Evaluation of Wall Deposits in Phloem Cells to Detect Little Cherry Disease in Sweet Cherry Trees

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ABSTRACT

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Wall deposits that were stained with acridine orange were found in phloem cells of petioles from 83% of sweet cherry trees with symptoms of little cherry disease and in about 20% of trees with normal fruit. They were found in 16 of 21 trees with symptoms that indexed positive and in four of seven that indexed negative on Sam indicator trees. Wall deposits in petioles collected in late summer from trees that bore normal fruit in orchards where diseased trees had been removed in midsummer of 1977 did not correlate with the development of little cherry symptoms in 1978 or in 1979. Although frequently associated, wall deposits in petioles may not be specifically related to the little cherry disease.

Little cherry disease was discovered in Kootenay Valley, British Columbia, in 1933. By 1950 it affected most cherry trees in most districts in the Kootenay region (5). It was found in the Okanagan Valley in 1969. Intensive surveys have been conducted annually, and all diseased trees and trees with suspected disease have been removed, in accordance with the British Columbia Plant Protection Act. In field surveys diagnosis of little cherry disease is limited to fruiting trees and is based on the small, uneven size, retarded maturity in color, insipid flavor, and in some cultivars, angular, pointed shape of the fruit. Some of these symptoms also may result from factors such as physical injury, poor pollination, nutrient excess or deficiency, and various infectious agents. Other methods are required to verify the disease and to detect it in trees without fruit.

Bud inoculation of fruiting cherry trees and subsequent development of similar symptoms was the initial means of determining that little cherry disease was caused by an infectious agent, presumably a virus (1). This method is costly in time, effort, and resources. Subsequently, graft inoculation of young test trees, such as Sam sweet cherry, on which the leaves turn red in late summer if infected with the virus, has been used to confirm the presence of the disease (4). Although less

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costly than using fruiting trees, this method is cumbersome and slow; one or two growing seasons are required for expression of symptoms in the field. Factors such as physical injury, water stress, and infectious agents also can cause leaf reddening that can mask or be confused with the interveinal reddening caused by the little cherry virus.

After a search for a light microscopic criterion for detecting little cherry disease, Verbeek (3) reported wall deposits in phloem cells of petioles from affected trees. The deposits were stained with acridine orange and could be observed with the fluorescence microscope. The deposits developed in the phloem of petioles during the first 2 mo after flowering. The phloem of petioles from healthy trees occasionally contained wall deposits but fewer of them.

We tested the fluorescence microscope technique as a supplement to bud indexing and to little cherry fruit symptoms found during field surveys in the Okanagan and Kootenay Valleys in 1976 and 1977. In addition, we used the technique to detect little cherry disease during the late summer of 1977 in trees remaining in orchards after trees with symptoms had been removed.

MATERIALS AND METHODS

In exploratory tests, wall deposits were not found in petioles collected from diseased trees before late June and were more abundant after mid-July; most samples were therefore collected after mid-July when fruit symptoms were evident.

After comparing 50 to 60 samples from different positions on each of 17 trees, we established guidelines for collecting samples as follows: six leaves were collected 2 or 3 m above ground on the south side of each tree. During July and early August, leaves were selected from the base of the terminal growth. Later, the leaves were selected near the middle of the current season's growth. A 5-mm section was cut with a sharp knife 10–15 mm from the base of each petiole and immersed in a fresh 1:1 mixture of solution A and solution B of Navashin fluid (2). The petioles were not split before sectioning.

Sections were cut 10 μm thick using a rotary microtome. Fifty to 60 entire cross sections from one petiole were placed on each glass slide for staining with acridine orange. We followed the procedure used by Verbeek (3), with minor alterations. Staining was done for 15–20 min, depending on the freshness of the acridine orange preparation, instead of 15 min, in all instances. The slides were then dipped in acetone momentarily and dried instead of being dipped quickly in acetone I, acetone II, and acetone-xylene (1:1) and held 1 min in each of three changes of xylene. Each slide was covered with diatex plastic mounting medium without glass cover slips. We examined 50–60 sections on a glass slide for each of two or three petioles from each tree; three or four petiole segments were reserved for use in further confirmation if desired. Only inclusions filling or partially filling the lumen of a companion cell were considered wall deposits.

For bud indexing, Sam test trees were prepared by field budding from Sam mother trees, in the virus-free Budwood Orchard, onto 1- or 2-yr-old Mazzard F12/1 rootstock from layer beds at Traas Nursery Ltd., Langley, B.C. In early August, two test buds were shield-budded on the rootstock below the established Sam buds. Observations for red leaf symptoms were made at weekly intervals through August and September of the following year. The diagnostic symptom was an interveinal reddening initially visible on the upper leaf surface and eventually also clearly visible on the lower surface. Various patterns of reddening sometimes developed from other causes including other pathogens, physical injuries, and various stresses, but generally they could be distinguished from the effects of little cherry virus. For reliable determinations, two or four test trees were inoculated with two buds each from each tree tested. Every fifth test tree in each row was kept as a self-budded or

Table 1. Wall deposits in petiole phloem cells in relation to little cherry disease on sweet cherry trees in 1976 and 1977

	Trees examined, wall deposits/section									
	1976					1977				
		<1		≥1			<1		≥1	
Little cherry fruit symptoms	Total	(no.)	(%)	(no.)	(%)	Total	(no.)	(%)	(no.)	(%)
Okanagan Valley										
Present	254	39	15	215	85	231	37	16	194	84
Suspected	17	1	6	16	94	83	27	33	56	68
Absent	54	40	74	14	26	62	49	79	13	21
Kootenay Valley										
Present	40	10	25	30	75	27	8	30	19	70
Suspected	20	18	90	2	10	32	25	78	7	22
Absent	24	24	100			20	15	75	5	25

Table 2. Wall deposits in cherry trees with and without fruit symptoms of little cherry disease in 1976 and results of indexing on Sam test trees

Trees with little cherry fruit symptoms		Red leaves on Sam index trees		Wall deposits per petiole section		
				0	<1	≥1
Present	28	Present	21	3	2	16
		Absent	7	3	0	4
Suspected	16	Present	12	9	2	1
		Absent	4	3	1	0
Absent	52	Present	0	0	0	0
		Absent	52	48	4	0

Table 3. Development of little cherry disease in 1978 and 1979 on sweet cherry trees sampled in late summer, 1977

Wall deposits per petiole	Trees examined for wall deposits in 1977			Trees with disease in 1978		Additional trees with disease in 1979	
	35 orchards with LCD trees removed	5 orchards with no diseased trees	Total	(no.)	(%)	(no.)	(%)
5	2	0	2	0		0	
4–4.9	3	0	3	0		0	
3–3.9	4	0	4	0		0	
2–2.9	35	1	36	1	2.7	0	
1–1.9	160	7	167	10	6.0	2	1.2
1	364	14	378	10	2.6	9	2.4
0	3,068	349	3,417	119	3.5	52	1.5
Total trees	3,636	371	4,007				
Trees diseased in 1978	135	5		140			
Trees diseased in 1979	63	0				63	

uninoculated control to assist in differentiating nonspecific leaf discolorations from those induced by little cherry virus.

RESULTS

Relation between wall deposits and symptoms. In 1976 and 1977, wall deposits were not found in samples collected in the Okanagan and Kootenay Valleys until late June. They were more frequent after mid-July when the fruit on most trees was mature and symptoms of little cherry disease could be recognized. The numbers of wall deposits varied among trees, samples from the same tree, and sections from the same petiole. They ranged from none in any of the samples from some trees to an average of 10 or more per section in other samples. The results with samples from the Okanagan and Kootenay Valleys in 1976 and 1977 are shown in Table 1. One or more wall deposits were found in thin sections from 70 to 85% of trees with characteristic symptoms and from 21 to 26% of trees with normal fruit.

Red leaf symptoms on Sam indicator trees. The results of examinations of petioles from trees with and without fruit symptoms that were indexed on Sam show relationships between the three criteria for little cherry disease (Table 2). None of the 52 trees with normal fruit induced red leaf on Sam test trees; only small numbers of wall deposits (avg. <1 per petiole section) were found in four of these trees. Of 28 trees with symptoms, 21 induced red leaves on Sam; averages of $\geqslant 1$ wall deposit per section were found in petioles from 16 of these trees. Seven of the trees with symptoms resembling those of little cherry disease did not induce red leaf symptoms on Sam, but wall deposits were found in four of them. The results from trees with symptoms suspected but not characteristic of little cherry disease varied. Twelve induced red leaf symptoms on Sam, but one or more wall deposits were found in only one.

Late summer sampling to detect disease. During July 1977, 314 cherry trees at 39 sites in the Okanagan Valley had symptoms indicative of little cherry disease (Yorston, *unpublished data*). All these trees were removed by 10 August 1977. Other trees remaining in these orchards might have been infected even though they did not show symptoms in 1977. During August and September, petiole samples were collected and processed for microscopic examination in an attempt to detect infected trees before surveys in July 1978. All orchards were mapped and the positions of trees carefully identified so that trees with wall deposits could be correlated with trees on which little cherry disease would be detected by symptoms in July 1978. The microscopic examinations were done on 4,007 trees from 40 orchards in the Okanagan Valley. These included 3,634 trees in 35 orchards in which diseased trees or trees with suspected disease had been removed in 1977 and 371 trees from five orchards in which the disease had never been found.

During the 1978 surveys in the Okanagan Valley, 193 trees had symptoms of little cherry disease (6). Of these, 140 were in orchards in which samples were collected from all trees late in the summer of 1977. The numbers of trees that developed symptoms of little cherry disease differed little between those with and those without wall deposits (Table 3).

In the 1979 surveys (Yorston, *unpublished data*), of 109 trees with symptoms of little cherry disease, 63 had been tested for wall deposits in 1977. As in 1978, there was no evidence of a positive correlation between the presence of wall deposits in 1977 and the development of symptoms of little cherry disease. None of nine trees with more than three wall deposits per petiole section in 1977 developed symptoms in 1978 or 1979.

DISCUSSION

The frequency of wall deposits in phloem cells of petioles from cherry trees with little cherry disease symptoms and the lower frequency in trees with normal fruit indicated a relationship between the disease and the development of wall deposits. Absence of wall deposits in some trees with symptoms could be interpreted to indicate that the symptoms were not caused by the little cherry virus or that wall deposits were present but missed because of inadequate sampling. Conversely, presence of wall deposits in trees with normal fruit could indicate that the trees were infected but symptoms on fruit were not yet evident. Similarly, the discrepancies between wall deposits in trees with suspected disease and development of red leaves in bud-inoculated Sam test trees could be explained by unreliability of detection by budding on Sam indicator trees.

The specificity of wall deposits as indicators of little cherry disease was brought into question most severely by the attempt at large-scale field use to detect diseased trees before the next crop. We had hoped that trees that would develop symptoms in 1978 could be detected by the wall deposits in leaf samples collected in the late summer and early fall of 1977. Because the incidence of symptoms in 1978 was not significantly greater among trees with large numbers of wall deposits than in trees with none, there appears to be no sound basis for using the technique to detect little cherry disease.

Unpublished results in conjunction with nutrient analysis of cherry trees with large and small fruit suggest that nutrient imbalance may affect the occurrence of wall deposits in petioles.

Our results show that although wall deposits occur frequently in trees that develop symptoms characteristic of little cherry disease, they are not exclusively associated with such symptoms. Unless a more specific relationship can be demonstrated, presence of wall deposits should not be relied upon as an indicator of the disease.

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