Stress Predisposes Young Filbert Trees to Bacterial Blight

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ABSTRACT

The effect of cultural and environmental stress on filbert tree mortality was evaluated in two newly planted orchards. Nonirrigated trees inoculated with *Xanthomonas corylina* had a high mortality during the second summer after planting. Irrigation significantly reduced mortality of the trees. Tree mortality did not differ significantly between treatments used to prevent sunburn of tree trunks and untreated controls at the Corvallis planting site. However, the use of a reflective paper collar to protect the trunk at Rickreall harmed the trees because sunlight reflected from the surface of this collar injured the trunk and resulted in greater mortality. Summer pruning of trees in the nursery enhanced callusing of the pruning wound of noninoculated trees, but resulted in trees of small diameter. More of the smaller summer-pruned trees died from blight infections after outplanting than did the larger winter-prune nursery trees. Sealing the pruning wound to prevent entry of the pathogen into noninoculated trees also enhanced callusing, but did not influence mortality of noninoculated trees exposed to naturally occurring inoculum. At Rickreall, *X. corylina* was recovered from discolored and apparently healthy tissues of trees 48 months after inoculation, but none was recovered from tissues of the noninoculated trees that were sampled. In conclusion, the most important factor in reducing mortality of newly established filbert trees infected with *X. corylina* is adequate irrigation the first 2 to 3 years after planting.

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Additional key words: Irrigation, canker, pruning wounds, sunburn, bacteria in healthy tissue.

Filbert (*Corylus avellana* L.) production in the United States is centered in the Willamette Valley of Oregon where there are 22,000 acres of trees. Bacterial blight caused by *Xanthomonas corylina* (P. W. Miller, et al.) Starr and Burkholder is a particularly serious problem in establishing new filbert orchards, because young trees may be killed by the disease. Trees more than 4 years old rarely die following infection, although nut yield may be reduced through loss of nut-bearing branches.

With a projected increase of filbert acreage, cultural and environmental factors that contribute to disease losses must be identified. Trees stressed by an unfavorable environment appear to be more susceptible to infection (8), and bacterial cankers often are associated with injuries from frost, sunscald, mechanical equipment, and pruning cuts.

The objectives of this study were to identify the cultural and environmental factors that enhance bacterial infection and later death of young filbert trees, and to suggest changes in cultural practices to avoid fatal infections of young trees.

MATERIALS AND METHODS.—Environmental and cultural factors that stress young filbert trees were imposed on inoculated and noninoculated trees to assess their effects on *X. corylina* infections and tree survival. Trials were conducted on trees growing at Rickreall and Corvallis, Oregon. At both sites, the cultivar Barcelona was used in the trials.

Inoculations with X. corylina.—Filbert trees were inoculated by pruning lateral branches with shears dipped in an aqueous suspension of *X. corylina* (10^6-10^7 bacteria/ml). The inoculum was prepared from a 24-hour culture grown in a broth containing 0.4% yeast extract, 2% dextrose, 0.4% peptone, and 0.5% ammonium sulfate. The broth cultures were centrifuged at 10,000 *g* for 10 minutes, and the cells were resuspended in sterile distilled water. When trees were inoculated in the summer, inoculum was transported to the field in an ice chest.

Recovery of X. corylina from trees.—After surface-disinfesting the trunk with 1.3% aqueous sodium hypochlorite and allowing it to air dry, we tried to isolate the pathogen from trunk tissue. The outer bark was cut away, a small section of tissue was removed down to the wood with a sterile razor blade and placed in 1-5 ml of sterile distilled water. Later a loopful of the water was streaked on King medium B (6), 3-ketolactose medium (1), and potato-dextrose agar (PDA) (Difco). Tissue samples were rated positive for the pathogen if colonies developed showing the characteristic yellow pigmentation of *X. corylina*. Questionable isolates were restreaked on PDA to determine colony pigmentation and morphology, tested for the ability to induce a hypersensitive reaction in leaf tissue (4), and checked for the diagnostic characters listed for *Xanthomonas* by Skerman (9).

Summer vs. winter pruning.—*X. corylina* may enter and infect uncallused pruning wounds of dormant filbert nursery stock, because the trees are customarily dug, pruned, and graded in December and then heeled-in outdoors where pruning wounds can be exposed to airborne inocula. To avoid possible infections through these wounds, 240 layered nursery trees were pruned during the summer to allow time for wound callusing and compared to 240 trees pruned as usual in the winter. On 19 June, 9 and 31 July, and 16 September, sixty trees each were summer-pruned. One-half of both the summer-and winter-pruned trees were inoculated and compared to noninoculated trees. In addition, wounds of one-half of the inoculated and noninoculated trees were sealed with a polyvinyl acetate pruning paint to protect the wounds and enhance callusing. All the trees were dug in December and stored in sawdust beds until planted in January (Corvallis) or March (Rickreall). Callus development at the pruning wounds, recovery of the pathogen, and tree death were recorded to evaluate the various treatments.

Sunburn protection.—The use of newsprint-mat collars to protect tree trunks from sunburn has been a standard cultural practice for many years. More recently, painting tree trunks with white latex paint has been adopted by many growers. Tree trunks of inoculated and noninoculated trees at Rickreall were painted with a white latex paint (diluted 1:1 with water) (7), or the trunks were fitted with collars made of heavy white paper. Each treatment was applied to four blocks of trees (five trees per block), and four more blocks were maintained with bare trunks to serve as controls. The same trunk treatments were applied to three blocks each (five trees per block) of inoculated and noninoculated trees at Corvallis. However, newsprint mat collars were fitted around the trunks, instead of the heavy white paper collars used at Rickreall. The newsprint mats have a dull finish in contrast to the highly reflective surface of the white paper. All trees were planted on a 4.6-m-square spacing.

Moisture stress.—At the Corvallis site, nursery trees were divided into two equal groups of 135 trees each. One group was irrigated three times each summer, and the other group was not irrigated, as a control. Each group was composed of randomized blocks of inoculated and noninoculated trees with sealed and nonsealed pruning wounds and trunk treatments to prevent sunburn.

Statistical procedures.—Before running an unbalanced four-factor analysis of variance (F-test), we used an arcsin square-root transformation of the data to normalize the variances. By this statistical analysis, we compared treatment interactions. When treatments differed by the F-test ($P = 0.05$), a least-significant-difference test of the single values was used to find significant treatment differences. The data from interacting treatments not differing by the F-test were averaged across several treatments.

RESULTS.—*Summer vs. winter pruning.*—Tree growth (stem diameter) was reduced when trees were pruned early in the summer. The average stem diameter of summer-pruned nursery trees was 15.8 mm (range, 11.4-20.4 mm). This diameter was 11% less than that of winter-pruned trees, which had an average stem diameter of 17.6 mm (range, 14.2-21.2 mm) after one season's growth at Rickreall. Mortality of summer-pruned trees was significantly greater than that of winter-pruned trees (Table 1). The numbers of dead trees among the four summer pruning dates did not differ significantly from each other.

Callusing of pruning wounds.—When lateral branches of noninoculated trees were pruned in the summer, wounds were smaller and callused more rapidly than those of winter-pruned trees. Rapid callusing reduced the exposure time of susceptible tissue to infection by *X. corylina*. Calluses developed most rapidly when the pruning cuts on noninoculated trees were sealed with polyvinyl-acetate pruning paint. In contrast, callus development on inoculated trees that were summer-pruned was retarded or stopped, even though the cuts were sealed with pruning paint. Tree mortality was significantly greater when wounds of summer-pruned trees were inoculated, but not sealed (Table 2). However, mortality was greatest among noninoculated trees when pruning wounds were sealed.

At Corvallis, when treatments were compared within either the irrigated or the nonirrigated groups of trees,

TABLE 1. Influence of summer vs. winter pruning of filbert nursery stock relative to fatal bacterial blight infection at Rickreall, Oregon

	Dead trees	
Pruning	Inoculated	Noninoculated
Summer	31[a]	7
Winter	14	4

[a]The number of dead trees out of 120, as assessed 4 years after planting. Each value differs significantly from the other, $P = 0.05$.

TABLE 2. Filbert tree mortality from bacterial blight at Rickreall, Oregon, as influenced by the sealing of pruning wounds

	Dead trees	
	Inoculated	Noninoculated
Sealed	16[a]	9
Nonsealed	29	2

[a]The number of dead trees out of 120, as assessed 4 years after planting. Each value differs significantly from the other, $P = 0.01$.

TABLE 3. Filbert tree mortality from bacterial blight at Corvallis, Oregon, as influenced by irrigation to prevent moisture stress

	Dead trees[a]	
	Irrigation	Nonirrigation
Inoculated and sealed	4 C[b]	22 A
Noninoculated and sealed	1 D	6 B,C
Noninoculated and nonsealed	1 D	7 B

[a]The number of dead tress out of 45, as assessed 3 years after planting.

[b]Values with a common letter do not differ significantly, $P = 0.05$.

TABLE 4. Filbert tree mortality from bacterial blight at Rickreall, Oregon, as influenced by trunk treatment to prevent sunscald

	Dead trees[a]	
Trunk protection	Inoculated	Noninoculated
Bare	8 B,C[b]	1
Painted	9 B	4 D
Collar	28 A	6 C,D

[a]The number of dead trees out of 80, as assessed 4 yr after planting.

[b]Values with a common letter do not differ significantly, $P = 0.05$.

mortality did not significantly differ between noninoculated trees with sealed or nonsealed pruning wounds (Table 3). However, there was significantly less mortality when the trees were irrigated than when nonirrigated. Significantly more trees died on which the pruning cuts had been sealed after inoculation than did the corresponding noninoculated trees.

Sealing pruning wounds appears to be of no value when natural populations of *X. corylina* are low and the trees are pruned in the winter.

Sunburn protection.—Installation of a protective white paper collar to prevent sunburn actually contributed to tree death at Rickreall, regardless of whether or not the trees were inoculated (Table 4), whereas painting to protect the trunk resulted in significantly more dead noninoculated trees. A major reason for the increased mortality with the collar treatment was the high reflectivity of the reinforced white paper. Reflected sunlight severely burned the tender tissues of some trees near the top of the collar. This white paper collar was chosen by the grower and differed from the newsprint-mat collar generally used by growers and used at Corvallis. The trunk treatments (paint or collar) at Corvallis did not differ significantly from the bare trunk control.

Moisture stress.—More of the nonirrigated young filbert trees died from *X. corylina* infections than did the irrigated trees at Corvallis (Table 3). This difference was not noticeable until the second year after planting (Fig. 1). Irrigation enhanced tree growth and significantly reduced mortality among trees inoculated with *X. corylina* (Table 3). The average stem diameter of inoculated and noninoculated trees after 3 years of irrigation was 43.6 mm (range 30.6 to 52.0 mm) and 44.8 mm (range 40.0 to 51.6 mm), respectively. In contrast, the average stem diameter of the inoculated and noninoculated trees that were nonirrigated was 29.2 mm (range 23.5 to 38.7 mm) and 30.4 mm (range 22.3 to 37.0 mm), respectively. Thus, growth decreased 32%.

Recovery of X. corylina from trees.—Although irrigation decreased the mortality of trees planted at Corvallis, stem cankers, other blight symptoms, (or both) showed on 44% and 52% of the inoculated and noninoculated trees, respectively, even though they were irrigated. Similarly, out of the nonirrigated block, 84% and 77% of the inoculated and noninoculated trees, respectively, showed symptoms. The Corvallis planting site was next to and downwind from a filbert orchard with many blighted trees. Disease symptoms and death of noninoculated trees in the experimental plot were attributed to natural infections. Isolations of *X. corylina* from these noninoculated trees substantiated this assumption. Samples of tissue were removed from 28 inoculated and 10 noninoculated trees in each of the irrigated and nonirrigated blocks. The bacterium was isolated more frequently from trees in the irrigated block (78% and 60% from the inoculated and noninoculated trees; respectively, as compared to 61% and 20% from the nonirrigated block). In the irrigated block, the bacteria were often isolated from healthy-looking tissues.

The Rickreall plot was remote from other filbert plantings, and blight symptoms were limited mainly to the inoculated trees. Tissue samples were removed from the scarred nodal area of the trunk, where laterals had been pruned away 48 months earlier. *X. corylina* was recovered from 13 of 32 inoculated trees, but from none of 32 noninoculated trees that were sampled. More than one half of the tissues that yielded *X. corylina* looked healthy, and thus the pathogen appeared to inhabit healthy-looking tissues for at least 48 months without causing visible injury.

Diagnostic tests on 45 cultures of bacteria isolated from the Rickreall and Corvallis test plots were in agreement with Skerman's scheme (9) of classification of *Xanthomonas*. Out of the 45 cultures, 36 showed the typical yellow colony pigmentation, four were a lighter yellow, and five were darker than typical. All the isolates were urease-negative, caused an alkaline peptonization of litmus milk, liquefied gelatin, did not produce indole, and could not ferment glucose. Forty-three of the 45 cultures produced a carotenoid pigment, 42/45 produced a weak acid reaction on glucose, 31/45 hydrolyzed starch, 44/45 did not reduce nitrate to nitrite, and 44/45 were oxidase-positive.

DISCUSSION.—A lack of irrigation significantly increased mortality of young filbert trees inoculated with *X. corylina*. Conversely, trees which were irrigated and inoculated grew vigorously with little mortality; i.e., they were tolerant to bacterial blight infection. Thus, trees stressed by a lack of irrigation might be predisposed to fatal infection. For fields without irrigation, we would suggest use of other methods of preserving moisture, such as mulching with sawdust, covering soil with black plastic, and controlling weeds. Also, large nursery trees with good root systems should be selected for planting.

Our results, based on tree mortality at Corvallis, show that trunks of inoculated trees protected against sunscald by collars or latex paint did not thrive better than bare trunks. However, sunburn can predispose tissues to blight infection, as shown by more tree deaths at Rickreall where reflective white paper was used for trunk protectors. Furthermore, the association of filbert blight infections with sunburned tissue has been observed in

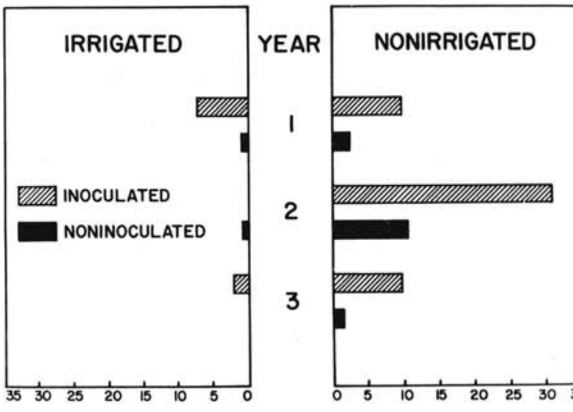

Fig. 1. Yearly mortality of filbert trees as influenced by irrigation and bacterial blight. Each bar represents the percent of trees killed in a given year out of 45 inoculated trees or 90 noninoculated trees.

orchards (8), although the occurrence was sporadic. This irregularity probably resulted from yearly fluctuations in the severity of climatic conditions. A climate less conducive to sunburn may have occurred the first 2 years of our field tests, thus explaining the lack of damage to unprotected tree trunks.

Sealing the pruning wounds with polyvinyl-acetate paint was expected to favor wound callusing and hinder ingress of *X. corylina*, yet more noninoculated trees died at Rickreall when the wounds were sealed. If the wounds of the noninoculated trees were contaminated at pruning and then sealed, the bacteria may have been protected. Sealed wounds may have provided a better infection site. Conversely, mortality was greatest among inoculated trees when pruning wounds were not sealed, especially of trees with the reflective white collar. Inoculated trees with sealed wounds and trunk collars may have been more tolerant to the sun's rays, hence under less stress. Mortality among the noninoculated trees at Corvallis was not influenced by sealing of the pruning cuts, even though pruning wounds had been exposed to inoculum, as shown by the occurrence of natural infections in the planting.

Many inoculated trees at Corvallis and Rickreall appeared to be tolerant of *X. corylina* infection, and the bacteria often were isolated from apparently healthy or only slightly discolored tissues. We suspect that a few bacterial cells reside in the tissues and act as a reservoir of inoculum which can become pathogenic when host defenses are weakened by environmental stress. This suspicion could explain the relatively high mortality when inoculated filbert trees with white paper collars were sunburned and developed girdling cankers.

The presence of the pathogen in apparently healthy tissue is a concern, because the pathogen may act as an inoculum source for infection of young nut-bearing twigs after the tree reaches maturity. The biology of *X. corylina* in symptomless tissue has not been studied, but the occurrence of bacterial pathogens in apparently healthy woody plants has been reported recently: *Pseudomonas syringae* can be isolated from the symptomless cherry tissue (2), *Erwinia amylovora* from healthy pear tissue (5), and *E. rubrifaciens* (10) (with a double drug marker) has been isolated from symptomless walnut tissue several hundred centimeters from the point of inoculation (3).

To reduce bacterial blight in young filbert plantings, Miller et al. (8) recommended fall or winter planting of first-quality nursery trees in deep well-drained soil, using a suitable trunk protector, disinfecting pruning tools, and spraying with a protectant bactericide. Besides these, we emphasize avoiding moisture stress during the first three summers after planting. In areas with limited summer rainfall, two or three summer irrigations are beneficial. If this good cultural practice is combined with others, the mortality of newly planted filbert trees will be reduced.

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