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Bahia Bark Scaling of Citrus: A Disease of Unknown Etiology

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ABSTRACT

In the 1960's a bark scaling disorder was identified in sweet orange and grapefruit trees in Brazil. It is characterized by yellow-beige to light brown scaling lesions on the trunk or on small branches on the upper part of the plant and followed by a significant exudation of water soluble gum. The disease affects all sweet oranges and some mandarins but it is especially severe on grapefruit varieties. Because of its regional occurrence in the states of Bahia and Sergipe, and being symptomatically very similar to psorosis A, this disease was initially referred to as Bahia type psorosis (tBa). However, accumulated data on inoculated indicator plants, histopathological studies as well as attempts of RT-PCR and molecular hybridization showed that it was a novel disease. The etiology of the disorder is still not elucidated and its nature remains unknown. Nevertheless, experimental data on its epidemiology showed that it is naturally transmitted, citrus diseased plants are the main inoculum source and its progress and spread is consistent with a disease vectored by an insect of limited dispersion ability. Currently the work is aiming at the identification of the pathogen and its vector.

Keywords: progress, psorosis, spatial pattern, *Toxoptera citricida*, transmission, virus-like

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SYMPTOMS AND IMPORTANCE

The first documented record of what was recognized as psorosis disease of citrus in Brazil seems to be the report by Fawcett & Bitancourt (1937) on the presence of psorosis disease in the States of Pernambuco, Bahia, Rio de Janeiro, São Paulo and Rio Grande do Sul. These authors stated psorosis to be "*undoubtely the most important citrus disease in the State of Bahia...in old orchards it is the primary cause of tree decay and low fruit yield ... mainly of Navel oranges*". Concerning the symptoms of the disease the authors found their characteristics identical to those already observed in California. They mention "*...bark lesions, pustules or scales, as well as chlorotic symptoms similar to a mosaic* (?), *on young leaves ... almost always rarely found, possibly due to the high temperatures that may mask them*".

Three decades later a report has been published on a disease inducing severe psorosis-like bark scaling and eruptive pustules on trunks of sweet orange, grapefruit and mandarin cvs. occurring in the main citrus area of Bahia and Sergipe States, especially Recôncavo Baiano region (Passos 1965). The author pointed out that grafted old clones or seedlings as well as nucellar clones were affected. In 1962, almost 100% of 'Bahia' sweet orange plants ('Washington Navel') in this region displayed bark scaling symptoms, but the disorder did not induce foliar symptoms (Passos 1965). These observations led to the rather general belief that it had some typical characteristics of an infectious, virus or virus-like disease, probably transmitted naturally as well as by seeds and that the absence of foliar symptoms was a consequence of the region's high temperatures (Passos and Cunha-Sobrinho 1971; Passos *et al*. 1974). Considering the similarity of symptoms to those described for psorosis A, the disease was, for a long time, designated as Bahia-type psorosis or tBA psorosis.

The intensity of the observed symptoms varies according to the variety or affected species and it is always more severe in grapefruit, where the symptoms can appear in plants two to three years of age. Initial symptoms in the youngest branches are characterized by darkening of the bark, swelling, and cracking, which evolve into scaling (**Fig. 1A**), with dieback often occurring. Adult plants show intense scaling of the main trunk and the oldest branches (**Fig. 1B**), eventually, followed by gum exudation. Affected plants can also show large lesioned areas as a result of the intensive scaling and the coalescence of the lesions (**Fig. 1C**). In sweet oranges, the symptoms are less severe than in grapefruits. However, in more sensitive varieties like 'Bahia', 'Baianinha' and 'Hamlin' large lesions covering an extensive area of the main trunk and the oldest branches are observed (**Fig. 1E** and **1F**), which are often concentrated in the intersection of the branch with the trunk (**Fig. 1D**). In these plants the symptoms appear frequently during the productive period. Varieties such as 'Natal' and 'Valencia' are less sensitive, showing either fewer or smaller lesions. In mandarins, the bark scaling symptoms are generally located

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Invited Mini-Review

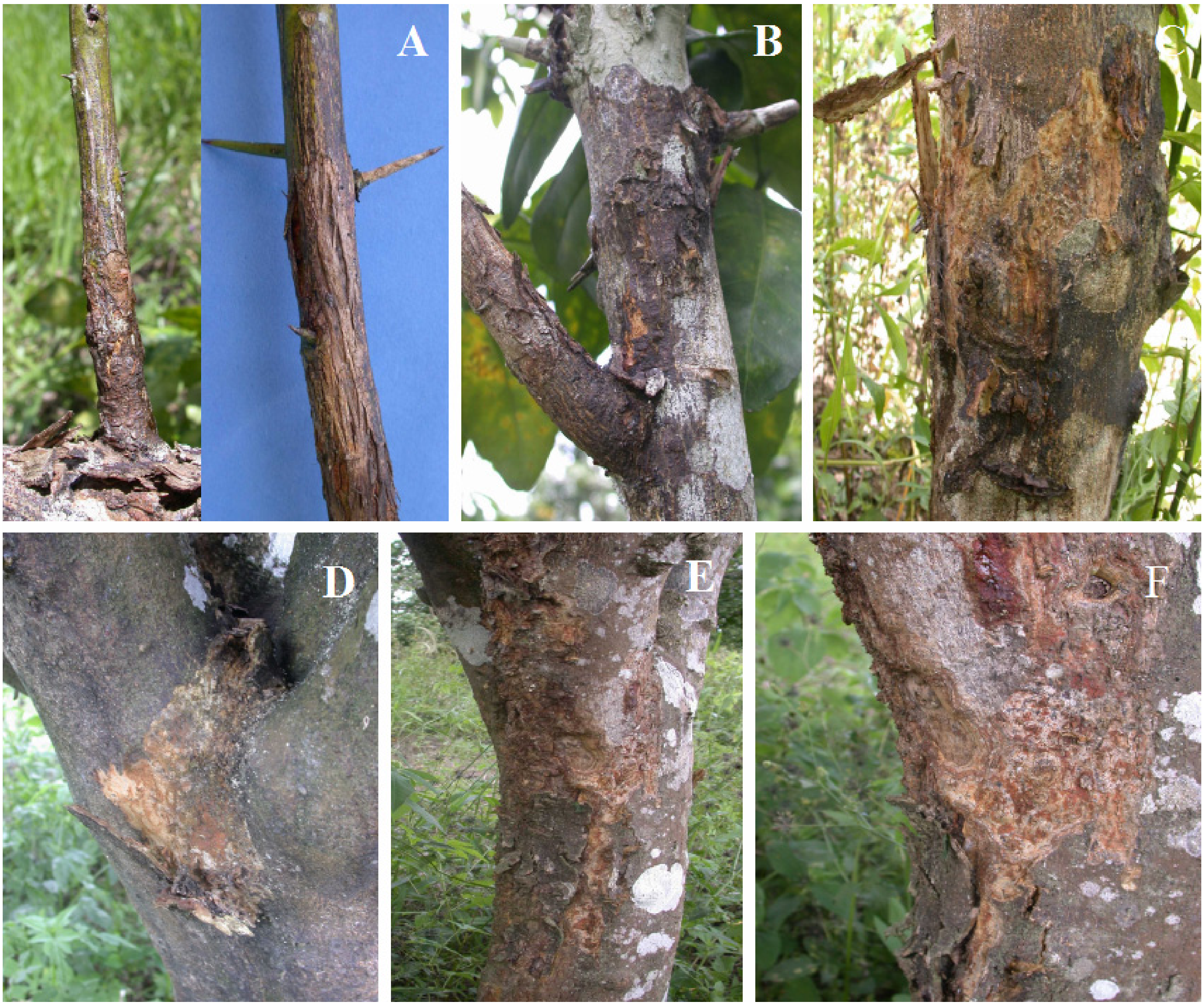

Fig. 1 BBS Symptoms in grapefruits. (**A**) Young branches showing darkening of the bark, swelling and cracking; (**B**) scaling of old branches; (**C**) large lesioned areas of the trunk of sweet orange cv. 'Hamlin'; (**D**) lesions at the intersection zone of the old branch with the trunk; (**E, F**) large lesioned area of the trunk.

on the trunk and at the intersection of the oldest branches.

Incidence and severity of Bahia Bark Scaling (BBS) symptoms, estimated in a scale from 0-3 (0 - no symptoms; 1 - small, isolated lesions; 2 - large lesions; 3 - large lesions spread on trunks and branches) were assessed in a study in a commercial orchard of sweet oranges older than 15 years, in the Recôncavo Baiano (Barbosa *et al.* 1999). The study, carried out in batches of 1,039, 854, 300 and 2,228 plants of 'Baianinha', 'Pêra', 'Natal' and 'Valencia' varieties, showed a correlation between incidence and severity, with the varieties rating 91, 90, 80 and 41% for incidence, and accumulating 86, 89, 80 and 41% of scores 1 and 2, respectively. The high incidence and severity of symptoms observed underlined BBS as an economically important dysfunction of citrus plants in this region and reinforced the hypothesis of the involvement of an efficient vector in its transmission and spread.

STUDIES ON TRANSMISSION

Transmission attempts by mechanical inoculations were performed in a field screenhouse with average temperature of 24°C and in an air-conditioned chamber at 25 ± 3°C. The trial was repeated several times using *Ocimum* sp., *Bidens pilosa*, *Canavalia ensiformis, Cassia occidentalis*, *Catharanthus roseus*, *Chenopodium quinoa*, *C. capitatum*, *C. bonus-henricus*, *C. amaranticolor*, *C. album*, *Crotalaria spectabilis*, *Cucumis sativus*, *Cucurbita pepo*, *Datura stramonium*, *Gomphrena globosa*, *Lycopersicum esculentum*, *Nicotiana tabacum* 'Xanthi', 'Samsun' and 'Hicks', *N. megalosiphon*, *Petunia hybrida*, *Phaseolus vulgaris* 'Red Kidney Bush', *Pisum sativum*, *Vigna sinensis*, *V. unguiculata* 'Early Ramshorn' cowpea, and *Zinnia elegans*. No symptoms were observed on any of these species. Leaf extracts (1:10, w/v) of heavily affected 'Marsh Seedless' grapefruits (*Citrus paradisi* Macf.) were used to inoculate leaves of young test plants by abrasion (Nickel 1990). Barbosa *et al.* (1998) used purified extracts of BBS-affected grapefruit plants to inoculate *C. ensiformis*, *C. quinoa*, *C. amaranticolor*, *Crotalaria spectabilis*, *C. ochloleura*, *C. anagiroides*, *Datura metel*, *Glycine max* cvs. 'Hardee', 'Braag' and 'FTT', *Nicandra physaloides*, *N. benthamiana*, *N. tabacum* 'TNN', *P. vulgaris* 'IAPAR-57', 'Manteiguinha' and 'Rico', and *Passiflora edulis*. The inoculum was obtained from leaf samples that were extracted in HB buffer (7 ml/g: 0.05 M Tris-HCl pH 8.0; 0.1% cysteine; 0.1% ascorbic acid and 0.5% 2-mercaptoethanol) by centrifugation and clarification with CCl_4 (Garcia *et al.* 1991). The obtained aqueous phase was used for the mechanical inoculation with carborundum 300 mesh in at least ten plants of each species. Groups of negative control plants were inoculated only with HB buffer. The inoculated plants were kept in a screenhouse and evaluated during 60 days for the presence of symptoms. The authors also used the brown citrus aphid *Toxoptera citricida* in an attempt to transmit the disorder to these hosts. Aphids were collected from grapefruit BBS-affected plants and transferred (30 aphids/plant) to groups of test plants for 48 hours under laboratory conditions. Then the plants were sprayed with insecticides and transferred to a screenhouse where they were inspected for symptoms up to 60 days after inoculation. An interveinal chlorosis followed by necrosis and shedding of leaves was evident only in two soybean cvs. inoculated either mechanically or by aphids. Symptoms developed 45 days after mechanical inoculation and 15 days after inoculation by aphids. Control plants remained symptomless (Barbosa *et al.* 1998). In subsequent studies, many of the extracts obtained from infected plants did not induce symptoms when inoculated in soybean, showing that the manifestation was erratic. The failure of the mechanical transmission attempt could be explained by an uneven distribution of the pathogen in the plants, a common feature of plant viruses in woody plants, or the environment influence on the pathogen concentration in the donor plants. In the samples of leaves, branches and roots of the inoculated soybean plants used for both transmission attempts as well as in tissues of the inoculum donor grapefruits no virus particles were found when analyzed by transmission electron microscopy using the leaf-dip method (unpublished results, C.J. Barbosa). An indirect ELISA test in which leaf samples of healthy 'Mexican' lime (*Citrus aurantifolia* (Christm.) Swingle) were used as a negative control and leaf samples of sweet orange (*C. sinensis* (L.) Osb.) infected by the CTV were used as positive control, showed that the symptoms observed in inoculated soybean plants were not caused by *Citrus tristeza virus* (CTV), which is endemic in Brazil.

Over the years a large number of citrus species and cultivars have been repeatedly tested for biological indexing of Bahia Bark Scaling. Nickel (1989) found that, except for 'Dweet' tangor (*C. sinensis* Osb. x *C. reticulata* Blanco) which showed an intense chlorotic spotting as a physiological reaction to environmental factors, 'Madam Vinous', 'Pineapple' and 'Parson Brown' sweet oranges, inoculated with buds from 'Marsh Seedless' grapefruits severely affected by BBS did not show a consistent and reliable symptomatic reaction. An experiment was designed to evaluate the influence of lower average temperatures on the potential of BBS inoculum to induce leaf symptoms as buds would sprout in the tropical "winter", the rainy season with average minimum temperatures in the night around 18°C, much more favorable for symptom expression. One single test plant of 'Madam Vinous' sweet orange showed a typical psorosis vein banding and flecking that persisted over 2 growing seasons and evaluation periods. Control plants that were grafted with their own buds remained symptomless (Nickel 1989). Based on these results, a group of test plants was inoculated with BBS and maintained in a screenhouse during the "cold" rainy season (night/day temperatures, 16-26°C/17-30°C). The other group was sorted in two sub-groups, one of which was kept in a cold chamber (day/night temperature 16-18°C) while the other was placed in a screenhouse under environment temperature (28-32°C). Two 'Dweet' tangor plants placed in the cold showed typical psorosis vein flecking. Plants left under environment temperature remained symptomless. However, when the latter were placed in a cold growth chamber conspicuous psorosis-like flecking developed on two 'Dweet' tangor plants and on one 'Madam Vinous' sweet orange test plant (Nickel 1990). The same two tangor plants also surprisingly displayed oak leaf patterns similar to those induced by citrus concave gum, which is a common contaminating component in psorosis-infected plants (Wallace 1968). In the search for test plants with reliable and reproducible reactions a wide range of sweet oranges, mandarins, trifoliate oranges, grapefruits as well as related genera either did not develop foliar symptoms or these were not reproducible (**Table 1**) (Nickel 1989; Santos Filho *et al.* 1990).

It seems generally established that lower temperatures usually have a positive effect on the development of viral symptoms in plants. Foster and Webb (1965) observed that the lowest temperature used (18.3°C) promoted the best symptom expression for 5 out of 6 melon viruses studied. The authors demonstrated that the lower temperature did not induce higher virus concentrations but apparently predisposed plants to better translate the virus effect into symptom intensity. Fridlund (1970) showed that lower temperatures are equally relevant for symptom expression of viruses of woody hosts. Additionally, BBS experiments in the cold would explore more adequately the thermic sensitivity range that exists among citrus indicator species. These results are in line with observation of Guirado (1992) who showed Madam Vinous sweet orange to be especially cold demanding. The author observed that increasing temperatures from 15 to 20°C lowered the number of plants showing psorosis leaf symptoms to 20%, and reduced the intensity of symptoms by 50%. Further increasing the temperature to 25°C completely prevented psorosis foliar symptom expression by 'Madam Vinous', while under the same conditions the reactions of 'Baianinha' and 'Do Céu' sweet oranges were unaltered or only slightly reduced but gave a definitely positive result of psorosis infection. BBS seems to feature its own specific characteristics relating to temperature. Although apparently weakly influenced by temperature, BBS did not change substantially its known behavior. Independently of the conditions under which test plants were maintained (room/low temperatures in growth chambers) the number of plants showing typical psorosis symptoms remained extremely low and erratic (Nickel 1989).

A range of atypical symptoms, as expected for a psorosis-like disease, "frequent in test plants" was observed by Santos Filho *et al.* (1988) when they inoculated micrografted 'Hamlin' sweet oranges with BBS. Contrary to plants inoculated with BBS, the authors noticed a constancy of symptomatic reactions of test plants inoculated with psorosis A. Conducting a similar experiment, Santos Filho *et al.* (1990) reported no symptomatic reaction of sweet oranges (*C. sinensis* Osb.), grapefruits (*C. paradisi* Macfad) and tangors (*C. sinensis* × *C. reticulata*) inoculated with either buds, bark pieces, leaves or pollen of BBS-affected sweet oranges and grapefruits.

The present knowledge on test plant candidates' behavior so far indicates that this disorder behaves differently from other known citrus diseases inducing bark scaling. The absence of foliar symptoms on BBS-affected field and experimentally inoculated plants is most probably not determined by climatic factors but represent an intrinsic feature of a disorder of apparently extremely low infectivity.

The very low number of inoculated plants reacting with some kind of symptoms could be explained by an ex-

Table 1 Foliar symptoms developed by *Citrus* spp., cultivars and related genera to the inoculation with Bahia Bark Scaling of Citrus.

Species, cultivars	Symptoms observed	References
'Madam Vinous' sweet orange	NR, CM, FL	1, 2
'Bahia' sweet orange	BL	3
'Dweet' tangor	NR, CS, FL, OLP	1, 2, 3
Citrus aurantium, (sour orange)	VCL, ST, LE, LR, VCR	2
'Mexican' lime	VCL, ST, DNG	2
C. jambhiri (rough lemon)	RSP	2
'Eureka' lemon	DW, LE	2
'Orlando' tangelo	TIS	2
'Temple' mandarin	RS, BL	2
Citrus hystrix	DW, SP, VCR	2
C. macrophylla	ST	2
C. excelsa	ST, SP	2
Aeglopsis chevalieri	EN, LE, LD, RS, ST, VCL, VTH	2, 4

BL, blisters on leaves; DNG, rapid, blackening dieback of new growth, shock reaction; DW, dwarfing; EN, enations; FL, flecking between secondary veins; LD, leaf distortion; LE, Leaf epinasty; LR, leaf rolling; OLP, oak leaf patterns; RS, ringspots, RSP, dark green ringspots on the first flush p.i. which persist in mature leaves, second flush symptomless; SP, stem pitting; ST, Stunting; TIS, tumours at inoculation sites; VCL, vein clearing; VCR; Vein corking; VTH, vein thickening; NR, symptomless.

1, Santos Filho *et al.* (1990); 2, Nickel (1989); 3, Nickel (1990); 4, Nickel *et al.* (1998).

tremely non-uniformly distributed agent in plant tissues, even when inocula were carefully taken only from symptomatic tissues. Standard transmission studies under conventional experimental conditions were not adequate to produce consistent etiological information on BBS, although some experiments seemed to indicate that a weakly transmissible and very elusive component may be associated with BBS-affected plants. *Citrus tristeza virus* (CTV), being endemic in the region of occurrence of BBS adds to the complexity. In some susceptible test plants CTV may interfere with the effect of the BBS inoculum, mask it or prevent its expression (**Table 1**). The reactions of CTV-sensitive species *C. hystrix*, *C. macrophylla* and *C. excelsa* are examples of such interference. However, some of the test plants used may deserve further attention in studies on BBS indexing: 1. 'Eureka' lemon (*C. limon* (L.) Burm. f.) and Sour orange (*C. aurantium* L.) show a tolerant reaction to common Brazilian isolates of CTV and reacted with conspicuous symptoms to the BBS inoculum. Both are indicators of the closely related *Citrus ringspot virus* (CRSV) and *Citrus psorosis virus* (CPsV) of wide occurrence in Argentinian citrus groves. 2. 'Mexican' lime (*C. aurantiifolia* (Christm.) Swingle showed typical CTV veinlet clearing in young leaves, which is expected since the inoculum usually contains CTV, but, additionally, also a remarking rapid, blackening dieback of new growth characteristic of a shock reaction (unpublished results, O. Nickel). The latter is not documented as a reaction of 'Mexican' lime to common isolates of CTV but to citrus psorosis. The same applies to dark green blisters on leaves of Bahia sweet orange inoculated with BBS (**Table 1**) (Roistacher 1993). 3. Ringspots recorded on mandarins (*C. reticulata* Blanco), are of relevance and should be given some attention. 4. Finally Chevalier's aeglopsis (*Aeglopsis chevalieri* Swingle) displayed a wide spectrum of foliar symptoms when inoculated with either buds or root pieces of BBS-affected plants (**Table 1**). CTV seems to be non-transmissible to *A. chevalieri* by grafting (Knorr 1956). Due to the nature of the inocula of BBS, generally complexed with CTV, it fitted well the search for indicators that this species could permit filtering CTV out of the complex. Since indexing of inoculated plants for CTV presence by the 'Mexican' lime test, ELISA and electron microscopy was negative (Nickel *et al.* 1998) symptoms induced in *A. chevalieri* may plausibly be associated with the BBS-inoculum.

RELATIONSHIPS OF BBS AND CITRUS PSOROSIS VIRUS, CPsV

Samples of young and mature leaves and bark of branches of grapefruit affected by BBS were analyzed by TAS-ELISA (Triple Antibody Sandwich - Enzyme-Linked Immunosorbent Assay) at the Instituto Valenciano de Investigaciones Agrarias (IVIA, Spain) and the Instituto Nacional de Tecnología Agropecuaria (INTA, Argentina) using the monoclonal antibodies 13C5 and 2A3 (Alioto *et al.* 1999; Martín *et al.* 2002a). In none of the tests was it possible to detect the CPsV (unpublished results). The absence of a serological relation between BBS and CPsV corroborates the erratic results of the biological tests previously carried out (Nickel 1989, 1990; Santos Filho *et al.* 1990; Nickel *et al.* 1998) with indicator plants for psorosis. Martín *et al.* (2002b) observed the existence of a correlation between the biological indexing and detection of CPsV by ELISA. The authors noticed that previously untested field trees correlated perfectly with psorosis diagnosis based on biological indexing, specifically with the capacity of those sources to cross-protect against challenge inoculation with psorosis B. Thus, trees without bark scaling were shown to be psorosis-infected by biological indexing and to contain CPsV by serological tests; trees showing psorosis-like bark or leaf symptoms in the field were shown to be psorosis-free by biological indexing and also CPsV-free by serology.

Leaf samples of BBS-affected grapefruit analyzed in IVIA by dot-blot hybridization and RT-PCR tested negative for CPsV infection (Martín *et al.* 2004). These results demonstrated that BBS is not caused by CPsV, since isolates with great differences of nucleotides identity are amplified with primers of the coat protein of CPsV and react in ELISA with the monoclonal antibodies against this virus (Alioto *et al.* 1999; Martín *et al.* 2002a, 2006).

Some other disorders such as Rio Grande Gummosis (Powell *et al.* 1998), Flaky Bark Scale (Roberts *et al.* 2007) and a disorder described in Spain (Martín *et al.* 2002b) can cause psorosis-like bark scaling (Roistacher 1993). In the work of Martín *et al.* (2004) the analysis of others sources from different geographic origins (Florida, India, Polynesia and Dominican Republic), presumed to be psorosis-affected, showed that most of them were CPsV-free. Many of these had been collected from bark-scaled field trees and others induced young leaf patterns in indicator plants, but identification of these symptoms as psorosis by the cross protection test could not be done. Based on symptom observation psorosis has been presumed to be a widespread disease in most citrus areas and the authors suggest that the incidence of CPsV might be more limited than initially thought and that symptoms reported in some cases might be induced by other causes than CPsV alone.

Of several criteria proposed as a consensus for judging a disease as belonging to the psorosis group (Roistacher 1993), BBS does not fulfill the most fundamental such as: *i*. Plants with BBS usually do not show leaf symptoms and/or ringpattern on fruits and leaves of field trees; *ii*. Bark scaling is common but is never associated with wood staining; *iii*. Cross-protection against psorosis B has not been tested; *iv*. BBS lesion inoculum did not induce psorosis B type symptoms; *v*. Shock reaction to BBS inoculum was observed in 'Mexican' lime but not, as with psorosis A, in sweet orange, mandarin, citron or lemon; *vi*. Blisters were observed on 'Temple' mandarin and 'Bahia' sweet orange leaves, but not on their stems and thorns; *vii*. Mechanical transmission to *Chenopodium quinoa* failed, despite numerous attempts, using different inoculation strategies, host species, buffers and additives, neither has a 48 kDa protein been detected in BBS-infected tissues; *ix*. BBS is neither recognized by CPsV-primers in RT-PCR nor by antibodies against CPsV (Alioto *et al.* 1999; Martín *et al.* 2004).

SIMILARITIES BETWEEN BBS AND OTHER CITRUS DISORDERS

The most striking feature of BBS, besides the conspicuous bark scaling, is a profuse gum exudation and impregnation of the wood. The externally oozing brownish-yellow gum is related to areas of gum deposition in the wood that generally form concentric rings. Internally it is a viscous bright-yellow exudate beneath the bark. As cambial activity forms new xylem these gum rings are buried progressively deeper into the wood. Microscopically these rings correspond to an anatomical disorder of the xylem and its elements. Apparently pathological changes begin with the collapse of vessels, the disintegration of middle lamellae and the subsequent dissolution of parenchymatous cells, leading to the formation of pockets or cell-free spaces which are, in the sequence, filled with gum, composing the periodic concentric rings. The number and size of xylem vessels in wood of affected plants are significantly reduced (Nickel and Costa 1991). The collapse of the xylem and the formation of gum-filled rings in the wood caused by BBS in sweet oranges and grapefruits are unique in their striking similarity and indistinguishable from symptoms induced in sweet oranges affected by psorosis (Webber and Fawcett 1935; Schneider 1969). It should, however, be taken into account, that gum production, histological disorders and the resulting reduction of the water flow capacity of the xylem vessels (Nickel 1992), are not a very specific reaction in citrus plants. They may be induced by several abiotic and biotic factors including several citrus diseases that do not induce bark scaling such as impietratura, concave gum and cristacortis (Safran 1969; Cartia and Catara 1974; Vogel and Bové 1975).

EPIDEMIOLOGY

Until 2006, very little was known about BBS epidemiology. Symptoms were occasionally reported as occurring on nucellar adult trees (Passos *et al.* 1974) and on micrografted plants (Barbosa, unpublished). High incidences of BBS has been observed in commercial plantations formed by nucellar mother plants and it was concluded that the high percentage of affected plants was an indication of the possible involvement of one or more vectors with the natural spread of BBS (Barbosa *et al.* 1999). Nevertheless, there was no experimental evidence of such a process. Moreover, along with the lack of knowledge about the putative pathogen, information about the progress or spatial patterns, infection and spreading processes were conjectural.

These issues were addressed by Laranjeira *et al.* (2006) with a specifically designed experiment. They established an area with healthy grapefruit plants downwind and alongside of an older symptomatic grove. Some of the test plants were confined in cages with anti-aphid screens during the entire experiment. After seven years, almost all exposed plants were diseased but none of the caged trees showed any BBS symptoms. This result showed that BBS has an infectious nature and that the putative causal agent is naturally transmitted from plant to plant unlike some citrus diseases such as exocortis whose pathogen (*Citrus Exocortis Viroid*) is transmitted only by infected buds or contaminated cutting tools. Moreover, as no caged plant had symptoms, it is clear that BBS is caused by an organism that needs to be vectored, probably by an insect. This rationale also excludes mites, soilborne fungi or nematodes as vectors of BBS pathogen.

First symptoms on exposed trees were observed 27 months after planting and half of them were symptomatic after four years. Considering that, the incubation period can be estimated ranging from two to four years, at least for grapefruit plants. That, and the fact that BBS took seven years to reach 98% of the plants shows that we are dealing with polyetic epidemics, as also observed for an epidemic of psorosis B in grapefruits in the USA (Timmer and Garnsey 1980). This fact impacts decisions concerning certification programs as well as on possible management practices at a commercial field level. Basically, if certification programs can not guarantee BBS-free budwood, it is possible to imagine a scenario where growers would find that their trees were infected only years after establishing their groves. Also, considering that up-to-date regional surveys are not available and the substantial reduction in water flow in BBS-affected plants (Nickel 1992) it is reasonable to consider a correlation of the disfunction with the relatively low productivity of Bahia's mainly non-irrigated citrus industry (10 t/ha compared to 20 t/ha in São Paulo State). However, the real importance of BBS could only be assessed by yield damage studies.

The progress of a BBS epidemic was characterized as following a sigmoid pattern. Also, the gradient analysis showed evidences of secondary foci inside the experimental plot. Together, these facts point to the existence of a plant-to-plant transmission process that is not dependent upon external inocula.

Spatial patterns of BBS were examined at three hierarchical levels: dependency between adjacent plants; detection of aggregation within quadrats; and spatial relationships between different areas of the experimental plot. When the interaction of adjacent plants was analyzed, it was found that the proportion of aggregated sequences of symptomatic trees was low for both directions, within planting rows or downwind. Nevertheless, the latter proportion was almost four times higher than that observed for the former. It is evident that wind plays an important role in this pathosystem. These observations are consistent with a hypothesis of dissemination by a wind-dependent vector with limited dispersion.

Within quadrats of the experimental plots, Laranjeira *et al.* (2006) detected very significant aggregation between affected plants, no matter the size of the sub-areas. Also, based on results of a binary form of the Taylor law they stated that the epidemic started aggregating and kept this tendency throughout its course, pointing out a positive relationship between aggregation and disease incidence.

Analyzing the patterns of affected plants in the experimental plot as a whole, Laranjeira *et al.* (2006) found that the first foci appeared at the edge near the older grove (inoculum source) and coalescence of these foci occurred before further spread. They reported secondary foci but isopath maps never showed isolated foci at the other borders of the experiment. Hence, they were not associated to other external sources of inoculum. Considering that such secondary foci appeared as close as 10 m apart from the old grapefruit grove, they concluded that the influence of the original inoculum source was limited. Moreover, they considered these observations as evidence that the putative vectors of BBS have low mobility or that they have a tendency to form colonies in *Citrus* plants and, eventually, migrate from one plant to another. Also for citrus psorosis in Argentina and Uruguay, evidence of natural dispersion raised the hypothesis of the involvement of a vector (Pujol and Beñatena 1965). Several attempts failed to transmit it (Portillo and Beñatena 1981) until it was experimentally transmitted from diseased to healthy *Citrus* spp. by *T. citricida*, *T. aurantii* and *Aphis citricola* (Portillo and Beñatena 1989).

CONCLUDING REMARKS

Despite new findings and scientific advances in the diagnosis of plant pathogens Bahia Bark Scaling of Citrus remains a disease of unknown etiology. The evidence of its natural spread and transmissibility shows that it is infectious and that its causative agent is vectored, probably by an insect. Accumulated information consistently indicates that similarities of BBS with diseases of the psorosis group might not be more than a coincidence. The dissimilarities are substantial and convincing. Research on BBS should therefore focus on producing data supporting the hypothesis of the infectious insect-vectored agent and fulfilling Koch's postulates. Probably the advance in the knowledge about the epidemiology of BBS will widen the spectrum of research approaches. The search for a vector as well as the focus on the biological and molecular characterization of the pathogen and the development of sensitive and reliable detection methods should be leading on the road to the elucidation of the nature of this astonishing and intriguing pathosystem.

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REFERENCES

Alioto D, Gangemi M, Deaglio S, Sposato S, Noris, E, Luisoni E, Milne RG (1999) Improved detection of citrus psorosis virus using polyclonal and monoclonal antibodies. *Plant Pathology* **48**, 735-741

Barbosa CJ, Valverde, PRH, Santos-Filho, HP, Vilarinhos AD, Nickel O (1998) Reaction of soybean cultivars to inoculation with tBA citrus psorosis. *Programme and Abstracts of the Conference of the International Organization of Citrus Virologists* **14**, 144

Barbosa, CJ, Valverde, PRH, Almeida RS, Santos-Filho HP, Nickel O, Vilarinhos AD (1999) Incidência e severidade de sintomas de sorose tipo Bahia em variedades de laranjas doces. *Revista Brasileira de Fruticultura* **21**, 382-384

Cartia G, Catara A (1974) Studies on impietratura disease. *Proceedings of the Conference of the International Organization of Citrus Virologists* (*IOCV*) **6**, 123-126

Fawcett HS, Bitancourt AA (1937) Relatório sobre as doenças dos citros nos Estados de Pernambuco, Bahia, São Paulo e Rio Grande do Sul. *Rodriguésia* **3**, 213-236

Foster RE, Webb RE (1965) Temperature effects on symptom expression and concentration of six muskmelon viruses. *Phytopathology* **55**, 981-985

Fridlund PR (1970) Temperature effects on virus disease symptoms in some *Prunus*, *Malus* and *Pyrus* cultivars. *Washington State Agricultural Experiment Station Bulletin* **726**, 6 p

Garcia ML, Arrese EL, Grau O, Sarachu AN (1991) Citrus psorosis disease agent behaves as a two component ssRNA virus. *Proceedings of the Conference of the International Organization of Citrus Virologists* (*IOCV*) **11**, 337-344

Guirado N (1992) Efeito de choque térmico na expressão de sintomas da sorose dos citros em quatro laranjas doces indicadoras. MSc Thesis, Piracicaba, Escola Superior de Agricultura Luiz de Queiroz Universidade de São Paulo, 59 pp

Knorr LC (1956) Suscepts, indicators and filters of tristeza virus, and some differences between Tristeza in Argentina and in Florida. *Phytopathology* **46**, 557-560

Laranjeira FF, Barbosa CJ, Santos-Filho HP, Gonçalves TF, Nickel O (2006) Progress, spread and natural transmission of Bahia Bark Scaling of Citrus in Brazil. *Annals of Applied Biology* **148**, 187-195

Martín S, Alioto D, Milne R, Garnsey S, Garcia ML, Grau O, Guerri J, Moreno P (2004) Detection of citrus psorosis virus by ELISA, molecular hybridization, RT-PCR and immunosorbent electron microscopy and its association with citrus psorosis disease. *European Journal of Plant Pathology* **110**, 747-757

Martín S, Alioto D, Milne RG, Guerri J, Moreno P (2002a) Detection of *Citrus psorosis virus* in field trees by direct tissue blot immunoasay in comparison with ELISA, symptomatology, biological indexing and cross-protection tests. *Plant Pathology* **51**, 134-141

Martín S, Garcia ML, Troisis A, Rubio L, Legarreti G, Grau O, Alioto D, Moreno P, Guerri J (2006) Genetic variation of populations of Citrus psorosis virus. *Journal of General Virology* **87**, 3097-3102

Martín S, Milne RG, Alioto D, Guerri J, Moreno P (2002b) Psorosis-like symptoms induced by causes other than Citrus psorosis virus. *Proceedings of the Conference of the International Organization of Citrus Virologists* **15**, 197-204

Nickel O (1989) Transmissibilidade da sorose tipo Bahia por enxertia. *Fitopatologia Brasileira* **14**, 272-275

Nickel O (1990) Attempts to transmit Bahia-type psorosis. *Revista de Investigaciones Agropecuárias* **22**, 99-109

Nickel O (1992) Anatomia patológica e fluxo hídrico de *Citrus* spp. afetados pela sorose tipo BS. *Fitopatologia Brasileira* **17**, 407-411

Nickel O, Costa N (1991) Pathological changes in the wood of grapefruit and sweet orange trees affected by Bahia-type psorosis. *Proceedings of the Conference of the International Organization of Citrus Virologists* (*IOCV*) **11**, 373-379

Nickel O, Müller GW, Chagas CM (1998) Evidence that *Aeglopsis chevalieri* is not infected by the *Citrus tristeza virus* through grafting. *Fitopatologia Brasileira* **23**, 68-70

Passos OS (1965) Absence of young leaf symptoms of psorosis in the state of Bahia, Brazil. *Proceedings of the Conference of the International Organization of Citrus Virologists* (*IOCV*) **3**, 167-169

Passos OS, Coelho, YS, Cunha-Sobrinho AP (1974) More information on psorosis disease in Bahia, Brazil. *Proceedings of the Conference of the International Organization of Citrus Virologists* (*IOCV*) **6**, 135-138

Passos OS, Cunha-Sobrinho AP (1971) Sorose em clones nucelares de Citrus no Estado da Bahia. *Ciência e Cultura* **23**, 208

Portillo MM, Beñatena HN (1981) Intentos de transmisión de psorosis por insectos. *Congreso Nacional de Citricultura* **2**, 295-303

Portillo MM, Beñatena HN (1989) Transmisión de psorosis de citrus a citrus por áfidos. *Revista de la Sociedad Entomológica Argentina* **45**, 299-305

Powell CA, Pelosi RR, Sonoda RM, Lee RF (1998) A psorosis-like agent prevalent in Florida's grapefruit groves and budwood sources. *Plant Disease* **82**, 208-209

Pujol AR, Beñatena HN (1965) A study of psorosis in Concordia, Argentina. *Proceedings of the Conference of the International Organization of Citrus Virologists* (*IOCV*) **3**, 170-179

Roberts P, Gottwald TR, Sieburth PJ, Garnsey SM, Hilf M (2007) Investigation of a Flaky Bark Disease on grapefruit. http://swfrec.ifas.ufl.edu/plant/pubs/pres/

Roistacher CN (1993) Psorosis - a review. *Proceedings of the Conference of the International Organization of Citrus Virologists* (*IOCV*) **12**, 139-154

Safran H (1969) Anatomical changes in citrus with *impietratura* disease. *Phytopathology* **59**, 1226-1228

Santos-Filho HP, Paguio OR, Silva MJ (1990) Transmissão comparativa de sorose A e sorose tipo Bahia. *Revista Brasileira de Fruticultura* **12**, 69-73

Santos-Filho HP, Silva MJ, Busetti EPC (1988) Sintomatologia do vírus da sorose em cultivares de laranja Hamlin (*Citrus sinensis* Osb.) sob condições controladas de casa de vegetação. *Fitopatologia Brasileira* **13**, 101

Schneider H (1969) Pathological anatomies of citrus affected by virus diseases and by apparently-inherited disorders and their use in diagnosis. *Proceedings of the International Citrus Symposium* **1**, 1489-1494

Timmer LW, Garnsey SM (1980) Natural spread of citrus ringspot virus in Texas and its association with psorosis-like diseases in Florida and Texas. *Proceedings of the Conference of the International Organization of Citrus Virologists* (*IOCV*) **8**, 167-173

Vogel R, Bové JM (1975) Studies on the cause of leaf symptoms associated with cristacortis disease of citrus. *Proceedings of the Conference of the International Organization of Citrus Virologists* (*IOCV*) **6**, 131-134

Wallace JM (1968) Recent development in the citrus psorosis diseases. *Proceedings of the Conference of the International Organization of Citrus Virologists* (*IOCV*) **4**, 1-9

Webber IE, Fawcett HS (1935) Comparative histology of healthy and psorosis-affected tissues of *Citrus sinensis*. *Hilgardia* **9**, 71-109