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The effect of irrigation on development of citrus variegated chlorosis symptoms

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ABSTRACT

The incidence and severity of citrus variegated chlorosis (CVC) caused by *Xylella fastidiosa* are higher in the northern region of Sao Paulo, Brazil than in the southern region. This phenomenon is partly due to differences in the climate, especially rainfall, as the northern region tends to be drier than the southern region. The progress of CVC under three levels of irrigation was assessed in a grove located in the northern region of Sao Paulo. Trees (10-year-old Natal sweet orange) were arranged in a randomized complete block in a 3 × 2 factorial scheme with three levels of irrigation and two methods of infection with *X. fastidiosa*. The disease incidence in branches and the number of symptomatic fruits per tree were evaluated for three years. A monomolecular model was used to describe the progress of CVC incidence for all treatments. Irrigation reduced CVC symptoms in trees, especially the number of symptomatic fruits per tree. Based on this significant reduction, citrus irrigation can be used to reduce the main negative effect of CVC: reduced fruit size. Our results also explain the higher incidence of CVC in the northern region of Sao Paulo, where a dry season occurs.

1. Introduction

Citrus variegated chlorosis (CVC), caused by *Xylella fastidiosa* Wells, has been recorded in Brazil for at least 20 years (Rossetti et al., 1990). CVC is considered one of the most important diseases affecting the Brazilian citrus industry, and economic losses due to CVC can reach $120 million per year (Bové and Ayres, 2007). In an effort to reduce losses, additional regulations have been placed on the production and commercialization of citrus seedlings. In 2003, it became mandatory in Sao Paulo that citrus be propagated in protected, screened houses, increasing the cost of production (Carvalho, 2003). CVC has also been reported in Argentina, Paraguay, and Costa Rica (Aguilar et al., 2005), but it has not been found outside of Latin America.

CVC is a vascular disease with a long incubation period; the bacterium proliferates only in xylem vessels, roots, stems and leaves. Symptoms may appear in one branch or in the whole canopy, but they rarely kill the plant. Affected trees exhibit reduced vigor and growth; leaves with interveinal chlorosis (resembling zinc deficiency) on their upper side and light brown, slightly raised gummy lesions on their lower side; and stem dieback. The fruit size is significantly reduced, and the fruits become hard, with the same color of a ripe healthy fruit, but with reduced juice content (Rossetti et al., 1990). Affected fruits are the main problem because hardened fruits can damage juice extraction equipment.

The incidence and severity of CVC are higher in the northern region than in the southern region of Sao Paulo (Ayres et al., 2001; Fundecitrus, 2012). Surveys of CVC showed a possible environmental influence on the expression and development of the disease, as the climate in the north is predominantly warmer and dryer than that in the south (Ribeiro et al., 2006). However, the presence of the pathogen in asymptomatic trees is higher in the south than in the north (Laranjeira et al., 2003). This pattern indicates that the pathogen is present in the southern region, but the disease intensity is less pronounced in the south than in the north. A survey of CVC vectors in different regions of Sao Paulo showed the year-round presence of leafhoppers (Hemiptera: Cicadellidae) (Roberto and Yamamoto, 1998).

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Several studies in non-citrus hosts have shown that water stress increases the disease severity and progress of *X. fastidiosa* in plants (Choat et al., 2009; McElrone et al., 2001; McElrone and Forseth, 2004). Although important epidemiological studies have been carried out to explain the spatial distribution of CVC in Sao Paulo (Laranjeira et al., 2003, 2004; Roberto et al., 2002), some gaps remain. Some studies suggest that water deficiency increases the severity of symptoms caused by CVC (Habermann and Rodrigues, 2009; Machado et al., 2007). However, in these studies only the photosynthetic variables (e.g., CO_2 assimilation rates, stomatal conductance, transpiration rate, photochemical activity) of citrus trees were considered, and the intensity of the disease under irrigation was not quantified. If the intensity of CVC is related to a water deficit, then irrigation can be used as an additional measure of disease control, especially in areas prone to CVC. Our objective was to evaluate the effect of different irrigation levels on CVC intensity in the northern region of Sao Paulo.

2. Materials and methods

2.1. Experimental design and treatments

The experiment was conducted in a citrus orchard planted in February, 1999 located at an experimental station in the northern region of Sao Paulo, Brazil (20°53′16″S and 48°28′11″W). ‘Natal’ sweet orange [*Citrus sinensis* (L).Osb.] trees grafted on Rangpur lime (*Citrus limonia* Osbeck) were spaced 6.0 m × 4.0 m between and within rows, for a total of 576 trees in an area of 13,824 m^2. The experimental trees were arranged in a randomized complete block in a 3 × 2 factorial design with the following treatments: no irrigation and irrigation with 50% and 100% of the crop's evapotranspiration (ETc) combined with natural infection and artificial inoculation of *X. fastidiosa*. The six treatments were randomly distributed in four blocks, where each plot was composed of 24 trees. The six trees located in the central row in the middle of the plot were evaluated. Artificial inoculation was accomplished by grafting infected buds on the presumably uninfected trees 10 months after planting. The efficiency of artificial inoculation was confirmed six months later using PCR with specific primers to detect *X. fastidiosa* (Pooler and Hartung, 1995). Natural infection occurred via the resident population of insect vectors of *X. fastidiosa* that are present in the experimental planting area (Roberto and Yamamoto, 1998). CVC is endemic in Sao Paulo; this characteristic prevents the use of a negative control treatment in any experimental area of the state. We selected this region because the temperature and water stress are higher there than in other regions of the state (Ribeiro et al., 2006).

2.2. Assessments of disease incidence and fruit with CVC symptoms

For each of the six trees per treatment, the CVC incidence was evaluated in six branches randomly chosen from the lower part of the plant, six from the upper part and six in the middle on two sides of the plant (between rows), totaling 36 branches evaluated, as described by Gonçalves et al. (2011). The assessment consisted of identifying the presence or absence of symptoms (leaves or fruit) on a 20 cm section measured from the apical part of the branch. The disease incidence within each tree was characterized by the percent of symptomatic branches per tree, as determined by the average of three raters who assessed all 144 trees in July 2006, July 2007, March 2008 and November 2008. The number of symptomatic fruits per tree was counted for three harvests: November 2006, 2007 and 2008. Hard and orange-colored fruits, typical of CVC symptoms, with a diameter smaller than 50 mm (inadequate for juice extraction) were considered symptomatic (Franco et al., 2008).

2.3. Data analysis of temporal progress

The monomolecular model $[y(t)=b_1-(b_1-y_0)^*e^{(-r*t)}]$ was fitted to the disease incidence, separately considering (i) the whole tree; (ii) each vertical strata of the canopy (lower, middle and upper); and (iii) the number of symptomatic fruits per tree. For this model, $y(t)$ is the disease incidence at time t (months); b_1 is the maximum (asymptotic) disease incidence (proportion); y_0 is the initial inoculum; and r is the monthly rate of disease progress (Madden et al., 2007). A non-linear regression of the data was performed using STATISTICA software (StatSoft, Tulsa, OK, EUA) and used to generate polyetic disease progress graphs. The parameter values were compared separately (two-by-two) for each treatment by t-tests, and comparisons of the number of symptomatic fruits from each treatment were made with Tukey's HSD.

2.4. Multivariate data analysis

Three-dimensional response surface models were fit to the disease incidence as a function of time and the height of symptomatic branches. The data from these response surfaces were subjected to nonlinear regression analyses (Table Curve 3D for Windows, Version 4, SPSS, Inc., Chicago, IL). The appropriateness of each model was determined by examining the coefficient of determination (R^2), the standard error, and biological relevance. Comparisons were made among the response surfaces via the Kolmogorov–Smirnov asymptotic (KSA) test statistic in SAS (SAS, Inc., Cary, NC). The KSA test provides a measure to evaluate differences (from both location and shape) between two response surfaces. The null hypothesis was that the responses are two samples drawn from the same distribution. The probability of a greater KSA (measure of the discrepancy between response surfaces) was calculated via a nonparametric analysis of ranks' variance (Gottwald, 1995).

3. Results

3.1. Number of symptomatic fruits

The number of symptomatic fruits was reduced by 69% (mean of natural infection and artificial inoculation) for trees under 100% irrigation compared to trees under non-irrigated conditions (Fig. 1). The number of symptomatic fruits was intermediate for trees subjected to 50% ETc irrigation. The incidence of diseased fruits was significantly higher in artificially inoculated trees compared to those naturally infected only under non-irrigated conditions (Fig. 1). Artificially inoculated trees had 30% more symptomatic fruits than naturally infected trees. The coefficients of determination (R^2) for the fitted model ranged from 31 to 82% (Table 1 and Fig. 2) and showed predicted values very close to the observed values. The maximum disease incidence (b_1) was significantly lower in trees that received 100% ETc irrigation than for the other treatments for artificially inoculated trees (Table 1). Under non-irrigated conditions, naturally infected trees had a lower predicted maximum incidence than artificially inoculated trees (Table 1). All treatments had similar initial inoculum (y_0) levels (Table 1).

3.2. Incidence of symptomatic branches – whole tree

The monomolecular model showed a good fit ($R^2 \geq 0.75$) in five of the six treatments (Fig. 3; Table 2). With artificial inoculation,