Etiology of Almond Leaf Scorch Disease and Transmission of the Causal Agent

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ABSTRACT

Marginal scorching of leaves, appearing after mid-June, is one of the most diagnostic characteristics of the disease. Electron microscopic examination of ultrathin sections of the mid-veins of scorched leaves from naturally and experimentally infected trees revealed the presence of rod-shaped bacteria in the xylem vessels. The bacterial cells had an average diameter of 0.4 μm and length up to 1.9 μm and they exhibited multilayered, rippled, and convoluted walls. No organism was found in the xylem of healthy almond trees. Leafhoppers *(Draeculacephala minerva)* transmitted the bacterium from naturally infected almonds to healthy Mission almond seedlings and rooted cuttings of Carignane grapevines in the greenhouse. The almond and grape indicators developed typical leaf symptoms of almond leaf scorch and Pierce's disease, respectively, within 2 months after exposure to infective leafhoppers. Control grape and almond plants exposed to leafhoppers that had fed on healthy almond shoots remained symptomless, and no bacteria were observed in the leaves of any control plants. The almond leaf scorch bacterium was readily graft-transmitted by buds, bud chips, or stems from naturally or experimentally infected to healthy 1- and 2-year-old almond trees.

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A leaf scorch disorder of unknown etiology in almond was recently described (11). The most characteristic symptom of almond leaf scorch (ALS) is leaf scorching followed by decreased productivity, general decline, and subsequent death of affected trees. Newly infected trees usually exhibit leaf symptoms in a single terminal branch (Fig. 1-A) and then in adjacent branches; within 2-5 years the entire tree may develop leaf symptoms. Scorched leaves first appear in affected trees about mid-June. The leaves at first show chlorotic areas either on the tip or on the sides of the lamina that subsequently desiccate and die. The scorched areas gradually enlarge over entire lamina. Severity and pattern of leaf scorch depend on the stage of disease, inherent character of the cultivar and climate (Fig. 1-B). The scorched leaves remain on the trees until fall defoliation. The affected trees become less productive, decline progressively, and often die within 3-8 years after the onset of leaf symptoms (Fig. 1-C).

A disorder resembling ALS was observed in the mid-1930's on a few almond trees near Riverside, California (L. C. Cochran, *personal communication*) and in the early 1950's ALS was observed in a few randomly scattered trees in Los Angeles and Contra Costa counties (11); presently ALS is known to occur in commercial almond orchards in 14 different counties throughout California (14). Occurrence of affected trees in groups within an orchard, gradual progression of symptoms through affected trees, and apparent spread of ALS from affected to surrounding healthy trees suggest that some infectious agent may be involved. However, previous attempts to transmit causal agent by buds (11) or grafts (L. C. Cochran, *personal communication*) from naturally affected to healthy almond nursery trees gave negative results.

This study was undertaken to determine the etiology of ALS and possible means of transmission of the causal agent from diseased to healthy almond trees. A short account of this work has been reported (9).

MATERIALS AND METHODS.—*Search for causal agent(s) in affected trees.*—Electron microscopic examinations were made on ultrathin sections of leaf tissue taken from naturally infected and apparently healthy, symptomless almond *(Prunus amygdalus* Batsch) orchard trees. Pieces of leaf tissue, 2 × 2 mm, were cut from the mid-vein portions of leaves with characteristic symptoms and from similar leaves of the healthy trees. These tissues were fixed (4) and then embedded in Spurr's medium (15). With a diamond knife, ultrathin sections were cut from the mid-vein pieces both across and parallel to the xylem elements, mounted on copper grids, stained (4), and examined with an electron microscope.

Leaves from both healthy and infected trees also were examined for the presence of bacteria with phase-contrast microscopy (3). Serial cross sections, approximately 15- to 12-μm thick, were cut from leaves by a hand microtome. They were placed in drops of 0.1 M KOH on microscope slides and examined for the presence of bacteria within the xylem vessels and in the mounting solution.

Histochemical diagnosis of almond leaf scorch.—Diagnosis of ALS by visual observation of orchard trees is unreliable during the period from leaf fall to mid-summer. Diagnosis may be complicated also by

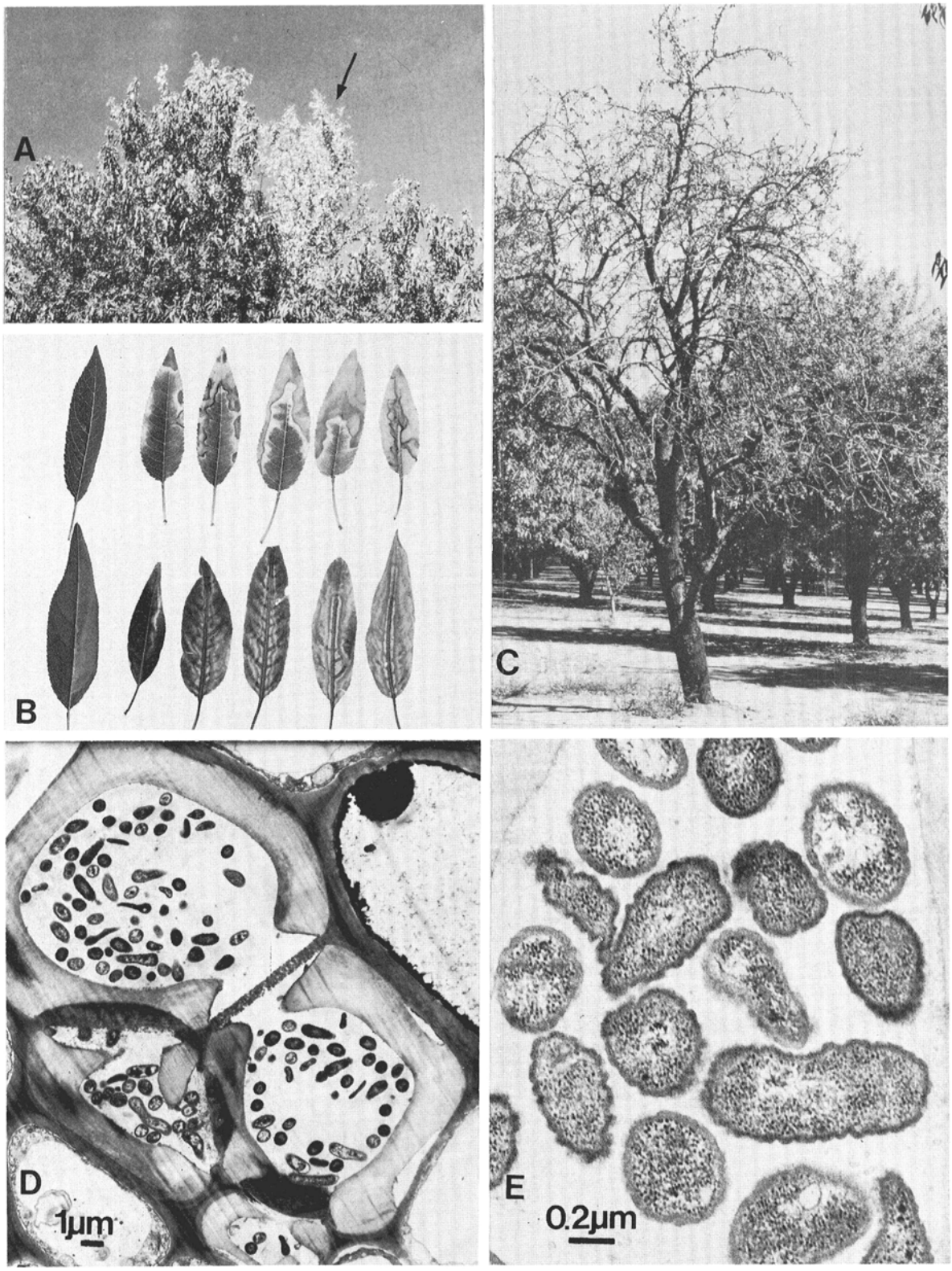


Fig. 1-(A to E). A) A newly infected orchard almond tree with leaf scorch symptoms in a single terminal branch (arrow). **B)** Leaves of Long IXL (upper row) and Mission (lower row) almond trees showing different degrees and patterns of leaf scorching. **C)** Almond tree in an advanced stage of the disease with dead spurs, dieback of terminal shoots and general decline. **D-E)** Electron micrographs of cross sections of leaf xylem vessels from affected trees showing bacteria within the lumina; four adjacent vessels contain the etiologic agent surrounded by xylem elements free of bacteria (D) and the cell walls of bacteria in the lumen of a xylem vessel are rippled (E).

the varying leaf scorch pattern produced by different cultivars in different climates, and symptoms of ALS may be confused with symptoms caused by excess salt and some herbicides. Because of morphological similarity between the bacterium associated with ALS-affected almond trees and the one associated with peach phony disease (5, 12), we evaluated the suitability of the phony peach disease chemical test (6) for diagnosis and detection of ALS in almond trees. The following procedure was used: Four to five pieces of 2- to 3-year-old wood (approximately 25-cm long) were collected at random from branches of trees suspected to be affected with ALS. A series of longitudinal sections (approximately 40- to 50-mm long and 1- to 2-mm thick) were cut from each branch wood piece and immediately submerged in approximately 20 ml acidified methyl alcohol (absolute methyl alcohol, 500 ml and concentrated hydrochloric acid, 5 ml) in a 100 × 1.5-mm diameter petri dish. The wood sections were observed for development of well-defined purplish-red or dark pink spots or streaks in the woody cylinder as reported for phony peach test (6).

Leafhopper transmission experiments.—Adult leafhoppers *(Draeculacephala minerva* Ball), had been maintained for several generations on barley *(Hordeum vulgare* L.) plants in the greenhouse. The leafhoppers were fed for 3 days on excised terminal almond shoots showing typical leaf symptoms collected from naturally infected Long IXL almond trees. Two insects were transferred onto each of four healthy seedlings of the almond cultivar Mission, peach (*Prunus persica* L. Batsch 'Lovell') and four rooted cuttings of grapevine *(Vitis vinifera* L. 'Carignane'). Control plants each received two insects that had fed for 3 days on shoots from healthy Long IXL orchard almond trees. All indicator plants were grown for 4 months in steam-pasteurized soil in 25-cm diameter clay pots in the greenhouse. Then they were placed individually in an insect cage and exposed to infective or noninfective leafhoppers. The leafhoppers were allowed to feed on indicator plants for 10 days, then the insects were destroyed. The indicator plants were removed from the cages, placed on a bench in a greenhouse with ambient temperatures ranging from 22 to 31 C, and observed for development of symptoms.

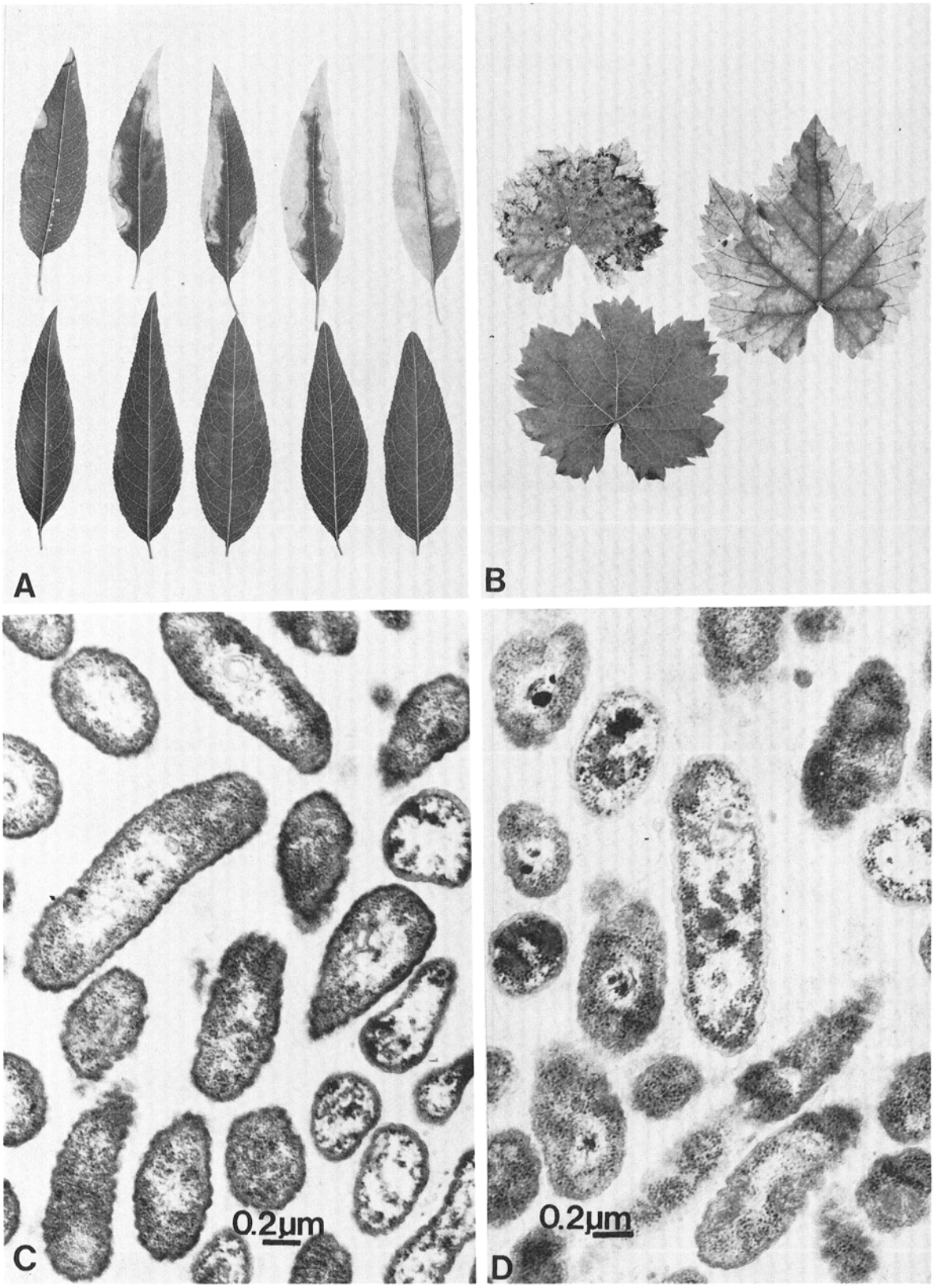
Nursery and lathhouse transmission experiments.—In one experiment, 8-month-old Long IXL almond trees on Nemaguard rootstock were inoculated in nursery rows. Buds with small amounts of woody tissue, taken from three different sources of either naturally infected or healthy Long IXL orchard trees (Table 1), were used to inoculate 20 nursery trees per inoculum source. On 28 August 1973, four buds were inserted in T-cuts on trunks of each nursery tree. Ten indicators for each bud source were transplanted (30 January 1974), either into steam-pasteurized soil in 20-liter containers on a bench in a lathhouse, or in a field experiment plot (1.8 × 36 m apart) at the University of California, Davis. These indicator plants were observed for appearance of leaf scorch symptoms until October 1974, when final data on transmission were collected.

In another experiment, the relative efficiency of graft inoculation was compared with that of bud inoculation in transmitting the causal agent from naturally infected Long IXL and Mission trees to healthy almond trees (Table 1). Two-year-old Long IXL trees growing in steam-pasteurized soil in 20-liter containers were whip grafted with two or three scions each from the previous year's growth of either naturally infected or healthy almond trees. The scions from diseased and healthy trees were grafted to lateral branches of each of 10 indicator plants on 30 January 1974, and then placed on a bench in the lathhouse. Final data on transmission were collected in October 1974.

TABLE 1. Relative efficacy of buds and stems in transmitting the causal agent of almond leaf scorch from orchard almond trees to 1-year-old Long IXL almond trees

Source of graft material (cultivars[a])	Type of graft material	Fraction[b] of indicators with almond leaf scorch symptoms	
		Lathhouse experiment	Field experiment
Infected trees - with leaf symptoms[c]			
Long IXL	Buds	4/10	6/10
	Stems	7/10	
Mission	Stems	2/10	
Infected trees - without leaf symptoms[d]			
Long IXL	Buds	0/10	0/10
	Stems	8/10	
Mission	Stems	8/10	
Noninfected trees, controls			
Long IXL	Buds	0/10	0/10
	Stems	0/10	
Mission	Stems	0/10	

[a]Orchard almond trees.
[b]Number of indicators with symptoms per number of indicator plants inoculated.
[c]Graft material from branches with scorched leaves; bacteria observed in the leaves by electron microscopy.
[d]Graft material from branches without leaf symptoms that reacted positively for almond leaf scorch in acidified methyl alcohol; bacteria were not observed in the leaf tissue at the time of grafting but the leaf symptoms appeared and bacteria were observed in the leaves of these graft sources 8 months later.


A
B
0.2µm
C
0.2µm
D

RESULTS.—*Observation of the bacterium associated with infected almond trees.*—Electron microscopic examination of ultrathin mid-vein sections of leaves with ALS symptoms collected from naturally or experimentally infected trees consistently revealed the presence of rod-shaped bacterial cells within the lumina of xylem vessels (Fig. 1-D, E). The vessels with bacteria usually were in groups. Only 10-15% of vessels in the leaf midrib contained the bacterium. The number of bacterial cells in sections of invaded vessels varied from few to many densely packed in the lumina. The bacterium was not observed in any of the numerous leaf samples collected from healthy symptomless almond trees. Typically, the bacterial cells are elongated, with rounded ends. An occasional cell showed tapered ends, but only minor differences in morphology were observed (Fig. 1-E, 2-C, D). In cross section, the ALS bacterium was spherical or ovoid. Measurements of 542 cells revealed an average diameter of 0.4 μm and a maximum length of 1.9 μm.

In longitudinal sections, the outer cell wall of the bacterium appeared convoluted, strikingly rippled, multilayered, thick, and ridged (Fig. 1-E, 2-C, D). The contents of the cells, in general, were similar to those found in other bacteria. Cytoplasmic membrane, ribosomes, and osmophilic, granular nuclear regions with strands of DNA plus other undetermined electron-dense cytoplasmic organelles were common. General morphology of the ALS organism resembles that of the bacterium which causes Pierce's disease of grapevines (2, 4, 10), the bacterium found in the xylem of peach trees affected with phony peach disease (5, 12), and the bacterium associated with plums affected with plum scald disease (8).

Phase-contrast microscopy revealed rod-shaped bacteria in almond leaves from both naturally and experimentally infected almond trees. No bacteria were observed in the leaves of healthy almond trees. Cross sections of midveins of infected leaves revealed gum and aggregates of bacteria in the lumina of xylem vessels. The bacteria from the xylem vessels were readily released from infected leaf tissue into the mounting solution of 0.1 M KOH, often in great abundance. The bacteria were very similar in size and shape to those detected in the same leaves by electron microscopy; they also occurred only in the xylem. Immediately after release from the infected tissue into the KOH mounting solution, bacterial cells appeared often in short chains of two, and occasionally three cells. When subjected to Gram's staining method, the cells released from leaves with incipient ALS symptoms were gram-positive. We failed to obtain pure cultures of the bacterium by direct isolations from the mounting solution or by plating surface-sterilized infected leaf tissue on 523 and D2 (minus LiCl) agar media (7).

Although the bacteria were readily observed in leaf tissues of infected almond trees, they were not detected in the rootlets of the same trees by either electron or phase-contrast microscopy.

Histochemical diagnosis of almond leaf scorch.—The wood sections from branches infected with the ALS bacterium developed well-defined purplish-red or dark pink streaks in the woody cylinder within 10-30 minutes after the sections were submerged in the acidified methyl alcohol. The wood sections from uninfected branches or trees remained whitish and free of the colored streaks (Fig. 3-C). However, if the samples were allowed to dry, or the wood sections were kept too long in the acidified methyl alcohol, a uniform pink color developed throughout the woody cylinder of both infected and uninfected trees. Tests were positive in each of 148 samples from known infected almond trees and in none of 98 samples from trees known to be free of the ALS bacterium. The chemical test provided consistent confirmation of ALS with each positive diagnosis by electron microscopy and transmission tests.

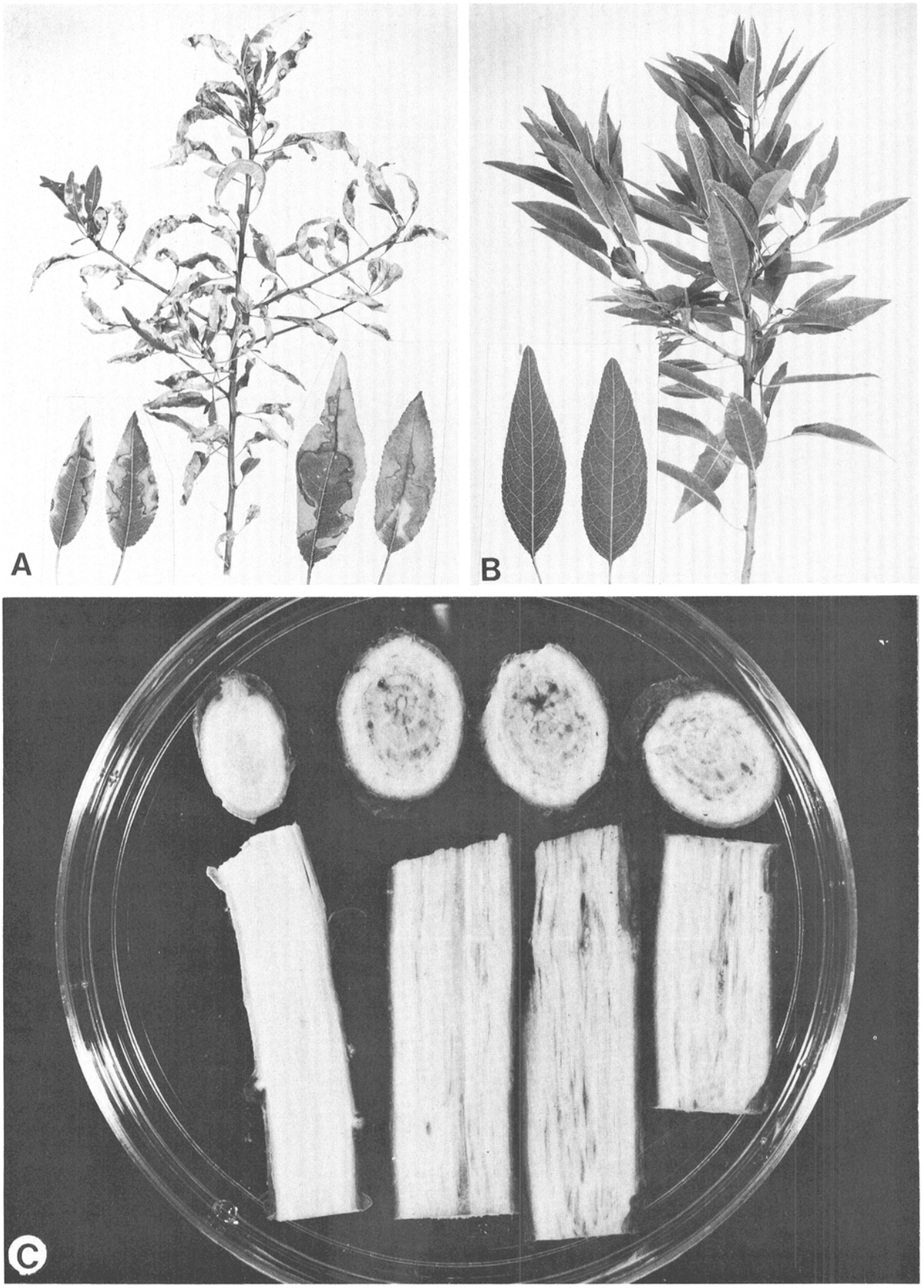
Transmission by leafhoppers.—Mission almond seedlings exposed to *Draeculacephala minerva*, which had fed for 3 days on leaves of naturally infected almond trees, developed leaf symptoms identical to those of the inoculum source trees (Fig. 2-A). Typical ALS symptoms were observed in all almond plants exposed to infective leafhoppers within 10 weeks in the greenhouse. Likewise, four cuttings of Carignane grapevine exposed to leafhoppers that had fed on the same almond inoculum source developed symptoms typical of Pierce's disease (13) within 8 weeks in the greenhouse (Fig. 2-B).

None of the four Lovell peach seedlings exposed to infective leafhoppers developed leaf scorch symptoms after 8 months of observation in the greenhouse. Almond, peach, and grapevine control plants exposed to leafhoppers that fed on leaves of healthy almond orchard trees remained symptomless for 8 months (Fig. 2-A, B).

Electron and phase-contrast microscopy revealed the presence of the bacteria in vessels of almond and grapevine indicator plants exposed to infective leafhoppers (Fig. 2-C, D). The bacterial cells in indicator plants were very similar to those observed in naturally infected almond trees (Fig. 1-E, 2-C, D). The bacterium was not observed in Lovell peach exposed to infective leafhoppers or in any of the almond or grapevine controls that were exposed to the noninfective leafhoppers.

The ALS bacterium was readily returned from insect-inoculated almond seedlings to healthy almond seedlings by bud-chip inoculation in the greenhouse. Five plants each of Long IXL almond, Lovell peach, and Nanking cherry (*Prunus tomentosa* Thunb.) were inoculated with bud chips from Mission almond seedlings that had been exposed to infective leafhoppers and had developed typical ALS symptoms. Each indicator plant received

Fig. 2-(A to D). **A)** Symptoms of leaf scorch disease on leaves of Mission almond seedlings (upper row) and symptomless leaves of control seedlings (lower row), after an inoculation with *Draeculacephala minerva* that had fed on leaves of naturally infected and healthy almond trees, respectively. **B)** Symptoms of Pierce's disease on leaves of Carignane grape (upper row) and leaf from control grape (lower row) exposed to the same vector as almonds in A. **C)** Electron micrograph of bacterial cells present in vessels of almond leaves shown in A (upper row). **D)** Bacterial cells in vessels of grape leaves shown in B (upper row); bacterial cells in C and D are similar.


A
B
C

four bud chips from the inoculum source. Long IXL almonds served as almond leaf scorch indicators, whereas Lovell peach and Nanking cherry were used as indicators for possible virus or viruses that might not be detected directly in the almond tissue by electron microscopy. The indicator almond plants developed symptoms typical of ALS 8 weeks after inoculation. The ALS bacterium was readily observed by electron microscopy in the leaves of all bud-chip-inoculated almond plants. None of the inoculated Lovell peach or Nanking cherry seedlings developed leaf scorch symptoms or any symptoms resembling those produced by any known viruses during the 8 months in the greenhouse. These results indicate that the bacterium alone can induce symptoms of ALS disease in almond plants.

Transmission by budding and grafting in nursery and lathhouse.—The ALS bacterium was transmitted with buds from naturally infected almonds with typical leaf symptoms to 10 of 20 inoculated almond plants. Likewise, 7 of 10 indicator plants became infected upon graft inoculation with stems from the same inoculum source (Table 1). However, buds from infected trees that showed no leaf symptoms failed to transmit the bacterium, even when the trees showed a typical positive reaction for ALS disease in acidified methyl alcohol tests when inoculum was collected. In contrast to bud inoculation, stem grafts from the same inoculum sources effectively transmitted the bacterium to healthy Long IXL almond trees (Table 1).

The first typical leaf symptoms in indicator plants, inoculated on 28 August 1973, with buds from naturally infected trees, were observed in mid-June 1974 in both field and lathhouse experiments (Fig. 3-A). The indicator plants which were graft-inoculated with stems in the lathhouse on 30 January 1974, showed the first leaf symptoms in mid-June, 1974. All inoculated plants that developed leaf symptoms in mid-June also showed a positive reaction for almond leaf scorch in acidified methyl alcohol (Fig. 3-C) approximately 6 weeks before the first leaf symptoms developed. None of the controls developed leaf symptoms of ALS (Fig. 3-B) or reacted positively for the disease in acidified methyl alcohol by the end of December 1974. Bacteria similar to those associated with naturally infected almond trees were readily observed by both electron and phase-contrast microscopy in all indicator plants with ALS symptoms.

DISCUSSION.—Our study indicates that ALS is a specific disease caused by a bacterium that is transmissible by budding, grafting, and leafhoppers. The constant presence of rod-shaped bacterial cells in the xylem vessels of only naturally and experimentally infected almond trees, plus the repeated failure to detect any other known pathogen in naturally or experimentally infected almond trees, strongly suggests a causal relationship between the bacterium and almond leaf scorch disease.

Morphological similarity between the bacterium associated with ALS-affected almond and the bacterium that causes Pierce's disease in grape (4, 10) suggests a possible relationship between these two organisms. Furthermore, the ALS bacterium was transmitted from almond to both almond and grape, resulting in ALS and Pierce's disease symptoms, respectively. In addition, almond seedlings exposed to leafhoppers that had fed on grapes experimentally infected with the Pierce's disease bacterium developed almond leaf scorch symptoms (1). Thus, ALS and Pierce's disease may be caused by the same, or very closely related strains of the same, bacterium.

The bacteria associated with the phony peach disease (5, 12) and plum leaf scald disease (8) appear to have some morphological similarity with the ALS organism. Hutchins et al. (6) reported graft transmission of the phony peach causal agent to almond, but they observed no leaf scorch symptoms. Likewise, Kitajima et al. (8) reported that almond *(Prunus amygdalus)* can be invaded by the plum leaf scald agent, but it remained symptomless. In our investigations, the ALS bacterium was readily detected in the leaves of naturally and experimentally infected almond trees, but we have never observed the organism in the peach or almond roots of the same trees. In contrast, the phony peach organism is found readily in the roots but with difficulty in the leaves of peach trees (5, 12). A relationship between phony peach and ALS is not well established, but these diseases have a common vector and react identically to the phony peach chemical test (6).

Pierce's disease of grape (2, 4, 10, 13), phony peach (5, 6, 12), plum leaf scald (8), and ALS diseases have several characteristics in common; however, the exact relationship between the causal agents of these diseases remains to be experimentally determined.

Wilting did not precede the onset of leaf scorching symptoms upon inoculation of almond and grape with the ALS bacterium, and no wilting was noted even in severely diseased trees. Usually less than 15% of xylem vessels contained bacterial cells in trees showing severe leaf scorching symptoms. Furthermore, in experimentally infected plants, chlorosis followed by marginal scorching of leaves frequently preceded the detection of bacteria in the scorched leaves by several weeks. Our observations suggest that the leaf scorching symptoms were more likely induced by both occlusion of vessels and a toxin or toxins produced by the bacterium rather than by a simple occlusion only of vessels with bacterial masses or tyloses.

The almond leaf scorch disease may destroy the productivity of an orchard tree within 3-5 years. It is widely distributed in California, although the incidence of infected trees in surveyed commercial almond orchards is generally low, except for two districts (14). This disease has devastated several commercial orchards in Los

Fig. 3-(A to C). **A)** Terminal shoot and leaves of 2-year-old Long IXL almond tree showing leaf scorch symptoms after an inoculation with buds from a naturally infected almond tree. **B)** Terminal shoot and leaves of control plant that received buds from healthy tree. **C)** Transverse and longitudinal sections of wood from branches of healthy and infected almond trees 20 minutes after immersion in acidified methyl alcohol; from left to right: healthy, naturally infected, experimentally infected with infective leafhoppers and experimentally bud inoculated; dark streaks and spots are in the woody tissue from trees infected with the almond leaf scorch bacterium.

Angeles County during the last 10 years. ALS has been observed in 10 different almond cultivars that collectively represent 86% of the total almond acreage in California. In addition, our results suggest that the causal agent of ALS may be effectively disseminated by leafhoppers as well as plant propagation. Therefore, ALS has attributes of a serious disease with the potential to limit the almond industry in certain almond-producing regions.

The ALS bacterium can be transmitted from diseased to healthy almond trees by budding and grafting. Therefore, control measures should include careful selection of propagating material to avoid affected trees. One or more insect vectors probably spread the ALS agent within orchards. Research is needed to determine the vectors of the causal agent in the orchards and on the efficacy of vector control on spread of the disease. Research on control of almond leaf scorch disease by chemotherapy is in progress.

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