DECLINES OF UNKNOWN ETIOLOGY

Spatial and Temporal Analysis of Citrus Blight
Incidence in ‘Valencia’ Orange Groves
in Central Florida

R. K. Yokomi, S. M. Garnsey, R. H. Young, and G. R. Grimm

ABSTRACT. Increase of incidence of citrus blight in two ‘Valencia’ on rough lemon
groves in central Florida over an 8 and 13 year period was evaluated. Blight distribution
was nonrandom: trees next to affected trees developed blight more frequently than
nonadjacent trees. Tree-to-tree spread increased with planting density. Increases in
disease incidence from 9 to 31 and 26 to 77% were linear with time. Blight increase in
14 other central Florida groves on rough lemon was also linear with time. From the
analyses, we believe that any possible pathogen is likely to be soilborne or have very
limited aerial movement.

Index words. citrus, decline rates, quantitative epidemiology, randomness tests, disease-
progression curves.

Citrus blight is the most serious
disease problem of Florida citrus.
Blight has been known to occur in
Florida for over 100 years, but its
etiology remains unknown. Visual
symptoms of affected trees include
zinc deficiency of leaves, wilting
of a sector or entire canopy of the
tree, delayed flush, thin foliage,
dieback, and production of water
sprouts (21). Symptoms of diseased
trees become progressively worse
with time, but trees usually do not
die. Affected trees are nonproduc-
tive and must be replaced. Because
a number of factors can cause simi-
lar symptoms (9), citrus blight is
diagnosed by reduced uptake of
water when it is injected into the
large roots and the trunk (5), and
accumulation of zinc and water-
soluble phenolics in trunk wood
(22, 25).

Blight symptoms can appear on
trees of any age once they reach
bearing age (30). Sweet orange and
grapefruit are varieties most se-
verely affected by blight (8). Inci-
dence of citrus blight varies con-
siderably among rootstocks. Trees
on rough lemon are much more sus-
ceptible than trees on other com-
monly used stocks in Florida (21).
In evaluations of current root-
stocks, trifoliate orange, Carrizo
citrange and Alemow (24, 28, 29)
were found to be moderately sus-
ceptible.

All attempts to transmit or re-
constitute blight from affected trees
have been unsuccessful. No causal
agent has been shown to cause
blight. A fastidious xylem-limited
bacteria (2, 12, 23) and soilborne
factors (3) are two popular hypo-
theses which have been proposed
as possible causal agents.

An analysis of the distribution
and rate of increase of diseased
plants often provides useful infor-
mation about the type of agent
causing the disease (32). In this
study, we surveyed blight inci-
dence and distribution annually in
two central Florida groves. These
data were analyzed to determine if
the probable type of causal agent
of the disorder could be deter-
MATERIALS AND METHODS

Survey procedure. A 16.2-ha nucellar Valencia/rough lemon grove, planted in 1965 near Winter Garden, Florida, was selected as the primary site for this study. The grove is under excellent management and is on deep Astatula fine sand (hyperthermic, uncoated typic quartzipsamments). Declining trees were first noticed in 1974. The first survey was taken in November 1975. Each tree was rated as follows: healthy; blight affected; a replant; or other (lightning damaged, foot rot, heart rot, freeze damaged, etc.). Since low levels of foot rot and lightning injury were the only factors besides blight contributing to young tree loss in this grove, replant records were used to estimate number of blight-affected trees from 1970-74. Trees were planted with 7.6 x 7.6 m spacing. Surveys were taken annually except in 1976.

We also analyzed blight incidence and distribution from a 'Valencia'/rough lemon grove near Avon Park, Florida. This grove was planted in 1955 on Pomello sand (sandy, siliceous, hyperthermic arenic haplohumod). Tree spacing was 4.6 x 9.1 m. The grower first became aware of the blighted condition of trees in 1964. Annual surveys were made in a 5.6-ha portion in the southwest corner of the grove from 1965-77. The tree rating system was the same as for the Winter Garden grove.

Additional blight incidence recorded by Grimm et al. (11) from 14 groves in central Florida was also analyzed for comparison with our data. Scion varieties in these groves included Valencia and Hamlin oranges and Duncan and red grapefruits. All varieties were on rough lemon rootstock.

Blight diagnosis. Zinc levels of trunk wood and water uptake were determined by previously described methods (5, 27, 31) to confirm visual diagnosis of tree condition. Representative healthy trees and trees in early stages of blight were tested.

Statistical analyses. The assumption made for this study was that blight is caused by a pathogen and the analyses employed were methods used for plant diseases. Growers replaced blight-affected trees with young, healthy replants annually. These replants were considered as blight-affected tree sites in subsequent surveys. Distribution of blight was analyzed by two tests for randomness. In the doublet analysis, a doublet is two adjacent diseased plants (32). When more than two adjacent infected plants occur, the number of doublets is one less than the number of diseased plants in a row. Our study sites were divided into smaller, more homogenous subunits and doublets were counted. The expected number of doublets was determined by

\[ \text{E(Dc)} = \frac{[N-r][m(m-1)]}{N(N-1)} \] (1)

where \( \text{E(Dc)} \) is the number of expected doublets corrected for the 'missing' trees when all rows are extended into a single row, \( r \) is the number of rows combined, \( m \) is the number of diseased plants, and \( N \) is the total number of plants (16). The standard deviation was calculated as

\[ \sigma = \sqrt{\text{E(Dc)}} \] (2)

(32).

The other test of randomness was the ordinary runs analysis. "With two types of symbols, a run is defined as a succession of one or more identical symbols which is preceded and followed by a different symbol or no symbol at all" (10). The expected number of ordinary runs is determined by

\[ \text{E(U)} = 1 + 2m(N-m)/N \] (3)
where \( E(U) \) is the observed number of ordinary runs, \( m \) is the observed number of infected plants in a row and \( N \) is as described above. The standard deviation of \( U \) is

\[
\sigma = \frac{[2m(N-m)][2m(N-m)-N]}{[N^2(N-1)]^{1/2}} \quad (4)
\]

For the doublet and the ordinary run analyses, the test for randomness was

\[
Z = \frac{[(\text{Observed} + 0.5) - \text{Expected})/\sigma} \quad (5)
\]

Distribution is nonrandom if \( Z > 1.64 \) \((P = 0.05)\) and 2.33 \((P = 0.01)\). Distribution is clumped when \( Z \) is positive for doublets and negative for ordinary runs.

Influence of tree spacing was ascertained by comparing the degree of nonrandomness by ordinary runs determined within a row versus that observed between the rows in the two study sites. \( Z \) values were determined for each year’s data and differences compared by the Student’s \( t \)-test.

Several transformations were employed to evaluate disease progression with time. According to van der Plank (33), disease can increase in time in several ways. If the pathogen multiplies within the field and spreads from leaf to leaf or plant to plant logistically, it is called a “compound interest” disease (CID). CID progression is linearized against time by

\[
Y = \ln[y/(1-y)] \quad (6)
\]

where \( y \) is the proportion diseased. If the pathogen does not spread directly from plant to plant within the field, it is called a “simple interest” disease (SID) and disease progression is linearized by

\[
Y = \ln[1/(1-y)] \quad (7)
\]

where \( y \) is as described above.

In addition, the Gompertz transformation as described by Berger (1) was used since it is useful when disease increase is skewed to the right and is intermediate of the CID and SID models. This transformation equation is

\[
Y = \ln[-\ln(y)] \quad (8)
\]

where \( y \) is as described above.

RESULTS

Declining trees in the Winter Garden and the Avon Park groves exhibited typical blight symptoms. Visual diagnosis was confirmed by water uptake and wood zinc tests. Trees with early blight symptoms took up 181 ml water/24 hr and had 11 ppm zinc in trunk wood; while healthy-appearing trees took up more than a liter of water/24 hr and had 3 ppm zinc.

The incidence of blight-affected tree sites in the Winter Garden grove increased from 9.3% in 1975 to 31.4% in 1982 (Table 1). The average annual rate of increase was 3.2%, with a range of 1.7-5.3%. Blight incidence in the Avon Park grove increased from 26.2% in 1965 to 79.4% in 1977. The observed annual increase ranged from 0.6 to 6.7% and averaged 4.4%. Blighted trees were distributed nonrandomly (i.e. infected trees were clumped) in the grove, based on \( Z \) values \((P = 0.01)\) for both the doublet and ordinary runs analyses (Table 1). More trees adjacent to infected trees or replants became infected than nonadjacent trees. No significant correlation was found when the degree of clumping (\( Z \) values) was regressed against blight incidence as might be expected if a pathogen were spreading from plant to plant.

When blight spread was compared at the Avon Park grove within a row versus between rows, nonrandomness \((P = 0.01)\) was greater within a tree row (trees 4.6 m apart) than between rows (trees 9.1 m apart) (Table 2). In the Winter Garden grove, which had a uniform plant spacing of 7.6
TABLE 1

COMPARISON OF TWO TESTS FOR RANDOMNESS OF BLIGHT-AFFECTED TREES IN TWO VALENCIA/ROUGH LEMON GROVES IN CENTRAL FLORIDA

<table>
<thead>
<tr>
<th>Year</th>
<th>Affected (%)</th>
<th>Doublet† Exp.</th>
<th>Obs.</th>
<th>Z</th>
<th>Ordinary run‡ Exp.</th>
<th>Obs.</th>
<th>Z*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Avon Park, FL§</td>
<td></td>
<td></td>
<td>Winter Garden, FL¶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1965</td>
<td>26.2</td>
<td>110.8</td>
<td>130</td>
<td>1.9</td>
<td>523.1</td>
<td>431</td>
<td>-6.4</td>
</tr>
<tr>
<td>1966</td>
<td>30.9</td>
<td>141.3</td>
<td>165</td>
<td>2.0</td>
<td>562.4</td>
<td>478</td>
<td>-5.5</td>
</tr>
<tr>
<td>1967</td>
<td>37.6</td>
<td>202.2</td>
<td>246</td>
<td>3.1</td>
<td>633.3</td>
<td>508</td>
<td>-7.2</td>
</tr>
<tr>
<td>1968</td>
<td>43.4</td>
<td>259.4</td>
<td>304</td>
<td>2.8</td>
<td>652.8</td>
<td>536</td>
<td>-6.5</td>
</tr>
<tr>
<td>1969</td>
<td>47.3</td>
<td>284.4</td>
<td>346</td>
<td>3.7</td>
<td>661.8</td>
<td>544</td>
<td>-6.5</td>
</tr>
<tr>
<td>1970</td>
<td>52.6</td>
<td>350.8</td>
<td>412</td>
<td>3.3</td>
<td>664.0</td>
<td>552</td>
<td>-6.1</td>
</tr>
<tr>
<td>1971</td>
<td>56.3</td>
<td>386.1</td>
<td>466</td>
<td>4.1</td>
<td>657.4</td>
<td>526</td>
<td>-7.3</td>
</tr>
<tr>
<td>1972</td>
<td>62.1</td>
<td>456.0</td>
<td>541</td>
<td>4.0</td>
<td>633.8</td>
<td>528</td>
<td>-6.8</td>
</tr>
<tr>
<td>1973</td>
<td>66.3</td>
<td>552.2</td>
<td>603</td>
<td>3.5</td>
<td>601.7</td>
<td>516</td>
<td>5.2</td>
</tr>
<tr>
<td>1974</td>
<td>69.3</td>
<td>563.8</td>
<td>655</td>
<td>3.8</td>
<td>573.1</td>
<td>500</td>
<td>4.6</td>
</tr>
<tr>
<td>1975</td>
<td>74.5</td>
<td>646.0</td>
<td>748</td>
<td>4.0</td>
<td>514.6</td>
<td>451</td>
<td>4.5</td>
</tr>
<tr>
<td>1976</td>
<td>78.8</td>
<td>719.7</td>
<td>822</td>
<td>3.8</td>
<td>456.6</td>
<td>404</td>
<td>4.2</td>
</tr>
<tr>
<td>1977</td>
<td>79.4</td>
<td>742.1</td>
<td>850</td>
<td>4.0</td>
<td>447.5</td>
<td>382</td>
<td>5.3</td>
</tr>
</tbody>
</table>

*Distribution nonrandom if |Z| > 1.64 (P = 0.05) and 2.33 (P = 0.01).
†Doublets calculated by method of van der Plank (32). Distribution is clumped when Z is large and positive.
‡Ordinary run calculated by method described in Madden et al. (16). Distribution is clumped when Z is large and negative.
§No. trees = 1328, row spacing 4.6 x 9.1 m.
¶No. trees = 2756, row spacing 7.6 x 7.6 m.

TABLE 2

RELATION OF TREE SPACING AND RANDOMNESS OF BLIGHT-AFFECTED TREES IN TWO VALENCIA/ROUGH LEMON GROVES IN CENTRAL FLORIDA DETERMINED BY ORDINARY RUNS ANALYSIS

<table>
<thead>
<tr>
<th>Tree spacing (m)</th>
<th>Direction</th>
<th>Avg. Z value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avon Park</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>North-south</td>
<td>-6.75</td>
</tr>
<tr>
<td>9.1</td>
<td>East-west</td>
<td>-5.84</td>
</tr>
<tr>
<td>P value*</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Winter Garden</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.6</td>
<td>North-south</td>
<td>-9.52</td>
</tr>
<tr>
<td>7.6</td>
<td>East-west</td>
<td>-9.63</td>
</tr>
<tr>
<td>P value*</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

*P value for significant differences by Student's t test.

High correlations resulted when blight incidence was regressed against time in both the Winter Garden (r² = 0.97) and the Avon Park (r² = 0.99) groves (Table 3). These coefficients were as high as any resulting from CID, SID, or Gompertz transformations. Slopes of regression lines of blight incidence resulted in rate increases of 4.5 and 3.5%/yr (Fig. 1) in the Avon Park and Winter Garden grove, respectively. This corresponds with the average annual rate increase already mentioned (Table 1). However, when incidence was adjusted to compensate for differences in tree spacing, disease progression at Avon Park...
TABLE 3
COMPARISON OF THREE TRANSFORMATIONS OF BLIGHT INCIDENCE DATA FROM TWO VALENCIA/ROUGH LEMON GROVES IN CENTRAL FLORIDA BY LINEAR REGRESSION ANALYSIS

<table>
<thead>
<tr>
<th>Grove</th>
<th>Nontransformed</th>
<th>CID</th>
<th>SID</th>
<th>Gompertz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avon Park</td>
<td>.992</td>
<td>.994</td>
<td>.987</td>
<td>.996</td>
</tr>
<tr>
<td>Winter Garden</td>
<td>.974</td>
<td>.988</td>
<td>.964</td>
<td>.986</td>
</tr>
</tbody>
</table>

*Transformations were CID = \ln\left[\frac{y}{1-y}\right]; SID = \ln\left[\frac{1}{1-y}\right]; and Gompertz = -\ln\left[-\ln(y)\right]; where y = proportion diseased.

decreased to 3.3%/yr and was, therefore, essentially the same as that in the Winter Garden grove.

Blight incidence from 14 other central Florida citrus groves reported by Grimm et al. (11) was analyzed and compared with our results. In all groves, there was a high correlation of linearity of blight incidence with time (Table 4). When the proportion of blight-affected tree sites was transformed by the CID, SID and Gompertz formulas, the transformations with time did not appreciably increase statistical fit. Slopes of regression lines plotted over grove age were also observed to be similar regardless of incidence, although results were more variable than those in our study groves.

DISCUSSION

Determination of disease patterns and rates of spread in the field can reveal much information on the etiology of a plant disease. If a random pattern of infected

Fig. 1. Relation of blight incidence with time in two ‘Valencia’/rough lemon groves in central Florida.
TABLE 4
LINEAR REGRESSION ANALYSIS OF BLIGHT INCREASE DETERMINED IN
ANNUAL SURVEYS IN 14 GROVES IN CENTRAL FLORIDA

<table>
<thead>
<tr>
<th>Site no.</th>
<th>County</th>
<th>Scion variety</th>
<th>No. trees</th>
<th>Grove age (yr)</th>
<th>Years of data</th>
<th>Affected t₀ - t₉⁺ (%)</th>
<th>Avg increase per yr (%)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lake</td>
<td>Valencia</td>
<td>551</td>
<td>30</td>
<td>6</td>
<td>21.1-33.9</td>
<td>2.1</td>
<td>0.92</td>
</tr>
<tr>
<td>2</td>
<td>Lake</td>
<td>Valencia</td>
<td>1583</td>
<td>19</td>
<td>7</td>
<td>1.7-11.8</td>
<td>1.4</td>
<td>0.97</td>
</tr>
<tr>
<td>3</td>
<td>Lake</td>
<td>Valencia</td>
<td>775</td>
<td>13</td>
<td>7</td>
<td>4.4-20.1</td>
<td>2.2</td>
<td>0.96</td>
</tr>
<tr>
<td>4</td>
<td>Orange</td>
<td>Valencia</td>
<td>1462</td>
<td>19</td>
<td>7</td>
<td>5.0-17.6</td>
<td>1.8</td>
<td>0.94</td>
</tr>
<tr>
<td>5</td>
<td>Polk</td>
<td>Valencia</td>
<td>428</td>
<td>29</td>
<td>7</td>
<td>6.1-38.8</td>
<td>4.7</td>
<td>0.97</td>
</tr>
<tr>
<td>6</td>
<td>Polk</td>
<td>Hamlin</td>
<td>558</td>
<td>29</td>
<td>7</td>
<td>9.0-49.5</td>
<td>5.8</td>
<td>0.99</td>
</tr>
<tr>
<td>7</td>
<td>Polk</td>
<td>Red grapefruit</td>
<td>262</td>
<td>29</td>
<td>6</td>
<td>21.8-39.3</td>
<td>2.9</td>
<td>0.96</td>
</tr>
<tr>
<td>8</td>
<td>Polk</td>
<td>Duncan grapefruit</td>
<td>178</td>
<td>30</td>
<td>6</td>
<td>11.2-28.1</td>
<td>2.8</td>
<td>0.90</td>
</tr>
<tr>
<td>9</td>
<td>Highland</td>
<td>Valencia</td>
<td>1054</td>
<td>24</td>
<td>5</td>
<td>20.0-50.4</td>
<td>6.1</td>
<td>0.95</td>
</tr>
<tr>
<td>10</td>
<td>Highland</td>
<td>Valencia</td>
<td>862</td>
<td>24</td>
<td>7</td>
<td>0.5-17.3</td>
<td>2.4</td>
<td>0.91</td>
</tr>
<tr>
<td>11</td>
<td>Highland</td>
<td>Valencia</td>
<td>900</td>
<td>24</td>
<td>7</td>
<td>0.6-19.9</td>
<td>2.8</td>
<td>0.92</td>
</tr>
<tr>
<td>12</td>
<td>Highland</td>
<td>Valencia</td>
<td>460</td>
<td>16</td>
<td>6</td>
<td>28.0-49.6</td>
<td>3.6</td>
<td>0.98</td>
</tr>
<tr>
<td>13</td>
<td>Highland</td>
<td>Valencia</td>
<td>316</td>
<td>16</td>
<td>6</td>
<td>19.9-35.8</td>
<td>2.6</td>
<td>0.96</td>
</tr>
<tr>
<td>14</td>
<td>Highland</td>
<td>Valencia</td>
<td>500</td>
<td>16</td>
<td>6</td>
<td>1.4-6.2</td>
<td>0.8</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*Incidence data taken from Grimm et al. (11).
†All groves were on rough lemon rootstock.
‡t₀ = percentage of blight at first survey; t₉ = percentage of blight at final survey.
plants occurs, adjacent plant-to-plant spread is minimal, whereas if aggregations of infected plants occur, adjacent plant-to-plant spread should be suspected (16). We observed nonrandom distribution of blight-affected trees in the two groves. Trees adjacent to infected trees or replant sites became blighted much more frequently than non-adjacent trees, and adjacent tree-to-tree spread is suspected. When tree spacing was considered, blight spread was more prevalent in the direction of the closer plant spacing. Proximity of trees and root systems or direction of cultivation in the wide middles may influence patterns of blight spread.

DuCharme (8) also observed aggregations of blighted trees as disease incidence increased, but noted that clusters remained random in the grove. Rhoads (20) observed that blight was severe on soils with low moisture-holding capacity and soils with poor drainage. Nemec et al. (17) reported that blight incidence was greater on shallow pan soil than on deep sands. They suggested that soil moisture stress under these conditions predisposed root systems to infection by 

Fusarium solani

and this resulted in blight. We did not find a clay layer associated with blight in either study site. Cohen (6) observed nonrandom blight distribution in several Florida flatwoods groves but found it was not consistently related to soil type except in areas with high organic soil. Such areas remained relatively free of blight. Extensive aggregations of blight-affected trees in our study were not associated with obvious differences in soil characteristics. We observed areas within the survey groves where blight incidence was low and distribution appeared random. In subsequent surveys of the same area, however, we found that trees adjacent to infected trees frequently became infected.

In a similar study, Lima et al. (14) observed nonrandom distribution of blight in the Mogi-Guaçu area of São Paulo, Brazil, in Valencia/Rangpur lime over a 5-year period. They also observed an increase in adjacent diseased trees within a row (4.9 m) versus between rows (8.5 m). Llanos et al. (15) concluded that blight incidence in the Isla de la Juventud, Cuba, was random based on the nearest neighbor analysis. However, we found this method inappropriate as described because it assumes that trees are distributed randomly in the field (as trees in a forest) and a correction factor must be used.

Since nonrandom distribution of disease usually suggests adjacent plant-to-plant spread, an examination of disease-progression curves was conducted to determine the way blight was spreading. Disease incidence in our annual surveys were found to be linear in relation to time. Different transformations commonly used to analyze disease progression did not increase statistical fit and, thus, were not useful in elucidating the nature of blight. Similar rates of blight increase were observed in the two study groves despite large differences in incidence. No known pathogen exhibits this type of progression. It is possible that the disease cycle was extremely slow or the logistic phase of blight increase was not detected by our sampling method. Our analyses of blight incidence recorded by Grimm et al. (11) in a number of orange and grapefruit groves in central Florida also resulted in a high correlation of linearity of blight increase in relation to time. Therefore, it appeared that the same mechanism of blight increase had occurred in all groves analyzed. Lima et al.
(14) found that the blight incidence rate was directly proportional to the number of blighted trees present when the grove was surveyed 3 years earlier. We did not observe this numerical response in our annual surveys from the Winter Garden and Avon Park groves ($r^2 = 0.05$ and 0.54, respectively).

Blight in our study seemed to be moving from tree-to-adjacent-tree, presumably via soil or by very limited aerial dispersion, rather than by long distance movement from external sources of inoculum. This conclusion was made because proximity to blight-affected trees influenced incidence, whereas rates of spread were slow and linear in relation to grove age.

If an efficient aerial vector is involved, as has been postulated (2, 12, 13, 23), it is unlikely that the observed rate of spread would be linear. Rather, disease incidence would be either more random or dispersed in a gradient from principal sources of inoculum as has been described for Pierce's disease (18) and peach yellow leaf roll (19).

Citrus blight remains one of the most perplexing problems in citriculture. It occurs throughout the citrus-growing areas of Florida. Blight or blightlike declines also occur in Brazil, Argentina, Uruguay, Cuba, South Africa (7), and Australia (4, 27, Wutscher, personal communication). Incidence often appears to be highest in vigorous groves receiving good care (21). If blight is soil-associated, nutritional and edaphic factors (3, 17, 20, 26) may play a role in its etiology. We feel that further careful examination of patterns of blight incidence in different locations and situations, particularly when decline first appears in a grove, is necessary and will contribute to characterizing the cause of blight.

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