BACTERIAL PHLOEM CANKER OF PERS

RECOMMENDATIONS: BACTERIAL PHLOEM CANKER OF PERSIAN WALNUTS

The following recommendations are from current data and may change as new information is gathered from tests currently in progress.

CONTROL

Control with protective sprays: Bacteria present on the tree can be killed with copper sprays. However, four years of field testing, beginning in 1967, showed that while a postharvest Bordeaux spray killed the bacteria, it did not prevent disease spread. First year's results (1969) with a preharvest fixed copper spray (3 lbs/100 gal) showed a significant (5% level) decrease in the number of new cankers in treated rows. Two additional plots were established in 1970.

ERADICATION

Eradication: Cankers can be surgically removed but this is practical only on trees less than 10 years old or with small cankers on older trees. The disease must be detected at an early stage. When cutting out the diseased tissue all the bark must be removed 6 inches beyond the upper and lower extent of the canker, and 1-inch to each side. Cutting tools should be sterilized between each cut, the removed tissue discarded, and the exposed, healthy tissue sterilized and left exposed to promote healing. For further details, consult your local farm advisor, or the Plant Pathology Department, University of California, Davis.

Bark cracking and exudate seen on walnut tree above, are external symptoms of bacterial phloem canker in late summer.

SIAN WALNUT . . . *development and control factors*

A requisite for development of bacterial phloem canker in walnuts (caused by Erwinia rubrifaciens) was the presence of the highly susceptible Hartley cultivar; although, when interplanted with Hartley, the Franquette and Payne cultivars were sometimes also attacked by the disease. The recently developed cultivars Gustine and Howe developed active cankers when inoculated, but not as extensive as those in Hartley. The age of the plant part was important to the development of the disease. The only parts of the tree developing the complete disease syndrome were the trunks and primary (scaffold) branches. Extension of the cankers in the tree was most rapid during the summer when the temperature was high. This was correlated with the effect of temperature on multiplication and growth of the bacterium in culture. Another requisite to development of the disease in these tests was the presence of openings in the thick phelloderm of the trunks and branches through which the pathogen can enter the inner bark. Of the several types of breaks commonly occurring, those produced by mechanical harvesting equipment and by sap-sucking birds were found to be infection sites. The pathogen occurred in large numbers in a slimy substance which exudes through cracks and accumulates on the bark of infected trees. They survived for at least 123 days in the exudate and were disseminated laterally as far as 20 ft in wind-blown rain. In addition, the exudate with viable bacteria was picked up on the pads of mechanical harvesting equipment.

N. W. SCHAAD • E. E. WILSON

THE DISEASE OF PERSIAN WALNUT (*Juglans regia* L.) caused by the bacterium *Erwinia rubifaciens* (Wilson, Zeitoun & Frederickson, 1967) was named "phloem canker" because the symptoms occurred in the phloem region of the inner bark of the tree. Histological studies showed that the pathogen first infects the inner nonfunctional sieve tubes and later enters the adjacent parenchymatous tissue. The absence of symptoms in the outer phloem and cortex distinguished this disease from the bark canker disease.

Phloem canker most commonly affects the Hartley cultivars of Persian walnut (*Juglans regia* L.). It is occasionally found in Payne and Franquette but has not been observed to occur as the result of natural infection in any other Persian cultivar or in the black walnut species, *J. hindsii* and *J. californica*, or in the Paradox hybrid (black walnut X Persian walnut).

Several recently developed Persian cultivars that are being introduced were tested for relative susceptibility by artificial inoculation. Several five-year-old trees available for testing were growing in a plot at the Kearney Horticultural Field Station at Reedley. The planting consisted of three of the newer cultivars (Chico, Gustine, and Serr), the Ashley, two strains of Franquette, the Marchetti, Howe, Payne, and Hartley.

Table 1 gives the results of inoculations which were made July 13, 1967, and examined July 13, 1968. In all instances an area of blackened tissue extended up and down the trunk from the inoculated area. The areas in most cultivars were several centimeters long; however, those in the Chico, Serr, Ashley, Franquette (Wilson strain), Marchetti and Payne were dry and without signs of recent activity. *E. rubrifaciens* was isolated, however, from the dark areas on all cultivars. In Howe, the affected areas were large and moist, resembling those in Hartley. In Gustine and the Schrasch strain of Franquette evidence of continued activity was variable. Similar results were obtained in inoculation tests conducted on these cultivars in two other years.

Initial infection of the tree was usually confined to the trunk and primary (scaffold) branches but the cankers may extend upward into secondary branches. Naturally occurring infections of small branches, twigs, or leaves have never been found. When current-season shoots of mature trees were inoculated with the pathogen, fairly extensive blackening of the vascular bundles occurred within three to four weeks. Although no further symptoms developed after one year, the pathogen could be isolated from the blackened vascular elements. Furthermore, when 20 six-month-old seedlings and 20 one-year-old grafts of Hartley were inoculated, no internal or external symptoms developed after one year—and the pathogen could be isolated only from the inoculated sites.

Distribution

Since phloem canker commonly infects only the Hartley walnut, its occurrence and distribution is primarily dependent on the presence or absence of this cultivar. Though the Hartley is grown to a limited extent in the northern coastal districts (Santa Clara, Hollister, Napa, and Sonoma valleys) the disease has not been found there. Hartley trees with the disease were found, however, in the Ojai Valley, within 20 miles of the coast in southern California.

Prevalence of the disease differed greatly between different parts of the Central Valley, being widespread in the southern half (San Joaquin Valley) but occurring much less frequently in the northern half (Sacramento Valley). One

possible explanation was that Hartley orchards in the northern half were usually considerably younger than those in the southern half and, consequently, had been exposed to infection by phloem canker for the shorter time.

70% infected

Seventy per cent of the trees in some San Joaquin Valley orchards were found affected by phloem canker. Numerous cracks had developed in the bark over the cankered areas on trunks and primary branches and extensive black streaks and bands occurred in the inner bark. Though some trees were dead and others had lost large limbs, the evidence was that the disease progresses quite slowly in the tree. This was partially confirmed by inoculating 18-year-old trees in August 1966. One year later external symptoms in the form of bark cracks had developed in only three of nine inoculated sites. After two years external symptoms were present on all nine trees. The average cankered area extended 1.5 meters up and down the inner bark of the trunk; however, none of the inoculated trees appeared seriously affected by the disease.

Liquid exudate

Liquid exudate accumulated in the infected bark, which had escaped to the surface through cracks, contained about 1×10^{10} viable *E. rubrifaciens* cells/ml. While small amounts of this bacterial slime may exude from the bark throughout the winter months, the greatest exudation occurs during the summer. The exudate runs down the surface of the bark, some of it falling to the soil beneath, but most of it dries and remains on the bark of the trunk and scaffold limbs. Eventually, however, most of it is washed from the surface of the bark by fall and winter rains.

To determine whether bacteria were present during the winter in the cracks of overlying cankers, monthly samples were taken by making washings between October 1967 and April 1968. The results from 25 sites showed that at least 75 per cent of each month's samples contained viable cells of *E. rubrifaciens*. In no case, however, was the pathogen recovered from the surface of adjacent trees free of phloem canker.

Whereas the number of viable cells in the liquid exudate varied very little from sample to sample, the number of viable cells in the dried, gummy exudate varied greatly, that is, from 0 to about 1×10^8 cells per mg.

TABLE 1. INOCULATION REACTION OF SEVERAL 5-YR-OLD WALNUT TREES TO PHLOEM CANKER BACTERIA, ERWINIA RUBRIFACIENS

Variety	Canker length cm*	Reaction type†
Chico	34	Dry-inactive
Gustine	72	Variable
Serr	30	Dry-inactive
Ashley	24	Dry-inactive
Franquette (Schrasch strain)	63	Variable
Franquette (Wilson strain)	43	Dry-inactive
Marchetti	31	Dry-inactive
Howe	75	Moist-active
Payne	21	Dry-inactive
Hartley	105	Moist-active

* Inoculated July 13, 1967, measured July 13, 1968.
† Dry-inactive = the discolored areas of tissue had dried up and showed no evidence of recent extension. Moist-active = the discolored area with bacterial exudate and with evidence of continual extension. Variable = varied from inactive to active type.

TABLE 2. EFFECTIVENESS OF PREHARVEST FIXED COPPER SPRAY IN CONTROL OF BACTERIAL PHLOEM CANKER OF WALNUT, 1969–1970 SEASON

	Number of diseased trees					
	Sprayed†			Unsprayed		
Plot*	1969	1970	increase	1969	1970	increase
1	6	6	0	2	2	0
2	7	7	0	4	7	3
3	3	3	0	2	3	1
4	3	5	2	6	7	1
4	5	5	0	8	9	1
6	6	7	1	5	5	0
7	2	2	0	1	2	1

* Each plot consisted of two paired rows of 19 trees each.
† *Trees were sprayed preharvest with 3 lb Kocide/100 gal water.*

Pathogen survival

To find out how long the pathogen survived in dried exudate, a recent accumulation of exudate on the trunk of a 20-year-old tree was selected. Additional exudate was prevented from replenishing the sampling site by gluing a piece of tinfoil to the bark just below the crack from which the exudate was oozing.

The first samples, taken on August 17, 1970, were from the slimy ooze in the crack while successive samples were collected from the dried gummy exudate below the tinfoil, 6, 13, 21, 30, 41, and 62 days later. Sampling was discontinued on October 16 because the remaining exudate was washed from the bark by rain on October 20 and 22.

The results showed that the original concentration of 1.4×10^9 viable cells/mg had decreased only to 1.0×10^7 cells/mg after 41 days and to 8.6×10^6 after 62 days (graph 1). Therefore 0.71 per cent of the bacteria were still viable after 41 days. When expressed as half-life the survival rate during the 41 days is equal to 5.7 days. That is, it took 5.7 days for one-half the population to die.

Similar data were obtained from a second experiment. In this case, survival of the bacteria in exudate placed on a healthy tree was compared with survival in exudate stored in the laboratory. The results showed that the environment was important. For example, half of the laboratory stored bacteria died in 5.6 days, whereas half of the bacteria placed on a tree died in only 3.8 days. The most significant result of both experiments, however, was that viable bacteria were still present under each of the conditions for over 120 days.

Dissemination

Tests conducted during the fall of 1968–69 showed that the pathogen can be carried, presumably in wind-blown rainwater, for some distance from the inoculum source. During each rain prior to December 22, 1968, the rainwater caught several feet from infected trees contained the pathogen in considerable numbers. Tests have also shown that the pathogen may be spread from tree to tree by mechanical shakers. *E. rubrifaciens* has been isolated from rubber pads carrying some of the gummy exudate associated with diseased trees.

Infection

Before the pathogen can gain entrance to the inner bark, breaks in the thick phelloderm (outer bark) must occur. The most common type of breaks are: growth cracks, pruning cuts, sap sucker feeding sites, and shaker wounds. The most consistent relationship to canker presence has been with shaker wounds. No experimental results are available; however field plots have been established to determine if shaker wounds are related to infection.

Temperature

Judging from external signs (exudation of bacterial slime), disease activity in the form of canker extension is greatest during the summer. This was partially confirmed by inoculating trunks of 24-year-old Hartley trees on February 9, April 1, and June 18, 1968, and examining the inoculated areas 60 days after inoculation. Cankers resulting from the winter and spring inoculations extended at the rate of about 2 mm per day as contrasted to 9 mm per day for the June inoculations. As shown in graph 2, the average maximum temperature from February 9 to June 1 was 70°F whereas the average maximum temperature following the June inoculations was 93°F.

As noted earlier the disease has not been found in the coastal districts where the summertime temperature is much lower than in the Sacramento and San Joaquin valleys. This may be due in part to the dependence of the pathogen on

relatively high temperature for its growth and disease production. We also found a difference between the two interior valleys in the prevalence of phloem canker. Whereas the disease is widespread in the San Joaquin Valley it is less prevalent in the Sacramento Valley.

Studies showed both the minimum and maximum air temperatures were higher at Reedley than at Davis. Furthermore, the temperature remained above 66°F for 21.2 hours at Reedley as contrasted to 14.5 hours at Davis. Because a temperatures above 66°F have been shown to result in a significant increase in the growth of the pathogen, it appears that temperature conditions for growth were more favorable at Reedley than at Davis. Inoculation tests also showed length of cankers produced in the five-year-old trees at Reedley averaged 222 mm in 30 days whereas those in the 12-year-old trees at Davis averaged only 90 mm.

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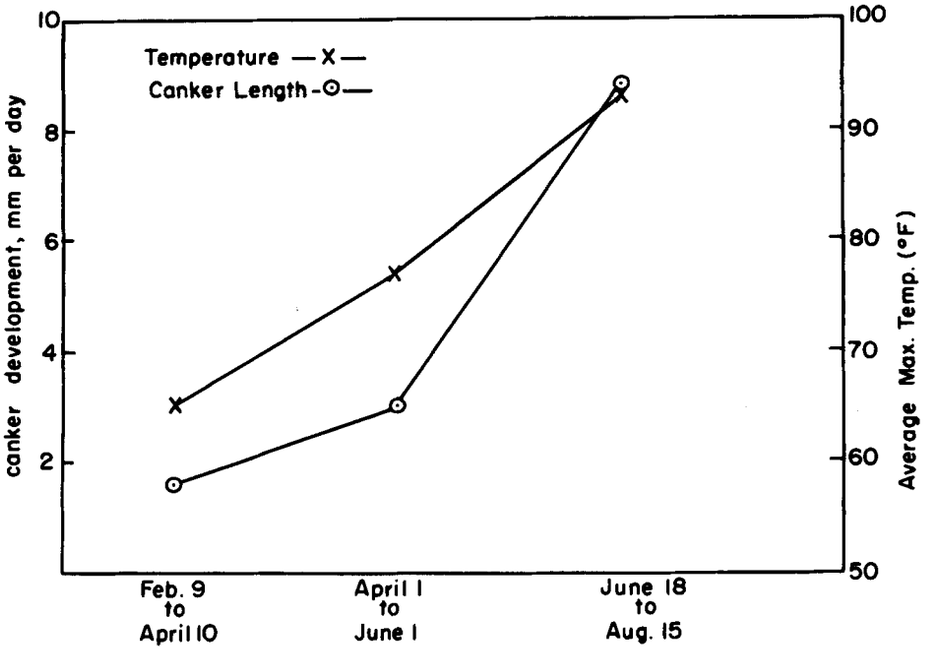
GRAPH 2. RELATION OF TEMPERATURE AND SEASON TO DEVELOPMENT OF BACTERIAL PHLOEM CANKER (CANKER LENGTH IS AVERAGE OF FOUR INOCULATIONS)



Internal symptoms of bacterial phloem canker are shown in this section cut from apparently healthy bark.

GRAPH 1. SURVIVAL OF E. RUBRIFACIENS IN NATURAL EXUDATE (AVERAGES OF TWO EXUDATE SAMPLES ASSAYED IN TRIPLICATE)