

Navelina ISA 315: A cultivar resistant to citrus variegated chlorosis



André Luiz Fadel ^{a,*}, Eduardo Sanches Stuchi ^b, Sérgio Alves de Carvalho ^c,
Maria Teresa Federici ^d, Helvecio Della Coletta-Filho ^c

^a Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo (ESALQ/USP), Av. Pádua Dias, 11, Piracicaba, 13418-900, São Paulo, Brazil

^b Estação Experimental de Citricultura de Bebedouro, Rodovia Brigadeiro Faria Lima, km 384, Bebedouro, São Paulo, Brazil

^c Centro APTA Citros Sylvio Moreira, Rodovia Anhanguera km 158, Cordeirópolis, São Paulo, Brazil

^d Unidad de Biotecnología, Estación Experimental Wilson Ferreira Aldunate, INIA Las Brujas Dirección: km 10 de la Ruta 48, Rincón del Colorado, Uruguay

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ABSTRACT

Citrus variegated chlorosis (CVC), caused by *Xylella fastidiosa*, is a bacterial disease of great importance to the Brazilian citrus industry. CVC is transmitted by grafting and by leafhoppers of the Cicadellidae and Cercopidae families. There is little information about CVC tolerant sweet orange cultivars (*Citrus sinensis* L. Osbeck). However, previous studies have indicated some resistance to CVC in the ‘Navelina ISA 315’ cultivar. Based on such information, this study has been carried out to determine the resistance of ‘Navelina ISA 315’ to CVC observing disease symptoms in the field and in the greenhouse, associated with the presence and quantitation of *X. fastidiosa* in plant tissue by PCR and quantitative real time PCR (qPCR). In agreement with previous information, the results show that ‘Navelina ISA 315’ is resistant to CVC, on the grounds that almost no symptoms and low bacterial concentrations were found.

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1. Introduction

Citrus variegated chlorosis (CVC), caused by *Xylella fastidiosa* (Wells et al., 1987), was first observed in 1987 throughout citrus orchards in southern Minas Gerais State and the northern region of São Paulo State, Brazil (Rossetti and De Negri, 1990).

CVC symptoms can be described as leaf yellowing in the mid and upper canopy, followed by further spread throughout the plant; symptoms of zinc, boron and potassium deficiencies in leaves; chlorotic blemishes on the ventral surface of leaves, which in older leaves appear as gummy circles on the dorsal surface; and small, hard fruits, unfit for trade (Rossetti and De Negri, 1990). *X. fastidiosa* infection leads to a noticeable reduction in transpiration rate and photosynthesis in leaves due to physiological changes (Machado et al., 1994). Decrease in CO₂ assimilation, stomatal conductance, transpiration, and sap flow was observed in ‘Natal’ sweet orange plants (*Citrus sinensis* L. Osbeck) expressing CVC symptoms (Machado et al., 2006). Such CVC symptoms are directly related to water stress, which is the most accepted mechanism of

pathogenicity caused by obstruction of the xylem by bacterial aggregates, gums and tyloses (Purcell and Hopkins, 1996).

CVC can be transmitted by bud grafting (Nunes et al., 2004), natural root grafts (He et al., 2000) and by leafhoppers (Hemiptera: Cicadellidae) (Yamamoto et al., 2002a). Management is based on three main methods: the use of healthy nursery trees, pruning and removal of symptomatic plants (Coletta-Filho et al., 1997) and chemical vector control (Yamamoto et al., 2002b).

Regarding susceptibility to CVC, all sweet orange cultivars evaluated so far were susceptible to a greater or lesser extent. On the other hand, plants from other citrus species and other related genera, such as *Fortunella* spp. and *Poncirus trifoliata*, showed no symptoms or did not exhibit the bacterial presence usually found in natural conditions (Laranjeira et al., 2005; Silva et al., 2004; Laranjeira and Pompeu Junior, 2002; Li et al., 2000; Laranjeira et al., 1998). There are some differences between sweet orange cultivars in terms of symptom expression. ‘Valência’, ‘Pêra’, and ‘Barão’ were the most susceptible (Machado et al., 1992), whereas ‘Rubi’, ‘Westin’ and ‘Ovale’ showed fewer leaf symptoms (Laranjeira et al., 2005).

Simple leaf symptom observation in each cultivar is not totally adequate to determine the degree of susceptibility because it is necessary to observe symptoms expressed in fruit as well as to characterize yield loss (Laranjeira and Pompeu Junior, 2002). A study of 15 sweet orange cultivars in relation to CVC involving the variables mentioned above has proposed the following cultivar

* Corresponding author.

E-mail addresses: afadel@usp.br, tujufadel@ig.com.br (A.L. Fadel), eduardo.stuchi@embrapa.br (E.S. Stuchi), sergio@centrodecitricultura.br (S. Alves de Carvalho), maritefe@inia.org.uy (M.T. Federici), helvecio@centrodecitricultura.br (H. Della Coletta-Filho).

classification: highly susceptible: 'Barão', 'Pêra', 'Lima', 'Rubi', 'Cadenera 17', 'Cadenera 51', 'Berna', and 'Valência'; susceptible: 'Gardner', 'Pineapple', 'Sunstar', 'Folha Murcha' and 'Baianinha'; moderately susceptible: 'Lue Gim Gong' and 'Westin' (Laranjeira and Pompeu Junior, 2002). Although 'Folha Murcha' sweet orange had positive infection as determined by PCR, this cultivar did not express symptoms as quickly as 'Pêra' sweet orange did (Nunes et al., 2006). A possible explanation for this is that the bacterium takes longer to colonize the plant.

The cultivars 'Navelina ISA 315', 'Navelina SRA 332' and 'New-hall navel SRA 343' were asymptomatic hosts of *X. fastidiosa* (Souza et al., 2006). Later studies reported that only 'Navelina ISA 315' remained symptom-free after seven years in the field, even under high inoculum pressure or after artificial inoculation by grafting on symptomatic plants (Stuchi et al., 2007). 'Navelina ISA 315' (*Citrus sinensis* L. Osbeck) was originated from Italy in 1976, as a result of a clone recovered by *in vitro* culture of undeveloped ovules (Starantino and Russo, 1985) which showed a good performance in several areas of meridional Italy (Rapisarda et al., 2000). It was introduced in Brazil and established in the field in 2000, for the early studies of CVC resistance. Through biological indexing, 'Navelina ISA 315' was found to be infected with HSDv (Hop stunt viroid) (Stuchi et al., 2007). Subsequently, it has been proven by biochemical tools that this citrus variety carried both HSDv (cachexia variant) and CDVd (citrus dwarfing viroid) (Eiras et al., 2013).

Based on initial findings (Souza et al., 2006; Stuchi et al., 2007), the aim of this current study was to determine the resistance of 'Navelina ISA 315' cultivar to CVC, by visual observation of typical symptoms in the field and in the greenhouse, and by presence and quantitation of *X. fastidiosa*.

2. Materials and methods

Three different experiments were designed to evaluate the resistance of 'Navelina ISA 315' against *X. fastidiosa*. All the experiments were carried out in Bebedouro, São Paulo State, Brazil (20° 53' 16" S; 48° 28' 11" W).

2.1. Experiment 1. Topworked 'Navelina ISA 315' in the field

In November 2006, eight 'Pêra' sweet orange trees showing typical CVC symptoms, including small fruits in at least 50% of its branches, were top pruned. Altered branches were painted with kaolin to prevent sun damage (Fig. 1a). In March 2007, shoots at the ideal point to be grafted – 0.7–1 cm in diameter – were grafted in an inverted "T" using branches from asymptomatic 'Navelina ISA 315' trees (Fig. 1b), derived from three original plants established in the field in 2000, which were originally budsticks from Italy. Despite being exposed to natural infection for seven years, those

'Navelina ISA 315' plants showed no symptoms of CVC, but tested positively for the presence of *X. fastidiosa* by PCR (Stuchi et al., 2007).

In December 2008, a new grafted 'Navelina ISA 315' canopy was grown, consisting of this combination: 'Rangpur' lime (rootstock), 'Pêra' sweet orange (interstock), and 'Navelina ISA 315' sweet orange (scion). Each remaining branch was labeled, with respective identification code and total number of grafted branches. The total number of grafted branches was variable per plant: plant No. 1, nine branches; plant No. 2, fifteen branches; plant No. 3, seven branches; plant No. 4, thirteen branches; plant No. 5, ten branches; plant No. 6, twelve branches; plant No. 7, twenty branches; and plant No. 8, eleven branches. Cultural management practices were used according to Mattos Junior et al. (1998), but no vector control activities were used.

Evaluations of CVC symptom incidence and severity and *X. fastidiosa* quantitation were carried out in summer (January) and winter (August) of 2009 and in autumn (April) and spring (November) of 2010.

Incidence and severity were determined on the leaves of all the topworked 'Navelina ISA 315'. All leaves with the same severity scale, formed a group (sample). In order to standardize leaf sampling within branches that had leaves with different degrees of severity, only the severely infected leaves were collected and clustered as a group. The leaves without symptoms composed a sample, classified as degree zero, according to the diagrammatic scale proposed by Amorim et al. (1993). When the absence of symptoms was observed on all the leaves of one plant (0% on the diagrammatic scale), the sampling was carried out by collecting one leaf from each branch of the asymptomatic tree. For all samples, *X. fastidiosa* quantitation was estimated by real-time quantitative PCR (qPCR) as described in 2.4.

2.2. Experiment 2. Nursery 'Navelina ISA 315' plants inoculated by cell suspension under screenhouse

Between December 2006 and February 2007, 18 'Navelina ISA 315' plants, about 10 cm high, grafted onto 'Rangpur' lime, were artificially inoculated with *X. fastidiosa* suspension and kept under a screenhouse. Twenty μL of *X. fastidiosa* strain 9a5c suspended in PBS at a concentration of 10^9 cells/mL was deposited on the stem of each plant following by successive punctures with a No. 1 entomology needle. Two plants were inoculated with the same volume of PBS for a negative control. In total, 20 plants were inoculated and the success of infection was monitored six and 45 months after inoculation by using traditional PCR as described in 2.4.

2.3. Experiment 3. Original and micrografted preimmunized 'Navelina ISA 315' nursery plants inoculated by approach grafting

Two different clones of 'Navelina ISA 315' were used in this experiment. One, called "original" 'Navelina ISA 315' that is infected with HSDv (Cachexia variant) and CDVd. The other one, "micrografted and preimmunized" 'Navelina ISA 315', is the same Navelina genotype but subjected to clonal sanitization by micrografting followed by preimmunization with a protective mild isolate of *Citrus Tristeza Virus* (cross protection). Twenty plants from each clone grafted onto 'Rangpur' lime were used in this experiment that began in April, 2009. Ten nursery trees of each clone ("original" 'Navelina ISA 315' and "preimmunized" 'Navelina ISA 315') were established in a greenhouse, plus another ten trees of 'Pêra' "IAC" sweet orange as controls. All the plants were inoculated with *X. fastidiosa* as described below.

To obtain the inoculum source a segment of branch of CVC diseased sweet orange "Pêra" (donor plant) and the stem from

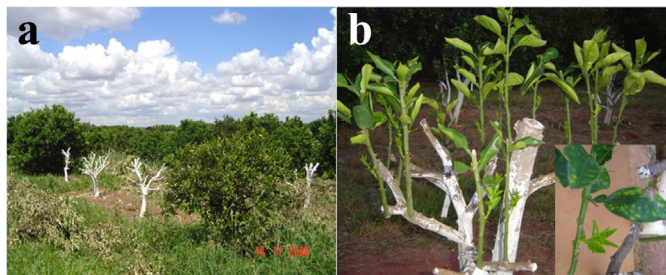


Fig. 1. Experiment 1 - Partial view of Pêra cultivar plants after top pruning (a), after grafting the branches from 'Navelina ISA 315' onto interstock of Pêra cultivar (b), with detail of a sprouted bud on a branch of Pêra cultivar with symptoms of CVC.

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