ANNUAL PROGRESS REPORT
ALMOND LEAF SCORCH DISEASE
PROJECT NO. 78-R4

PERSONNEL:
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1. OBJECTIVES:

To determine the host range of the causal bacterium; to determine how the disease spreads in commercial orchards; to develop effective, economic control measures for the disease.

2. INTERPRETIVE SUMMARY:

Almond leaf scorch (ALS) disease is caused by a bacterium transmitted by certain sucking insects that feed predominantly in the water-conducting (xylem) tissues of plants. The same bacterium may infect many species of plants and causes severe disease in European bunch grape varieties and alfalfa. The effect of ALS on almond nut yields has not been determined, but severely infected trees have extremely low yields of mature fruit.

The causal bacterium appears to spread slowly in almond trees from the point of initial infection -- moving only a few feet per year at most. Pruning out incipient infections in smaller branches by removing branches with ALS foliar symptoms at a point about three feet below the lowest symptomatic leaves seems to permanently eliminate recent infections. Once the ALS bacterium has invaded larger scaffold branches or trunk tissues, however, eradicating the disease from an infected tree by pruning is not possible. Injections of bacteriacides such as tetracyclines into trees with ALS has been demonstrated to be therapeutic. Symptoms of the disease are usually greatly reduced or eliminated by annual treatments, but these treatments so far have not completely eliminated the causal organism and must be repeated annually to be effective. During the past year three new bacteriacides were tested with promising results. This control method is not registered for use in almonds at present.

All important commercial almond cultivars are susceptible to ALS, but cultivars appear to vary widely in relative susceptibility and tolerance to ALS, as measured by the speed of movement of symptoms and the degree and severity of ALS symptoms. A test of varietal resistance to ALS was begun in 1976 with the establishment of a test planting of 16 commercially important almond varieties at U.C. Davis. In addition other Prunus species such as peach, apricot, and plum were established. Half of the trees of each variety have been inoculated with stem and chip grafts from ALS-affected trees and rated yearly for their response. Many of the

inoculations made in 1977 were unsuccessful and most trees in this trial were reinoculated in 1978. Future observations in this experiment should yield useful information on differences in varietal susceptibility to ALS.

The isolation on artificial media of the ALS bacterium from trees with ALS demonstrated a high correspondence between successful isolations and disease symptoms. Isolation thus can serve as a reliable means to confirm ALS diagnosis, which may be valuable in confirming reports of ALS from areas of the state where the disease is not present. The causal bacterium appears to be previously undescribed bacterium, as yet unnamed, that does not belong to any established grouping (genera) of plant pathogenic bacteria.

Repeated mappings of ALS in designated orchards in Contra Costa County have been made each year since 1974. While this data has not been thoroughly analyzed, the disease does seem to have spread each year in this district. Contra Costa County has experienced severe effects from the extensive spread of ALS, unlike the remainder of the state, in which the appearance of ALS has been sporadic and rare. Especially puzzlying is the apparent complete absence of ALS from the extensive almond acreages in the counties of Madera, Fresno, Tulare, King, and most of Kern. The pattern of disease spread is consistent with the hypothesis that an insect vector is responsible for all field spread of the disease. However, the observed pattern (or patterns) of disease spread is not consistent with the hypothesis that ALS is spread by any insect species known to be important in infecting grapevines or alfalfa with the same bacterium that causes ALS. Surveys and insect trap collections in orchards with actively spreading ALS have not yet implicated a suspect insect species. Which insects are important in the natural spread of ALS is still unknown.

In March and April of 1978 almond trees in an established planting at Kearney Horticultural Field Station and the Westside Field Station were inoculated with bacteria isolated from diseased grapevines from northern Tulare County. Inoculations of bacteria were made with a hypodermic needle and with leafhoppers first fed upon diseased grape. A high percentage of mechanically inoculated branches developed ALS symptoms by late summer. Insect-inoculated branches developed only mild and very localized ALS symptoms - usually on only a few leaves. A greater percentage of insect-inoculated branches yielded bacteria upon isolation from inoculated leaves, showing that the causal bacteria were present but not producing symptoms. These results indicate that natural infections develop more slowly under field conditions than in previous greenhouse experiments. Continued observation of these inoculation sites should provide information on survival of the pathogen through the winter dormant period.

3. EXPERIMENT PROCEDURE

Tolerance of almond varieties and spread of ALS in artificially infected trees.

Ten trees each of 16 principal varieties of almond and one variety of each plum, peach, cherry, and apricot planted in the fall of 1975 were

graft inoculated with ALS causal agent in the field experimental plot. In January 1976 one of three or four scaffold branches of each tree received the stem graft that contained ALS bacterium and showed typical symptoms of the disease in the fall of 1975. The rate of spread from ALS-infected grafts into inoculated scaffold branch, the subsequent rate of spread throughout inoculated trees, and the severity of ALS damage in different almond cultivars and Prunus spp. is being monitored. Ten uninoculated trees of each almond variety and Prunus spp. that are interplanted with the inoculated trees are used to study rate of natural spread from diseased to healthy trees.

The rate of spread of ALS from the single point of inoculation in artificially inoculated susceptible cultivars has been studied. Twenty, two-year-old Long IXL trees were planted in a single row of which six trees were artificially inoculated and showed symptoms in one terminal branch in 1974. The rate of spread and effect of ALS on growth and dieback of terminals in ALS-affected trees were observed.

Mapping of natural spread.

During August and September, designated portions of five orchards were mapped for ALS. Each quadrant of every tree was rated for ALS symptoms on a severity scale of 0 (no symptoms) to 4 (most severe).

Pruning for disease removal.

In 1977, trees in mapped orchards with varying ALS severity ratings in individual branches were selected for trials of the effectiveness in pruning out symptomatic branches in preventing spread throughout the tree. Branches were pruned about three feet below the lowest visible leaf symptoms and tagged. Unpruned diseased branches were tagged as controls. The trees were monitored in 1977 and 1978 during August and September for ALS symptoms.

Chemotherapy

During the dormant season, beginning in December, almond trees rated for ALS that fall were treated with injections of the tetracycline Terramycin® and three additional experimental bacteriacides: TC 2216, KT bacteriacide, and CA-AFN. Injections were made with one to two liters of aqueous solutions of each material under 150 psi pressure into holes drilled into the main trunk or major scaffold branches. Disease ratings were the sum of each severity rating (0 to 4) of the four quadrants of each tree.

Vector surveys.

Rectangular (12 x 14 in, 6 in deep) plastic water pans were placed in three Contra Costa orchards that had been periodically mapped for ALS. A total of nine yellow and six green traps were set out April 13, 1978

and checked weekly or biweekly until July 6, 1978. Each pan was filled with about 4 in. of water with several drops of detergent added as a wetting agent. Traps were attached to upper tree branches at heights of seven to twelve feet above ground. Three of the yellow traps were located on shed roofs in or near orchards. Twelve 5 x 10 inch yellow sticky board traps were also placed in the same orchards. Leafhoppers and spittlebugs were collected from the traps and preserved in alcohol for identification.

On three occasions, sweep net collections of insects in marsh vegetation bordering both sides of the San Joaquin estuary from Antioch to Oakley were made and all leafhoppers and spittlebugs preserved for later identification in the laboratory.

Inoculation of almond with bacterial suspensions and leafhoppers

An experiment was designed to test whether or not central San Joaquin Valley isolates of the causal bacterium from grapevines with Pierce's disease could produce ALS under field conditions in the central San Joaquin Valley. Five trees in an established almond variety block at the University of California Kearney Horticultural Field Station were selected for an inoculation trial using bacteria isolated from grapevines with Pierce's disease from Tulare County. Six mature NePlus trees were similarly selected in an established variety trial at the Westside Field Station. In late March and again in mid-June, half of the trees at each site were inoculated mechanically with hypodermic needle injections of bacterial suspensions into green twig tips and with green sharpshooters (Draeculacephala minerva) previously fed on grapevines with Pierce's disease. Heavy bacterial suspensions were prepared by washing agar media with phosphate buffer. Three bacterial isolates were used in March and two of the same isolates in June. Four to six sites on each tree were used for each isolate and for leafhopper inoculation. Both small plastic and mesh clip cages and organdy sleeve cages held the leafhoppers onto the leaves of terminal growth. Each inoculation site was tagged and periodically monitored for ALS symptoms. In June, July, and September isolations on artificial media were attempted from leaves from inoculation sites.

Isolation technique: efficiency in diagnosis

The efficiency of isolating the causal bacterium on JD-3 agar medium was tested in 1977 and 1978 with leaves from trees with ALS symptoms in two Contra Costa orchards. One orchard plot consisted of approximately 50 year old Long IXL almonds with several of the Drake and Mission variety. The second orchard had approximately 20 year old Jordonola and Mission trees with less severe symptoms than in the first orchard. Surface sterilized leaf petioles were squeezed to express sap onto plates of agar media (JD-3). The plates were incubated for six days, sometimes longer, and any resulting colonies of ALS bacterium confirmed by serological agglutination tests. The success of isolating the ALS bacterium was compared to field diagnosis based on symptoms.

Serological tests to identify the causal bacterium

Since serological techniques are sensitive, usually highly specific, and rapid methods to positively identify bacteria, a number of established serological techniques were tested using the ALS bacterium. Antisera were prepared to several geographical isolates of bacteria isolated from grapevines with Pierce's disease, from alfalfa with alfalfa dwarf disease, and from almond with ALS. The antisera were produced in rabbits using various preparations of cultured bacterial cells grown in quantity in liquid media and previously tested for pathogenicity to grape or almond. The following serological techniques were adapted and tested for use with the ALS bacterium:

1. agglutination - precipitation
2. fluorescent antibody
3. gel double-diffusion
4. immunoelectrophoresis

Antibiotic sensitivity

Antibiotic sensitivity test discs impregnated with known concentrations of various antibiotics were placed on agar media previously inoculated with bacteria. The resulting zones of inhibition of bacterial growth after two weeks were related to relative sensitivity to each antibiotic.

Toxin production

The possibility that a translocatable toxin produced by the ALS bacterium could be responsible for some of the symptoms of ALS was evaluated. Fractions from column chromatography of aqueous extracts of cultures of the ALS bacterium were assayed for toxicity to several plant species. Detached leaves or stems of almond, grape, and other plant species were placed with petioles immersed in tubes of these extracts. Extracts of uninoculated media served as controls.

4. RESULTS:

Tolerance of almond cultivars and spread of ALS in artificially inoculated trees.

Twenty months after inoculation 98 of 160 trees of 16 almond cultivars and 21 of 40 trees of Prunus spp. had growing inoculum scions from ALS-infected trees. However, only 15% of the growing inoculum scions showed ALS symptoms ten months after inoculation. This suggested that ALS bacterium may not survive well in majority of almond terminal shoots smaller than 3/8 inch in diameter during the dormant period of almond trees. The symptomless inoculum scions were inoculated in March 1978 with bud chips from ALS-infected trees growing in a greenhouse. In October 1978 an additional 46% of the reinoculated almond trees showed ALS. By October 1978 ALS spread from the inoculum into the inoculated

scaffold branch of Thomson, Peerless, Carmel, Drake, Davey and Milo cultivars. No spread of ALS from the inoculum into the scaffolds of Price, Harvey, Mission, Ruby, Long IXL, Merced, Neplus, Fritz, Carrion and Nonpareil cultivars have been observed as yet. However, it is premature to draw any conclusion from these data on the relative resistance of the almond cultivars to ALS. We expect to obtain more definite data on the relative resistance of almond cultivars to ALS within three years.

Spread of ALS in Long IXL from the single point of inoculation ranged from 16.3 to 11.2 feet during the four-year period of observation. During this period five-year-old Long IXL trees (five to seven scaffold/tree) were completely invaded with ALS. However, only branches which showed ALS symptoms every year since 1974 developed severe dieback of terminals in 1978. The trunk circumference 6 inches above the union of infected trees ranged from 16.5 - 10.5 inches (avg. 14.5 inches) as compared to 21.0 - 13.5 inches (avg. 17.4 inches) for the noninfected trees. There was no natural spread from the infected to adjacent healthy trees in this experiment indicating that natural spread of ALS in commercial orchards is dependent on the presence of efficient vectors.

Mapping of natural spread of ALS

Maps of the five orchards surveyed have been completed,but the tabulations of disease ratings and comparisons with previous years are not completed.

Pruning for disease removal

A very low percentage of branches with disease ratings of 1 (mild) to 3 (severe) that were pruned out showed ALS symptoms in 1978. Symptoms on unpruned control branches were in general more severe.

Chemotherapy

The results of tree injections are summarized in the following table:

		Average rating	
Material	No. of trees	1977	1978
Terramycin (10 grams)	22	4.6	5.5
Terramycin (15 grams)	13	7.6	7.9
TC 2216	10	9.5	9.0
KT bacteriacide	23	11.2	9.9
CA - AFN	14	9.5	6.2
Check (untreated)	5	10.8	14.6

Previous tests have demonstrated the effectiveness of Terramycin, thus new experimental materials can be compared to results with Terramycin. The three new materials tested this year should be evaluated at different dosages following this year's trials.

Vector Surveys

Both water pan traps and sticky traps captured leafhopper species that are not suspected as vectors of ALS bacterium because of their feeding behavior or negative results from previous tests of their transmission ability. The predominant leafhopper species collected were species common to the weed cover or adjacent crops (e.g. grape leafhopper). A single known leafhopper vector species, Draeculacephala minerva and a single spittlebug species were collected during the survey. Other species collected are listed in the following table.

	Number caught	
Leafhopper	Water pan	Sticky trap
Amblysellus grex	36	107
Empoasca species	9	144
Dikraneura species	4	9
Balclutha species	0	1
Erythroneura eleagantula	3	184
Colladonus montanus	2	11
Euscelidius variegatus	71	60
Known vectors		
Draeculacephala minerva	0	1
Cercopid (spittlebug)	1	0
Unknown	3	5

Euscelidius variegatus occurred in above normal levels compared to previous years and to other almond areas where surveys have been completed in the past.

Sweep net surveys of marsh vegetation in Contra Costa County totally failed to collect any xylem-feeding leafhoppers or spittlebugs.

Inoculation of almond with bacterial suspensions and leafhoppers

The results of leafhopper and mechanical (needle) inoculation attempts were assayed by recording visual symptoms of ALS and isolating from leaf petioles from the infection sites (terminal leaves). These results are given in Table A.

Table A. Inoculation of almond by insect transmission and syringe injection with the PD-ALS bacterium at the Westside and Kearney Field Stations. Trees were inoculated on March 28 and June 16, 1978, and diagnosis of symptoms and reisolation of the pathogen were conducted in October, 1978.

Inoculation technique	% Inoculated trees with symptoms	% Inoculated branches with symptoms	% Reisolation from inoculated branches	% Reisolation from inoculated branches with symptoms
Syringe Injection	90.9 (10/11)	31 (29/94)	44 (41/94)	83 (24/29)
Insect Transmission	18.2 (2/11)	5 (2/37)	22 (8/37)	50 (1/2)

The trees were inoculated in March and June of 1978. By October, 1978, ALS symptoms were apparent in 10 of 11 trees injected with the pathogen and 2 of 11 trees inoculated by insect transmission. All of the trees (6/6) inoculated by injection in March showed symptoms and four of five trees injected in June showed symptoms. One tree each of the March and June insect transmissions showed symptoms. Regardless of symptoms, the pathogen was reisolated in October from 44% of the branches inoculated by injection and 22% of the branches inoculated by insect transmission. A much higher percentage of reisolation was obtained from inoculated branches with symptoms (Table A).

These results suggest that if the pathogen survives the winter in the inoculated branches that a much higher percentage of trees will have symptoms by the end of next summer. The insect transmissions did not appear to be as efficient as the syringe inoculations; however, they may take longer to progress due to lower initial amounts of the pathogen introduced into the trees. The data does prove that trees growing in Fresno and Tulare counties are susceptible to isolates of ALS from that area. Whether the failure of the disease to become established in these counties is due to the absence of a suitable insect vector, the failure of the pathogen to survive the winter in infected trees, or some other factor may be more evident next year.

Isolation techniques: efficiency in diagnosis

Positive bacterial isolations were obtained from 80% (12/15) of the diseased trees sampled in one orchard and 100% (5/5) from the second orchard. Isolations were attempted on six different dates (Table B) from 10 diseased trees in Orchard no. 1, and on four different dates from five diseased trees in orchard no. 2 resulting in 55% (33/60) and 95.7% (22/23) successful isolations, respectively. Monthly isolations from June through October did not result in significantly different numbers of successes.

Table B. Summary of isolations for 1977 and 1978 from orchards no. 1 and no. 2.

	Orchard no.	% Trees with positive isolations	Positive isolations Attempted isolations
Diseased	1	80 (12/15)	33/60 (55%)
Healthy	1	0 (0/11)	0/58
Diseased	2	100 (5/5)	22/23 (95.7%)
Healthy	2	0 (0/6)	0/22

Eighty-one percent (22/27) of the unsuccessful isolation attempts in orchard no. 1 were from four of the 10 trees sampled. Isolations were obtained from two of these four trees, but only once in six attempts each. No differences in symptoms or general appearance were observed when these trees were compared with other trees which readily produced bacterial isolates. Possibly the distribution of bacteria in these trees is different, and the symptoms seen in the branches sampled were caused by a translocatable toxin. Healthy trees were also sampled in both orchards. No positive isolations were obtained from healthy trees.

Table C. Isolations from Long IXL almonds in orchard no. 1 with almond leaf scorch.

Tree no.	Date of Isolation 6/23/77	8/31/77	6/30/78	7/26/78	9/26/78	10/24/78	Total/tree
1	0	+	+	0	+	+	4
2	+	0	0	0	0	0	1
3	+	+	+	+	0	+	5
4	0	+	+	+	+	+	5
5	+	+	+	+	+	+	6
6	0	0	0	0	0	0	0
7	+	+	+	+	+	+	6
8	+	0	+	+	+	+	5
9	+	0	0	0	0	0	1
10	0	0	0	0	0	0	0
Total/ Orchard	6	5	6	5	5	6	33

+ = positive isolation

0 = negative isolation

Serological tests

All isolates of the causal bacterium from infected grape, almond, or alfalfa gave the same positive reaction in agglutination tests. Fluorescent antibody staining was also positive in tests with cultured bacteria but was not attempted with infected plant tissues alone. Gel double-diffusion and immunoelectrophoresis reactions of all isolates also reacted identitically. We have concluded that serology is a useful aid in more positively identifying cultured bacteria as the causal agent of ALS. Serology also supports the conclusion that the same bacterium is responsible for causing almond leaf scorch, Pierce's disease of grapevines, and alfalfa dwarf disease.

Antibiotic sensitivity

Additional antibiotics not previously evaluated are listed below on the basis of the growth inhibition on cultures of ALS bacterium. Revisions of evaluations previously reported as "variable" are marked with an asterisk.

Sensitivity to Antibiotics (impregnated disks)

Sensitive	Resistant
* Bacitracin (10 units)	* Ampicillan (2, 10 mg)
* Cosycycline (5 mg)	* Erythromycin (2 mg)
* Kanamycin (5 mg)	Cephaloglycin (30 mg)
Chlorampenical (30 mg)	Colistin (2 mg)
Methacycline (5 mg)	Nalidixtic acid (5 mg)
Nitrofurantoin (100 mg)	
Rifampin (5 mg)	

Toxin Production

Certain chromatographic fractions of aqueous extracts of ALS bacterium cultures did produce marginal drying and "scorching" of excised leaves of almond and grape. This reaction was noticeable within hours in some tests and overnight in others.

The toxin seems to be very stable, since dormant cuttings placed in these extracts would develop reactions typical of those induced by toxins even after several days. Brief boiling does inactivate the toxin. Toxin reaction of different almond cultivars could be tested as an assay for a component of varietal resistance to ALS.

V. DISCUSSION

Summary of major conclusions and accomplishments

1. Almond leaf scorch is caused by the same bacterium that causes Pierce's disease. Isolation of this bacterium can be confirmed with serological tests. The identity of this bacterium remains unresolved. It is likely to belong to a new grouping of plant pathogenic bacteria.

2. Isolation of the causal bacterium from trees with ALS symptoms is useful to confirm diagnosis of ALS. This procedure is not practical for routine rogueing of the disease by growers, however.

3. Three newly tested antibiotics show promise as chemotherapeutic materials for injection, but optimum dosages and residue levels are not known.

4. Chemotherapy is effective in rejuvenating older trees with severe ALS symptoms to the point of increasing nut production substantially, but this method requires registration before it can be recommended.

5. Pruning out recent infections continues to hold up as a practical control measure.

6. Vector surveys did not provide any substantial clues as to the identity of the insect responsible for field spread of ALS.

7. Experimental inoculations prove that ALS can develop in areas of California (e.g. Fresno County) where the disease has not yet been recorded, despite the frequent occurrence of Pierce's disease.

8. The leaf "scorching" symptom of ALS seems to be due to a translocatable toxin produced by the causal bacterium.

Plans for future research

The long term aspects of this study with respect to evaluation of varietal resistance and rates and patterns of severe disease spread will be continued. If climatic effects have a strong influence on the rate of spread of ALS, this basic information is essential.

New antibiotics which were initially tested this year should be evaluated at higher dosages and by using foliar applications.

Observations of ALS symptoms and the presence of the ALS bacterium should continue in Central Valley inoculation plots to assess the overwinter survival of the bacterium and the rate of symptom development of leafhopper infected branches.

Tests of the leafhopper Euscelidius variegatus as a potential vector of ALS bacterium to almond should be made.

PUBLICATIONS

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