Project Number 77-R3
Project Researcher: Purcell

Almond Leaf Scorch Disease (ALS)

What is it?

A disease caused by bacteria which multiply inside the water-conducting tissues (xylem) of the plant. The disease spreads throughout infected trees slowly over several years leading to a gradual decline in productivity and eventual death.

Symptoms

Beginning in late summer, the leaves of infected trees begin to take on a "scorched" or burned appearance. The margins of the leaves turn brown with a narrow zone of chlorotic (yellow) discoloration inside the dried-out margin. The dead margin of the leaf gradually enlarges until leaf fall. Leaves usually remain attached longer than in healthy trees. Symptoms usually appear in from one to several branches and spread slowly as much as a few feet per year from the original point. Almond varieties differ in symptoms and the speed with which symptoms spread within the tree. Certain varieties such as Long IXL are very susceptible, but all varieties tested so far can be infected.

The same bacteria that cause ALS seem to cause a disease of grapevines called Pierce's disease and a disease of alfalfa known as alfalfa dwarf. The bacteria can exist and may multiply in the xylem cells of many kinds of plants without producing disease symptoms.

How does Almond leaf scorch spread?

Certain leafhoppers called sharpshooters and other sucking insects known as spittlebugs can transmit the bacteria that cause ALS. Experimentally, the disease can be transmitted by grafting if enough xylem is included in the scion. There is no evidence that pruning tools or machinery can spread the disease.

Exactly which species of insect is important in spreading ALS is not known. In Fresno, Tulare, and Kern counties almonds planted next to alfalfa fields with alfalfa dwarf and next to vineyards with a high incidence of Pierce's disease have not yet shown any sign of ALS. The reason for this is not known.

How important is the disease?

Almond leaf scorch has been very important in two almond-growing areas: Contra Costa County from Antioch to Brentwood and in the Antelope Valley in Los Angeles County. It is only occasionally found in the Central Valley as far south as Merced. The majority of California's almond plants have thus far not suffered from this disease. If the disease were to become as common in the Central Valley as in the two parts of the state in which it has been serious, almond leaf scorch could be a serious threat. Epidemics of Pierce's disease of grapes, which is caused by the same bacterium, occur about every 10 to 20 years.

What can be done to control ALS?

If infected branches are cut off 2 to 3 feet below the point where leaf symptoms appear, the disease can usually be eliminated from the tree.

A method to keep severely affected older trees in production may be available soon. This approach uses pressure injections during autumn of an antibiotic solution to reduce the effects of the disease. It is not yet known whether antibiotic treatments can completely eliminate the disease. Research so far suggests that such treatment usually should be repeated every year.

ANNUAL PROGRESS REPORT
ALMOND LEAF SCORCH DISEASE
PROJECT NO. 77-R3

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1. OBJECTIVES:

To determine the cause and host range of the disease; to determine means of spread of the causal agent in commercial orchards; to develop an efficient and rapid diagnostic method for the disease; and to develop control measures that will be compatible with general practices in commercial orchards.

2. INTERPERATIVE SUMMARY:

Almond leaf scorch (ALS) is a disease caused by a bacterium transmitted by certain insects. The bacteria invade the water-conducting tissues (xylem) of infected plants, and gradually spread throughout the tree over a period of several years, depending on variety. Almond cultivars show a wide range of susceptibility to ALS, although all cultivars tested so far are susceptible to some degree. Trees inoculated with bud-chips from diseased almond grafted at the terminal shoots of young (2 yr. old) almond trees developed symptoms of ALS in all major scaffold branches within 4 years. The average rate of spread of symptoms was 4.2 feet per year from the point of graft-inoculation. Healthy check trees interplanted in varietal susceptibility plots have never developed ALS, which confirms previous conclusions that insect carriers (vectors) are responsible for natural spread.

The relatively slow spread of ALS within a tree from the point of infection suggests that pruning off branches with ALS symptoms should eliminate these infections. Results over the past two years show that so far cutting off branches two or three feet below the lowest point of ALS leaf symptoms is a very effective means of eliminating new infections. Once the causal bacterium reaches the main trunk of a tree, however, pruning is no longer effective. Injections of 7 to 10 grams of Terramycin antibiotic in a solution under pressure into diseased trees gave partial to complete recovery from ALS symptoms depending on how well the injected chemical was distributed into the tree. No residues were recovered from nut meats from trees treated with 10 grams of Terramycin the previous year. The long term effectiveness and economic feasibility of chemical treatments has not yet been established.

The bacterium that produces ALS also causes diseases in other plants such as grapes and alfalfa. Recently this bacterium was grown on artificial media for the first time. The bacterium was isolated consistently from diseased plants. When pure cultures of this bacterium were inoculated into

healthy grape and almond test plants, distinctive ALS symptoms resulted in the almond and Pierce's disease (PD) in the grape test plants. The bacterium could be reisolated from the inoculated plants. In addition to providing conclusive proof that ALS is a bacterial disease, culturing the bacteria on artificial media will allow further studies of the causal bacterium's susceptibility or resistance to antibiotics, and its nutritional and environmental requirements.

A major unanswered question is why isn't ALS found in the lower Central Valley in Fresno, Tulare, and most of Kern County? Pierce's disease of grapes and alfalfa dwarf disease are often severe in some portions of Fresno and Tulare Counties but adjacent almond orchards under observation for the past few years have so far shown no sign of ALS. The possibility that the strain of the bacterium found in these counties caused diseases in grape and alfalfa but not in almond was discounted after almond test plants inoculated with cultures or with leafhopper vectors from Fresno and Tulare counties developed ALS symptoms in greenhouse tests. A tentative conclusion is that the insects known to be important vectors of PD and alfalfa dwarf in the Central Valley do not spread ALS. Experiments have demonstrated that these insects are capable of transmitting ALS under greenhouse conditions. We do not know what insects are responsible for spreading ALS in those areas that have a high incidence of the disease.

3. EXPERIMENTAL PROCEDURE:

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

Ten trees each of 16 principal varieties of almond, plum, peach, cherry and apricot were graft inoculated with ALS causal agent. One of our scaffold branches of each tree received 3 scions from ALS-infected Long IXL trees. The spread of ALS from Long IXL grafts into inoculated trees and subsequent rate of spread through inoculated trees and severity of damage induced by ALS in different Prunus spp. and almond varieties will be monitored over a period of several years. Ten uninoculated trees of each Prunus spp. and almond cultivars are interplanted with the inoculated, and rate of natural spread from diseased into healthy trees will also be studied.

The rate of spread of ALS from the single point of inoculation in artificially inoculated, susceptible Long IXL cultivar also has been studied. Twenty Long IXL trees of which six were artificially inoculated were planted 6 feet apart in a single row at Davis in 1974 and have been observed for natural spread of ALS from infected to healthy trees.

Chemotherapy of the disease

Pressure injections of Terramycin solution at 3, 7, and 10 grams per tree were repeated in diseased trees treated the previous year and in additional diseased trees. Untreated diseased trees in the same orchards served as controls. In December, 1976 trees without ALS symptoms were treated with a 3 gram dose to see if this would affect natural spread.

Tree surgery

Trees with incipient ALS symptoms in 1975 were pruned below the lowest wood symptoms as revealed by the acid-alcohol test previously used for diagnosis. Incipient infections in additional trees were removed by pruning in 1976 to develop more data.

Culture and pathogenicity of causal bacterium

Bacteria were isolated from diseased almond trees in Contra Costa county and from grapes with Pierce's Disease in Napa and Tulare counties using media developed previously. Tops of almond seedlings were excised and a hose fitted over the cut end. A second cut was made in the stem several inches below the cut top exposing a piece of tissue. This piece of tissue was placed in a suspension of bacteria and the bacteria were sucked into the stem by vacuum. The tissue was then taped back in place using grafting tape. Test plants were also inoculated by injecting with a syringe, by dipping exposed roots, or by spraying leaves with a suspension of bacteria. We also used several geographic isolates of the ALS bacterium and tested a wide range of concentrations of bacterial suspensions in inoculation trials. Antisera for several isolates of ALS and PD bacterium were produced in rabbits and a standard immunofluorescent assay developed.

Scanning and transmission electron microscopy were used to compare the morphology of cultured bacteria with those in the tissues of diseased grape and almond.

Antibiotic sensitivity

The sensitivity of the cultured bacterium to an assortment of antibiotics was evaluated. Disks (6mm diam.) impregnated with the antibiotic were placed on the agar surface and zones of inhibition were measured after 3 weeks growth.

Leafhopper transmission

Isolates of PD from Fresno and Tulare County vineyards were used as source plants for the leafhoppers Draeculacephala minerva that were either originally colonized from Fresno County or collected in Fresno or Tulare counties. The insects were exposed to IXL seedlings for varying periods to see if PD isolates from these parts of the state could cause ALS. The relative transmission efficiency of D. minerva and Graphocephala atropuncta (= Hordnia circellata) to almond and grape seedlings was also evaluated.

Mapping of natural spread of ALS in Contra Costa orchards.

During September, plots in five orchards were mapped for ALS. The severity of symptoms were rated on a scale of 0 to 4 for each quadrant of each tree in each orchard. Note was also taken of salt burn symptoms.

4. RESULTS:

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

Ten months after inoculation of Prunus sp. and 16 almond cultivars 127 of 210 trees had growing inoculum-scions from ALS-infected trees. However, only 32 percent of inoculated trees developed ALS symptoms in the inoculum and no symptoms of ALS were observed outside the inoculum in any of the inoculated trees. We expect to obtain some indication on the relative resistance and severity of ALS in inoculated almond cultivar within 3 years.

Spread of ALS from the point of inoculation averaged 1.27 meters per year during a 3-year period. Two-year-old almond trees inoculated at terminal shoots of one scaffold branch with bud-chips containing ALS bacterium showed ALS symptoms in all scaffold branches (5-7 scaffold branches/tree) within 4 years. The maximum spread of disease from the point of inoculation was 4.0 meters from the point of inoculation. There was no natural spread of disease from infected to healthy trees in this experiment.

Chemotherapy

In general, the results were encouraging in that all treatments reduced the severity of the disease. In trees treated two consecutive years the results were not as spectacular the second as they were the first year. Whether this was due to the bacteria developing resistance to the chemical or to some other factor or factors is not known at present. Trees with light or moderate cases of disease accept the injection solution faster than heavily infected trees. However, the heavily infected trees that take the total dosage respond very well to the treatment. The problem is to get the solution into such trees. Trees that are only lightly or moderately diseased are easier to maintain in recovery condition by a second annual treatment than are trees originally severely diseased. The larger dose of chemical required to induce temporary recovery makes us wonder if the Terramycin treatment is economically feasible.

Among trees treated in 1976 were some old very large Nonpareil trees partially to completely infected. Most of these trees responded very well to treatments resulting in remission of symptoms except in certain branches which the chemical obviously did not reach.

One of the first effects of almond leaf scorch in a tree is marked reduction in crop. Treatments with Terramycin that resulted in symptom remission the season following treatment increased crop production the second year following treatment.

Prophylactic 3 gram doses in healthy trees failed to reduce natural spread, but additional data is needed to properly evalute the effectiveness of preventive treatment.

Tree surgery

Trees from which incipient infections had been removed in 1975 by making cuts below any evidence of wood symptoms remained free from disease in 1977 as they did in 1976 except for the single branch failure reported in 1976. Additional trees with incipient infections pruned last year also remained free of ALS symptoms.

Culture and pathogenicity of causal bacterium

Approximately 6-8 weeks after inoculation by suction with bacterial isolates from diseased almond and grape, symptoms typical of almond leaf scorch (ALS) were apparent. Results are noted in Table 1. ALS symptoms were also induced in almond seedlings when plants were inoculated by injecting the bacteria in the stem with a syringe. Dipping cut roots into a bacterial suspension and spraying the leaves of almond trees with bacteria did not cause disease.

Grape plants were also inoculated with the ALS isolates at different concentrations. The plants inoculated with high numbers of bacteria expressed symptoms sooner and a greater percent became infected than those plants inoculated at lower concentrations (Table 2). These studies indicate that the grape isolates and almond isolates are very similar in their ability to cause disease. Indeed the Tulare County grape isolate cannot be readily distinguished from the Contra Costa almond isolate and as noted in Table 1 is highly virulent on almonds. Additional studies involving a critical comparison of serological, physiological and biochemical characteristics will be necessary to definitively establish their true relationships. We cannot yet explain why almonds in Tulare county (and other almond growing areas) have not become infected with almond leaf scorch.

Bacteria were successfully isolated from numerous ALS diseased trees in Contra Costa County in 1977. However, the bacterium is inconsistently isolated from what appear to be infected trees. The variability of isolating the bacterium may be due to the presence of only low numbers of bacteria; the populations may fluctuate throughout the season; the techniques for isolating may not be sensitive enough; we may not be using the best tissue from which to isolate; or symptoms of ALS may be confused with other problems such as salt burn or root diseases.

The bacterium which causes ALS is not typical of any of the bacteria which are known to cause plant diseases. It will not grow on regular bacteriological media and therefore it cannot be readily identified using standard tests. We do not yet have enough data to establish a Latin binomial name. We are continuing some work in this area because a better knowledge of the physiological requirements of these organisms might be useful in control procedures. It is a Gram-negative, aerobic rod-shaped bacterium which grows best at a Ph between 6.5 and 7.5. Isolates can be stored for several months in phosphate buffer at 28C but not in water or 2% Bovine Serum Albumin. Isolates have been lyophilized (freeze dried) for long-term storage and for submission to the American Type Culture

Collection. The ALS and PD are sensitive to many of the common antibiotics in tests performed in the laboratory (Table 3). Results have not been conclusive because the bacteria grow so slowly that some of the antibiotics may break down and not be effective by the completion of the test.

Table 1

Inoculation of almond seedlings with bacteria isolated from plants with Almond Leaf Scorch and Pierce's Disease.

Source of Isolates	Almond Trees Infected	
	Number with Symptoms / Total Inoculated	%
Almond - Contra Costa County	$^{11}/_{25}$	44
Grape - Tulare County	$^{10}/_{10}$	100
Uninoculated Check	$^{0}/_{10}$	0

Table 2

Inoculation of grape cuttings with different concentrations of the Almond Leaf Scorch bacteria.

Approximate Number of Cells/Cutting	Number Infected/Number Inoculated	%
100,000,000	18/18	100
1,000,000	14/16	88
10,000	3/18	17
100	2/22	9

Antisera specific for the ALS and PD bacteria have proven valuable for the rapid identification of cultured bacteria. Immunofluorescent staining of xylem extracts from diseased plants has been variable. Gel double diffusion of ALS and PD isolates has been done to determine strain relationships. Some differences have been noted between isolates but additional research is necessary.

Antibiotic sensitivity

Table 3

Sensitivity of the Almond Leaf Scorch and Pierce's Disease bacteria to Antibiotics

Sensitive	Resistant	Variable
Bacitracin 10 units	Demeclocyline 5 ug	Ampicillin 2 ug
Carbenicillin 50 ug	Lincomycin 2 ug	Bacitracin 2 units
Chlortetracycline 5 ug	Nafcillin 1 ug	Doxycycline 5 ug
Gentamycin 10 ug	Oleandomycin 2 ug	Erythromycin 2 ug
Oxytetracycline 5 ug	Oxacillin 1 ug	Kanamycin 5 ug
Tetracycline 5 ug	Penicillin 2 units	Novobiocin 4 ug
	Streptomycin 2 ug	Polymyxin B 50 units
	Vancomycin 5 ug	
	Triple Sulfa 1 mg	

Leafhopper transmission

The green sharpshooter, Draeculacephala minerva collected from Tulare County and placed for 2 days on grapes with PD isolated from Fresno County transmitted ALS individually to 3 of 20 IXL seedlings on the first day, 2 of 18 the second day, and 3 of 18 seedlings the third day. Transmission by the same leafhoppers of PD to grape following the tests on almond produced similar results. Transmission by D. minerva of Contra Costa ALS isolates has been at about the same efficiency. Further tests of the meadow spittlebug, Philaenus spumarius indicate that insect is a much more efficient vector to almond than D. minerva, which is a grass-feeding species. Of the 18 D. minerva that survived for 5 serial daily transmission exposure periods, only 2 of 8 insects transmitted more than once. This demonstrates that the transmission process is inefficient in D. minerva rather than the acquisition process alone.

Mapping diseased orchards

Maps of disease spread in orchard plots have been completed, but the analysis of this data has not been completed. Because of extensive salt

burn last year and this year, we are cautious in interpreting results, but the rate of spread of ALS could have been as high as 20% per year in some orchards.

5. DISCUSSION:

Summary of major conclusions and accomplishments

1. Almond leaf scorch is caused by an as yet undetermined gram-negative bacterium.

2. This bacterium can be isolated on artificial media and when inoculated into healthy plants produces ALS.

3. Pruning incipient infections well below the lowest leaf symptoms seems to eliminate these infections.

4. Antibiotic injections will reduce symptoms of ALS and produce more normal tree growth in severely diseased trees. The effect is dramatic the first year and less marked the second year of injection. Resistance to antibiotics may be a problem, and the amounts of antibiotics necessary may be uneconomical in commercial production. Residues of injected antibiotics were not detected in assays of nut meats.

5. There is no evidence that the bacterium that causes ALS is not identical with the causal bacterium of Pierce's disease of grapes (PD), even in areas where PD is severe but ALS is totally absent.

6. The insect vectors important in the spread of PD in the Central Valley seem to be of no importance in the spread of ALS.

Plans for future research

Additional observations will be needed to realistically assess varietal resistance and rate of natural spread. The same can be said ot surveys for possible vectors, particularly if ALS spread is epidemic, that is changes greatly from year to year.

At least one additional year of research will be needed to assess the effectiveness of antibiotic therapy and tree surgery. The likelihood of antibiotic resistance needs further study. The relatively slow spread of the causal bacteria that makes pruning out young infections a promising technique also requires that we continue to evaluate this approach to ensure that it is a sound one.

The identity and many physiological needs and characteristics of the causal bacterium are not known. This is a most promising area for future basic research. Improvements to the culture medium may increase the usefulness of antibiotic sensitivity tests and isolation success. Serological tests are being developed to identify possible strain differences among bacterial isolates. Some differences have been noted but additional research is necessary.

There is no satisfactory explanation for why ALS is not more prevalent in the Central Valley. Future investigations of this question should center on two possibilities: (1) an unknown vector in areas where ALS is serious, and (2) the possible effect of climate in suppressing disease development.