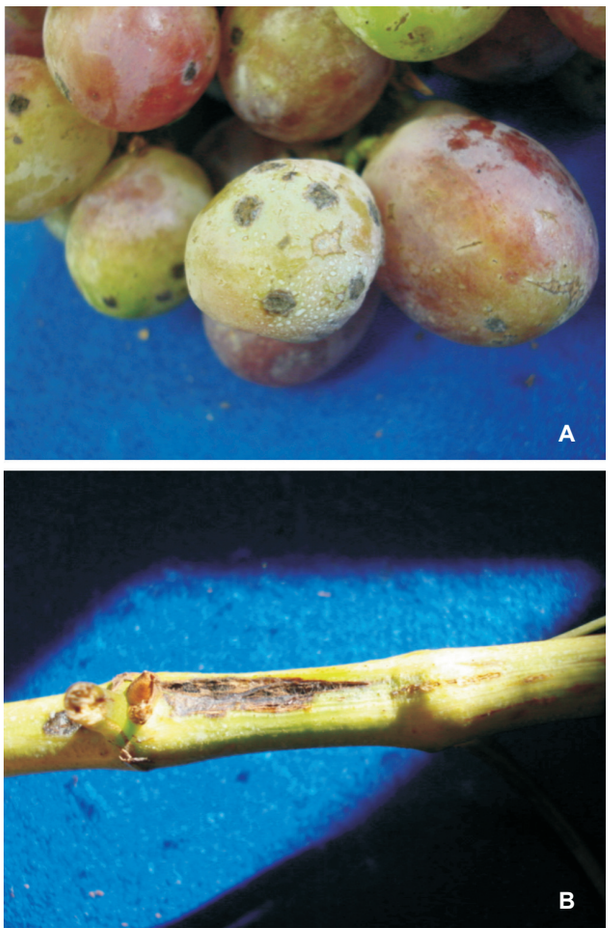

FIGURE 1 – A. Natural symptoms induced by *Xanthomonas campestris* pv. *viticola* on berries and **B.** twig.

immersed in a bacterial suspension (ca. 10^7 CFU/mL) on the lower surface of the leaves. Three weeks after inoculations similar symptoms were observed and identical bacteria were re-isolated.

Seven isolates were used in molecular tests. Genomic DNA was extracted as described by Pitcher et al. (1989). The PCR amplification of the 16S-23S rDNA spacer region was performed as described by Destéfano and Rodrigues Neto (2002). The amplification yielded a unique band of approximately 1.1 kilobase (kb) for all the strains tested. The restriction fragments were separated by electrophoresis in 3% agarose gels in 1X TAE buffer (Maniatis et al., 1982). The gels were stained with 0.1 μg per mL of EtBr and then photographed under ultraviolet transillumination using an Alpha Innotech 2200 digital system. The molecular weights of the fragments were determined by comparison with a 100 base pairs (bp) DNA ladder (GE Heathcare). PCR products (5 μL) were digested, individually, with each of the following restriction endonucleases *Dde* I, *Hinf* I and *Hpa* II under conditions specified by the manufacturer (Fermentas), and the results showed identical RFLP patterns of the isolates in comparison with the pathovar reference strain of *X. campestris* pv. *viticola* and with a bacterial strain isolated of plant material from Petrolina, State of Pernambuco, Brazil (IBSBF 1967^P and 1926, respectively) (Figure 2).

Grapevine cultivation is economically important for the country and bacterial canker may become a limiting factor for this crop, even having an impact on trade. For this reason *X. campestris* pv. *viticola* is presently considered a quarantine pest (A2) by the government and it is under regulation ("MAPA, Instrução Normativa n. 52", November/20, 2007). Efforts and control strategies carried out by the Coordination of Plant Protection of the State of Sao Paulo were based on intensive surveillance and eradication measures.

As a result of this eradication campaign, the above mentioned orchard was eradicated and approximately 4700 plants were destroyed, corresponding to five hectares of planting. Surveys were conducted in 2009/2010 in the State of São Paulo in grape-producing regions; a total of 68 orchards were inspected for detection of bacterial canker and no other infected orchard was observed. Thus, the situation of *Xanthomonas campestris* pv. *viticola* in the State of Sao Paulo can be described as follows**:** absent, detected in 2009 on one orchard located at Tupi Paulista county, eradicated and under official control.

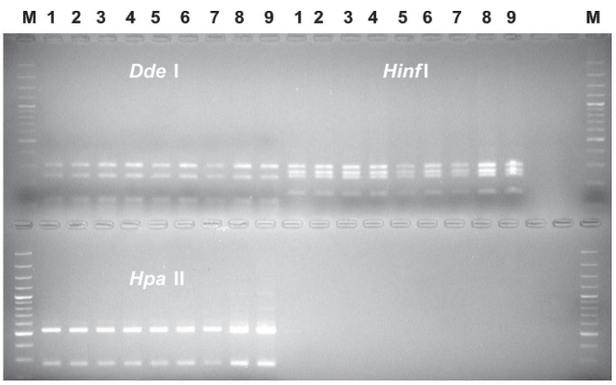

FIGURE 2 - PCR-RFLP of the 16S-23S rDNA spacer region digested with *Dde* I, *Hinf* I and *Hpa* II. (M) 100 bp marker (Fermentas), (1-3) bacteria isolated from leaves (IBSBF 2738, L21 and L-23, respectively); (4-5) from berries (IBSBF 2740, 2741); (6-7) from twigs (IBSBF 2739, TW17); controls: (8) IBSBF 1967^P and (9) IBSBF 1926. (^{P}= pathovar reference strain).

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