Project 75-R (New)

Title: Almond Leaf Scorch Disease

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I. Objectives and Goals:

To determine the cause and the host range of the disease; to determine means of spread of the causal agent in commercial orchards and to develop control measures for almond leaf scorch disease that will be compatable with general production practices in commercial orchards.

II. Abstract:

Our research on the nature of almond leaf scorch (ALS) revealed that this disease is caused by bacteria which invade water and mineral conducting tissues. The bacteria is spread and transmitted from diseased to healthy almonds by leafhoppers (Draeculacephala minerva) and by budding and grafting. The bacteria also can be transmitted by leafhoppers from almonds to grapevines. Our surveys of commercial orchards revealed that ALS is widely distributed in California. ALS has been observed in 11 almond cultivars, but differential tolerance to ALS was noted in naturally infected cultivars. This suggests that losses caused by ALS can be minimized by using tolerant cultivars in areas severely affected by ALS. In March 1975 experiments have been established to evaluate relative tolerance of 16 almond cultivars to ALS. Almond trees were artificially inoculated and they will be observed for severity of symptoms and the rate of spread of the disease from infected to uninfected control trees. Several other stone fruit species were also inoculated to determine their role as possible hosts and source of inoculum for infection of almonds. Mature orchard trees naturally infected with ALS have been sbujected to pressure infection with several antibiotics. The effectiveness of these chemicals in curing infected trees and economic feasibility of this control measure is being evaluated. Our research and field observations suggest that ALS is infectious, specific disease that has potential to inflict serious losses to the almond industry in certain almond producing districts.

III. Experimental Procedure

Nature and means of transmission of ALS--

Search of causal organism in infected trees was made by electron microscopic examinations of ultrathin sections of leaf tissue from naturally infected and healthy orchard trees. Pieces of leaf tissue were fixed and then imbedded in Spurr's medium. Ultrathin sections were cut with a diamond knife, mounted on copper grids, stained and examined with an electron microscope.

Leafhoppers (Draeculacephala minerva)

generations and then used for transmission of the ALS causal bacteria from naturally infected almond to almond, grape and peach. The leafhoppers were fed for 3 days on almond (Long IXL) shoots with typical leaf scorch symptoms. Two insects were transferred onto each of four healthy almond (Mission) peach (Lovell) seedlings and grapevines (Carignone) rooted cuttings (Table 1). Control plants each received two insects that fed on shoots of symptomless orchard almond trees. The insects were allowed to feed on indicator plants for 10 days. The indicator plants were maintained in a greenhouse and observed for development of ALS symptoms for 8 months.

Transmission of ALS from diseased to healthy almonds by budding and grafting was investigated. In one experiment, 20 1-year-old Long IXL almond trees on Nemaguard were inoculated with four buds each in nursery rows. In another experiment 20 2 year-old-Long IXL trees growing in 5 gal. cans in a lath house were graft-inoculated with inoculum from 2 different inoculum sources (Table 1). The indicator plants were inoculated with buds and grafts in September and February, respectively. Final data on transmission were collected in the following October.

Attempts were made to return the ALS bacteria from insect inoculated almonds to healthy almond seedlings in greenhouse experiments. Five indicator plants of each Long IXL almond, Lovell peach and Nanking cherry were inoculated with buds from a Mission seedling that had been previously exposed to infective leafhoppers and experimentally infected with the ALS bacteria (Table 1). Control plants received buds from almond seedlings exposed to noninfective leafhoppers.

Evaluation of almond cultivars and other Prunus trees for their tolerance to ALS.

Twenty trees each of 16 almond cultivars (Thomson, Peerless, Price, Harvey, Mission, Ruby, Merced, Milo, Neplus, Carmel, Fritz, Carion, Nonpariel, Drake, Davey and Long IXL) also Carolyn peach, Santa Rosa plum, Royal apricot and Mono sweet cherry have been planted in the test plot at UC Davis. In August, 1975, ten trees of each cultivar were artificially graft-inoculated with bud chips and buds from an almond tree naturally infected with almond leaf scorch. The inoculated trees of almond cultivars will be evaluated for their tolerance and resistance to almond leaf scorch on the basis of severity symptoms and the rate of spread of the disease in inoculated trees within a certain period of time. Non-inoculated trees are randomly distributed among inoculated trees within the test plot and they will serve for determination of possible natural spread and rate of spread of the disease from infected to healthy trees. Inoculated stone fruit trees other than almond will be observed for occurrence of almond leaf scorch disease, and they will also be examined by electron microscopy to determine whether or not they can harbor the almond leaf scorch organism as symptomless hosts and as such may serve as a constant source of inoculum for almond trees.

Control of ALS by chemotherapy--

erythromycin at varying dosages during the growing season and dormant season. Dormant season was chosen because the xylem transpiration stream is quiet and should permit maintaining high dosage of chemicals. Small branches with symptoms were pruned out of partially affected trees to see if this procedure would affect the spread of the disease within the trees. A chemical identification test to detect wood of infected branches was used to determine the extent of internal infection in the branches.

Gravity injections during the growing season were done as previously described for treating pear decline trees. Pressure injections were done with several types of equipment. Research was done on one type of injector. The Reil-type injector was used to make October, November and December injections in 1975. Three chemicals at four different concentrations were injected into trees each month using three replications for each treatment to find the best time to make treatments and the best concentration of chemical to use.

IV. Results:

Nature and means of transmission of ALS--

The ultrathin sections of scorched leaves collected from naturally infected almonds always contained rod-shaped bacterial cells within the lumina of xylem vessels. No bacteria were observed in the leaves from healthy symptomless almond trees. The bacterial cells were elongated, morphologically uniform with an average diameter of 0.4 m and a maximum length of 1.9 m. The outer cell wall of the bacteria appears rippled, multilayered and thick. Prominent bands or ridges on the outer membranes of the cells are distinguishing features of this organism. The organism multiplies by binary fission within the invaded xylem vessels. The contents of the bacterial cells, in general, resemble those reported for other bacteria. Four mission almond seedlings each exposed to two adult leafhoppers (Draeculacephala minerva) that had fed for 3 days on the excised branches of naturally infected almond trees became infected with the ALS bacteria and developed typical symptoms within 10 weeks (Table 1). Likewise, the Carignane grape cuttings exposed to leafhoppers that had fed on the same almond inoculum source developed symptoms typical of Pierce's disease of grape (Table 1). Almond and grape index plants contained bacteria identical to those in the inoculum source - naturally infected almonds. None of the Lovell peach seedlings exposed to infective leafhoppers developed observable symptoms within 8 months (Table 1). Almond and grape control plants remained symptomless. No bacteria were found in Lovell peach exposed to infective leafhoppers or in any almond and grape plants exposed to non-infective leafhoppers.

The ALS bacteria was transmitted by buds from naturally infected trees to 10 of 20 inoculated almond trees. Likewise, 15 of 20 and 10 of 20 almond trees became infected upon graft-inoculation with stems from Long IXL and Mission almond trees, respectively (Table 1). The first leaf scorch symptoms in trees bud inoculated in September and graft-inoculated in January developed in mid-June following the inoculation. Bacteria identical to those associated with naturally infected trees were readily observed by electron microscopy in all inoculated trees with ALS symptoms.

almonds to 5 of 5 almond indicator plants (Table 1). The almond plants developed typical symptoms of ALS within 10 weeks after inoculation in the greenhouse. The ALS bacteria were readily detected by electron microscopy in all bud-inoculated almond plants. None of Lovell or Nanking cherry seedlings that received the same inoculum developed ALS symptoms or any symptoms resembling those produced by any known virus during the 8 month period (Table 1). Likewise, no organism was detected by electron microscopy in any of the inoculated Lovell or Nanking cherry plants. These results showed that the bacteria alone may induce symptoms of ALS disease in almond plants.

Evaluation of almond cultivars and other Prunus trees for their tolerance to ALS

We observed apparent differential field tolerance among different almond cultivars grown in adjacent rows in the same commercial orchards. This observation suggests that use of tolerant varieties to ALS may be one of the control measures that will minimize losses in the areas severely affected with ALS. However, the experimental evidence is needed to conclude on the inherent tolerance of different almond cultivars to ALS. Experiments for evaluation of 16 almond cultivars for their relative tolerance to ALS were established in March 1975. A preliminary result from this experiment will be available in the fall on 1976.

Control of ALS by chemotherapy

Injections made during the growing season whether by gravity or pressure were not effective in preventing symptoms the following year. In some cases dormant pressure injections seemed promising and therefore this is the area we are concentrating on this winter.

V. Discussion:

Our research showed that the ALS is a specific disease caused by a bacterium that is transmissible by budding, grafting and leafhoppers. The constant association of rod-shaped bacterial cells with naturally and experimentally infected almond trees and the absence of any other pathogen in naturally and artificially infected almond trees, strongly indicates the causal relationship between the bacterium and ALS disease. Morphological similarities between the ALS bacterium and the bacterium causing Pierce's disease in grapes and capability of either bacterium to induce the almond leaf scorch in almond and Pierce's disease in grape suggest that these two diseases may be caused by the same, or very closely related strains of the same bacterium. However, during the course of this investigation, we repeatedly failed to culture the almond leaf scorch bacterium using the same procedures by which the Pierce's disease bacterium was isolated from infective leafhoppers. Thus, the exact relationship of the ALS organism to the bacterium causing Pierce's disease in grapes remains to be determined. Furthermore, Pierce's disease of grape, phony peach, plum leaf scald, and ALS have several characteristics in common; however, the exact relationship between the causal agents of these diseases remains to be experimentally determined.

ALS is widely distributed in California although its incidence except in two districts, is low. The disease may destroy productivity of an orchard

that collectively represent 86% of the total almond acreage in ... addition, the disease is caused by an infectious agent that can spread from infected to healthy trees. The causal agent can be disseminated either by leafhoppers or propagation materials from infected to healthy almond-producing areas. This disease has attributes of a serious plant disease with the potential to limit almond production in certain almond-producing regions. Therefore, control measures should include careful selection of propagating meterials to avoid affected trees. One or more insect vectors probably spread the disease within orchards. Research is needed to determine the vectors of the causal agent in the orchards and on efficiency of vector control on spread of the disease. Evaluation of almond cultivars and of advanced selections that may become commercial cultivars for their tolerance to ALS should be continued. Research on control of ALS by chemotherapy should also be continued.

Table 1. Transmission of the almond leaf scorch bacterium by buds, stems, and leafhoppers from naturally and experimentally infected almond trees to healthy almond and grapevine plants

Source of inoculum	Method[a] of transmission	Fraction[b] of indicators with leaf sc symptoms			
		Almond	Lovell peach seedling	Nanking cherry seedling	C
Naturally infected orchard almond tree cv. Long IXL	Budding	10/20			
	Grafting	15/20			
	Leafhopper[c]	4/4	0/4		
Naturally infected orchard almond tree cv. Mission	Grafting	10/20			
Experimentally inoculated open pollinated Mission almond seedling	Budding	5/5	0/5	0/5	
Healthy orchard almond tree cv. Long IXL	Budding	0/20	0/5	0/5	
	Grafting	0/20			
	Leafhopper[c]	0/4	0/4	0/4	

[a]See text for detailed description of the methods of transmission.

[b]Number of plants with symptoms per number of plants inoculated.

[c]Draeculacephala minerva.