Bacterial blight on Persian walnuts

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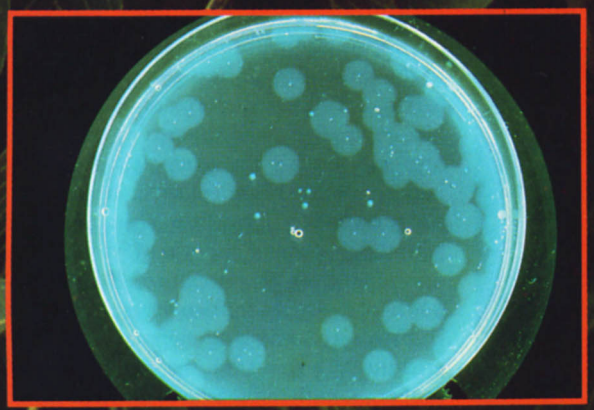


On selective medium, blight bacteria colonies are surrounded by pale blue zones of digested starch. Other colonies, mostly Pseudomonads, grow but do not produce the distinctive starch hydrolysis zone.

Blight bacterium attacks husks of developing nuts, causing discoloration of the shell and nut meat.

Bacterial blight of English (Persian) walnuts has been an economically important disease in California for nearly 85 years. The causal bacterium *Xanthomonas campestris* pv. *juglandis,* can infect leaves, catkins, green branches, and nuts. Blight reduces yield and frequently lowers quality of harvested nuts. Young nutlets infected early in the season generally fail to develop and drop prematurely. Late-season infections on maturing nuts can discolor the shell and nut meats, so that they are off-grade or unsalable at harvest. Although none of the English walnut cultivars appear to be resistant to blight, the disease is most serious on early varieties with bloom periods that coincide with spring rains. In years with extended spring rains, blight can reduce nut yields 50 to 80 percent.

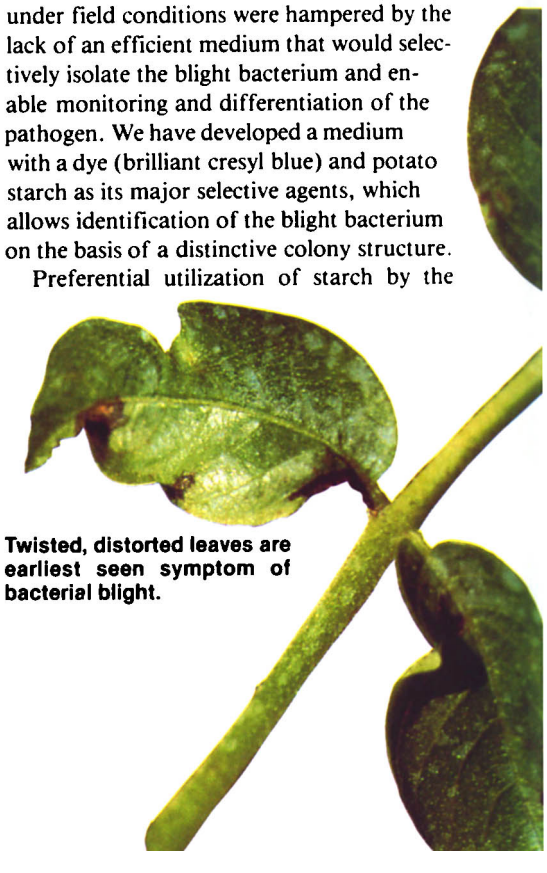
The blight bacterium, like most xanthomonads, is host specific, infecting only *Juglans* species. The lack of an alternate host has suggested to most researchers that the inoculum responsible for initiating spring epidemics must survive the winter on the walnut tree. Precisely where the bacterium survives has been a point of controversy among researchers since 1901, and studies have failed to establish conclusively how overwintering inoculum initiates blight epidemics.

In 1975 we began studying walnut blight to determine how and where the pathogen survives, and to gain greater insight into the ecology of the blight bacterium and disease epidemiology.

Selective medium

Previous efforts to study xanthomonads under field conditions were hampered by the lack of an efficient medium that would selectively isolate the blight bacterium and enable monitoring and differentiation of the pathogen. We have developed a medium with a dye (brilliant cresyl blue) and potato starch as its major selective agents, which allows identification of the blight bacterium on the basis of a distinctive colony structure.

Preferential utilization of starch by the



Twisted, distorted leaves are earliest seen symptom of bacterial blight.

walnut blight bacterium creates a distinctive hydrolysis zone around the pathogen colonies, making them easily distinguishable from some pseudomonads that also grow in the medium. The brilliant cresyl blue/starch (BS) medium provides a simple, effective means of isolating pv. *juglandis* from walnut tissue while eliminating 97 percent of the contaminating bacteria that grow on ordinary media. In laboratory tests, BS medium was also useful for isolating six other *Xanthomonas campestris* pathovars—pv. *begoniae,* pv. *campestris,* pv. *incanae,* pv. *malvacearum,* pv. *phaseoli,* and pv. *vesicatoria.*

Bud and catkin infestation

Isolations using BS medium were made from dormant and developing buds and catkins to determine if they were sites for survival of blight inoculum over winter. Apparently healthy dormant buds and catkins were collected from seven walnut cultivars, representing early, middle, and late blooming habits, in 29 orchards. Thirty to forty buds and an equal number of catkins from each orchard were individually assayed for presence of the pathogen. Every cultivar studied had some level of bud and catkin infestation. A partial summary of the results of these isolations appears in table 1. Statistical analysis of these data showed that buds were infested significantly more often than catkins.

Bud infestation was greatest on early-blooming cultivars, with infestation frequencies of 5 to 95 percent in the 18 Payne and Ashley orchards examined. In 8 of these orchards over 59 percent of the buds harbored the pathogen. Bud infestation in Serr and Eureka, the other early-blooming varieties, ranged from 0 to 30 percent and 0 to 20 percent, respectively. Infestation frequencies in Hartley and Franquette, late-blooming varieties, were 0 to 13 and 0 to 33 percent.

Cultivar blooming habits, rainfall patterns, and orchard design influenced the development and severity of blight and the subsequent infestation of buds and catkins. The incidence of bud infestation was greatest (mean = 41.6 percent) in the early-blooming varieties, Payne and Ashley, growing in the northern California area where spring rainfall occurs more frequently. In the drier southern counties, bud infestation averaged 9.7 percent on the same varieties. Middle and late varieties usually bloom after spring rains have subsided and have only minor nut and leaf blight during the season. The reduced blight severity is also reflected in low bud and catkin infestation on Hartley and Franquette.

The orchard of the Marchette cultivar, however, was an exception among the later blooming varieties in that blight caused major crop losses, and the incidence of bud infestation was correspondingly high. Walnut trees in this orchard were interplanted with plums; the resulting dense leaf canopy presumably restricted air movement and increased the frequency and duration of dews. Such conditions would be conducive to blight development and subsequent bud and catkin infestation.

Walnut trees produce both vegetative and fruitful (nutlet-bearing) buds. Depending on the variety, fruitful buds may occur as terminal buds, lateral buds, or both. Examination of terminal and lateral buds of six walnut cultivars of differing fruitful bud-bearing habits showed that location of buds and bud fruitfulness did not influence the incidence of bud infestation.

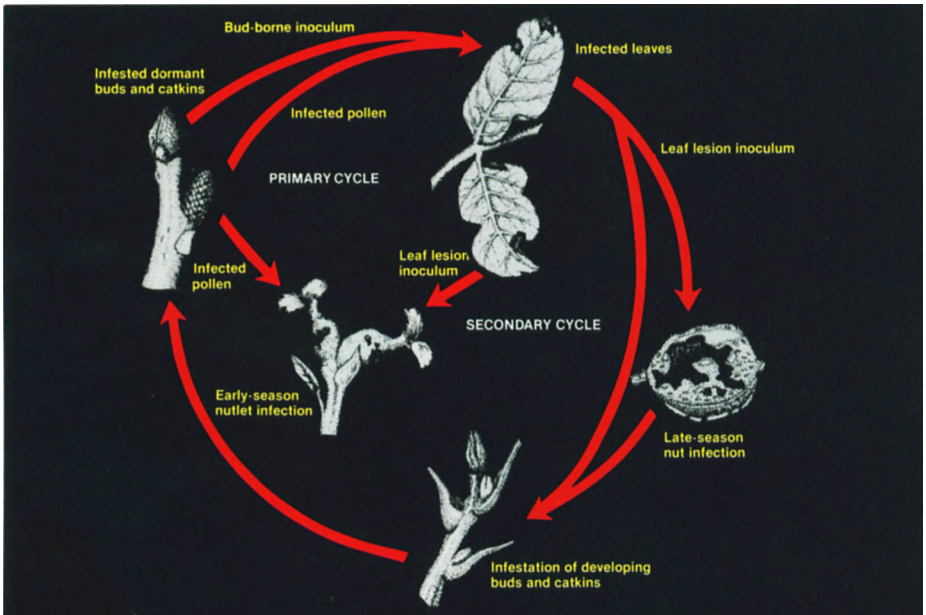
However, buds that developed on shoots with infected foliage became infected significantly more often that those on shoots with healthy leaves. Over 90 percent of infested dormant buds harbored two distinct populations of the pathogen—an epiphytic, or surface, population and an internal population. The epiphytic population is subject to attrition by such adverse environmental factors as drying and ultraviolet light. Internal populations of the pathogen are more protected and, in general, are 10 times greater than epiphytic populations.

The presence of a residual population of pv. *juglandis* within dormant walnut buds explains the aggregated, nonrandom distribution of infected leaves observed in spring. Early in the season some buds produce shoots with heavily infected leaves, while adjacent buds produce healthy leaves.

Catkins, whether healthy or diseased, drop from the leaf canopy following elongation. Nutlets infected in the early stages of development generally drop a few weeks after infection. Blighted leaves, regardless of when they become infected, remain in the canopy through the rest of the growing season and are the major source of inoculum for secondary spread of the pathogen. Blight bacteria populations exceeding 10^6 colony-forming units (CFU) were recovered from individual infected leaflets. Sprinkler irrigation water collected from beneath blighted trees after it had fallen through the leaf canopy contained 5×10^3 to 7.8×10^4 CFU of the pathogen per milliliter.

These data show how important infected leaves are to the progress and severity of blight epidemics. Inoculum from infected leaves, spread by any source of moisture (sprinkler irrigation, dew, rain), can infect other leaves or maturing nuts and contribute to the infestation of next season's buds and catkins. The infested dormant buds and catkins ensure that an adequate supply of inoculum will survive the winter to potentially initiate another blight epidemic in spring.

Twig cankers have long been associated with the overwinter survival of pv. *juglandis* on walnut trees. We monitored 20 fresh walnut blight cankers for two years. During the first season of canker development, we recovered the pathogen from the surface of all 20 cankers at both midseason (June) and before dormancy (September). These cankers never produced macroscopic exudations of bacteria or bacterial strands. We made isolations by wiping the surface of these cankers with a



The disease cycle for walnut blight emphasizes the importance of bud- and catkin-borne bacteria in blight epidemics in Persian walnuts.

sterile, moist cotton swab and then using this swab to inoculate plates of BS medium. Following the first winter dormancy, the pathogen could no longer be recovered from any of the 20 cankers, which appeared hardened and dry. *Alternaria* spp. were recovered from all these year-old cankers, and *Rhizopus stolonifer* was recovered from 17 of the 20.

The disease cycle summarizing results of our study (see drawing) shows only infested dormant buds and catkins as overwintering sites for pv. *juglandis* on walnuts. Further research is needed to determine if other sites on the trees are suitable for epiphytic survival of the pathogen. Our studies, however, failed to substantiate the role of twig cankers in the survival of blight bacteria through the winter dormancy of walnuts.

Control

The vast majority of published information on walnut blight deals primarily with the use of various copper compounds and spray schedules developed for disease control. The program recommended for blight control consists of at least one spray during catkin elongation and two to three sprays during pistillate bloom. This program does not provide reliable, consistent blight control. In dry years, nut blight losses can usually be held below 10 percent with two to three copper sprays. In rainy years, it is difficult, if not impossible, to keep nut blight below 30 percent regardless of the spray schedule or copper formulation used.

Our epidemiological studies suggested that reducing overwintering populations of blight inoculum might improve control. Field experiments were established to evaluate the effectiveness of dormant sprays. In one replicated field plot, single branches were sprayed to runoff with various copper compounds and an experimental antibiotic one week before bud break. Two weeks later, foliage samples were collected, and populations of pv. *juglandis* on leaves emerging from treated buds were determined. Populations of the pathogen were significantly lower on new growth in all five treatments than in treated controls (table 2). However, even with $CuSO^4 \cdot H_2O$, the most effective treatment, the mean population per shoot was 1.86×10^5 CFU.

In a second series of field plots, one or two dormant-season sprays were applied in conjunction with standard bloom-period sprays. For two consecutive years, addition of dormant sprays failed to show a statistically significant improvement in nut blight control when compared with bloom sprays alone. Dormant-season copper sprays appeared to reduce the number of surface-infested buds (table 3), but these reductions were not sufficient to have any effect on the overall progress of the epidemic and subsequent crop loss.

TABLE 1. Recovery of *Xanthomonas campestris* pv. *juglandis* from Dormant and Developing Walnut Buds and Catkins

Variety	County	Date sampled	Blooming habit*	Infested on surface: Buds†	Infested on surface: Catkins
				%	%
Ashley	Butte	June	Early	90	90
Ashley	Butte	Feb	Early	45	35
Ashley	Glenn	Oct	Early	73	20
Ashley	Tehama	March	Early	73	—
Eureka	Contra Costa	Oct	Early	10	20
Eureka	Tulare	Oct	Early	20	0
Franquette	Butte	April	Late	0	0
Franquette	Contra Costa	April	Late	33	16.6
Hartley	Butte	April	Middle	0	0
Hartley	Contra Costa	April	Middle	13	21
Marchette	Tulare	Nov	Middle	63	18
Payne	Contra Costa	Dec	Early	95	95
Payne	Contra Costa	Aug	Early	80	70
Payne	Contra Costa	March	Early	45	55
Serr	Contra Costa	Sept	Early	20	0
Serr	Tulare	Sept	Early	5	0

*Blooming habits were based on leafing dates; early, March 15; middle, April 1; and late, after April 15.
†On the basis of a paired t-test, buds were infested significantly more often than catkins.

TABLE 2. Populations of *Xanthomonas campestris* pv. *juglandis* on Young 'Ashley' Walnut Shoots* Following Dormant-season Application of Three Bactericides

Treatments†	Mean shoot population (× 10^5)‡
$CuSO_4 \cdot 5H_2O$) (25% Cu, 10 g/liter)	1.86 a
A-16886-b (100 ppm)§	4.43 a
A-16886-b (200 ppm)	9.03 a
Tribasic $CuSO_4$ (53% Cu, 5 g/liter)	23.33 a
Untreated	1,110.62 b

*Shoot = all new growth that emerged from an individual bud, including leaves, stems, nutlets, and developing buds and catkins.
†All treatments were applied on March 18, 1980, with a pressurized garden sprayer.
‡Means were based on isolations from five shoots per replication, each treatment replicated seven times in a randomized complete block design. Values followed by the same letter are not significantly different (P = 0.05), LSD = 767.05.
§Experimental bactericide provided by Eli Lilly Inc.

TABLE 3. Effect of Dormant-season Copper (Kocide) Sprays on Surface Bud Infestation and Nut Blight on 'Marchette' Walnut

Treatments*	Bud infestation after dormant sprays (Winter 1978)†	Nut blight at harvest‡: 1978	Nut blight at harvest‡: 1979
	%	%	%
2 dormant	58	22.4 a	ND§
1 dormant & 3 bloom	56	10.6 b	11.8 a
2 dormant & 3 bloom	66	21.6 b	ND
3 bloom	ND	12.2 b	12.9 a
Unsprayed	84	29.0 a	42.5 b

*Single tree treatments were replicated five times in a randomized complete block design. All Kocide treatments were applied to run off at 0.481 kg per 100 liters with a hand-gun sprayer.
†10 buds from each replication of treated and control trees were assayed for infestation seven days after the last dormant spray was applied January 27, 1978.
‡Values in column followed by the same letter are not signicantly different (P = 0.05).
§ND = not done.

Preventing bud and catkin infestation with multiple protective copper sprays would be an approach to reducing the amount of overwintering inoculum. However, this type of program is not yet economically feasible.

Copper materials do not effectively penetrate walnut buds and catkins and, therefore, cannot eradicate blight bacteria from these overwintering sites. Thus the inoculum within these structures escapes the effects of topically applied copper sprays and can, under favorable environmental conditions, multiply and infect host tissues. Until bactericides with eradicative or systemic activities are developed walnut blight will continue to be a difficult disease to control.

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The authors express their appreciation to Ross Sanborn, Steve Sibbett, Bill Olson, and Larry Fitch, Farm Advisors, Contra Costa, Tulare, Butte, and Glenn counties, respectively; Dr. Beth Teviotdale, Extension Specialist, Plant Pathology, Parlier; and Janet Barton, Research Assistant, U.C., Berkeley.