Pierce's Disease on Muscadine Grapes in North Carolina

R. D. MILHOLLAND, Professor, PI-YU HUANG, Research Cooperator, C. N. CLAYTON, Professor Emeritus, and R. K. JONES, Associate Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27650

ABSTRACT

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Bacteria with rippled cell walls averaging 0.5 × 1.4 μm were consistently observed in the lumen of xylem vessels of muscadine grape leaf veins and petioles with marginal leaf burn symptoms. The rickettsialike bacteria in the xylem vessels and successful transmission of an infectious agent by grafting indicates that the marginal leaf burn problem on muscadine grapevines in North Carolina is Pierce's disease. This is the first report of this disease on muscadine grapevines in North Carolina. The erratic symptom expression on the cultivar Carlos did not appear to be related to winter temperature.

Pierce's disease has been a serious problem of bunch grapes *(Vitis vinifera)* since the late 1800s (9). Affected vines show necrosis of the leaf margin, decline in vigor, reduction in yield, and eventually death. Several workers have associated a rickettsialike bacterium with the xylem tissue of grapevines with symptoms (2,3,5). Davis et al (2) recently isolated a Gram-negative, rod-shaped bacterium from grapevines with Pierce's disease and Koch's postulates were completed. Characteristically, the bacteria are confined to the lumen of xylem vessels, have a rippled cell wall, and measure 0.25–0.50 × 1.0–4.0 μm.

Symptoms of this disease were also reported for muscadine grapevines (*V. rotundifolia*) (4). Symptoms similar to those of Pierce's disease were first observed on muscadine grapevines in North Carolina during the summer of 1962. Necrosis or burning of the margin of older leaves appeared in July to September, but in contrast to bunch grapes, affected muscadine vines did not die and did not always show the symptoms each year. The problem was referred to as marginal leaf burn (MLB) (1), because of the leaf symptoms and the lack of proof of the etiological agent causing Pierce's disease in 1962.

The purpose of our studies was to examine the response of muscadine grapevines, particularly the cultivar Carlos, to MLB over a period of years, and to determine if bacteria were associated with MLB in North Carolina vineyards.

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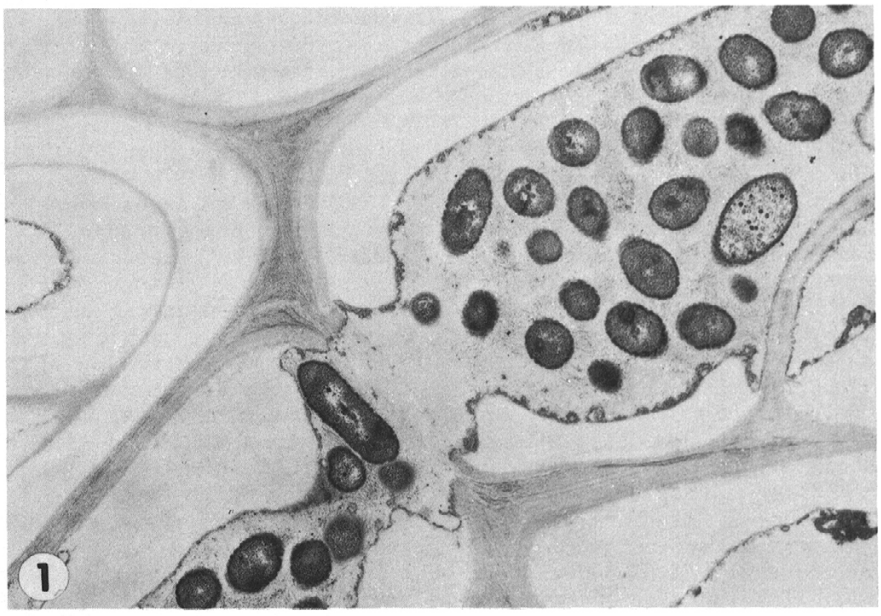
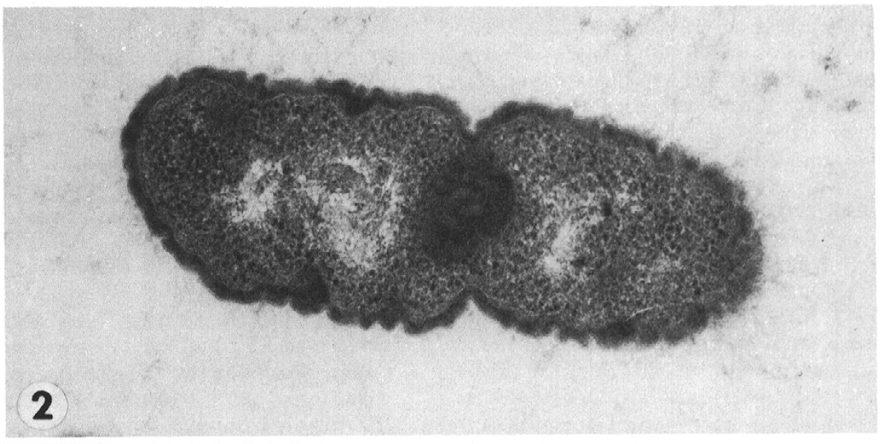
MATERIALS AND METHODS

Reaction of muscadine grape plants. Selected 4-yr-old Carlos plants in a vineyard in Pender County, NC, were rated for MLB severity in 1972 and 1973. Plants were grown on a Geneva double curtain trellis system, and each of the four arms were scored separately. Individual arms were rated in September on a scale 0 = no symptoms, 1 = 1–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100% of the leaves with MLB.

Eight-year-old Carlos plants were rated for MLB severity in an overhead





Figs. 1 and 2. Rickettsialike bacteria in lumen of xylem vessel in muscadine grape with symptoms of marginal leaf burn: **(1)** Bacteria with rippled cell walls in lumen of xylem vessels (×10,350). **(2)** A dividing bacterium. Note invagination of both cell wall and cell membrane (×52,900).

Table 1. Occurrence of bacteria with rippled cell walls in the petiole and leaf vein of Carlos grapevines with marginal leaf burn[a]

Symptoms	Petiole		Leaf vein	
	Vessels examined (no.)	Vessels with bacteria (%)	Vessels examined (no.)	Vessels with bacteria (%)
Severe	2,724	1.6	1,887	8.6
Moderate	1,490	2.1	1,415	5.8
Mild	2,152	1.3	1,407	5.3
Healthy	1,500	0.0	1,800	0.0

[a] Averages of 12 samples (four plants from three locations).

vineyard in Onslow County in September of 1977, 1978, and 1979.

Graft transmission. Twenty-three cuttings from 10 Carlos vines with MLB symptoms in 1974 were rooted in the greenhouse. Approach grafts were made between vines of Carlos plants and the vinifera grape cultivar Carignane, which is susceptible to Pierce's disease. Cuttings of Carignane, known to be free of Pierce's disease, were obtained from D. L. Hopkins, University of Florida, Agricultural Research Center, Leesburg. Two Carignane vines were used as controls. Symptoms were recorded after 4 mo.

Histology. Leaf samples from Carlos plants with 100% (severe), 50% (moderate), 5% (mild), and no marginal necrosis were collected in September 1979 from three vineyards in New Hanover, Pender, and Onslow counties. Four leaves in each severity class were collected from each location. Tissue samples (5 mm long) were excised from petioles and midveins of leaves. Alternate samples were fixed in formalin-propionic-propanol or glutaraldehyde fixative (4% glutaraldehyde in 0.06 M sodium cacodylate).

Samples fixed in formalin-propionic-propanol for light microscopy were dehydrated for 1 wk in an isopropyl alcohol series, infiltrated, and embedded in Paraplast (Sherwood Medical Industries, St. Louis, MO 63100). Specimens were softened for 48 hr in a solution of 90 ml of 1% sodium laural sulfate (Dreft) and 10 ml of glycerol and were sectioned on a rotary microtome to a thickness of 12 μm. Sections were mounted on slides with Haupts adhesive and stained with Harris hematoxylin (7).

Samples were prepared for electron microscopy as described by Huang and Goodman (6). Electron micrographs were taken with a Siemens Elmiskop 1-A electron microscope.

RESULTS AND DISCUSSION

Reaction of muscadine grape plants. In 1972, MLB was rated as severe on two of three C45-11 and C45-12 arms and mild on one arm. The percentage of leaves with MLB was high for all arms except C45-12-4, with 11–25% affected leaves. In 1973, the severity of symptoms changed in two of the arms from severe to mild (C45-11-2) and mild to severe (C45-12-4). The remaining arms were rated the same as in 1972. Incidence of MLB was drastically reduced in all three arms of C45-11 but remained relatively the same for the three C45-12 arms. This change in symptom expression from year to year has been observed in North Carolina vineyards for the past 10 yr.

The reaction of 20 Carlos plants over a 3-yr period to MLB in an overhead vineyard was also erratic. The percentage of the vine with MLB increased on some plants and decreased on others; several varied from year to year. A few plants remained symptomless during the 3 yr. The percentage of the vine with symptoms increased for 14 of 20 plants from 1977 to 1978 but was drastically reduced in 1979. Vines from one plant showed 50% MLB symptoms in 1978 but were symptomless in 1979.

This difference in symptom expression from year to year does not appear to be related to winter temperatures as suggested by Purcell (10), who reported that cold temperatures may act indirectly to induce recovery of grapevines with Pierce's disease. Later studies (11) indicated that cold temperature alone is unlikely to cause remission symptoms. In our studies, temperatures averaged much lower during the 1977-1978 winter months than during the 1978-1979 winter, yet the percent of vines with MLB increased in 1978 and decreased in 1979. The number of hours below 7 C was 2,059 from 1 October 1977 to 1 April 1978 but 1,563 hr for the same period in 1978-1979. The total hours at −10 to −4 C during the 1977-1978 winter months was twice the number that occurred during the 1978-1979 period. This tends to support the hypothesis that colder winter temperature alone is not therapeutic.

Graft transmission. MLB symptoms were observed on 17 of 23 Carignane vines 4 mo after grafting with the Carlos vines. The severity of symtoms ranged from none to complete leaf necrosis. Nine vines had mild MLB, seven vines were rated as severe, and all the leaves on one vine were dead. The two Carignane vines used as controls did not show MLB. Carlos plants from which cuttings were taken in 1974 were rated for MLB severity the following year; all of the vines showed MLB symptoms, with 5–50% of the leaves affected.

These results indicated that a pathogenic organism and not a nutritional deficiency (as once thought) is involved in MLB on Carlos. The range of symptoms on Carignane from none to death of the vine probably reflects the concentration of bacteria in the Carlos cuttings.

Histology. Bacteria were present in the tracheary elements of leaves and petioles with severe, moderate, and mild MLB from each of the three vineyards. Bacteria were not found in healthy leaf and petiole sections. The number of tracheary elements containing bacteria was greater in leaf vein sections than in the petioles for each severity rating (Table 1).

The percentage of leaf vein vessels with bacteria (8%) was much lower than the 22% reported by Mollenhauer and Hopkins (8) for muscadine grapes with Pierce's disease. This may be attributed to the fact that a more susceptible cultivar (Pride) was used in their studies.

Bacteria with rippled cell walls were consistently observed in the lumen of xylem vessels of grape leaf veins with symptoms of Pierce's disease but not in the tissue prepared from healthy controls (Fig. 1). Bacteria were rod-shaped and 0.35–0.7 × 1.0–2.3 μm (average, 0.5 × 1.4 μm). The rippled cell walls were 41 nm thick. Abundant microfibrils extended from the cell walls of most bacteria.

The bacteria appeared to divide by binary fission; ie, they were characterized by invaginations both of cell walls and cell membranes (Fig. 2).

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