"IS SABARÁ SWEET ORANGE IMMUNE OR RESISTANT TO CITRUS LEPROSIS?": ADDRESSING BITANCOURT'S 1938 QUESTION

JOSÉ CARLOS VERLE RODRIGUES[1]

SUMMARY

Citrus leprosis is caused by a virus transmitted by the mite *Brevipalpus phoenicis* Geijskes (Acari: Tenuipalpidae). Damages from the disease negatively affect yield and more than 60 million dollars per season are spent on chemical sprays to control the mite vector. Alternatives to chemical control, like the use of the genetic resistance to reduce the yield losses, are being sought. This search was initiated by a prominent Brazilian plant pathologist – Dr. Agesilau Antonio Bitancourt that conducted in the 1930's and 40's a series of experiments to understand the disease, which at the time was a growing problem of unknown etiology. Bitancourt did not observe symptoms of citrus leprosis on 'Sabará' during all his trials and therefore raised a question: "Será a laranjeira Sabará resistente ou imune à leprose?" ("Is Sabará sweet orange immune or resistant to citrus leprosis? ") (**Biológico**, v. 4, p. 300, 1938). This current work addressed Bitancourt's question through experimental leprosis virus transmission by mites, reciprocal crossings between this genotype and sweet orange Pêra and pollen analysis.

Index terms: Citrus leprosis virus; genetic resistance; plant breeding.

[1] University of Puerto Rico, College of Agricultural Sciences, Crop Protection Department, EEA HC - 04 Box 7115, Juana Diaz PR 00795, USA.

"SERÁ A LARANJEIRA SABARÁ RESISTENTE OU IMUNE À LEPROSE?" RESPONDENDO À PERGUNTA DE BITANCOURT FEITA EM 1938

RESUMO

A leprose dos citros, causada por um vírus e transmitida pelo ácaro *Brevipalpus phoenicis* Geijskes (Acari: Tenuipalpidae) é uma das doenças mais importantes na citricultura brasileira. Atualmente, seus danos podem levar a perdas significativas da produção e demandam um controle químico do ácaro vetor, que soma mais de 60 milhões de dólares por ano aos custos de produção. O alto custo e a grande quantidade de princípio ativo utilizados torna necessária a busca de alternativas de combate a essa importante doença, sendo a utilização da resistência varietal um componente extremamente essencial. Esforços nesse sentido foram iniciados pelo eminente fitopatologista Agesilau Antonio Bitancourt, que realizou uma série de experimentos nas décadas de 30 e 40 para entender a doença, que tinha, nessa época, etiologia incerta. Bitancourt não observou sintomas na variedade Sabará e levantou a seguinte questão: "Será a laranjeira Sabará resistente ou imune à leprose?" (**Biológico,** v. 4, p. 300, 1938). Neste trabalho, a questão posta foi analisada através de transmissões experimentais do vírus da leprose por ácaros virulíferos, de cruzamentos com a variedade Pêra (suscetível à leprose) e através de análise palinológica.

Termos de indexação: Citrus leprosis virus; resistência genética; melhoramento.

1. INTRODUCTION

The yield of citrus orchards is severely compromised by inadequate cultural techniques, weather, and especially by losses associated with phytossanitary problems. Wherever it occurs, citrus leprosis plays a major role in reducing the sweet orange yield of citrus orchards (RODRIGUES et al., 2003).

Citrus leprosis was first observed in Brazil in 1933 (BITANCOURT, 1938); before that it was reported in Argentina, Paraguay, Venezuela, and in Florida (EUA), where it later disappeared (CHILDERS et al., 2003a).

Recently the disease has been detected in Panama (DOMINGUEZ et al., 2001), Guatemala (MEJIA et al., 2002), Honduras (RODRIGUES et al., 2004), and Mexico (NAPPO, 2005). Leprosis is the major viral disease affecting citriculture in São Paulo (MÜLLER et al., 2002).

Citrus leprosis virus, cytoplasmic type (CiLV-C), has a single stranded sense positive bipartite RNA genome (+ssRNA) (PASCON et al., 2006) and it is transmitted persistently by the tenuipalpid mite *Brevipalpus phoenicis* (Acari: Tenuipalpidae). This virus can cause drastic reductions in the yield associated with premature fall of leaves and fruits, dieback of twigs, and death of plants in severe outbreaks (RODRIGUES et al., 2003). Unlike a majority of known plant viruses, CiLV-C causes local symptoms and stays restricted to the tissue immediately adjacent to and within the lesions. The vector is a polyphagous species, with a global distribution and broad host range and is found not only infesting citrus, but also other crops and ornamental plants (CHILDERS et al*.,* 2003b) where it becomes more difficult to control. In addition, other non-rutaceous plants have recently been reported as alternative hosts for CiLV-C (RODRIGUES et al., 2005)

There are few reports about citrus leprosis affecting tangerines and tangerine hybrids (i.e. tangor) in field conditions (BITANCOURT, 1955). Since leprosis was discovered in Brazil in the 1930's, few observations have been published on varietal resistance. The Pêra sweet orange, the main variety of the São Paulo citriculture, has been identified as one of the most susceptible varieties (RODRIGUES et al., 2003). BITANCOURT (1938) reported that "Sabará sweet orange" did not show symptoms of leprosis in field conditions. A series of experiments was conducted in the following years, and the results prompted to him the question: "Será a laranjeira Sabará resistente ou imune à leprose?" ("Is Sabará sweet orange immune or resistant to citrus leprosis?"). At that time, he also hypothesized that Sabará could be a hybrid between the sweet orange (*Citrus sinensis* Osbeck) and tangerine (*Citrus deliciosa* Tenore), with the tangerine parent as the source of resistance to leprosis. Now Sabará is recognized as a tangor and it is part of the citrus germplasm bank of Centro APTA Citros Sylvio Moreira (BAG-Citrus) in Cordeirópolis, State of São Paulo, Brazil (DOMINGUES & TULMANN, 2002). In addition, tangor Murcott does not develop leprosis symptoms

(RODRIGUES, 1995), although it allows mite vector colonization, thus corroborating the hypothesis that the tangors had possibly inherited some resistance to leprosis from the tangerine parent.

This work addresses the question posited by BITANCOURT (1938) by characterizing the response of the citrus accession Sabará present at the active bank of citrus germplasm of BAG-Citrus, in greenhouse conditions under infestation of viruliferous *Brevipalpus phoenicis* mites. Also, tentative reciprocal crossings were conducted between sweet orange Pêra and Sabará, and pollen morphology of Sabará was analyzed.

2. MATERIALS AND METHODS

Plant material - access CV 210 was used, originated from BAG-Citrus, and described as Sabará tangor (DOMINGUES & TULMANN, 1998). Seedlings with ten leaves were maintained in one-liter pots and kept in a greenhouse. Plants of mandarin, sweet orange and grapefruit were maintained in the same way and included in the experiment (Table 1).

Mite Transmission Assays - *Brevipalpus phoenicis* mites (Genbank accession no.- AY320027) had been transferred to leaves with symptoms of citrus leprosis and kept there for 48 hours for acquisition of the CiLV-C. The source of the isolated CiLV-C was a Cleópatra mandarin (*C. reshni* Hort. ex Tanaka) described in RODRIGUES et al. (2000). After 48 hours, the mites were transferred (30-40 mites per plant) to test plants (Table 1). Six

Table 1. Reaction of citrus plants 90 days after infestation with viruliferous *Brevipalpus phoenicis*, under greenhouse conditions

BAG-Citrus accession	Description	Plants	
		number [1]	%
CV 199	Mandarin Cleópatra	6/6	100
CV 70	Sweet orange Seleta Vermelha	5/6	83
CV 322	Grapefruit Marsh Seedless	0/6	0
CV 210	Sabará tangor	7/12	58

[1] - # /# - Plants showing symptoms/plants infested with viruliferous mites.

plants of each access were not infested and were kept as controls. The plants were kept in a greenhouse for 90 days after the infestation and checked for development of symptoms.

Crossings - reciprocal crossings were conducted with Sabará and sweet orange Pêra. Developed fruits were harvested and the seeds removed, washed, and sown in plastic trays for germination. The plants had been evaluated for the presence of hybrids, through screening with random primers (RAPD) according to CRISTOFANI (1997).

Pollen Analysis - palynologic analysis was conducted in order to see if Sabará pollen grains had the regular formation expected of viable grains. First pollen was subjected to acetolysis and then slides were prepared for morphological analysis using an optic microscope in accordance with BARTH (1998).

3. RESULTS AND DISCUSSION

Reaction to citrus leprosis - access CV 199 Cleópatra mandarin showed 100% of the plants with the symptoms (highest index observed in the assay). The origin of viral inoculum (from this species) could have played an important role in that high susceptibility. The Sabará tangor (CV 210), which appeared resistant or immune to leprosis in the assays conducted by BITANCOURT (1938, 1955), presented symptoms of the disease, although less severe than those observed in other genotypes (Table 1 and Figure 1). Virus-like particles were observed by electron microscope. These results could indicate a partial resistance in Sabará tangor, and could explain the absence of leprosis symptoms in this genotype in the field assays (i.e. "*field immunity"* - COOPER & JONES, 1983). The small size of the lesions observed in Sabará in this study (data not shown) can reflect a lower rate of expansion of the lesion (K_{exp} - radial lesion expansion rate (mm/day)) in this genotype when compared with others that are more susceptible. Perhaps at the cellular level, the virus moves less efficiently in this particular variety. This in turn could be an important component of leprosis epidemiology in the orchard, as suggested for some fungal foliar diseases (BERGER et al., 1997). Leprosis is a polycyclic disease in São Paulo environmental conditions.

The smaller lesions would provide less leaf area for infecting mites, and thus over the course of a season fewer mites would become viruliferous. Consequently, it would be less likely that the virus would persist in plants with this sort of resistance.

Figure 1. Biologist Rubem N. Tomita stands on the side of Sabará tangor at germplasm bank, Cordeirópolis, São Paulo, June 10th, 1996. Left superior - Symptoms of citrus leprosis in Sabará tangor 90 days after infestation with viruliferous mites.

The type of resistance to leprosis observed in Sabará and in other tangors could be useful if transferred to other citrus genotypes. Also, the quality of juice from Sabará is similar to sweet orange varieties (DONADIO et al., 1995). Also, grapefruit showed no symptoms of leprosis (both in this experiments and all field observations conducted by the author). However, other questions about the nature of these resistances must be addressed before crosses with this genotype are worth pursuing.

Crossings - RAPD analysis showed that the seedlings from the reciprocal crossings were all nucellars - no hybrids resulted. This could indicate: 1) incompatibility between the parental types; 2) infertility or malformation of the reproductive structures of Sabará, as Pêra, produces viable crossings with other citrus genotypes (CRISTOFANI, 1997); 3) lower competitive capacity of possible zygotic embryos compared with nucellar embryos.

Palinology analysis - Sabará pollen grains observed under an optic microscope showed a discontinuity and irregularity in the reticulate exine pattern in contrast to what is characteristic for other citrus species (RODRIGUES et al., 1998). This is a strong indication for a bad formation of the pollen grains (BARTH, 1998) and supports the hypothesis that Sabará is a hybrid.

Therefore, in response to Bitancourt, Sabará tangor is not immune to citrus leprosis, but appears to present some sort of resistance to the virus, which may restrict the persistence of the disease in the field.

ACKNOWLEDGEMENTS

I would like to thank the Centro APTA Citros Sylvio Moreira for plant material and greenhouse facilities. Thanks also to Dr. Mariangela Cristofani-Yaly for help with the plant crosses and Dr. Rubens N. Tomita for fruitful discussion during the development of this work. The author received scholarship from CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior at graduate program of University of São Paulo, Centro de Energia Nuclear na Agricultura.

REFERENCES

BARTH, O.M. - **Paleopalinologia** IGL - 791 - Universidade Federal do Rio de Janeiro, Centro de Ciências Matemáticas e da Natureza, Instituto de Geociências, Departamento de Geologia, Laboratório de Palinologia. Rio de Janeiro – (RJ), 1998 (Apostila).

BERGER, R.D.; BERGAMIN FILHO, A. & AMORIN, L. Lesion expansion as a epidemic component. **Phytopathology**, v.87, p.1005-1013, 1997.

BITANCOURT, A.A. Estudos sobre a leprose dos citros. I- Distribuição geográfica e sintomatologia. **Arquivos do Instituto Biológico**, v.22, p.161-218, 1955.

BITANCOURT, A.A. Será a laranjeira Sabará resistente ou imune à leprose? **Biológico** v.4, p. 300, 1938.

CHILDERS, C.C.; RODRIGUES, J.C.V.; DERRICK, K. S.; ACHOR, D.S.; FRENCH, W.J.V.; WELBOURN, W.C.; OCHOA, R. & KITAJIMA, E.W. Citrus leprosis and its status in Florida and Texas: past and present. **Experimental and Applied Acarology**, v. 30, p.181-202, 2003a.

CHILDERS, C.C.; RODRIGUES, J.C.V. & WELBOURN, W C. Host plants of *Brevipalpus californicus*, *B. obovatus*, and *B. phoenicis* (Acari: Tenuipalpidae) and their potential involvement in the spread of one or more viral diseases vectored by these mites. **Experimental and Applied Acarology**, v. 30, p. 29-105, 2003b.

COOPER, J.I. & JONES, A.T. Responses to plants viruses: proposals for the use of terms. **Phytopathology**, v.73, p.127-128, 1983.

CRISTOFANI, M. **Mapas de ligação de *Citrus sunki* Hort. ex. Tan. e *Poncirus trifoliata* Rubidoux e localização do gene de resistência ao vírus da tristeza**. Tese (Doutorado), Universidade de Sao Paulo, 1997.

DOMINGUES, E.T. & TULMANN, A.N. - Banco Ativo de Germoplasma de Citros e sua importância para o melhoramento. **Laranja**, Cordeirópolis, v. 19, p. 343-364, 1998.

DOMINGUEZ, F. S.; BERNAL, A.; CHILDERS, C. C., & KITAJIMA, E. W. First report of citrus leprosis in Panama. **Plant Disease,** v.85, p.228, 2001.

DONADIO, L. C. ; FIGUEIREDO, J. O. de; PIO, R. M.: Variedades cítricas brasileiras. Jaboticabal: FUNEP, 1995. 228 p.

MEJIA, L.; PANIAGUA, A.; CRUZ, N.; PORRAS, M. & PALMIERI, M. **Citrus leprosis, disease that endangers plantations in Guatemala**. Proc. 42nd Ann. Mtg. Amer. Phytopathol. Soc., Caribbean Div., Antigua, Guatemala, 17-19 June, 2002. (Abstr.)

MÜLLER, G. W; CARVALHO, S.A.; MACHADO, M.A. & RODRIGUES, J.C.V. **Controle das doenças causadas por virus e similares em citros**. In: ZAMBOLIM, L.; VALE, F.X. Ribeiro do; Monteiro, A.J.A. & Costa, H. (Org.). Controle de doenças de plantas - Fruteiras. Visconde do Rio Branco, 2002, v. 1, p. 247-277

NAPPO – North American Plant Protection Organization's. Detection of Citrus Leprosis Virus in the state of Chiapas, Mexico. In.: **Official Pest Reports** http: //www.pestalert.org/ 2005.

PASCON, R.C. et al. The complete nucleotide sequence and genomic organization of Citrus Leprosis associated Virus, Cytoplasmatic type (CiLV-C). **Virus Gene,** v.32, p. 289-298, 2006.

RODRIGUES, J.C.V. **Leprose dos citros: cito-histopatologia, transmissibilidade e relação com o vetor *Brevipalpus phoenicis* Geijskes (Acari:Tenuipalpidae).** Piracicaba, 1995. 79p. Dissertação (Mestrado) – Centro de Energia Nuclear na Agricultura, Universidade de São Paulo.

RODRIGUES, J.C.V.; LOCALI, E.C.; FREITAS-ASTUA, J. & KITAJIMA, E.W. Transmissibility of citrus leprosis virus by *Brevipalpus phoenicis* to *Solanum violaefolium*. **Plant Disease,** 89:911, 2005.

RODRIGUES, J. C. V.; MACHADO, M. A. & CARVALHO, S. A. Microscopia eletrônica de varredura de pólen de algumas espécies de *Citrus* e gêneros correlatos. **Rev. Brasileira de Fruticultura**, Jaboticabal, v. 20, n. 3, p. 382-386, 1998.

RODRIGUES, J.C.V.; MACHADO, M.A.; KITAJIMA, E. W. & MULLER, G. W. Transmission of citrus leprosis virus to mandarins by *Brevipalpus phoenicis* (Acari: Tenuipalpidae). In: **Conference of International Organization of Citrus Virologists**, 14, Campinas, 1998, Campinas. Proceedings of Conference of International Organization of Citrus Virologists p.174-178, 2000.

RODRIGUES, J.C.V.; MÜLLER, G.W.; NOGUEIRA, N.L. & MACHADO, M.A. Yield damage associated to citrus leprosis on sweet-orange varieties. In: **Proc. Int. Soc. Citricult**., IX Congr. 3–7 December 2000, Orlando, FL, v. II; p. 1055–1059, 2003.

RODRIGUES, J.C.V.; ZUNIGA, J.A.; CHILDERS, C.C. & GALLO-MEAGHER, M. New *Brevipalpus* haplotype from citrus in Honduras, Central America based on mitochondrial COI DNA sequences. In.: **International Congress of Entomology**. Brisbane, Australia. August 15-21. 2004.