BACTERIAL CANKER OF GRAPEVINE IN BRAZIL

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ABSTRACT

In early 1998, symptoms of stem canker and necrotic spots on leaves, leaf veins, petioles, rachis, peduncles, cap stems and berries were observed on plants in vineyards in the "Submédio" of the São Francisco Valley. Initially, the symptoms were observed on 'Red Globe' and seedless grape cultivars up to three years old, during the flowering and beginning of fruit bearing stages. The incidence of disease symptoms was 100% and in some cases yield losses were nearly total. Isolations from diseased plants yielded a bacterium which was identified as *Xanthomonas campestris* pv. viticola, after biochemical, physiological and pathogenicity tests. The disease has already been detected in vineyards in Petrolina county of Pernambuco State, Piauí State and also in Curaçá, Casa Nova, Sento Sé and Juazeiro counties in Bahia State.

Key words: Vitis vinifera, Xanthomonas campestris pv. viticola.

RESUMO

Cancro bacteriano da videira no Brasil

No início de 1998, observou-se em alguns parreirais do Submédio do Vale do São Francisco plantas com sintomas de manchas necróticas nas folhas, nervuras e pecíolos, na ráquis e pedicelo dos cachos e nos ramos, com posterior formação de cancros. Estes sintomas foram observados, inicialmente, em parreirais com menos de três anos de idade, principalmente na fase da floração e início da frutificação em 'Red Globe' e em cultivares de uva sem sementes, com incidência de até 100% e causando perdas totais na produção

The "Submédio" of São Francisco Valley, especially the irrigated areas in Petrolina and Juazeiro, are extremely favorable for the growth of grapevines (*Vitis vinifera* L.) due to its climatic conditions which allows 2.5 harvests per year. This region is the largest producer and exporter of grapes in Brazil. In 1996, the area occupied with vines increased over 65%, from 2,902 ha to 4,847 ha (IBGE, 1996), having produced, in that same year, approximately 27% of the country's total harvest. Viticulture has a major social importance in the region, employing as many as 3-4 people/ha/year.

In early 1998, symptoms of stem canker, leaf spots and necrosis were observed on 'Red Globe' vines in the São Francisco Valley. Leaves showed small necrotic lesions that started as interveinal angular spots and sometimes were surrounded by a yellow halo (Fig. 1A e 1B). At later stages, leaves would turn yellow and fall off. Symptoms also developed on leaf veins and petioles, on pedicels and rachis of grape clusters. On the stems, irregular spots coalesced to form long dark streaks that evolved to necrotic lesions and cankers. Longitudinal cracking of the stems was often obem algumas áreas. Nos isolamentos a partir de plantas infectadas, detectou-se a presença de uma bactéria, identificada como *Xanthomonas campestris* pv. viticola através de testes bioquímicos, fisiológicos e de patogenicidade. A doença já foi constatada em parreirais do município de Petrolina, no Estado de Pernambuco, no Estado do Piauí e nos municípios de Curaçá, Casa Nova, Sento Sé e Juazeiro, no Estado da Bahia.

served on stems exposing the underlying tissues (Fig.1C e 1D). Vascular discoloration was also observed. In the beginning of fruit set stage, grape clusters showed necrosis of pedicels and rachis (Fig.1E and 1F), followed by fruit wilting and drying All symptoms were initially observed on young vines, up to three years of age, during the flowering stage, beginning on fruit bearing at the pea-sized stage or after the first pruning.

Cultivars severely affected were 'Red Globe' and the seedless varieties originated from 'Thompson Seedless', on which the incidence was nearly 100%. In some areas, total loss in production was reported. Disease symptoms were detected with variable incidence on 'Italia', 'Festival', 'Piratininga', 'Patricia', 'Ribier', 'Catalunha', 'Brasil' and 'Benitaka'. Cultivar Italia showed tolerance to the disease under field conditions. Similar symptoms were observed in older vines of the same cultivars during the maturation phase. In some areas, disease symptoms became more severe after a heavy rain (100 mm) which occurred in mid-January. More severe and faster developing symptoms were observed on



FIG. 1 - Bacterial canker on grapevine, cultivar Red Globe, caused naturally by *Xanthomonas campestris* pv. viticola:
A - Leaf spots; B - Marginal necrosis; C and D - Cankers on canes; E - Dried inflorescences on a cluster;
F - Grape cluster showing necrosis on the rachis and on a berry.

vines conducted under different irrigation systems such as central pivot and overhead sprinkler irrigation. Bacterial canker had a direct effect on fruit yield and future losses could be predicted since the canes and leaves were highly infected. Disease control strategies such as pruning of the symptomatic side branches, leaving canes with 20 cm of length containing four buds, and also intensive copper spraying on infected 'Red Globe' plants did not prevent infection of new scion shoots. Symptoms were observed 50-80 days after pruning.

Disease occurrence was reported in Petrolina, state of Pernambuco, and in Juazeiro, Curaçá, Sento Sé and Casa Nova counties, state of Bahia. It was also detected in the state of Piaui (Malavolta Jr. *et al.*, 1999). The objective of this work is to report the occurrence of this new disease on grapevines and to study its etiology. Preliminary reports have been published (Lima *et al.*, 1998; Malavolta Jr. *et al.*, 1998).

Bacterial isolation: In order to isolate the causal agent of grapevine canker, stems, leaves and berries from infected plants were collected at different localities where 100% of

disease incidence had been observed. Small pieces of infected tissue were first examined under the microscope for the presence of bacterial oozing. Tissue was surface-disinfested (70% ethanol, 30 sec; 0.1% sodium hypochlorite 1-3 min.), rinsed twice with sterile distilled water, blotted, macerated in a sterile droplet of water with a glass rod and placed on NA (nutrient agar) or on Kado 523 medium (Kado & Heskett, 1970). Plates were incubated at 28-30 °C.

Tobacco hypersensitivity test: Single cell colonies of the bacterium were transferred to NA plates and incubated at 28-30 °C for two days. A bacterial suspension (10^9 CFU/ml) was prepared and infiltrated in young tobacco leaves with a needle. Plants were inoculated under greenhouse conditions during the morning and evaluated 24 hours later.

Pathogenicity tests: Pathogenicity of 26 bacterial isolates originated from diseased plants was evaluated on healthy 'Red Globe' plants grafted on 'IAC 576' as rootstock. Two inoculation techniques were used: (a) leaves were wounded with a sterile needle and sprayed with a 10⁷ CFU/ml bacterial

suspension on both sides (Mello *et al.* 1997) and (b) deposition of bacterial cells from 2-day-old cultures (Nayudu, 1972) at the base of the petioles after superficial wounding with a needle. With the latter method the inoculation point was covered with a small piece of aluminum foil to prevent dessication and two points were inoculated in each plant. Two plants per isolate were inoculated with each method. Plants were kept in a moist chamber for 48 hours before and 48 hours after the inoculation, in a greenhouse, where temperatures ranged from 18-43 °C.

Testing against *Xylophilus ampelinus* **antisera:** Due to the similarity of symptoms observed in infected plants to those caused by the bacterial blight causal agent, *Xylophilus ampelinus* (Panagopoulos) Willems *et al.* (Bradbury, 1991) all isolates and the crude extract from infected leaf tissue were tested with the specific *X. ampelinus*-monoclonal antibody. A DASI- ELISA (double antibody sandwich indirect ELISA) procedure was used according to the manufacturers instructions (REALISA Immunoenzymatic Methods for Diagnosis; Valencian Institute for Plant Research, IVIA, Valencia, Spain).

Identification of bacterial isolates: Identification tests were carried out at the Laboratório de Fitopatologia - UnB, Brasília, DF. Bacterial isolates were identified according to conventional biochemical and physiological tests (Schaad, 1988). The following isolates were included for comparative tests: *Xanthomonas* sp. pv. passiflorae (Pereira) Vauterin *et al.* (UnB 684); *X. axonopodis* pv. manihotis (Berthet & Bondar) Vauterin *et al.* (UnB 1037), *X. campestris* pv. vesicatoria (Doidge) Dye (UnB 823), *Pseudomonas cichorii* (Swingle) Stapp (UnB 846), *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* (UnB 1173 and 630) and *Pseudomonas syringae* pv. tabaci (Wolf & Foster) Young *et al.* (UnB 647).

Intense bacterial oozing from stems, fruit and leaf tissues from all different collection sites was observed. Isolations yielded, in most cases, pure cultures of round, mucoid, creamy-white, colonies of a bacterium. Growth on Kado 523 or NA plates was observed after 48-72 h at 28 °C and 33 °C. Tobacco hypersensitivity tests were negative or weak after 24 hours. Plants showed yellowing after 48 hours and necrosis after three days. However, all 26 isolates were pathogenic to 'Red Globe' plants. Symptoms developed 12-14 days after inoculation and varied according to the inoculation method used. Plants inoculated at the base of petioles showed necrosis, dark lesions and small cankers causing longitudinal crackings from the inoculation point. Twentyfive days after inoculation, lesions varied from 5 to 26 mm in size and necrosis had girdled more than 50% of the stems. Disease severity varied among the different isolates. Plants inoculated by spraying with bacterial suspension over the leaves showed necrotic spots developed at the wounded sites spreading later to new areas; lesions were more frequent at the lower surface. Leaf spots were occasionally surrounded by a chlorotic halo. Yellowing and premature falling off the leaves were observed 25 days after inoculation. Long, necrotic spots were seen on the petioles and on leaf veins. After longitudinal section of the stems, vascular discoloration was evident. Bacterial streaming was abundant on leaves and stems of inoculated plants. Bacterial colonies were recovered from the inoculated petioles and leaves that developed typical symptoms. Cultural characteristics of these isolates were similar to the original ones, thus fulfilling Koch's Postulates.

None of the isolates and neither the extract obtained from infected tissue reacted with the Xylophilus ampelinusspecific antibody. No positive reaction was detected 15, 30, 45 or 60 minutes after the substract was added. The bacterial isolates obtained from infected grapevine tissue showed the following properties: Gram-negative; metabolism strictly aerobic; negative production of fluorescent pigments on King's B medium; urease and oxidase activity not detected; asparagin not utilized as the sole source of carbon and nitrogen; poly-β hydroxybutyrate inclusions not produced; casein utilized, tolerance up to 2% NaCl but negative growth on Kado 523 medium amended with 5% NaCl; acid production from: glucose, mannose, galactose, trehalose, cellobiose and fructose. The negative results obtained with the DASI-ELISA test, the absence of urease acivity, fast growth (48-72 hs) at 28 °C and 33 °C, production of acid from glucose and the absence of a brown pigment on yeast-galactose-chalkagar (Panagopoulos, 1994) indicated that the isolated pathogenic bacteria was not X. ampelinus. According to biochemical, physiological and pathogenicity tests, the grapevine canker pathogen was identified as the non-pigmented Xanthomonas campestris pv. viticola (Nayudu) Dye. Considering the nomenclature proposed by Vauterin et al. (1995) the bacterium should be referred as Xanthomonas sp. pv viticola (Nayudu) Vauterin et al., since the pathovar viticola was not included in their reclassification of the genus Xanthomonas.

Pathogenic bacteria on Vitis spp. already reported are: Xylella fastidiosa Wells et al. causing Pierces disease, Agrobacterium tumefaciens (Smith & Townsend) Conn causing crown gall, Xylophilus ampelinus causing bacterial blight (Pearson & Goheen, 1994); Xanthomonas campestris pv. viticola (Nayudu, 1972), X. campestris pv. vitiscarnosae (Moniz & Patel) Dye (Moniz & Patel, 1958) and X. campestris pv. vitistrifoliae (Padhya, Patel & Kothastane) Dye (Padhya et al., 1965). In Brazil, the only known bacterial disease of grapevines is crown gall (Marques et al., 1994). Xanthomonas campestris pv. viticola was first detected in India in 1972 and it infects V. vinifera (Vitaceae), neem (Azadirachta indica A. Juss) (Meliaceae) and Phyllanthus maderaspatensis L. (Euphorbiaceae) (Nayudu, 1972). In Brazil, it has been suspected that the disease is present in the region since 1996 or 1997, however it remained undetected until 1998.

Considering the severity of the symptoms solely on young vines it is possible that the pathogen may have been introduced into the area through grafting with contaminated planting material, that was later distributed to other vineyards. Since the disease was later observed in older vines, it is possible that, in this case, pathogen dissemination occurred by the use of contaminated pruning tools. Disease development was probably favored by heavy rainfall in January, which caused leaf rupture allowing the penetration and spread of the bacterium. A similar situation occurred in India, where a higher incidence was reported during rainy periods, suggesting the ability of the bacterium to survive during dry periods (Nayudu, 1972).

Disease resistance, chemical control and cultural practices would be feasible measures to manage bacterial canker in the area. In field assays in India, it was observed that copper followed by Bordeaux mixture applications reduced intensity of the disease, but the treatment was less effective in areas with frequent rainfall (Chand et al., 1992). Management practices that minimize the risk of infection and disease spread during pruning have been proposed by Chand et al. (1991). During a screening for resistance to the pathogen, under artificial and natural infection conditions, Chand (1992) found that V. vinifera was highly susceptible while some genera in the family Vitaceae and some species of Vitis were highly resistant. Seedless cultivars of V. vinifera were more susceptible than the others. Assessment of resistance among grape cultivars currently grown in the São Francisco Valley, especially the seedless cultivars, is a necessary effort. Rootstock varieties commonly used in the region (IAC 572, 766 and 420A) have failed to show any symptoms so far, even when grafted with highly susceptible scion varieties. It is also important to evaluate their reaction to X. campestris pv. viticola for future recommendations.

In order to prevent the introduction of the bacteria into new areas and to control disease spread, the following measures have been recommended: destruction of infected canes and branches; application of a copper slurry in wounds resulting from pruning; copper sprays after pruning and budding; disinfection of pruning tools using a 2% active sodium hypochlorite or a 0.1% quaternary ammonia solution ('Quatermon'); use of healthy planting material; avoidance of twisting the branches before application of the growth regulator ('Dormex'); avoidance of overhead sprinkler irrigation which facilitates spread of bacteria and avoidance of traffic of vehicles and equipments. Finally, the importation and exchange of planting material and germplasm should follow the legal restrictions imposed by the Ministry of Agriculture (Embrapa-Semi Árido / Valexport, 1998).

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