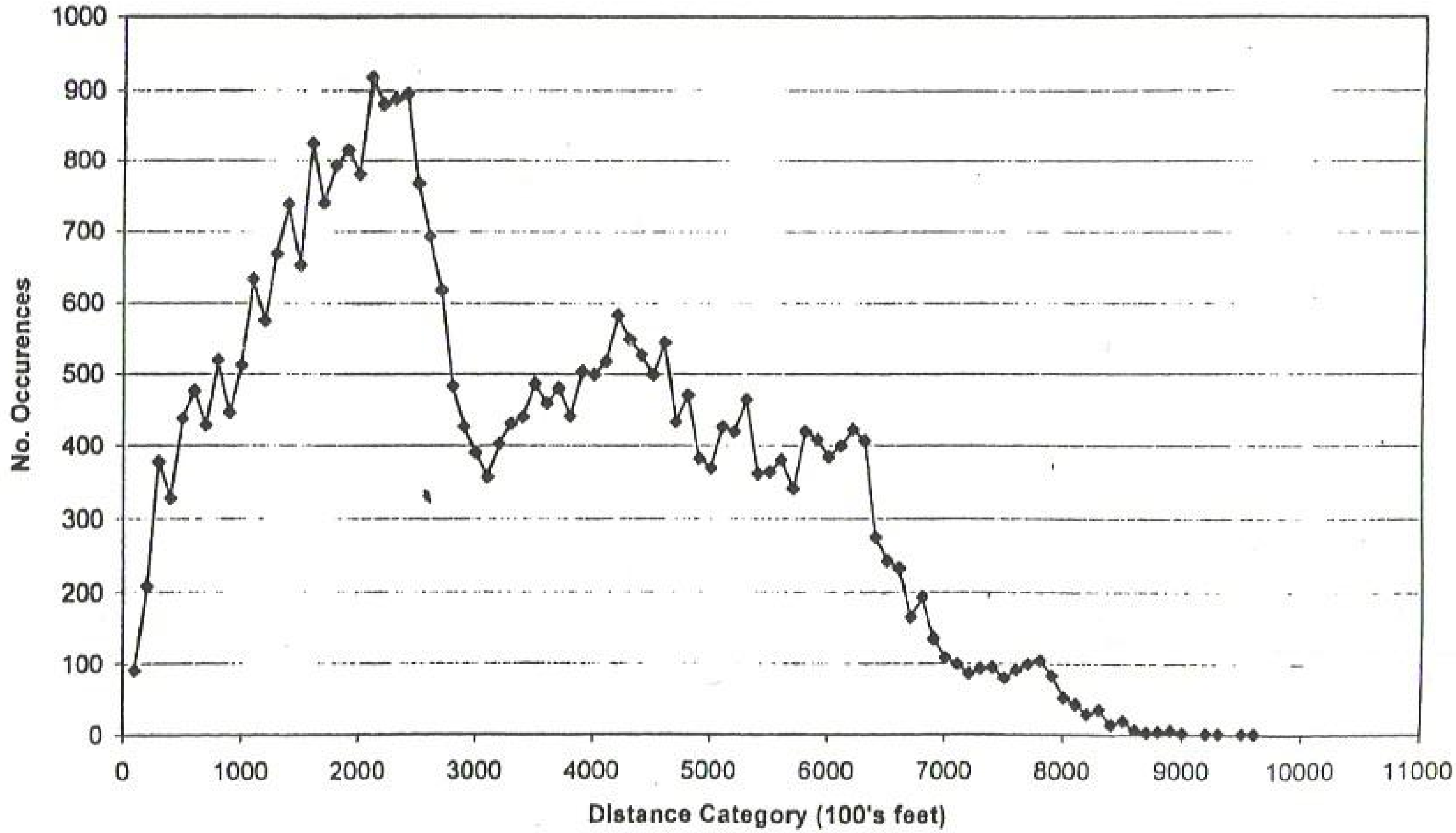


Fig. 4. Citrus Canker Epidemiology Spread Study in Urban Miami
Site 2: Distances from Focal Tree to Secondary-Infected Trees

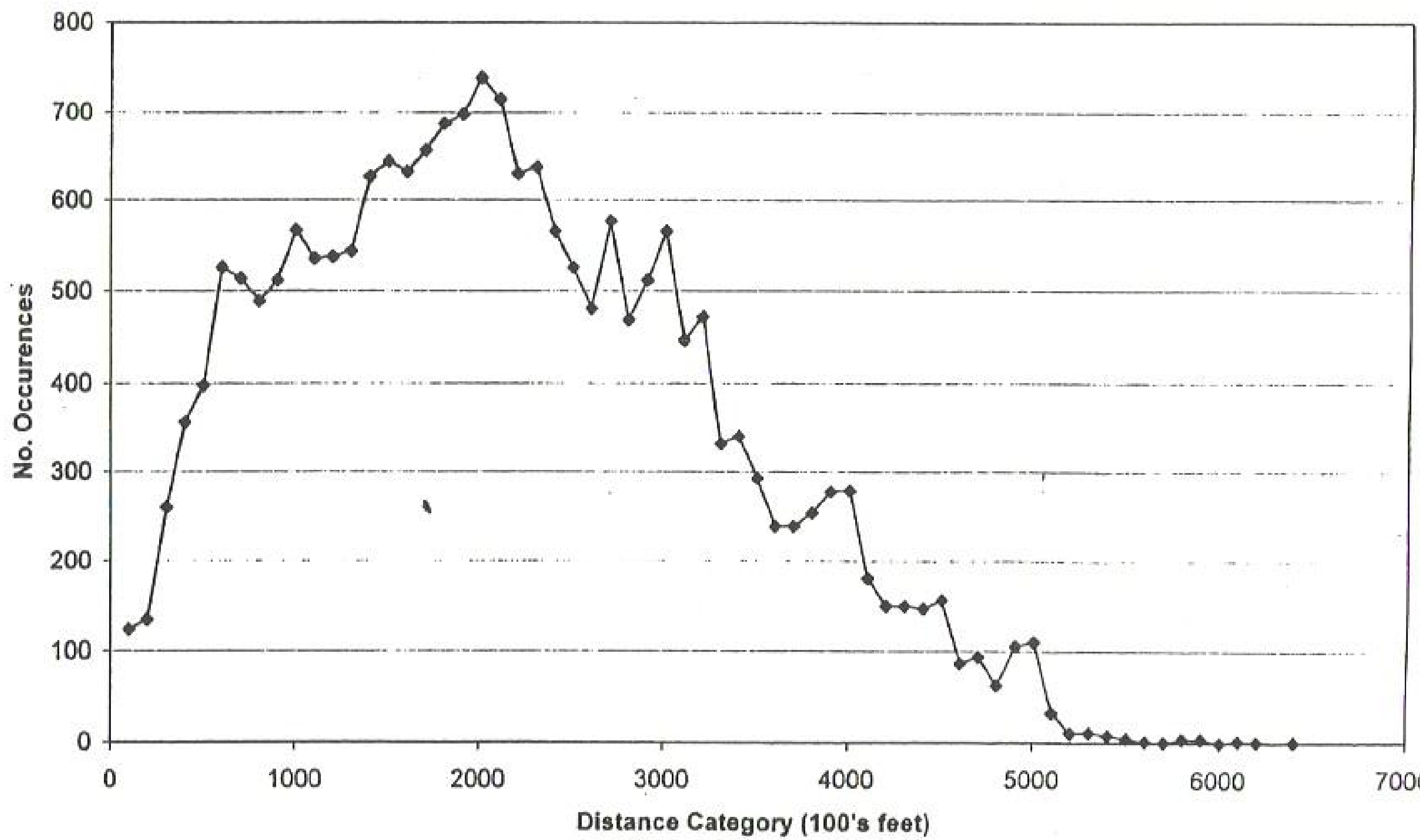


Fig. 5. Citrus Canker Epidemiology Spread Study in Urban Miami
Site 3: Distances from Focal Tree to Secondary-Infected Trees

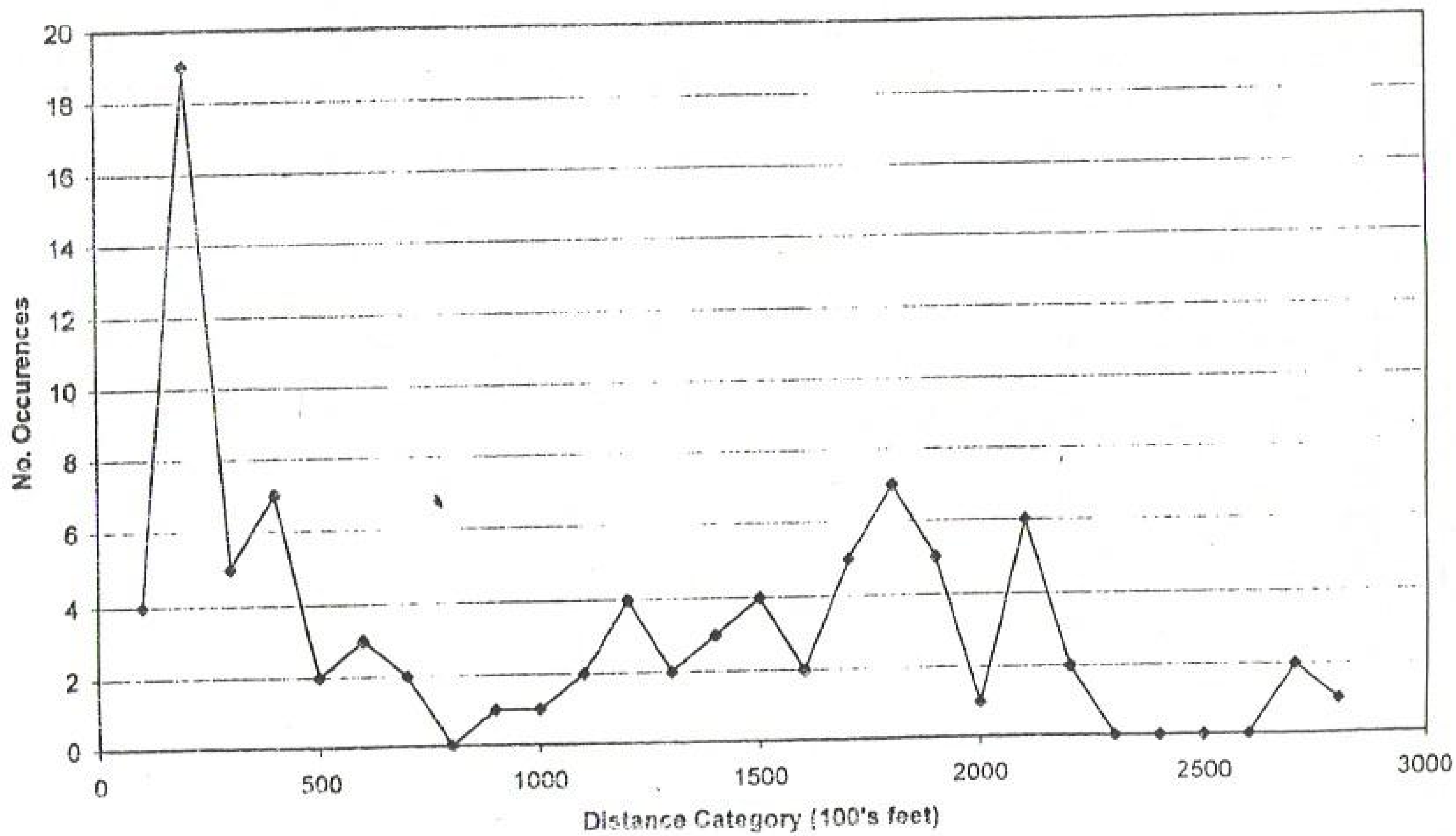


Fig. 6. Citrus Canker Epidemiology Spread Study in Urban Miami
Broward Co. Site Distances from Focal Tree to Secondary-Infected Trees

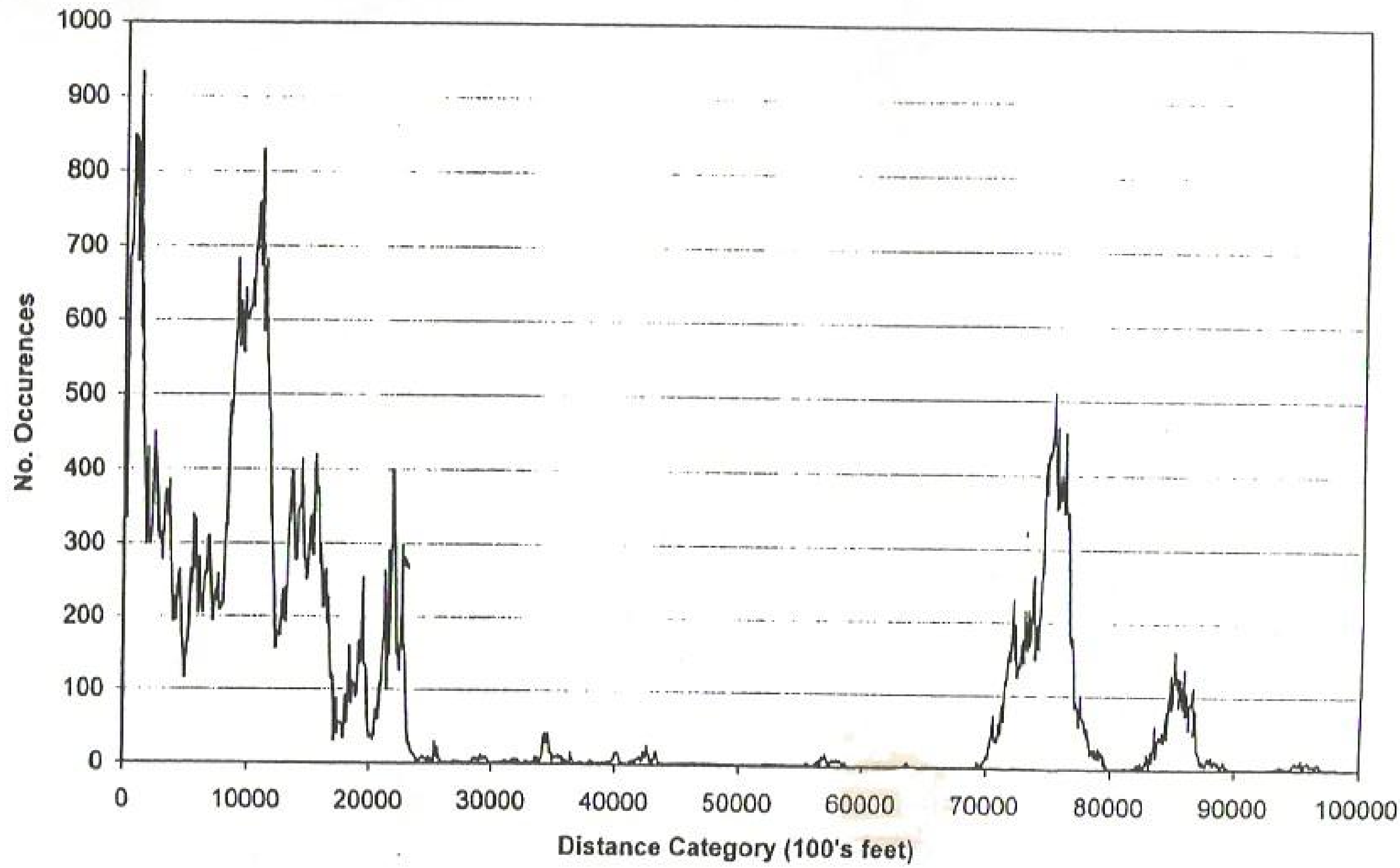
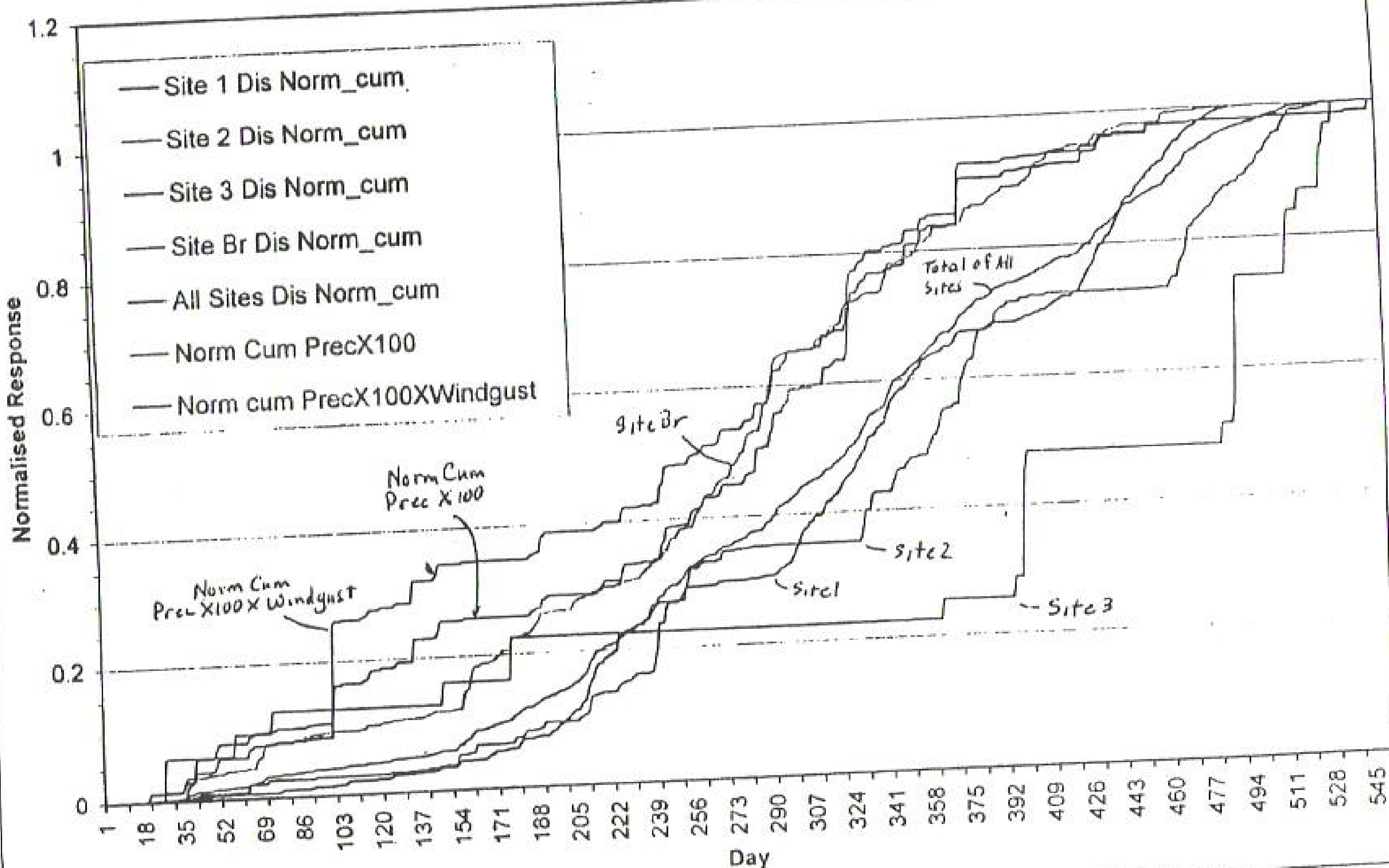


Fig. 7. ,Cross Correlation of Weather Parameters with Disease Incidence by Study Site



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