Pathological Changes in the Wood of Grapefruit and Sweet Orange Plants Affected by Bahia-type Psorosis

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ABSTRACT. Wood of 20 varieties of grapefruit and of two varieties of sweet orange affected by Bahia-type (tB) psorosis, was examined histologically. In stained 28-32-μ sections of trunks, scaffold and smaller branches, dissolution of parenchymatous cells and disintegration of the vascular bundles were observed, giving rise to concentric rings of gum-filled pockets. These histological alterations are similar to those caused by other citrus viruses such as psorosis A, impietratura, cristacortis and concave gum. In addition peduncles of diseased plants displayed extensive areas of xylem disintegration and necrosis of parenchymatous cells. Medullary ray cells showed atypical division and hyperplasia in the affected tissue regions.

Bahia-type psorosis (tB), first observed in 1961 (6), attacks mainly plants of sweet orange and grapefruit causing severe losses. In most cases affected plants are not killed but are left in an unthrifty condition, having their fruit bearing life considerably shortened. Cultivars affected include those most important commercially (7). Trunk symptoms are similar to those of psorosis A.

The first apparent external symptoms of the disease are tiny "pop corn lesions" or bark eruptions that expand and may coalesce, giving rise to larger lesions with generally longitudinal, loose, bark scales. "Pop corn lesions" also may commonly stop growing and disappear, and are covered by new, sound bark growth. Usually, a profuse exudation of water soluble gum also is visible in the lesions or their neighborhood on trunks, scafford limbs and small twigs; twig dieback is widespread. Grapefruit plants are the most sensitive and are also severely dwarfed.

For the past three decades there has been uncertainty about the etiology of psorosis tB. Establishing an etiologic link between psorosis tB and psorosis A has been difficult due to the absence of young leaf symptoms on inoculated test plants, as well as on field plants naturally affected by psorosis tB with severe bark symptoms. In one experiment leaf symptoms appeared, but only in a very low percentage of plants (3). Recently more promising results have been reported (4, 5), but the relationship of psorosis A and psorosis tB is still not confirmed.

The purpose of this study was to examine anatomical changes in tissues of psorosis tB affected plants and compare them with similar symptoms caused by psorosis A and other known citrus virus diseases, reported previously.

MATERIALS AND METHODS

Trunk, scaffold and small limbs. Eight-yr.-old Marsh Seedless, Camulos, Inman Late, and Shambar grapefruits and 12-yr-old Bahia and Baianinha sweet orange plants showing bark scaling and gum exudation as well as advanced twig dieback, were sampled in the CNPMF orchard, Cruz das Almas, Bahia, Brazil. The wood was cut transversally in pieces 2-3 cm long. Wood cores about 0.9 cm in diameter were immediately punched out transversally and longitudinally near the cambium and midway between cambium and the pith. Cores were fixed in FAA (37% formaldehyde, 50% alcohol, glacial acetic acid, 1:1:1) or sectioned unfixed in a freezing microtome after being trimmed to a suitable length. Sections 28-32 μm thick were stained by two alternative methods: progressively with haematoxylin by the method of

Schneider (10), modified by mordanting sections for 1 min in iron alum, suppressing the post-hematoxylin treatments and replacing these by washing of sections in ethanol and xylene before mounting in Canada balsam. Orange G also was used as a stain, not as a contrast with a dark stain (8). Hematoxylin imparts a blue to deep blue stain in gums and cell occlusions, necrotic, lignified cell walls and callus on sieve plates. By the treatment with iron alum-orange G any evidently pathologically altered, necrotic tissues are stained dark brown to black, whereas healthy ones remain yellow.

Peduncles. Grapefruit introductions from California, 6 yr-old and severely affected, were sampled, including the following varieties: Shambar, Tresca, Clason, Reed, Camulos, Joachimsen, Inman Late, Cecily, Wheeny, Red Blush 3, Little River Seedless, Garner, Healton, Imperial, Triumph, Starret, Howell Seedless, Alonso, Frost Nucellar Marsh, and Marsh Seedless. Peduncles were fixed or sectioned unfixed, stained and mounted as described above. Sections were made immediately below the sepals.

Two diseased trees for each variety were sampled and 2-3 samples per plant (8-12 sections per sample) were observed under the microscope. Psorosis- and CTV-free grapefruit wood controls were also examined. No CTV-free peduncles were used for comparison. Micrografted Natal sweet orange and psorosis-free Marsh Seedless grapefruit plants were used as healthy controls.

RESULTS

Wood from the trunk, scaffold and small limbs of sweet orange and grapefruit plants affected by psorosis tB displayed many evident pathological changes. The most striking feature besides the conspicuous bark scaling, was gum exudation and impregnation of the wood. The external oozing gum is related to extensive impregnation of the wood by gum that generally forms concentric rings (Fig. 1). Usually the gum was viscous in nature in the outer parts of the wood or in the cambial region and the

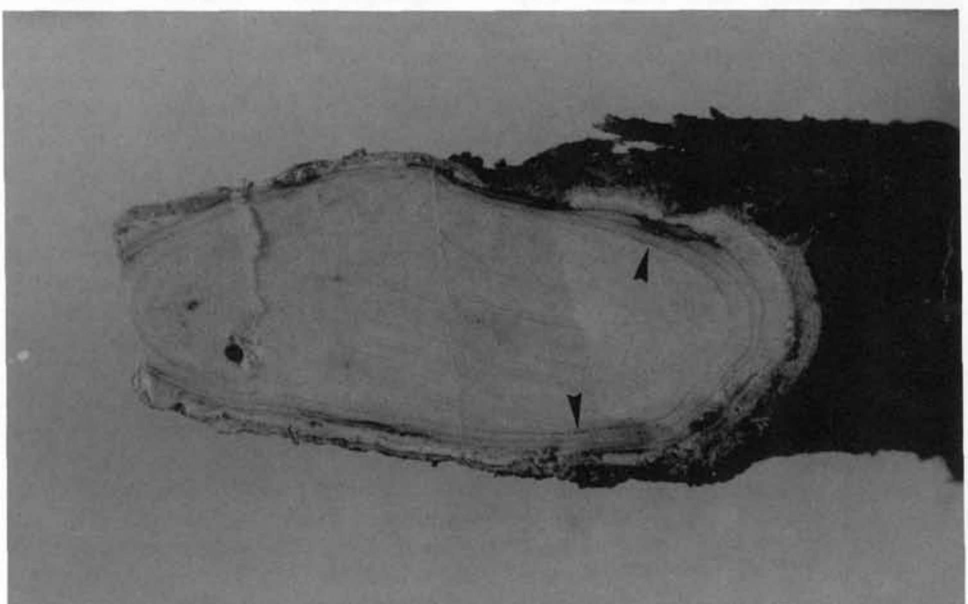

Fig. 1. Cross section of trunk of grapefruit affected by psorosis tB showing concentric gum rings and gum exudation (arrows).

youngest xylem. It was brownish-yellow and stained the wood amber immediately beneath the bark. With the seasonal activity of the cambium, new xylem is formed and these gum rings are buried deeper and deeper in the wood.

At the microscopic level these gum rings correspond to an anatomical disorder of the xylem and xylem elements that may reach tissues several centimeters away from the lesion site. Apparent pathological changes begin with the collapse of vessels, the disintegration of middle lamellae and the subsequent dissolution of some parenchymatous cells, while the non-disintegrated cells become isodiametric and only loosely linked to one another (Fig. 2). This cell dissolution leads to the formation of holes or pockets, i.e. cell-free spaces which are in turn filled with water-soluble gum (Fig. 3), these composing the periodic concentric rings. The number and size of xylem vessels in the affected areas are substantially reduced (Fig. 4). Generally, medullary ray cells are not affected.

Peduncles. In these tissues symptoms of a histopathological process differ from those previously mentioned. Dissolution of xylem elements with the formation of gum pockets does not usually occur, although it may be found occasionally.

All cultivars studied showed, to varying degrees of severity, a characteristic type of necrosis and collapse of vessels and xylem parenchyma cells, which appear crushed between the unaffected, normal ones (Fig. 5). Parenchymatous cells also may appear disarranged and show irregular shapes. Medullary ray cells become hyperplastic with their typical tangential mode of division erratically altered forming easily recognizable off-type ray cell groups (Fig. 6). Affected tissues appear as large, irregular to triangular patches of deformed and necrotic cells containing no functional xylem vessels.

DISCUSSION AND CONCLUSIONS

This is the first study on the anatomy of psorosis tB affected

Fig. 2. Cross section of scaffold limb of grapefruit affected by psorosis tB showing advanced dissolution of parenchymatous cells and isodiametric parenchyma cells loosely linked to one another (arrows, 320x).

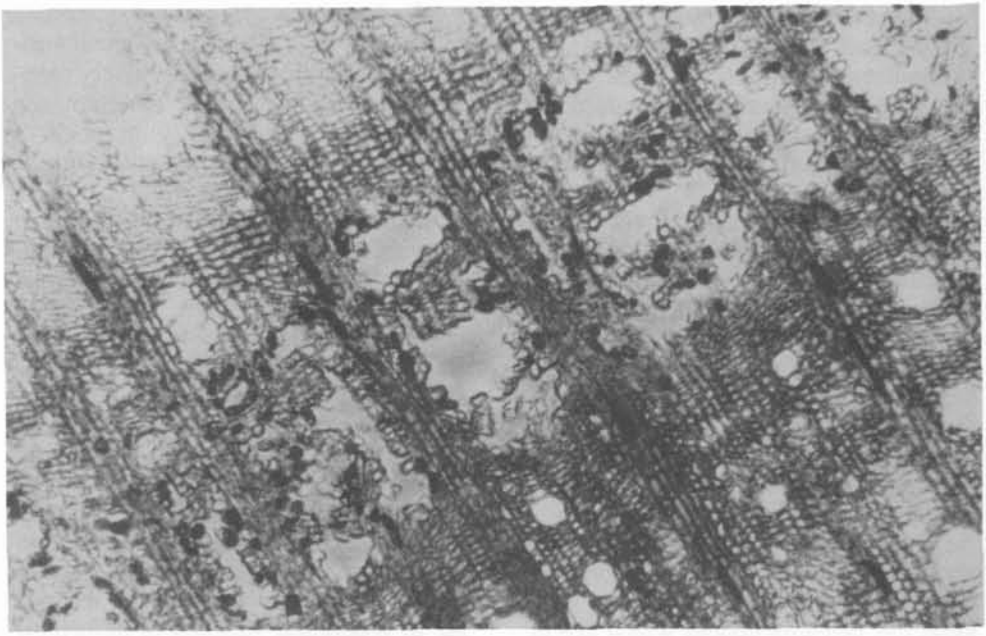

Fig. 3. Cross section of a grapefruit trunk affected by psorosis tB showing gum-filled pockets in the cambial zone (125x).

plants. Since it has been difficult to characterize the etiology of psorosis tB, this approach produced additional information on a suspected relationship to psorosis A and possibly to other virus diseases of citrus.

Psorosis A affected plants show two kinds of wood lesions (1). Primary

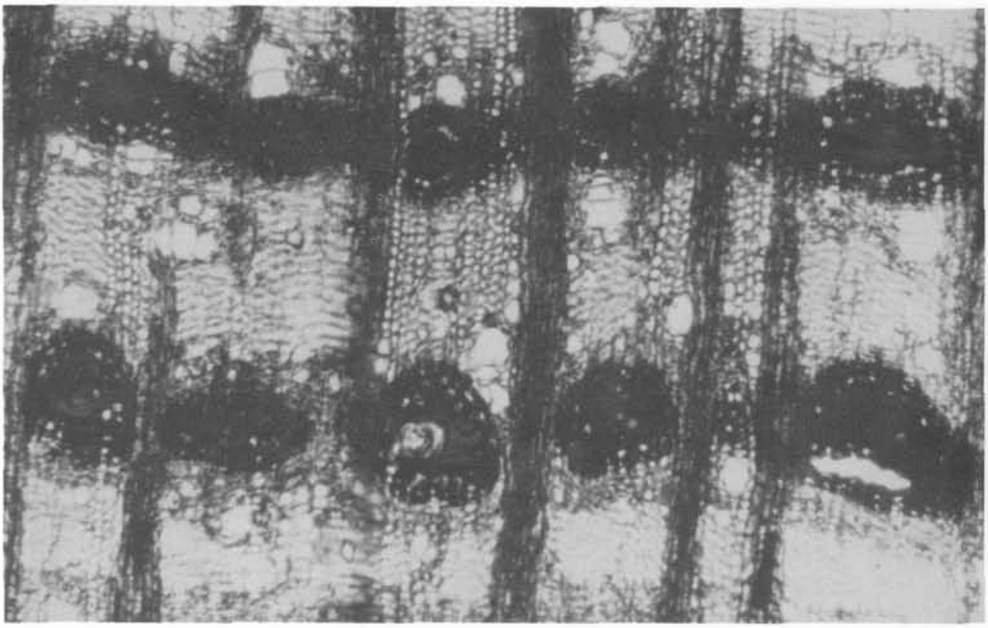

Fig. 4. Cross section of a grapefruit trunk affected by psorosis tB showing segments of three older concentric gum rings in the cambial zone, reduced number and size of remaining xylem vessels (160x).

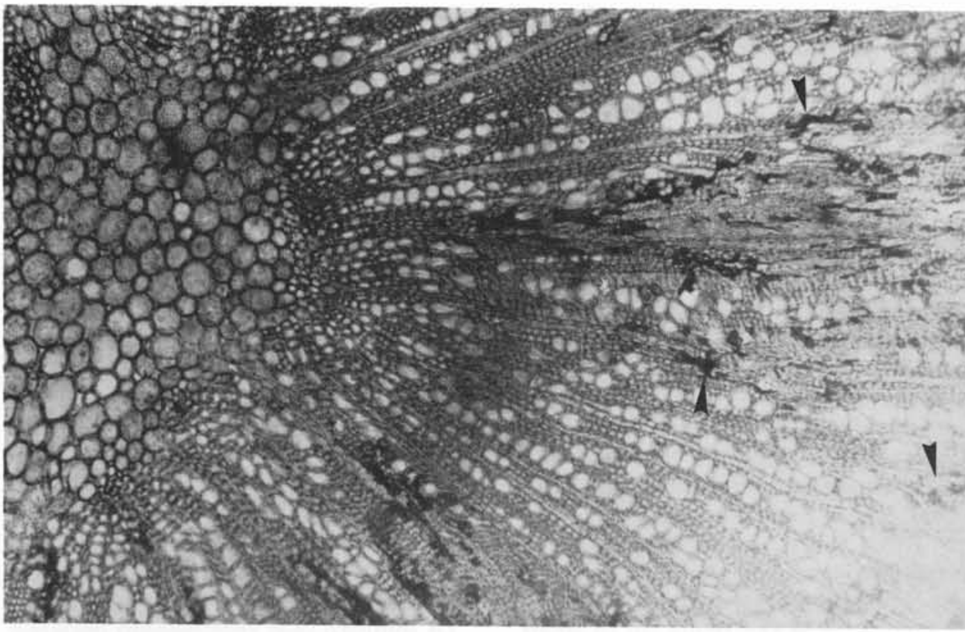

Fig. 5. Cross section of a grapefruit peduncle wood affected by psorosis tB showing triangular to irregular patches of necrotic or disarranged xylem and xylem elements (arrows, 120y).

lesions are characterized by gum layers or rings of gum pockets between layers of either normal or altered wood. Secondary lesions, a kind of staining of the inner wood, appear only several years after the bark symptoms become visible.

In this study several cultivars of grapefruit and sweet orange 8 to 20 yr-old, were examined for wood stain-

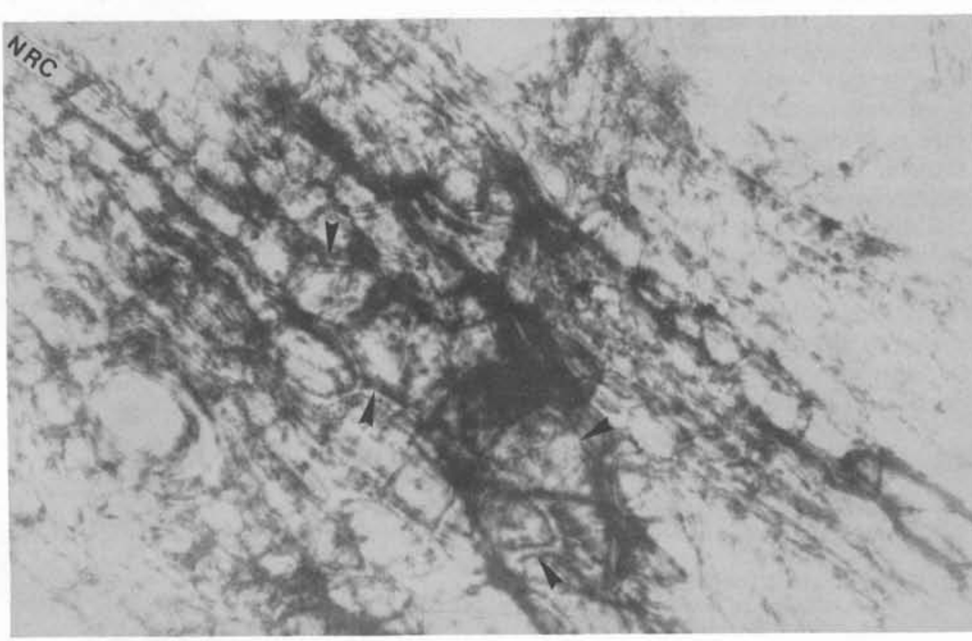

Fig. 6. Cross section of a grapefruit peduncle wood affected by psorosis tB showing hyperplastic ray cells (arrows, NRC, Normal ray cells, 500x).

ing. The typical discoloration of wood caused by psorosis A was not found. However, lesions that may be classified as primary were easily detected in the xylem of trunks, scaffold and small limbs of affected plants but not in healthy ones. The observed wood symptoms of psorosis tB are extremely similar to those described by Webber and Fawcett (13) for psorosis and identical to those rings of gum-filled pockets observed by Schneider (11) for psorosis affected sweet orange.

In peduncles a kind of tissue deterioration was found that had not been reported previously for any other citrus virus diseases.

These necrotic patches free of functional vascular bundles were usually associated with strong symptoms of scaling from the trunk up to small twigs and more or less advanced stages of twig dieback and tree decline, but also occurred in peduncles close to tissues without the scaling or gum exudation of psorosis tB.

Since the citrus tristeza virus (CTV) is endemic in the area and no other specific symptoms were observed that could be attributed to psorosis tB, and since no CTV-free peduncle controls were examined, an interference with CTV in these symptoms cannot be ruled out. Therefore it is suggested that the wood of trunks, scaffold and limbs but no peduncles should be used for differential diagnosis of psorosis tB until this point is cleared up; such a study is in progress.

At this rather preliminary stage of research on psorosis tB it is important to take into account that gum production in citrus may be induced by several abiotic and biotic factors including several viruses. Accordingly, Cartia and Catara (2) and Safran (9) reported on ring-like necrosis and secretion of gum into pockets or cavities formed by collapsing xylem vessels in impietratura diseased Volkamer lemon and grapefruit. Furthermore, formation of concentric gum rings as observed here on naturally infected plants was induced experimentally by concave gum virus and cristacortis virus in Washington Navel sweet orange and Orlando tangelo by Vogel and Bové (12).

Definite conclusions on these diseases is difficult at this time. However, similarities of external trunk symptoms as well as the identity of the xylem deterioration in scaffold branches and trunks of psorosis A and tB indicate that relationship between both diseases or even to other citrus virus diseases may not be casual. Furthermore, experiments in progress also show that environmental conditions for foliar symptom expression of psorosis tB may be improved (5). Studies on the anatomical conditions of phloem of affected plants may add relevant information on this puzzling issue.

The present results stress the urgent need for more basic research on psorosis tB.

ACKNOWLEDGMENT

The author thanks Dr. J. L. Bezerra for making photomicrographs possible at CEPLAC Laboratories, Itabuna, Bahia and Dr. H. P. Santos Filho (CNPMF, Bahia) and M. J. Beretta (IB, São Paulo), for supplying psorosis-free materials.

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