Seasonal Concentration of the Pierce's Disease Bacterium in Grapevine Stems, Petioles, and Leaf Veins

D. L. Hopkins

Professor of Plant Pathology, University of Florida, Agricultural Research Center, Leesburg 32748.
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ABSTRACT

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Stem, petiole, and leaf vein tissues were collected five times during the season from cultivar Schuyler grapevines infected with Pierce's disease and were examined by light microscopy for bacterial occlusions in the xylem. Bacteria were not found in the 29 March samples of current season tissues, but were present 21 April. The highest concentration of bacterial occlusions occurred 7 June and high concentrations also were present 28 July. By 10 October, bacterial concentrations were greatly reduced. The number of vessels occluded per cross section was not an accurate indication of the amount of plugging in the tissue. Values obtained by serial sectioning of 0.5 cm of tissue were 4–12 times those obtained per cross section. Leaf veins from leaves with marginal necrosis had 80% of their vessels completely plugged in 0.5 cm of leaf vein tissue. Bacterial infestations in leaf veins were highly correlated with leaf marginal necrosis. Seasonal concentrations of bacterial plugs were closely correlated with symptom development and with seasonal vector transmission data.

Pierce's disease (PD), the major limiting factor in the production of bunch grapes in the southeastern USA, is caused by a small, Gram-negative bacterium that has been referred to as rickettsialike (2,5,8). Symptoms of the disease include decline of vigor, marginal necrosis or scorching of leaves, wilting and drying of fruit, uneven maturation of canes, delayed growth in the spring, and usually death of the grapevine. These symptoms suggest a dysfunction of the water conducting system (6,14).

Although bacterial aggregates, gums, and tyloses definitely block vessels and restrict water movement in grapevines with PD (4,11,12), it is questionable that this physical blockage is sufficient

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to cause the disease symptoms. In cross sections of petioles and veins of grape leaves with marginal necrosis, rarely more than half of these are totally occluded (12). In stems, even fewer vessels are blocked. Only 15% of the xylem vessels in almond trees with almond leaf scorch contained rickettsialike bacteria (10). It was postulated that almond leaf scorch symptoms resulted from both occlusion of vessels and the action of a bacterial toxin. Subsequently, the PD bacterium was reported to produce a phytotoxin in culture which reproduced, at least in part, the disease symptoms in grapevine (9).

Epidemiological studies in Florida indicated that leafhopper vectors collected from infected grapevines are not naturally infective until 8 wk, or longer, after spring budbreak in the vines. The most efficient transmission of the disease by the vectors occurred during May, June, and July. Transmission between July and November was sporadic (1).

The purpose of this study was to monitor the seasonal buildup of the PD bacterium in various tissues of susceptible grapevines and possibly to correlate these observations with the results of earlier transmission studies. The possible relationship of the bacterial buildup to symptom development also is discussed. A preliminary report was published (7).

MATERIALS AND METHODS

A 4-yr-old planting of *Vitis labrusca* L. 'Schuyler' at the Agricultural Research Center in Leesburg, FL, was used in this study. Individual vines showing marginal necrosis of the leaves during the preceding year were selected for sampling in 1977. Two of the vines died during midsummer, so comparable vines were substituted in the last two samples.

Three stem, petiole, and leaf vein samples were collected from the vines on 29 March, 21 April, 7 June, 28 July, and 10 October. Leaves with marginal necrosis were sampled when available. Samples were cut into 2- to 3-mm pieces, fixed in FAA (formalin-alcohol-acetic acid) for 48 hr, dehydrated in tertiary butyl alcohol, and embedded in Paraplast. Sections 15 μm thick were cut with a rotary microtome and mounted on slides with adhesive. The sections were stained with Harris' hematoxylin and orange G (13) which stained the bacteria dark blue against the orange-stained grape host tissue.

Values given for bacterial infestation are averages of data from three-to-five infected vines. A minimum of 27 cross sections of stem, petiole, or leaf vein were counted for each value. The number of xylem vessels infested with bacteria—this includes vessels with very few bacteria to those completely plugged—was determined; of these, the vessels completely plugged with bacteria were noted separately (Fig. 1). The values for both infested vessels and completely plugged vessels were expressed as a percentage of the total vessel count.

Five-mm long pieces of stem, petiole, and leaf vein samples were collected from the Schuyler vines and used to get a three-dimensional perspective of bacterial plugging. Samples were prepared for sectioning as previously described. Contiguous sections were cut from the 5-mm length with a microtome. Drawings were made of three vascular bundles selected at random from the sections. The bacterial incidence and vessel plugging was then determined from the 5 mm of contiguous sections and incorporated into the drawings. The percent plugging within the 5-mm piece was obtained from the composite drawing of the sections.

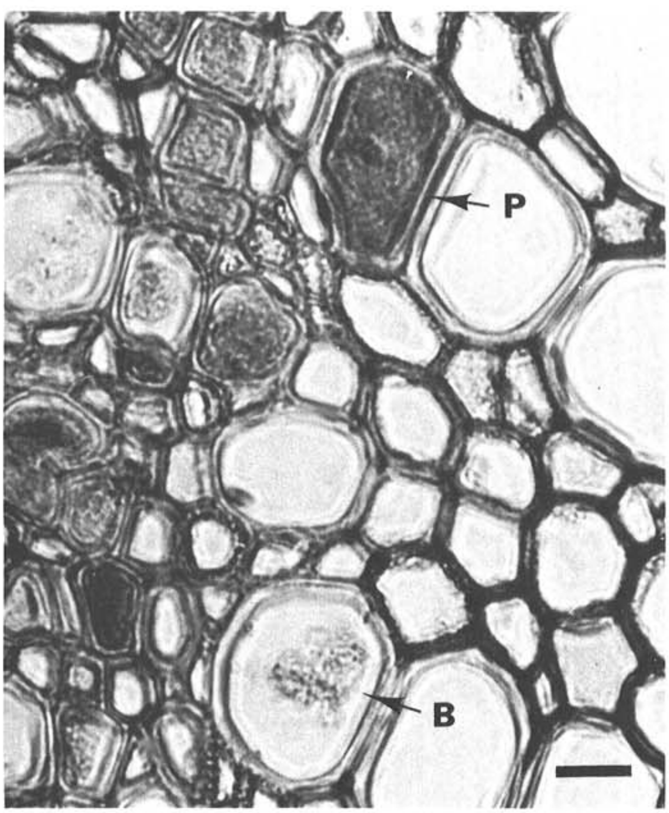

Fig. 1. Photomicrograph of xylem vessel cross sections used in determining bacterial concentrations in grapevine tissues. Completely plugged vessels (P) and vessels infested with bacteria (B) but not plugged are shown. Bar represents 10 μm.

RESULTS

New growth of cultivar Schuyler grape occurred approximately 1 March 1977. On 29 March, bacteria were detected by light microscopy only in stem tissue from the previous year (Table 1). No bacteria were detected in the basal leaves on the current year's growth. On 21 April, the vines were still symptomless; however, bacteria were present in all three tissues sampled, with highest concentrations in leaf petioles.

Marginal necrosis of the leaves was first observed on 15 May. Between 21 April and 7 June the major increase in percentage of xylem vessels plugged with bacteria occurred in the leaf vein, with the highest number of bacterial occlusions occurring at the 7 June sampling date when leaf symptoms were rapidly developing (Table 1). Bacterial infestation of xylem vessels was still high on 28 July, but many of the older leaves had already fallen from the diseased vines. By 10 October bacterial concentrations had declined from midsummer values, but the leaves sampled were from new growth because PD had almost completely defoliated the vines in June, July, and August.

Average percentage of infested and plugged vessels per 15-μm cross section of grapevine stem, petiole, and leaf vein tissue was compared with the total percentage infested or blocked vessels over a 5-mm length of tissue (Table 2). The variability in percentage of bacteria-infested and plugged vessels per cross section was high, ranging from a low of zero in all tissues to a high of 32.7% infestation in a leaf vein cross section. The percentage of vessels containing bacteria in a 5-mm length of stem tissue was 10 times the

TABLE 1. Seasonal concentration of Pierce's disease bacteria and bacterial plugs in cultivar Schuyler grapevines

	Vessels infested with bacteria[a] (%)			Vessels completely plugged with bacteria[a] (%)		
Date	Stem	Petiole	Leaf	Stem	Petiole	Leaf
29 March	3.0[b]	0	0	0.3[b]	0	0
21 April	0.1	16.1	5.0	0.1	9.0	0
7 June	3.8	15.4	17.9	0.6	2.6	10.9
28 July	1.2	12.7	14.1	0.1	3.2	5.5
10 October	1.3	10.9	0	0.4	8.2	0

[a]Values given are the average obtained from three-to-five infected vines. Each value is the average obtained from counting infested vessels in a minimum of 27 cross sections.

[b]Stem tissue from previous season; all other samples are tissue from the current season.

TABLE 2. Comparison of the percentage bacteria-infested and plugged xylem vessels in a single cross section with the percentage infested and plugged over a 0.5 cm length of grape tissue

Sample	Vessels infested with bacteria (%)			Vessels completely plugged with bacteria (%)		
	Stem	Petiole	Leaf	Stem	Petiole	Leaf
Cross section[a]	4.8	13.6	10.1	2.1	9.1	6.7
Total 5 mm	46.2	55.6	52.3	27.2	41.8	47.0
Range over 5 mm[b]	0–22.2	0–30.0	0–32.7	0–18.5	0–21.8	0–26.5
Ratio (cross section/total)[c]	0.10	0.24	0.19	0.08	0.22	0.14

[a] Data are given as average percentage of infested or completely plugged xylem vessels per cross section over 5 mm of tissue and the total percentage of infested or completely plugged vessels anywhere in the 5 mm of tissue.
[b] Range in bacterial concentration found in cross sections from a 5-mm length of tissue.
[c] Average percentage of infested or completely plugged vessels per cross section divided by the total percentage of infested or completely plugged vessels over 5 mm of tissue.

average percentage of vessels containing bacteria in a single 15-μm cross section, in petiole tissue it was four times the cross section value, and in leaf veins it was more than five times the cross section value. Ratios are calculated to convert bacterial infestation per cross section to infestation over a 5-mm length of tissue.

Seasonal concentrations of PD bacterial infestation expressed on the basis of a 5-mm length of tissue showed that 94.2% of the leaf vein vessels contained bacteria at the time of leaf marginal necrosis symptom development in late May and early June (Table 3). Approximately 80% of the leaf vein vessels were completely occluded somewhere in the 5-mm length of tissue of leaves with marginal necrosis.

On 7 June, the percentage of bacteria-infested vessels in symptomless tissue was compared with that of tissue from leaves with marginal necrosis (Table 4). The only significant difference was in the leaf veins; only very small differences were detected in petioles. The largest difference in totally plugged vessels was found in the leaf veins in which eight to nine times as many vessels were plugged in leaves with marginal necrosis as in symptomless leaves.

TABLE 3. Seasonal concentration of Pierce's disease bacteria and bacterial plugs calculated to occur in a 5-mm length of Schuyler grapevine tissue

Date	Vessels infested with bacteria[a] (%)			Vessels completely plugged with bacteria[a] (%)		
	Stem	Petiole	Leaf	Stem	Petiole	Leaf
29 March	30[b]	0	0	3.8[b]	0	0
21 April	1	67.1	26.3	1	40.9	0
7 June	38	64.2	94.2	7.5	11.8	77.9
28 July	12	52.9	74.2	1.3	14.5	39.3
10 October	13	45.4	0	5.0	37.3	0

[a] The ratios obtained in Table 2 were used to convert concentrations per cross section to concentration over a 5-mm length of tissue.
[b] Stem tissue from the previous season; all other samples are tissue from the current season.

TABLE 4. Percentages of bacteria-infested and completely plugged xylem vessels from grapevine tissue with leaf marginal necrosis (MN) compared with percentages from symptomless (SL) tissue

Tissue	Vessels infested with bacteria[a] (%)				Vessels completely plugged with bacteria[a] (%)			
	Cross section		Total 0.5 cm		Cross section		Total 0.5 cm	
	MN	SL	MN	SL	MN	SL	MN	SL
Stem	3.1	2.6	31.0	26.0	0.5	0.5	5.0	5.0
Petiole	15.4	12.5	64.2	52.1	2.6	2.5	11.8	11.4
Leaf vein	17.9	9.7	94.2	51.1	10.9	1.3	77.9	9.3

[a] Data are given as the average percentage of infested or completely plugged xylem vessels per cross section and the total percentage over the 5 mm of tissue. Values were obtained from four infected vines on 7 June. The samples from symptomless tissue came from the same vines as did the samples from areas of leaf marginal necrosis. These symptomless areas were from 8–10 leaves located acropetally from basal leaves showing MN symptoms; they probably would have developed MN symptoms 2–3 wk later.

DISCUSSION

The seasonal concentrations of PD bacteria in Schuyler grape tissue correlated very well with PD symptom development. PD bacteria were not detectable in current season vine growth during the first 4 wk after the beginning of new growth, but were detected in old wood. Bacterial concentrations were at a maximum in late spring and early summer when leaf marginal necrosis was most severe. By October, bacterial concentrations had declined in the leaves. This probably occurs because severely infected plants often are defoliated in midsummer to late summer, and regrowth takes place. Therefore, the leaves sampled in October were symptomless and were of comparable age to those sampled in April.

Data for the seasonal concentrations of PD bacteria in susceptible grapevines in Florida also may explain natural infectivity of vectors and transmission of PD from grapevine data in 1977 (1). In March and April when bacterial populations were low, leafhopper vectors could not transmit the bacteria. Bacterial concentrations in the plant were highest and vector transmission was greatest during June. Later in the season—August to October—when there was a lot of new plant growth and bacterial concentrations were lower, transmission was erratic.

In our studies, a maximum of 33% of the vessels in a leaf tissue cross section contained bacteria; rarely do more than 40% contain bacteria (12). Plants show no water stress with a much higher percentage of nonfunctioning vessels (3). However, serial sectioning demonstrated that different vessels contain bacterial plugs in different cross sections. The percentage of occluded vessels over a 5-mm length of stem, petiole, and leaf vein was 4–12 times that in a single cross section. Therefore, there may be sufficient blockage over the length of a petiole or leaf vein to produce sufficient water stress to result in leaf marginal necrosis. This could certainly be true if lateral water movement between vessels also is inhibited.

Bacterial plug concentrations, expressed as percentage of vessels infested, was much lower in stems than in leaves. In leaves with marginal necrosis symptoms, a higher percentage of vessels in leaf veins contained bacteria than in petioles. Bacterial infestation and vessel plugging in leaf veins were highly correlated with leaf marginal necrosis. Totally blocked vessels in the leaf veins were found only in the June and July samples, when leaves showed marginal necrosis symptoms. There was actually a higher percentage of blocked vessels in petioles in April, prior to symptom development. Totally blocked vessels in veins of leaves with marginal necrosis were much more prevalent than in leaves that were still symptomless. These symptomless leaves which would probably develop symptoms in 2–3 wk already had as many occluded vessels in the petioles as did the leaves with marginal necrosis. This plugging in the leaf veins appeared to be sufficient to account for the water stress symptoms of the disease in the leaves. However, studies on the water relations of the PD-infected grapevine are needed to define the roles of phytotoxin (9) and of physical blockage—by bacteria or by emboli (15)—of xylem vessels during disease development.

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