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## Temporal and Spatial Comparisons Between Epidemics of Citrus Blight and Citrus Tristeza Virus\*

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ABSTRACT. Separate blocks of sweet orange scion on four different rootstocks (sweet orange, cleopatra mandarin, trifoliate orange and Troyer citrange) in a single grove were mapped for incidence of citrus blight from age 10 to 22 yr. Separate blocks in another grove of sweet orange scion on sour orange rootstock were mapped when trees were 14 to 21 yr of age for incidence of citrus tristeza. Temporal increase in trees with blight was described best by a linear model. Temporal increase in trees with tristeza symptoms was described best by a logistic model. In geostatistical analysis of trees with blight, the condition of a tree, whether blighted or healthy, was related to the condition of trees surrounding it. In geostatistical analysis of trees with tristeza symptoms, the condition of a tree was independent of the condition of neighboring trees.

Citrus blight is a decline of unknown etiology that has been a problem in Florida for over 100 yr (20). Recently, blight was induced on symptomless trees by root grafting to blight affected trees (23). Rhoads (18) considered blight to be a non-parasitic disorder while fastidious xylem-limited bacteria (4, 11, 12, 22) and soil borne factors (5, 17) have been suggested as causal agents.

Visible symptoms of affected trees include wilting of the foliage, delayed flush, thin foliage, zinc deficiency of leaves, production of water sprouts dieback (20).Diagnostic symptoms include reduced uptake of water when injected into the trunk and accumulation of zinc and watersoluble phenolics in wood (8, 27). Rootstock susceptibility ranges from high in rough lemon, Rangpur lime, and Carrizo citrange to moderate in Cleopatra mandarin to slight in sour orange (20, 25).

In recent years, groves planted on sour range rootstock as a measure to avoid losses due to blight have been seriously affected by citrus tristeza virus (CTV). The causal agent of CTV is a phloem-restricted closterovirus measuring 2000 x 10-12 nm (1). CTV is disseminated through infected propagation material by several aphid species in a semi-persistent manner (2). A severe decline reaction can

occur in infections of sweet orange, mandarin, or grapefruit trees on sour orange rootstock.

The decline and loss of trees to citrus blight and CTV have had a major impact on citrus production in Florida. Annual losses to blight alone can reach 500,000 trees (26). If epidemics of blight and CTV are compared, the presence of similar dispersal mechanisms can be discerned. A major drawback in research on spread of blight is that symptoms do not appear on trees less than 5-8 yr old and it takes several additional years for the epidemic to progress to significant levels.

During a period from 1970 to 1985, the incidence of blight and tristeza were mapped in two citrus groves in the flatwoods production area of southeast Florida. Data from these maps were analyzed to determine the temporal increase and spatial patterns of symptomatic trees.

#### MATERIALS AND METHODS

Survey. Incidence and pattern of blight were analyzed for the following scion/rootstock combinations: Pineapple sweet orange/trifoliate orange, Pineapple sweet orange/Troyer citrange, Pineapple sweet orange/cleopatra mandarin, and Valencia sweet orange/sweet orange. All combinations were grown in separate blocks

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within the same grove located in the flatwoods area west of Ft. Pierce in Southeast Florida. Each block consisted of six to ten rows with 32 or 33 trees per row giving a total of 192 to 330 trees per block. Spacing within the row was 6 m and spacing between rows varied from 9 m to 11 m. Rows were situated on raised beds with two rows per bed. Each bed was separated by a water furrow.

Trees were planted in 1963 and rated for incidence of blight in 1973, 1978, 1980, 1981, and 1985. The condition of each tree was rated as being healthy, blight affected, replant, or other (foot rot, heart rot, freeze damaged, etc.). Representative healthy trees and blighted trees were tested for zinc levels in wood and water uptake to confirm diagnosis (8, 27).

Incidence and pattern of CTV were analyzed in separate blocks in another grove in the same area containing sweet orange scion on sour orange rootstock. Each block consisted of 10 to 12 rows with 25 trees per row giving a total of 250 to 300 trees per block. Tree spacing was 6 m within the row and 9 m between rows. Rows were arranged on raised beds in a manner similar to the grove surveyed for blight.

Trees were planted in 1960 and rated for incidence of CTV in 1970, 1971, 1973, 1974, and 1975. Additional ratings in one block were made in 1978 and 1981. Each tree was rated as healthy, CTV affected, replant, or other. Representative healthy trees and blighted trees were tested using Mexican lime as an indicator host.

Statistical analyses. Ordinary runs analysis was used to determine the nonrandomness of affected trees in the 0° direction (down row) (15). A run is defined as a succession of one or more identical symbols followed and preceded by a different symbol. Symbols used were 0 for a healthy, replant, or other and 1 for a tree with blight or CTV. Under the null hypothesis of randomness, the expected value (E) of U is given by:

E(U) = 1 + 2m(N-m)/N

where U represents the total number of runs, m is the number of affected trees and N the total number of trees. The observed number of runs will be less than E(U) if there is a clustering of affected trees. For this analysis, the rows were combined to form a single row with length equal to the total number of trees. The standard deviation of U is given by: Su = (2m(N-m)[2m(N-m)-N]

 $/N^{2}(N-1))^{1/2}$ 

The standardized U is given by Zu = [U + 0.5 - E(U)]/Su

The value of Zu will be a large negative number if there is clustering (15). The test for nonrandomness (clustering) is one-sided and the left tail probability is used. A row of trees was considered to have a nonrandom sequence of infected and healthy trees if Z was less than -1.64 (P=0.05).

Geostatistics was used to measure variability in the spatial structure of blight- or CTV-affected trees in four directions (0°, 45°, 90°, and 135°). This technique can compensate for variable distances between plants and has been used to measure spatial variability of diseased plants (6). If h is used to represent a particular distance between samples and their relative orientation, and if the difference between the values of each sample is assumed to depend only upon h, then the mean difference for all pairs separated by a specific h is defined as m(h):

 $m(h) = (1/n)\Sigma[g(x)-g(x+h)]$  where g indicates the measure of a value, x denotes the position of one sample in the pair, x+h the position of another sample h units away, and n the total number of pairs separated by this distance. The variance of these differences is defined as:

 $V(h) = (1/2n)\Sigma[g(x)-g(x+h)]^2$  The term V(h) is called the semi-variance and is a measure of the expected difference between all values separated by a distance h in a selected direction. When the semi-variance is determined for as many different distances and directions as possible and plotted against sample distance, a pic-

ture of the spatial structure for the sampled area is obtained. This picture is called a semi-variogram. As the distance between samples is increased slightly, some difference between sample values is expected and the semi-variogram will show a small positive value, As the distance between samples increases, differences in the values of samples will increase until the samples become independent of each other at which time the semi-variogram levels off (7).

The temporal increase in blight or CTV affected trees was analyzed using the logistic model, Gompertz model, and a linear model (3, 28). Parameters for all three models were obtained using PROC GLM in SAS (19).

#### RESULTS

Measurements of water uptake for healthy and blighted trees on cleopatra mandarin or sweet orange rootstock are presented in table 1. Sweet orange rootstocks with blight took up twice as much water than blighted trees on cleopatra mandarin rootstock. Water uptake of 500 ml per 24 hr was considered normal for a healthy tree. One tree on sweet orange rootstock that received a healthy

TABLE 1
WATER UPTAKE OF HEALTHY AND
BLIGHT-AFFECTED TREES ON
SWEET ORANGE AND CLEOPATTRA
MANDARIN ROOTSTOCK

Rootstock	Tree condition	Wate uptake (ml/24 hr) <sup>2</sup>	
Cleopatra mandarin	blight	12	
Cleopatra mandarin	healthy	500	
Cleopatra mardarin	blight	5	
Cleopatra mardarin	blight	8	
Cleopatra mardarin	blight	12	
Cleopatra mardarin	blight	15	
Sweet orange	blight	43	
Sweet orange	blight	27	
Sweet orange	healthy	151	
Sweet orange	blight	24	
Sweet orange	healthy	503	
Sweet orange	blight	23	

<sup>&</sup>lt;sup>2</sup>Water uptake determined in September, 1978 using a 24 hr gravity injection method (8).

rating in 1978 took up only 151 ml of water over a 25 hr time period (table 1). This tree was diagnosed as having blight in a subsequent survey.

Ten years after planting, the incidence of CTV in the three separate blocks ranged from 3% to 9% (fig. 1). Five years later, the incidence of trees with CTV had increased to 49% to 74%. The temporal increase in CTV was best described using the logistic model table 2.

Ten years after planting, blight incidence in separate blocks ranged from 7% to 18% (fig. 1). Five years later, incidence in the same blocks ranged from 18% to 48%. The incidence of blight was highest in the with the Troyer citrange rootstock and lowest in the block with cleopatra mandarin rootstock. When blight incidence was regressed against time, a linear model provided a good estimation of the observed values (table 2). The logistic or Gompertz models did not provide an appreciably better description of the observed data based on the pattern of residual errors. The incidence of blight in-

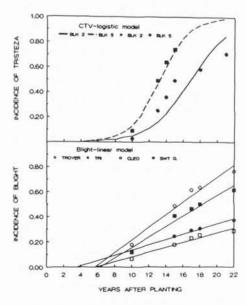


Fig. 1. Incidence of blight or citrus tristeza virus affected trees over time. Symbols represent observed values and line are predicted values using the appropriate model.

Decline	Scion/rootstock <sup>z</sup>	Linear		Gompertz		Logistic	
		Rzy	rx	$R^2$	r	$\mathbb{R}^2$	r
Blight	Pine/tri	.99	.018	.95	.094	.87	.306
Blight	Pine/Troy	.95	.039	.99	.159	.93	.392
Blight	Pine/cleo	.94	.015	.97	.085	.90	.295
Blight	Val/swt	.94	.031	.99	.131	.93	.361
Tristeza	Val/sour	.83	.038	.93	.160	.94	.418
Tristeza	Val/sour	.76	.051	.90	.216	.99	.574
Tristeza	Val/sour	.45	.029	.71	.153	.90	.500

TABLE 2
REGRESSION ANALYSIS OF DISEASE INCIDENCE OVER TIME

"tri = trifoliate orange, Pine = Pineapple sweet orange, Troy = Troyer citrange, cleo = cleopatra mandarin, Val = valencia sweet orange, swt = sweet orange, sour = sour orange.

xrate parameter, per yr.

creased at a linear rate of 1.5% to 3.5% per yr.

In block two, newly affected trees with CTV appeared in clusters but these clusters were usually in one section of the block and not necessarily in the proximity of established infections (fig. 2). Similar patterns of increase were observed in other blocks.

When runs analyses was used over time, a different trend was present in each of the three blocks surveyed for CTV. In block 2, runs analyses indicated that the pattern of affected became increasingly more aggregated up to 15 yr after planting, and then reverted towards random pattern in later years (fig. 3). In block 5, the initial foci of trees with CTV was significantly aggregated. As the incidence of CTV increased, the pattern of affected trees became random. In block 6, there was no significant aggregation of CTV affected trees on any of the sample dates.

In semi-variograms of CTV affected trees in block 2, no spatial dependence was detected in any direction (fig. 4). Thus, the presence of a tree with CTV appeared to have little or no effect on the condition of its immediate neighbors. Similar results were obtained in semi-variograms of other blocks with CTV.

In maps of the spatial progress of blight on the Pineapple sweet orange/ trifoliate orange combination, newly affected trees usually appeared in clusters in close proximity to established foci (fig. 5). Similar trends were evident in maps of other blocks with blight.

At 10 yr after planting, runs analysis detected significant aggregations of blighted trees only in the block con-

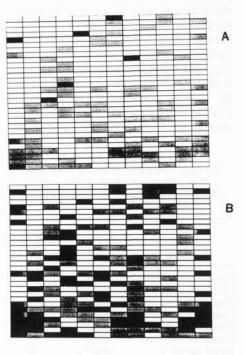


Fig. 2. Spatial progression of citrus tristeza virus decline in block 2. Dark shaded areas represent CTV affected trees at the earlier sample date, lighter shaded areas represent trees affected at the latter sample date, and white areas healthy trees. A) progression from 10 to 13 yr after planting, B) from 13 to 14 yr after planting.

yRegression coefficient comparing observed and predicted levels of disease incidence.

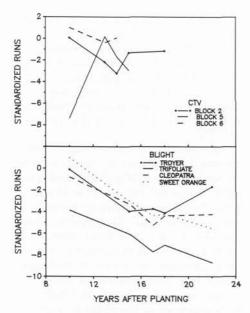


Fig. 3. Standardized runs (Zu) versus time of citrus blight and tristeza (CTV) in the down row (0°) direction. Values < -1.64 represent significant clustering.

taining the Pineapple sweet orange/ trifoliate orange combination (fig. 3). As epidemics of blight progressed, the pattern of blighted trees became increasingly aggregated in all of the blocks surveyed.

In semi-variograms of blight-affected trees 10 yr after planting, the block with the Pineapple sweet orange/trifoliate orange combination revealed some spatial dependence in the 45° direction (fig. 6). In subsequent years, a strong trend of spatial dependence was evident in all directions. In the 45° and 90° direction spatial dependence extended as far as 55 m. Thus, the condition of a tree with blight was related to the condition of its immediate neighbors and this relationship extended out for some distance depending on direction. Similar results were obtained in the other three blocks surveyed.

#### DISCUSSION

Epidemics of CTV differed from epidemics of blight spatially and temporally. For CTV, disease development over time was described best by the logistic model. A logistic model was also used to describe epidemics of CTV in Argentina, Brazil, Florida, and California (2). This model is used

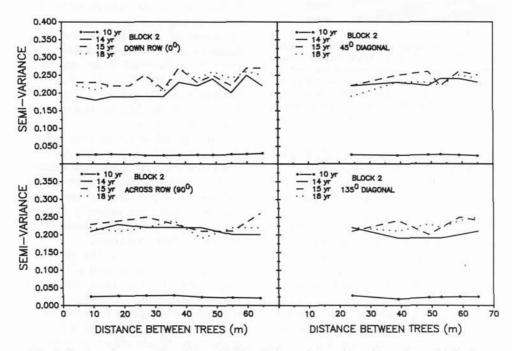


Fig. 4. Semi-variograms of spatial variability of citrus tristeza virus affected trees in Block 2.

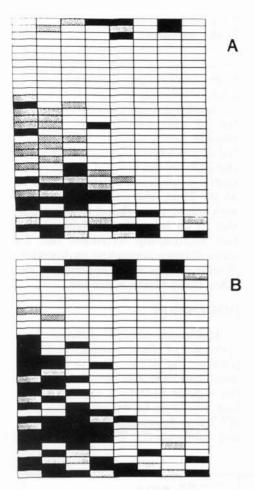


Fig. 5. Spatial progression of citrus blight on trifoliate rootstock. Dark shaded areas represent blight affected trees at the earlier sample date, lighter shaded areas represent trees affected at the latter sample date, and white areas healthy trees. A) progression from 10 to 15 yr after planting, B) from 15 to 17 yr after planting.

to describe epidemics in which multiple infection cycles take place. Although multiple infection cycles of CTV appear to take place, no distinct patterns of disease spread could be detected with geostatistics or runs analysis. The variability in the standardized runs was similar to results obtained in a study of maize dwarf epidemics (16). The authors suggested that variation was due to a combination of within-field spread and spread from outside sources. In this study, differences in efficiency of transmission of CTV isolates and

presence of mild CTV isolates in planting material may also contribute to the variability in spread of disease (2). In the analysis of patterns of CTV-affected trees with geostatistics, the lack of spatial dependence suggests that spread from adjacent trees was not a principal factor in the increase of CTV.

Epidemics of citrus blight progressed in a different manner. The linear increase in blight-affected trees over time is not consistent with examples of epidemics where an aerial vector is involved (21). In addition, linear disease progress curves have not been reported in epidemics of known pathosystems in which a biological agent has been proven. A linear increase was also found in blight affected groves in the ridge production area of central Florida (24).

Blight appeared to spread out radially from established foci. Aggregation of blighted trees appeared to increase over time and the condition of a tree, whether blighted or healthy, appeared to be related to the condition of trees surrounding it. These results differ from those obtained by Yokomi et al (24) where initial patterns of blighted trees were random in three of four blocks surveyed. Aggregated patterns of blighted trees were also observed in several other studies (9, 10, 13, 24). However, Llanos et al (14) reported that blight incidence in the Isla de la Juventud, Cuba, was random.

The spatial and temporal pattern of blight was similar in the four rootstock/scion combinations studied. All four combinations showed a linear increase in blight-affected trees over time with the most recently affected trees occurring in close proximity to previously blighted trees.

From the analyses, the processes responsible for the spread of CTV are much different from those for blight. Spread within and between groves appears to take place in epidemics of CTV resulting in more explosive epidemics. Blight-affected trees increased at a linear rate and areas af-

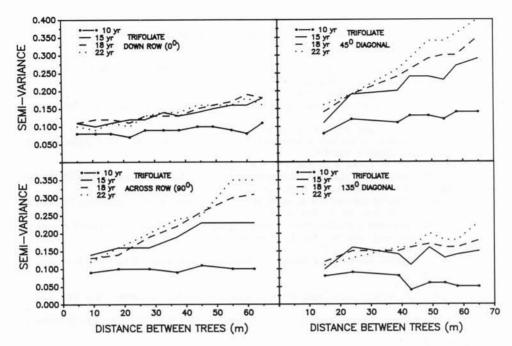


Fig. 6. Semi-variograms of spatial variability of blight-affected trees on trifoliate orange rootstock.

fected by blight extended radially from previously affected trees. This

type of disease development is most unusual.

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