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Project Title: Almond leaf scorch epidemiology

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Rationale

Almond leaf scorch (ALS) is a persistent disease that gradually increases in severity from year to year until trees are no longer productive (4). The disease has occurred at a relatively low incidence in most almond producing areas, but Farm Advisors have reported sharp increases in the occurrence of ALS in the last two years. The disease is caused by an insect-transmitted bacterium, *Xylella fastidiosa*. Strains of *X. fastidiosa* that cause Pierce's disease of grapevines have been thought to be identical to those that cause ALS (2, 4), but the two diseases do not overlap broadly (5). Over the past 20 years I have made numerous observations of Pierce's disease outbreaks in vineyards near almond orchards in which we could never find a single tree with ALS. Conversely, in other regions with a high incidence of ALS (e. g. Contra Costa and San Joaquin counties), the incidence of Pierce's disease adjacent to orchards with high amounts of ALS was low or zero. These observation suggest that different strains of *X. fastidiosa* must be involved in the two diseases, but tests in the greenhouse indicate that bacteria isolated from grape cause ALS and vice versa. We have developed molecular data (random amplified polymorphic DNA or RAPD) that suggest that most strains of the bacterium isolated from almond with ALS are genetically distinct from strains isolated from grapevines with Pierce's disease but that some ALS strains are very closely related to PD strains.

The appearance within the last three years of the first substantiated cases of ALS in Fresno and Tulare counties indicate that ALS may now pose a threat to almonds in the San Joaquin Valley. To evaluate this threat and devise containment or control strategies, it would be very helpful to understand why ALS has not occurred in the southern Central Valley. Our best guess is that strains of *Xylella fastidiosa* that cause almond leaf scorch disease survive winter dormancy in almond much better than do strains that cause Pierce's disease. Late season (after June) infections of grapevine with *X. fastidiosa* do not survive dormancy in grape very well. Such a difference between strains in overwinter survival rates could explain why ALS has not been found until recently in the southern San Joaquin Valley where severe PD has occurred in vineyards adjacent to almond orchards.

To test this idea, we inoculated four ALS strains and four PD strains into new shoots of mature almond trees in experimental plots at U. C. Davis and Kearny Agricultural Center (KAC) in April, 1997, and in August-October, 1997 we recorded ALS symptoms (leaf scorching) and attempted to culture *X. fastidiosa* from leaf bases (petioles) to determine which shoots had been infected. In the fall of 1998 we again will diagnose all inoculated sites for symptoms and the presence of *X. fastidiosa*. A much higher incidence of infection of ALS strains compared to Pierce's disease strains would indicate that Pierce's disease strains of *X. fastidiosa* have probably infected almond in areas such as Fresno and Tulare counties, where ALS has not occurred until the last few years, but have not caused visible damage to trees because the bacteria do not survive winter dormancy in almond.

A recent development in southern California may have important consequences for the future severity of ALS: the establishment of a vector species, the glassy-winged sharpshooter

(*Homalodisca coagulata*), that is new to California. This insect has been detected in moderate to heavy populations on citrus, avocado, and numerous woody ornamentals from San Diego through Ventura. It is considered to be one of the most important vectors of *X. fastidiosa* to peach (phony disease) (6) and grape (Pierce's disease) (1) in the southeastern states. Because this newly introduced species is now unusually abundant in some habitats in southern California and is likely to eventually spread to northern California, we would like to verify our predictions that it will be able to transmit ALS strains of *X. fastidiosa* to almond.

Objectives:

1. Determine if strains of *Xylella fastidiosa* that cause almond leaf scorch (ALS) in the northern Central Valley survive winters in almond at a higher rate than do strains from grape from the southern Central Valley.

2. Evaluate new ALS strains of *X. fastidiosa* for their potential to spread more quickly within almond trees compared to reference ALS strains where the disease progresses slowly within the tree.

3. Evaluate the ability of the newly introduced *Homalodisca coagulata* sharpshooter to transmit ALS strains of *X. fastidiosa* to almond.

Methods:

We inoculated four ALS strains and four PD strains by needle into new shoots of eight mature Nonpareil almond trees each in experimental plots at U. C. Davis and Kearny Agricultural Center (KAC) in April, 1997. Uninoculated shoots were tagged as controls. We used artificial media to culture the bacterium (3) from leaves (petiole and leaf vein) from inoculated twigs, from mid-July through early October. We made 2 or 3 repeated attempts to culture the bacterium from twigs that were initially negative for bacteria. Some inoculated twigs could not be found, and a few died. We also noted symptoms from all inoculated shoots by recording the number of scorched leaves on each shoot and their relation to the inoculation point.

Adult glassy-winged sharpshooters were collected from citrus groves at the University of California, Riverside in November, 1997 and taken to Berkeley for laboratory experiments under a permit from the California Department of Food and Agriculture. For transmission tests, we caged the sharpshooters on grape infected with ALS strains of *X. fastidiosa* for two or more days, then transferred the insects to almond seedlings for 4 or more days. Because of the poor survival of the insects before we began our tests, we put 12 sharpshooters on an almond test plant. Three months later, we cultured *X. fastidiosa* from the test plant. In later tests with single glassy-winged sharpshooters fed on grapevines with Pierce's diseases, the insects transmitted *X. fastidiosa* to one of ten grapevines. We plan to repeat our laboratory transmission trials with glassy-winged sharpshooters on almond test plants in June, 1998 when additional almond seedlings should be available.

Results

About half of the inoculated sites at each location proved to be infected by *X. fastidiosa.* We cultured *X. fastidiosa* from 48% of 160 inoculated twigs at Davis and 55% of 170 twigs at KAC (Table 1). We recovered three ALS strains and one PD strain at below these average rates. Seventeen percent of the stems from which we cultured the bacterium had no leaf scorch symptoms. Where we made more than one culturing attempt from the same stem, 131 were negative for all (2 or 3) attempts; 12 were positive at the first attempt but negative at a later attempt; 20 were positive only from a later attempt; only 9 were positive for two attempts (only a few sites were sampled after proving to be positive). The 9 shoots from which we cultured the

bacterium in our first attempt but did not culture bacteria in a second attempt probably reflect some inefficiency in detecting infection by culturing or an irregular distribution of bacteria among leaves on the same stem. These results suggest that we probably did not detect bacteria in additional infected shoots.

The extent and degree of leaf scorching symptoms caused by the ALS strains were not noticeably more severe than those of the PD strains, although one ALS strain (Tulare) was cultured and caused symptoms more frequently (67 and 79% at UCD and KAC, respectively) than average. In no case did we observe symptoms below the inoculation site. Most symptoms on infected twigs were not so conspicuous that they would have been noticed in routine orchard surveys. Natural infection by insect vectors typically are even slower to develop than needle inoculations, so growers are unlikely to notice most infections made in the current growing season.

Table 1. Summary of successful culturing (August-October) of *Xylella fastidiosa* from almonds inoculated at two locations in April, 1997.

	UC Davis			Kearney Agric. Center		
STRAIN	NUMBER POSITIVE	NUMBER OF ATTEMPTS	% success	NUMBER POSITIVE	NUMBER OF ATTEMPTS	% success
Almond leaf scorch strains						
Oakley	2	21	10%	8	24	33%
Dixon	4	19	21%	10	23	43%
Manteca	7	20	35%	5	21	24%
Tulare	14	21	67%	19	24	79%
	27/81 = 33%			42/92 = 46%		
Pierce's disease strains						
Traver	8	19	42%	16	19	84%
Conn	11	20	55%	13	19	68%
UCLA	14	19	74%	10	21	48%
Fresno	17	21	81%	12	19	63%
	50/79 = 63%			51/78 = 65%		
Totals	**77**	**160**	**48%**	**93**	**170**	**55%**

In an experiment not included in this proposal but relevant to *X. fastidiosa* in almond, we needle-inoculated almond seedlings with strains of *X. fastidiosa* cultured from oleanders with a newly described disease from southern California, oleander leaf scorch. Four of the ten inoculated trees developed leaf scorch among the seven of the ten almond plants from which we cultured *X. fastidiosa.*

Significance and Future Plans

There was no evidence that ALS strains spread more rapidly or caused more severe symptoms than did Pierce's disease strains. Growers and Farm Advisors report that ALS seems to be moving in trees more rapidly than in previous years, but our results suggest that this is probably not because of more almond-virulent strains of *X. fastidiosa.*

The most important results from our experiments will be our assessments of the survival rate of the bacterium after the winter of 1997-98 and the extent of ALS symptoms in the inoculated

stems in the summer and fall of this year. After our diagnoses, we will prune the branches below the extent of symptoms on each shoot. If we can retain the trees an additional year (this may not be possible at Kearney), we will again survey the trees for symptoms to see if pruning effectively eliminated the bacterial infections. Pruning is now recommended as control method where ALS symptoms are limited to an isolated branch or branches within a tree.

Even though our transmission tests to date were limited to a single plant, successful transmission verifies that the glassy-winged sharpshooter is a vector of an ALS strain to almond. We plan to repeat these tests this summer when we have sufficient almond seedlings available. Our findings that the new oleander strain of *X. fastidiosa* can cause disease in almond in the greenhouse may be significant for future studies.

References

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