Until blackline virus kills large portions of tissue around graft union, few symptoms are visible. Advanced stage of disease apparent in this tree — yellowing of foliage, small leaves, dieback, and profuse suckering — leaves little doubt.

Suckering near the graft union is a more common symptom of blackline disease on black walnut rootstock than on Paradox, but is not always present. Small holes or cracks at the graft union (below) are also more common on black walnut rootstock.

Incidence of walnut blackline disease in California's commercial orchards

Wilbur O. Reil □ Gordon A. Rowe □ David E. Ramos □ Sreko M. Mircetich

Early detection is vital but difficult

Walnut blackline disease was first seen in Contra Costa County in 1929 and probably was present earlier. A few years ago, S. M. Mircetich and co-workers determined that the disease is caused by a graft-, seed-, and pollen-transmissible virus identified as a strain of cherry leafroll virus. Inoculation experiments have also shown that the virus moves slowly within the tree — 5 to 30 inches vertically and 2 to 3 inches horizontally per year. This slow movement means that the tree may be infected with the virus for several years before blackline shows at the graft union.

The typical symptom, from which the disease received its name, occurs at the graft union of English walnut scion on either black or Paradox (black-English hybrid) rootstock. Black walnut and Paradox are hypersensitive to the virus, which causes the death of cambial cells at the graft union. The dead tissue turns black and forms a narrow line on black walnut. On Paradox rootstock, a canker usually forms, starting at the graft union and extending downward.

Until the virus kills a relatively large portion of tissue around the graft union circumference, few or no symptoms are visible. The decline of the tree occurs because of girdling that eventually starves the tree.

Before the 1950s, the disease was observed only in the California counties surrounding San Francisco Bay; it was assumed to be limited to coastal areas and to be incapable of affecting walnut trees in the hot interior valley. In the 1950s and 1960s, blackline began to appear in San Joaquin, Stanislaus, and Yolo counties, indicating possible spread into the valley. Now, blackline-affected trees can occasionally be found in many counties of the Central Valley, and considerable numbers are showing symptoms in San Joaquin, Stanislaus, Merced, and Yolo counties, in addition to the coastal counties of Contra Costa, Alameda, Santa Clara, and San Benito. The walnut industry now considers blackline a serious threat in the entire state.

Identification of the virus that causes walnut blackline and more recent work on the methods of transmission and movement within the tree by Dr. Mircetich helped explain some of the field observations. At the same time, an ELISA test (enzyme-linked immunosorbent assay) was developed and shows promise as a diagnostic tool for early detection of the virus.

In 1982, at the request of the Walnut Marketing Board, we conducted a blackline survey to: (1) estimate the incidence and distribution of blackline-affected

TABLE 1. Blackline survey report by county

County	Acres surveyed	Trees checked	Good	Blackline	Replants	Missing
			%	%	%	%
Butte	581.0	4,782	97.40	0.18	1.50	0.89
Colusa	177.3	1,167	97.85	0.25	1.71	0.17
Contra Costa	190.2	1,978	21.63	53.99	8.99	15.36
Fresno	182.2	1,206	99.58	0.41	0.00	0.00
Glenn	245.4	2,076	93.40	0.04	5.39	1.15
Kings	103.9	835	96.76	0.95	1.79	0.47
Lake	359.2	1,998	99.34	0.15	0.10	0.40
Merced	373.4	3,347	90.05	3.61	4.27	2.06
San Benito	188.8	1,914	76.12	18.33	4.23	1.30
San Joaquin	996.8	6,815	68.20	18.79	9.49	3.50
Stanislaus	959.8	8,740	85.68	9.48	3.90	0.92
Sutter	556.6	3,555	88.18	0.33	7.08	4.38
Tehama	462.2	4,405	96.57	0.06	2.11	1.24
Tulare	1,011.2	9,923	95.97	0.14	2.22	1.65
Yolo	324.3	3,062	92.45	4.50	1.99	1.04
Yuba	286.5	2,406	96.71	1.12	1.57	0.58
Totals	6,998.8	58,209				

TABLE 2. Blackline survey report by variety

Variety	Acres surveyed	Trees checked	Good	Blackline	Replants	Missing
			%	%	%	%
Ashley	673.2	7,317	93.98	4.08	1.21	0.71
Eureka	624.9	3,916	65.83	21.73	8.04	4.39
Franquette	814.0	3,423	95.76	0.99	2.01	1.22
Hartley	1,825.4	13,074	88.06	3.30	5.40	3.22
Payne	1,338.6	12,725	74.42	17.49	5.32	2.75
Serr	1,198.4	11,721	96.33	0.25	2.32	1.07
Tehama	356.1	4,411	95.94	0.02	2.92	1.11
Vina	168.2	1,622	98.52	0.00	0.98	0.49
Totals	6,998.8	58,209				

trees in major walnut-producing counties in California; (2) identify areas with very low disease incidence where a blackline eradication program might be considered; and (3) determine the feasibility of serological testing (ELISA) of pollen in estimating the extent of symptomless trees that may be infected with blackline virus in diseased orchards.

Survey

We obtained a list of walnut orchard blocks in the major producing counties from the U.S. Department of Agriculture (USDA) Statistical Reporting Service. These counties accounted for 90 percent of the walnut-bearing acreage in 1981. To facilitate the survey, we further restricted the sample to orchard blocks of 10 acres or larger planted in 1974 or earlier. Although younger trees have been seen with the disease, we assumed that orchards younger than eight years old probably would not have trees expressing symptoms and should not be considered in the survey.

For greater standardization, the survey included only the following varieties: Ashley, Eureka, Franquette, Hartley, Payne, Serr, Tehama, and Vina. These varieties represented 89 percent of the bearing acreage for 1981; all others were considered either regional in distribution or too low in numbers to provide useful data. For the survey, we selected 3 to 5 percent of these blocks in each county, using a random sequential sampling method.

Property owners of the selected orchards were contacted for permission to inspect a 20 percent subsample in each orchard. Every effort was taken to standardize techniques used in the field. Four inspectors were trained in the examination procedures and worked as a team; each inspector surveyed approximately one-fourth of the trees in each orchard. The supervisor spent considerable time each week with each inspector to ensure uniformity.

Each subsample tree was visually examined for blackline symptoms during the late spring and summer of 1982. Criteria used to determine a blackline-suspect tree were: (1) general tree appearance, including yellowing, sparse foliage, lack of vigor, and dieback; (2) suckers occurring from the rootstock; (3) budunion pinholes, cracking, or bleeding; (4) bleeding and cankers on the rootstock; and (5) depression or flattening of the scion or slight bulging of the rootstock immediately below the graft union.

The inspector cut a small observation window across the graft union through the bark to the wood of each suspect tree and removed the patch to examine the cambial area. Blackline was positively diagnosed when a definite black line or, with Paradox rootstock, a canker showed at the graft union.

After the examination, the orchard was mapped to show all blackline-affected trees as well as missing and replanted trees. The information was then computerized and analyzed. A total of 58,209 trees in 266 blocks were examined in 16 major producing counties.

Survey results

The survey showed that 3,873 trees or about 6.7 percent of the 58,209 trees sampled had blackline at the graft union. Replants totaled 2,276 or 3.9 percent of the trees sampled, of which 1,451 or 2.5 percent were in the six counties of highest blackline incidence. If one assumes the replanted trees in those six counties had replaced blackline-killed trees previously in those positions, the blackline-affected trees and replants would add up to an observed incidence of 9.1 percent. The addition of another 748 or 1.3 percent for missing, probably blackline-killed, trees in those six counties means the total incidence of blackline in surveyed orchards is more likely closer to 10.4 percent than 6.7 percent. In counties with low blackline incidence, a few of the spaces where trees were missing or replanted were possibly losses to blackline. This estimate is probably conservative, since tests of pollen by ELISA have shown that the number of trees actually infected with the blackline virus may be much higher than the number showing blackline at the graft union.

Incidence of trees with blackline at the graft unon ranged from a low of 0.04 and 0.06 percent in Glenn and Tehama counties, respectively, to a high of 53.9 percent in Contra Costa County (table 1). The numbers of missing and replant trees were generally high in the counties with a high incidence of blackline, and most of these losses were probably due to blackline. The high numbers of replant and missing trees in Sutter County, however, could have resulted from causes other than blackline.

There were significant differences among varieties, with Eureka and Payne showing the highest incidence of blackline (table 2). One must take into consideration, however, the prevalence of those varieties in certain counties. Although 28 percent of the sampled trees were Eureka and Payne, 82 percent of the sampled Eureka and Payne trees were in Contra

Costa, San Joaquin, San Benito, and Stanislaus counties, which also had the highest incidence of blackline among the 16 counties surveyed. In these four counties, the incidence of blackline was 18.1 percent, while 21.9 percent of the Eureka and Payne trees were affected. Therefore, the incidence of blackline in Eureka and Payne trees was probably related to county or location and not to the variety. If there were a varietal relationship, the incidence of blackline would be high in Eureka and Payne trees, regardless of the county. A high incidence of affected trees in the four counties is perhaps related to their proximity to the original site of blackline occurrence in California. It was incidental that Eureka and Payne were the dominant varieties in those counties.

We also considered age of the block as a variable and rejected the hypothesis that age was associated with the incidence of blackline. In each of the four most severely affected counties, the orchard with the highest incidence of blackline was planted in 1950 or later. The highest incidence was 94 percent (1950), 41.7 percent (1955), 65.5 percent (1957), and 37.5 percent (1954) for Contra Costa, San Benito, San Joaquin, and Stanislaus counties, respectively.

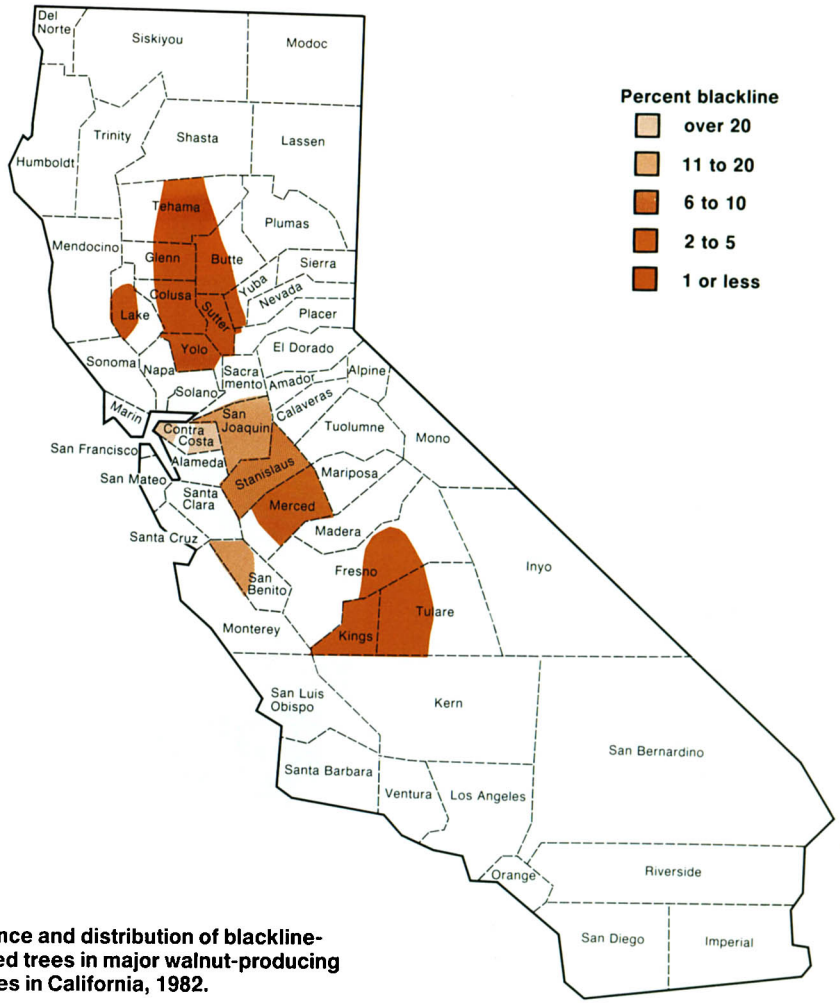
Pollen ELISA test

In the spring of 1982, catkins were mechanically shaken from 100 walnut trees in each of several orchards surveyed for blackline the previous fall. Orchards were selected on the basis of low blackline incidence but at least one blackline tree identified among the test trees. All available (up to 200) catkins were hand-collected from under each sampled tree. The catkins from each tree were kept separate and dried in a walnut bin dryer. Dried samples were crushed and sieved. The resulting samples of pure pollen, ranging from less than 1 gram to more than 50 grams per tree depending on individual tree and orchard, were then assayed by ELISA for blackline virus presence or absence.

Because of the extremely wet spring in 1982, several problems occurred during pollen collection. Many orchards were too wet for shaking equipment to operate until after the prime pollen-shedding period of trees was over. The amount of pollen collected from most of the trees was therefore insufficient to provide a reliable sample for detection of blackline virus by ELISA, even though the tests showed that some of the symptomless trees were already infected with blackline virus.

TABLE 3. Percent blackline found in orchards planted in different years in Contra Costa, San Benito, San Joaquin, and Stanislaus counties.

Year planted	Blackline	Orchards surveyed
	%	
1974-70	4.16	17
1969-65	20.01	16
1964-60	19.62	16
1959-55	25.46	16
Before 1955	32.52	30



Incidence and distribution of blackline-affected trees in major walnut-producing counties in California, 1982.

Discussion and conclusions

Because walnut pollen is wind-borne, long-distance spread from diseased to healthy orchards may follow the prevailing wind pattern. How far viable pollen will travel and infect healthy trees is not known, but wind-blown diseased pollen may have been responsible for the introduction of blackline into the interior valleys from the San Francisco Bay area, where it first appeared. Considering the slow movement within trees before visible symptoms occur and the number of cases reported in the early 1930s, it is quite possible that the blackline virus was introduced into California a long time before blackline-affected trees were discovered in 1929.

Blackline has now been identified thoughout the Central Valley, although incidence decreases with distance from San Francisco Bay. The disease progression suggests that the virus has gradually moved during the last 60 or 70 years from the original source of infection to a distance of approximately 200 miles from San Francisco Bay.

Statistical estimates of incidence of blackline and replants in relation to county, variety, and age showed significant differences between counties. Contra Costa (53.9 percent), San Benito (18.3 percent), San Joaquin (18.8 percent), and Stanislaus (9.5 percent) were all above average in incidence; statistical estimates show that generally they were significantly higher (with a probability of 0.99) than all other counties.

A further breakdown within counties showed that western San Joaquin County orchards had a much higher incidence of blackline (36.2 percent) than did the eastern area of the county (13.3 percent). Likewise, the western area of Stanislaus County was higher in blackline than the rest of the country (14.2 vs. 11.2 percent). Merced County also had more disease in its western than in its eastern region (7.3 vs. 2.3 percent). While orchards were se-

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lected randomly throughout the county and not selected as to location, the arbitrary separation of eastern and western sections of a county also suggests a progression of disease from the west near San Francisco Bay.

A high incidence of blackline was observed in Eureka and Payne varieties. However, these two varieties are mainly grown in Contra Costa, San Benito, San Joaquin, and Stanislaus, the same counties with the highest blackline incidence.

If tree age is considered in the four counties with the highest blackline incidence, a trend is observable. Orchards with trees 8 to 12 years old showed a low incidence of blackline (4.2 percent) in these four counties as compared with those that were 13 to 17 years old (20 percent) or 18 to 22 years old (19.6 percent) (table 3). Orchards with trees 23 to 28 years old in these four counties averaged 25.7 percent blackline and those older than 28 years showed 32.5 percent. Although the variation between blocks was too great for statistical validity, the low incidence found in the 8- to 12-year-old orchards supports the contention that blackline occurs in bearing orchards, with disease increasing over time.

The blackline virus is not uniformly distributed within infected trees, and individual catkins may only be partially infected with the virus. Thus, catkins yielding at least 50 grams of pollen are required from each tree to give a reliable ELISA test for an early virus infection. Collection and processing of samples this large would be difficult and extremely time-consuming on a commercial scale. Spring weather conditions can also hinder pollen collection. Serological testing (ELISA) of pollen to determine the extent of symptomless trees infected with blackline virus in commercial orchards thus does not appear to be practical at this time.

Current technology also appears to be too costly and tedious for successful eradication of blackline from areas of low incidence. Such eradication would require ELISA testing of each tree within the targeted area and removal of all infected trees. The potential for reintroduction of diseased pollen from sources outside the area would make it necessary to monitor orchards regularly and would severely limit any chance of success.

The ELISA, however, is an efficient, reliable, and rapid procedure for detection of the blackline virus in nursery trees, English walnut seedlings and seed, and graftwood and budwood. The ELISA may be effectively used for indexing propagation material for the presence of the blackline virus when and if needs arise for these control measures.

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Cutaway view shows black line at the junction of black walnut rootstock and English scion. Canker usually occurs with blackline on Paradox rootstock (below).

Jack Kelly Clark