Leaf Spot of *Vitis vinifera* L. Caused by *Xanthomonas* Sp.

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ABSTRACT

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A bacterial leaf spot was identified on the grape cultivar Cabernet Sauvignon in 1978 on Long Island, NY. Numerous interveinal, water-soaked lesions, which often coalesced into large necrotic areas, were apparent on infected leaves. The pathogen was identified as a *Xanthomonas* sp. biochemically and physiologically different from *X. ampelina*, a known pathogen of grapevines that causes similar leaf-spotting symptoms. Greenhouse pathogenicity tests were positive on the cultivars Cabernet Sauvignon and Thompson Seedless.

Bacterial leaf spots and blights of *Vitis vinifera* L. have been reported in Greece, France, South Africa, India, Argentina, and New Zealand. Various pathogens, including *Xanthomonas ampelina* Panagopoulos (9), *Pseudomonas viridiflava* (Burkholder) Dowson (10), *P. syringae* Van Hall (7), and *P. viticola* Nayudu (8), have been documented as causal agents of these sometimes highly destructive diseases.

In the fall of 1978, a leaf spot of the grape cultivar Cabernet Sauvignon was observed in a 10-acre vineyard on Long Island, NY. This report describes the disease, and a *Xanthomonas* sp. is shown to be the pathogen. This is the first report of a bacterial leaf spot of *V. vinifera* L. in the United States.

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MATERIALS AND METHODS

Isolation and characterization of the pathogen. Water-soaked lesions from infected grape leaves were macerated in a drop of sterile, distilled water. Several loopfuls of the solutions were examined microscopically and streaked on plates of King's medium B (5), which were then incubated for 48 hr at 28 C.

The following tests were used to characterize the isolates: gram stain; production of cytochrome oxidase, catalase, and urease (3), indole, acetoin, and H_2S (2); a yellow, water-insoluble pigment (9), a fluorescent pigment (5), a brown pigment on 1% yeast extract, 2% galactose, 2% $CaCO_3$, and 2% agar (YGCA) (9); inhibition by triphenyltetrazolium chloride (TTC) (1); acid from glucose and galactose (4,9), xylose and sucrose (2,9); nitrate reduction (2); hypersensitive response on tobacco (6); growth on nutrient agar (Difco) (9); oxygen requirements (2); maximum growth temperature (9); ability to liquefy gelatin and to hydrolyze starch and esculin (3); and tolerance to NaCl (9).

Each of five isolates was grown on Difco potato-dextrose agar (PDA) for 48 hr before testing; all tests were done at least twice.

Bacteria were examined for size, shape, and flagellation by transmission electron microscopy. Isolates were grown for 48 hr in one-third strength nutrient broth (Difco) before examination.

Pathogenicity tests. Pathogenicity of isolates was tested by inoculating 3-mo-old Cabernet Sauvignon and Thompson Seedless grapevines in the greenhouse. Inoculum was grown on PDA for 48 hr at 28 C, and water suspensions containing approximately 10^8 colony-forming units/ml were made. The inoculum was atomized onto upper and lower leaf surfaces, and plants were then covered with plastic bags for 48 hr. Six vines of each cultivar were inoculated with each of the five isolates.

Green shoots of the same cultivars were stab-inoculated with a thick smear of bacteria and covered with moist cotton for 48 hr. Two shoots on each of three vines were inoculated for each isolate. Check inoculations were made with sterile, distilled water. Final disease ratings were made 3 wk after inoculation.

RESULTS

Description of disease. Numerous interveinal, water-soaked lesions, which became necrotic and often coalesced into large necrotic areas, were apparent on infected leaves (Fig. 1). Only the basal one to six leaves of primary shoots (no leaves of lateral shoots) were infected. Ten to 70% of the older leaves of about half of the vines in the vineyard were infected. When the vineyard was observed the following spring, no cankers were present on trunks or shoots.

Isolation and characterization of the pathogen. A nonfluorescent bacterium that produced small white colonies in 48 hr on King's medium B was consistently isolated. The bacterium was gram-negative, rod-shaped, about 1.75 μm $\times$ 0.6 μm, and motile by a single polar flagellum. A hypersensitive response on tobacco was produced; however, this reaction was variable and often took 3–4 days before it appeared. Isolates were positive for H_2S production and produced a negative reaction for nitrate reduction as well as production of indole and acetoin. None of the isolates was able to grow anaerobically or was inhibited by 0.1% TTC. Table 1 compares other test results with published results for *X. ampelina*, which causes "Tsilik marasi," a serious bacterial disease of grape in Greece (9). Except for the lack of inhibition by TTC, our isolates fit the description of a *Xanthomonas* sp. given in Bergey's manual (1). Although our isolates resemble *X. ampelina* in some respects, significant differences are also apparent.

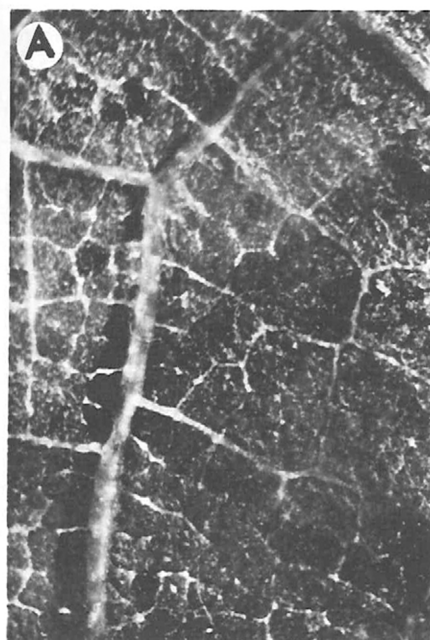
Pathogenicity tests. Lesions similar to those observed in the field developed on both Cabernet Sauvignon and Thompson Seedless leaves inoculated in the greenhouse (Fig. 2). Infections occurred only on the basal five or six leaves of each plant. Because leaf susceptibility increased with leaf age, plants at least 3 mo old were used for pathogenicity tests. Water-soaked lesions, visible 3 days after inoculation, later became necrotic, and the pathogen was easily reisolated from greenhouse infected plants.

Darkened areas developed around shoot inoculations; however, they did not spread and no shoots died.

Table 1. Comparison of biochemical and growth characteristics of *Xanthomonas* sp. from grape and *X. ampelina*

Test	*Xanthomonas* sp.	*Xanthomonas ampelina*[a]
Production of yellow, water-insoluble pigment	−	+
Growth on nutrient agar	+	slow[b]
Pigment on YGCA	−	+
Oxidase	−	+
Catalase	+	+
Urease	+	+
Gel liquefaction	−	−
Starch hydrolysis	−	−
Esculin hydrolysis	−	−
Acid from glucose	+	−
Acid from xylose	+	−
Acid from galactose	+	+
Acid from sucrose	−	−
NaCl tolerance	4%	1%
Maximum growth temperature	37 C	30 C

[a] Data from Panagopoulos (9).
[b] Colonies of *X. ampelina* are barely visible (0.2–0.3 mm diam) on nutrient agar after 6 days at 26 C, while *Xanthomonas* sp. colonies are visible in 48 hr.



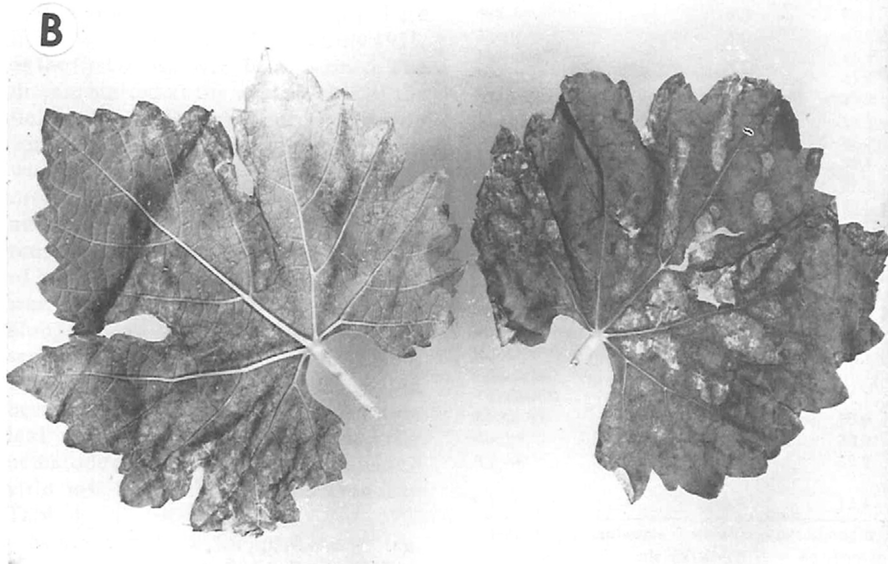


Fig. 1. (A) Water-soaked lesion and **(B)** necrotic areas on naturally infected Cabernet Sauvignon leaves.

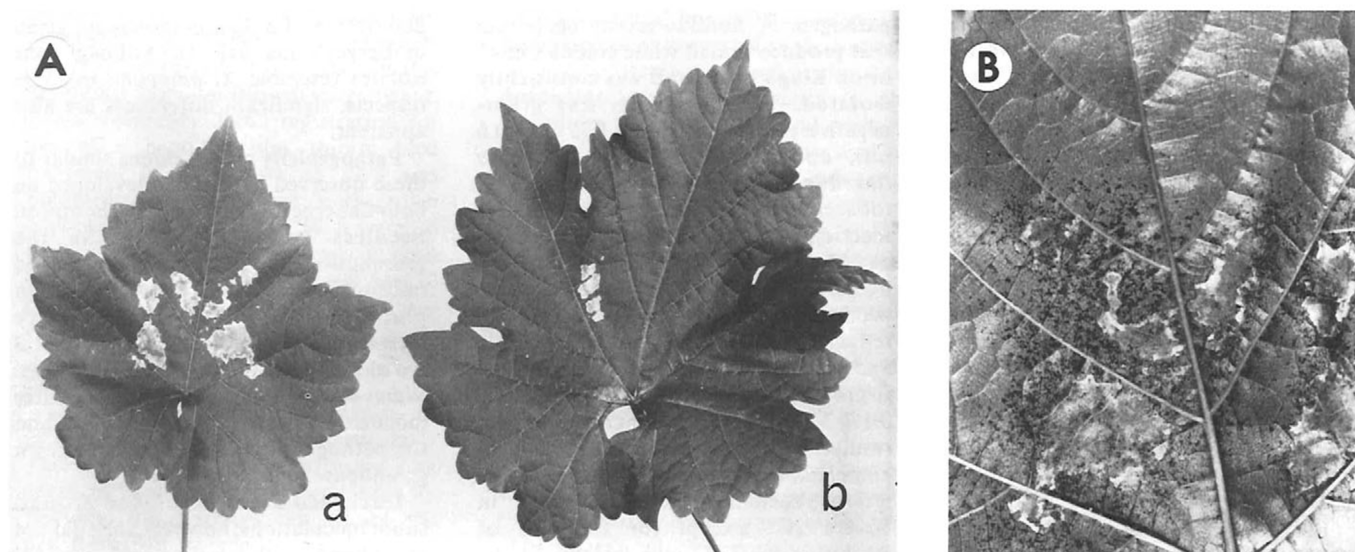


Fig. 2. (A) Necrotic lesions on artificially inoculated Thompson Seedless (a) and Cabernet Sauvignon (b) leaves. **(B)** Close-up of necrotic areas.

DISCUSSION

The *Xanthomonas* sp. isolated from grapevines in New York State differs in several biochemical and physiological ways from *X. ampelina* (9) and other bacterial pathogens reported to cause leaf spot and blight symptoms on *Vitis vinifera* (7,8,10). Known isolates of *X. ampelina* were not obtained and compared with our isolates in laboratory tests because we did not want to risk introducing the pathogen into our vineyards. It seems unlikely, however, that the differences we found between *X. ampelina* and our isolates could result from differences in laboratory techniques.

Field observations also suggest that "Tsilik marasi" caused by *X. ampelina* differs from the leaf spot we observed. The symptoms of "Tsilik marasi" include swelling and death of infected spurs, cracking and cankering of infected shoots, and a haloed spotting of leaves (9). We observed none of these symptoms except leaf spotting; however, differences in environmental conditions and cultivar susceptibility may account for the lack of some symptoms.

Xanthomonas leaf spot was not observed during the 1979 growing season. We speculate that the unusually dry July and August, in contrast to the wetter season of 1978, contributed to the disease's absence. The impact of this disease on New York State grape production cannot yet be predicted.

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