Infectivity, Persistence, and Host Susceptibility of Almond Varieties to Almond Leaf Scorch Disease

Project No.: 02-BK-00

Project Leader: Bruce Kirkpatrick, Dept. of Plant Pathology, UC Davis
Ken Shackel, Ted DeJong, Dept. of Pomology, UC Davis

Cooperating Personnel: Joe Connell, Tiesen Cao, Tom Gradziel

Introduction

Xylella fastidiosa (Xf) (Wells et al, 1987) is an insect-transmitted and xylem-limited bacterium that can cause Pierce's disease of grapevines (PD), citrus variegated chlorosis, almond leaf scorch, as well as diseases of alfalfa, peach, forest trees and other plants (Hopkins, 1989; Purcell and Hopkins, 1996). Almond leaf scorch (ALS) is a persistent disease that gradually increases severity over time until trees are no longer productive (Mircetich et al, 1976; Purcell, 1998). Xf is transmitted from infected to healthy trees by several species of xylem-feeding sharpshooters including the newly introduced glassy-winged sharpshooter (Purcell, 1999) and spittle bugs (DeLong and Severin, 1950; Purcell, 1976). ALS has occurred at a relatively low incidence in most almond producing areas, but it has increased somewhat in recent years (Purcell, 1998). Numerous observations on the incidence of PD and ALS suggest that there are some biological differences in Xf strains that cause ALS and PD because there are several instances in which no ALS was found on almond trees near a vineyard with severe PD and PD incidence was low or zero in a vineyard adjacent to almond with ALS (Purcell, 1998). However, tests in the greenhouse indicate that bacteria isolated from grape can cause ALS and vice versa (Purcell, 1998). In this study, we used a combined suspension of three Xf strains (two grape strains and one almond strain) to evaluate the susceptibility of 11 almond varieties and the persistence of Xf in almond host.

Objectives

1. Determine the overall susceptibility of the nine almond varieties to ALS.
2. Determine what time of the growing season almond trees are susceptible to Xf infection and develop persistent Xf infections.
3. Determine the relative resistance or susceptibility of almond/peach hybrids to Xf infection.
4. Determine whether Xf can pass through a high-worked peach rootstock to infect individually grafted almond scions.
5. Map and determine variety susceptibilty in a commercial almond orchard located near Chico that had an epidemic incidence of ALS in 2002.

Materials and Methods

To accomplish objective 1, twenty-seven 13-year-old almond trees growing in the Plant Pathology field area at UC Davis were used. Nine varieties including Butte, Carmel, NonPareil, Padre, Price, Solano, Sonora, Texas Mission, and Thompson were inoculated with three separate Xf strains by a pinprick inoculation procedure (Hill and Purcell, 1995) on July 15, 2000. Nine green and matured shoots of each tree were inoculated with three Xf strains with three shoots per strain. Three trees of each variety were evaluated. A total of 243 shoots have been evaluated. The inoculated shoots were evaluated 3 months, 14 months, and 26 months after inoculation.

To accomplish objective 2, eighty-eight 1-year-old almond trees of 11 varieties (Butte, Carmel, Mission, Neplus, NonPareil, Padre, Peerless, Price, Solano, Sonora and Thompson) were planted in Plant Pathology field plot on February 6, 2002. The trees were irrigated every 7 to 10 days and fertilized twice during the growing season of 2002. We planned to inoculate the 11 varieties starting from April to October on a monthly basis. But we did not begin the inoculation until June because of the lack of growth material due to transplanting. We also missed the Xf inoculation in September due to the lack of growth of an almond strain of Xf. So during the growing season of 2002, we had four Xf inoculations implemented on June 15, July 15, August 30, and October 15, plus a control inoculation with sterilized D.I. water. On each date of inoculation, four green and matured shoots from one tree of each variety were inoculated and labeled. Symptomatic ALS scorching leaves on the inoculated shoots and the farthest symptomatic leaf from the inoculation site were recorded biweekly. On September 21 and October 15, two symptomatic leaves or a symptomatic leaf plus an asymptomaitc leaf immediately above the symptomatic leaf were collected from the shoots inoculated with Xf on June 15 and July 15, respectively, and tested for Xf by immuno-capture PCR.

To accomplish objective 3, we inoculated about 15 almond/peach hybrid potted trees in Pomology screenhouse on June 15, 2002. Because it is rare to see any ALS symptoms on shaded trees, we transplanted 44 potted almond/peach hybrids to the field in Plant Pathology field plot on June 27, 2002. Since the inoculation was made on June 15, 2002, before the transplanting, it was difficult to make observations on all of the inoculated trees due to defoliation and death of several of the trees. So we evaluated 15 trees with inoculation of Xf. By the end of September, symptomatic leaves from each inoculated shoot were collected and confirmed with immuno-capture PCR.

To accomplish objective 4, on February 28, 2002, we whip-grafted two almond varieties (cv. Peerless and Neplus) on 1- or 2-year-old branches of 26 peach trees (Queencrest/ Nemaguard). For each tree we grafted 2 to 6 peach branches with either Peerless or Neplus. Eleven Peerless and 15 Neplus shoots were successfully grafted. On June 15, two green and matured almond shoots for each tree were inoculated with Xf, and two similar almond shoots were used as control with no Xf inoculation. Symptomatic leaves on Xf inoculated shoot and the control were recorded every three weeks after Xf

inoculation. Two symptomatic leaves or a symptomatic leaf plus an asymptomatic leaf were collected in September for Xf testing using immuno-capture PCR.

In mid-August it was clear that an epidemic outbreak of ALS had occurred in several almond orchards north of Chico. In September, 2002, we mapped and rated ALS trees in a portion of one orchard. Three rating scales, 0 (healthy), 1 (only limb diseased with ALS), 2 (more than 1 limb diseased with ALS), 3 (all limbs within the tree diseased with ALS) were used to rate each individual tree. A total of 1895 trees were mapped and rated. Leaf samples were also collected for immuno-capture PCR detection. We also labeled 53 trees with only one ALS-diseased limb and established a severe pruning experiment to determine the efficacy of severe pruning for stopping the spread of Xf within a tree.

Results and Discussion

The relative susceptibility of nine almond varieties to ALS were evaluated by inoculating 3 different Xf strains in 2000. As previously reported, very few leaves developed ALS symptoms 3 months after inoculation. Fourteen months later (last year), we found 78% of the varieties had more than 20% shoots with ALS symptoms. Unfortunately, we were not able to confirm the leaves with PCR in 2001.

In 2002, we evaluated the inoculated shoots in 2000 and rated all the branches with a 0 to 3 rating scale. It indicated that only Carmel, NonPareil, Padre and Sonora developed ALS symptoms (Table 1). The three positive samples were all from the branches inoculated with the Xf strain Traver which is a grape strain that is very closely related to an almond strain Manteca (Hendson et al, 2001). No branches inoculated with UCLA and Fetzer were detected PCR positives. This appears to support the hypothesis that PD strains of Xf do not survive well in winter in almond host.

Table 1. Disease rating and confirmation by immuno-capture PCR on nine 13-year-old almond varieties (09/25/02).

Cultivar	No. of shoots inoculated in 2000	No. of shoots diseased in 2002	Disease rating[1]	No. of diseased shoot PCR positive	No. of shoot missing in 2002
Butte	27	0	0		6
Carmel	27	3	1.3	0	1
NonPareil	27	7	1.3	3	2
Padre	27	1	1	0	5
Price	27	0	0		2
Solano	27	0	0		2
Sonora	27	3	2.3	0	6
Texas Mission	27	0	0		2
Thompson	27	0	0		7

1. Disease ratings: for the inoculated branch, 0-healthy; 1-less than 1/3 leaves with ALS; 2-less than 2/3 leaves with ALS, but more than 1/3 leaves with ALS; 3-more than 2/3 leaves with ALS.

In 2002, we set up another experiment to evaluate the susceptibility of 11 almond varieties to ALS and determine what time of the growing season almond trees are

susceptible to Xf infection and showing ALS symptoms. We used a combination of three Xf strains including two grape strains Temecula and Fetzer, an almond strain Dixon to inoculate 11 almond varieties at different time of the growing season. We found that August was the best time to evaluate ALS symptoms for shoots that were inoculated in June (Table 2). About 10 weeks after Xf inoculation in June, all 11 almond varieties developed typical ALS symptoms. It appears that Xf can move 215 to 570 mm (8 to 24 inches) from the inoculation site within a period of 10 weeks (Table 1). By the end of the growing season, Xf moved up about 1 meter (3 feet) in some varieties. In some cases we observed a few symptomatic leaves below the inoculation site. For trees inoculated in July, most of the inoculated shoots began to show symptoms in the early part of September about 2 months after Xf inoculation (Table 1). We also observed a few symptomatic leaves on shoots inoculated in late August (Table 1). But no symptomatic leaves were observed on shoots inoculated in October. In some cases, we did observe scorched leaves on control plants which were inoculated with sterilized D.I. water; however, none of these leaves tested positive by PCR.

Table 2. Total number of symptomatic ALS leaves of the four inoculated shoots and the longest distance (mm) of the farthest symptomatic leaf from the inoculation site

Variety	Date of inoculation	06/30		07/15		07/30		08/15		08/30		09/15		09/30		10/15	
		L	D	L	D	L	D	L	D	L	D	L	D	L	D	L[1]	D[2]
Butte	06/15/02	0	0	0	0	0	0	10	385	27	640	34	705	53	820	34	905
Carmel	06/15/02	0	0	0	0	0	0	1	(25)	11	360	27	570	28	570 (65)	24	780 (65)
Mission	06/15/02	0	0	0	0	0	0	23	122	35	578 (90)	57	1000 (90)	65	1000	65	1120
Neplus	06/15/02	0	0	0	0	0	0	4	300	19	553	25	553	13	660	13	900
NonPareil	06/15/02	0	0	0	0	0	0	13	102	13	250	17	250 (28)	18	250	18	250
Padre	06/15/02	0	0	0	0	0	0	3	100	25	460	34	660	53	780	50	1035
Peerless	06/15/02	0	0	0	0	0	0	0	0	3	570	14	808	9	810	9	1020
Price	06/15/02	0	0	0	0	0	0	4	430	13	380 (20)	22	610	51	710	50	710
Solano	06/15/02	0	0	0	0	0	0	18	87	24	672	40	672	58	692	58	980
Sonora	06/15/02	0	0	0	0	0	0	12	120	15	513 (50)	23	513	33	615	25	700
Thompson	06/15/02	0	0	0	0	0	0	1	340	14	556	20	670	36	870	21	965
Butte	07/15/02					0	0	0	0	0	0	2	205	2	205	14	610
Carmel	07/15/02					0	0	1	(45)	2	120	3	200 (60)	4	30 (110)	6	510
Mission	07/15/02					0	0	0	0	3	(140)	6	420	8	520	15	605
Neplus	07/15/02					0	0	0	0	0	0	4	370	11	560	12	560
NonPareil	07/15/02					0	0	7	62	2	230 (45)	4	15 (100)	6	280	7	410
Padre	07/15/02					0	0	0	0	1	270	3	320	7	605	11	790
Peerless	07/15/02					0	0	0	0	1	360	1	360	9	790	9	870
Price	07/15/02					0	0	0	0	1	210	2	210	5	70 (35)	6	345
Solano	07/15/02					0	0	0	0	0	0	2	18 (50)	7	275	16	415
Sonora	07/15/02					0	0	0	0	0	0	1	160	7	445	10	445
Thompson	07/15/02					0	0	0	0	1	85	4	270	8	160 (120)	16	415
Butte	08/30/02											9	250 (150)	9	250	11	250
Carmel	08/30/02											7	(190)	10	220	11	230
Mission	08/30/02											7	275 (340)	9	275	11	275
Neplus	08/30/02											0	0	0	0	1	(10)
NonPareil	08/30/02											8	(110)	9	8	9	8
Padre	08/30/02											1	(330)	1	(330)	1	(330)
Peerless	08/30/02											0	0	0	0	0	0
Price	08/30/02											0	0	0	0	4	(70)
Solano	08/30/02											0	0	0	0	0	0
Sonora	08/30/02											7	130 (85)	9	130	9	175
Thompson	08/30/02											4	50 (160)	6	165 (220)	8	165

1. L---Average number of symptomatic ALS leaves per shoot;

2. D---Average distance of a symptomatic ALS leaf from the inoculation site, data in parentheses refer to distance of the farthest symptomatic leaf below inoculation site.

Two symptomatic leaves or a symptomatic leaf plus an asymptomatic leaf were collected 3 months after Xf inoculation. We found that the immuno-capture PCR was sensitive enough that a single leaf was sufficient for a test; however, for security purposes, we tested 2 leaves for each sample by PCR. PCR results showed that for shoots inoculated in June, Xf can move up 235 to 730 mm within 3 months after inoculation. For shoots inoculated in July, Xf can move up 0 to 680 mm within a period of 3 months after inoculation (Table 3).

Table 3. Distance of Xf movement within the inoculated stem 3 months after inoculation as verified by immuno-caputure PCR[1].

Variety	Date of inoculation	Average distance above inoculation site (mm)	Range of Xf movement (mm)	No. of stems PCR positive	No. of stems inoculated
Butte	06/15/02	560	285 – 735	4	4
Carmel	06/15/02	405	380 – 430	2	4
Mission	06/15/02	730	390 – 990	4	4
Neplus	06/15/02	445	440 – 450	2	4
NonPareil	06/15/02	235	120 – 330	3	4
Padre	06/15/02	654	460 – 895	4	4
Peerless	06/15/02	668	400 – 810	3	4
Price	06/15/02	441	210 – 350	4	4
Solano	06/15/02	485	290 – 765	4	4
Sonora	06/15/02	425	345 – 550	3	4
Thompson	06/15/02	600	550 – 670	3	4
Butte	07/15/02	440	270 – 610	2	4
Carmel	07/15/02	490	470 – 510	2	4
Mission	07/15/02	481	270 – 605	4	4
Neplus	07/15/02	383	130 – 520	3	4
NonPareil	07/15/02	410	410	1	4
Padre	07/15/02	527	335 – 790	3	4
Peerless	07/15/02	680	490 – 870	2	4
Price	07/15/02	0	0	0	4
Solano	07/15/02	355	285 – 415	3	4
Sonora	07/15/02	290	290	1	4
Thompson	07/15/02	315	315	1	4

1. Two most distant symptomatic leaves or a symptomatic leaf plus an adjacent asymptomatic leaf were used for immuno-capture PCR detection.

Unfortunately, we missed the inoculation in April, May, and September due to improper growth of the plants and Xf almond strain. We will perform inoculations during these months next year.

One of our objectives was to evaluate the relative susceptibility of almond/peach hybrids to Xf infection. We inoculated 15 almond/peach hybrid trees on June 15. By the end of September, we collected all symptomatic leaves on each of the shoots inoculated with Xf and tested these with immuno-capture PCR assay. All almond/peach hybrids were infected with Xf (Table 4). We didn't take any leaf samples from hybrids that were 50/50

almond/peach because no symptomatic leaves were observed on these hybrids. This data confirms previous observations that peach is not a host of Xf.

Table 4. Susceptibility of almond/peach hybrids to Xf infection.

Hybrid type	Hybrid property	No. of shoots PCR positive	No. of shoot tested
F7,5-9+10	94% almond, 6% *P. webbii*	4	7
F7,1-9+10	94% almond, 6% peach	1	5
F8,4-153	90% almond, 10% peach	2	7
F7,3-7	82% almond, 18% peach	4	5
F8,7-179	70% almond, 30% *P. webbii*	1	1

To test the hypothesis that peach rootstocks that were high worked with almond scions may limit the spread of Xf within a tree, we whip-grafted almond scions onto 26 limbs of peach trees. In June and July, two shoots of each tree were inoculated with Xf and two shoots were used as control without Xf inoculation. There were varying distances of peach stock between the Xf-inoculated almond scion wood and uninoculated almond shoot grafted on the same peach rootstock. The trees were observed every three weeks for symptomatic ALS leaves. In September and October, symptomatic leaf samples from both Xf inoculated shoots and control shoots were taken for PCR testing. If there were no symptomatic leaves on control shoots, then the closest 2 or 3 asymptomatic leaves were taken for PCR detection. Three samples from uninoculated control shoots yielded positive PCR results in Peerless and 5 in Neplus, which suggests that Xf moved through the almond/peach graft union to spread to another uninoculated almond scion wood.

Table 5. PCR result for almond high-worked trees on peach (Queencrest/Nemaguard) in Pomology teaching orchard (10/20/02)

Variety	Treatment	No. of trees PCR positive	No. of trees PCR negative	Total number of trees
Peerless	Xf inoculation	9	2	11
	Control	3	8	11
Neplus	Xf inoculation	12	3	15
	Control	5	10	15

The distance between a symptomatic leaf to the almond/peach graft union, the distance in peach stock portion, and the distance from the peach/almond graft union to the leaves sampled for PCR on the control shoot were determined. Although not statistically significant, there was a general trend that indicated that Xf did not move through long distances of peach rootstock and infected the uninoculated almond shoot. We are planning to further investigated the movement of Xf through peach rootstock by grafting almond scions at greater distances apart on peach rootstock this coming spring.

Table 6. Distance of the inoculation site on almond stem to the almond-peach graft union in the Xf inoculated stems.

Variety	Category	Distance of the inoculation site to the graft union on inoculated stem*
Peerless	Trees with PCR positive leaf on uninoculated almond stem	120 ± 46 a
	Trees with PCR negative leaf on uninoculated almond stem	178 ± 74 a
Neplus	Trees with PCR positive leaf on uninoculated almond stem	170 ± 95 A
	Trees with PCR negative leaf on uninoculated almond stem	153 ± 76 A

* Means followed with the same letter were not significantly different.

In mid-August, 2002, Joe Connell documented severe outbreaks of ALS in several orchards north of Chico. In mid-September, we mapped and rated 1895 14-year-old almond trees in one portion of an orchard. Four varieties were planted in this orchard, including 50% NonPareil, 25% Peerless, 12.5% Butte, and 12.5% Carmel. The overall incidence of ALS in this portion of the orchard was about 17% based on visual disease rating (Table 8). We also randomly took 24 symptomatic samples for PCR testing to authenicate our visual ratings; all 24 samples were PCR positive. The incidence of ALS showed that Peerless had the highest incidence (32.4%), next was NonPareil (16%). Butte and Carmel only had 2.3% and 0.4% ALS incidence, respectively (Table 9). These data clearly showed that Peerless and NonPareil are more susceptible to Xf infection than Butte and Carmel.

Table 7. Incidence of almond leaf scorch (ALS) in Chico orchard containing NonPareil, Peerless, Butte and Carmel varieties in 2002 (Anita Rd. & Hwy 99).

Disease rating	NonPareil	Peerless	Butte	Carmel	Total
Healthy	794	329	217	240	1580
1	63	53	3	0	119
2	54	26	2	0	82
3	34	79	0	1	114
Total ALS trees	151	158	5	1	315
Total	945	487	222	241	1895

Table 8. Disease incidence (% ALS infected) for each almond variety in 2002 (Anita Rd. & Hwy 99).

Disease rating	NonPareil	Peerless	Butte	Carmel
Healthy	84.0	67.6	97.7	99.6
1	6.7	10.9	1.4	0
2	5.7	5.3	0.9	0
3	3.6	16.2	0	0.4
Total ALS trees	16.0	32.4	2.3	0.4
Total	100	100	100	100

In addition, we marked 53 almond trees that had only one limb diseased with ALS. In cooperation with the grower, these limbs were removed in October, and next summer we will observe and test the remaining portion of the tree to determine whether severe pruning can limit the spread of Xf within a tree.

References

Davis, M.J., French, W.J., Schaad, N.W. 1981. Axenic culture of the bacteria associated with phony peach disease and plum leaf scald. Curr. Microbiol. 6:309-314

DeLong, D.M., and Severin, H.H.P. 1950. Spittle-insect vectors of Pierce's disease virus. I. Characters, distribution, and food plants. Hilgardia 19:339-355

Hendson, M., Purcell, A.H., Chen, D., Smart, C., Guilhabert, M., and Kirkpatrick, B. 2001. Genetic diversity of Pierce's disease strains and other pathotypes of *Xylella fastidiosa*. Applied and Environmental Microbiology 67(2):895-903

Hill, B. L.; Purcell, A. H. 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. Phytopathology 1995. 85 (11): 1368-1372

Hopkins, D.L. 1989. *Xylella fastidiosa*: A xylem-limited bacterial pathogen of plants. Annu. Rev. Phytopathol. 27:271-290

Mircetich, S.M., Lowe, S.K., Moller, W.J., and Nyland, G. 1976. Etiology of almond leaf scorch disease and transmission of the causal agent. Phytopathology 66:17-24

Purcell, A.H. 1976. Seasonal changes inhost plant preference of the blue-green shapshooter *Hordnia circellata* (Homoptera: Cicadellidae). Pan-Pac. Entomol. 52:33-37

Purcell, A.H. 1998. Almond leaf scorch epidemiology (project annual report).

Purcell, A.H. 1999. Almond leaf scorch epidemiology (project final research report)

Purcell, A.H. and Hopkins, D.L. 1996. Fastidious xylem-limited bacterial plant pathogens. Annu. Rev. Phytopathol. 34:131-151

Purcell, A. H.; Saunders, S. R. 1999. Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. In: Plant Disease Sept., 1999. 83 (9): 825-830.

Wells, J.M., Raju, B.C., Hung, H.Y., Weisberg, W.G., Mandelco-Paul, L., and Brenner, D.J. 1987. *Xylella fastidiosa* new-genus new-species gram-negative xylem-limited fastidious plant bacteria related to *Xanthomonas* spp. J. Syst. Bacteriol. 37:136-143