Effects of *Xylella fastidiosa* Group, Almond Cultivar, and Climate on the Establishment and Persistence of Infections Causing Almond Leaf Scorch

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Project Leader: Kent Daane
Department of Environmental Science
Policy and Management (ESPM)
University of California
Berkeley, CA 94720
(559) 227-9670
daane@uckac.edu

Project Cooperators: Christina Wistrom, ESPM, UC Berkeley, CA 94720-3114
Bruce Kirkpatrick, Department of Plant Pathology
UC Davis, CA 95616

Interpretive Summary:

Almonds are one of the most widely-grown crops infected by the plant pathogenic bacterium *Xylella fastidiosa*, which causes almond leaf scorch (ALS) disease. *X. fastidiosa* must survive multiple winters in an almond tree to reach sufficient populations for vector acquisition and economic impact in the orchard. However, there have not been controlled studies on the effect of dormancy and winter cold on *X. fastidiosa* infection survival in almonds. Previous growth chamber and field studies in grapevines showed that the degree of plant dormancy, and cold exposure, affected *X. fastidiosa* survival, with extremely cold winter temperatures curing infections. Later studies with almonds indicated that there was some infection curing during dormancy, depending on the *X. fastidiosa* strain, but the amount of cold was not noted.

In this study, the effects of cold temperatures on *X. fastidiosa* infections were measured in field and greenhouse-grown almonds, using multiple *X. fastidiosa* strains, and ALS-resistant and ALS-susceptible varieties. Potted trees infected with *X. fastidiosa* were over wintered outside, or in cold rooms at 1.7°C or 7°C, for 1, 2, or 4 months. Cold exposure time negatively influenced potted tree recovery from *X. fastidiosa* infection, while cold intensity did not: 21% of trees recovered after 1 month, 13% recovered after 2 months, and 7% recovered after 4 months. In the field, trees at UC Davis (UCD) and Intermountain Research and Extension Center in Tulelake (IRC), were inoculated with either grape or almond-subspecies *X. fastidiosa*. Initial infection rates and bacterial populations were similar at UCD and IRC, although ALS symptoms were much more severe at UCD, especially in 'Peerless' trees. At UCD, 10% of trees inoculated with almond-strain *X. fastidiosa* were infected, vs. 78% of trees inoculated with grape-strain

X. fastidiosa. Both strains infected trees at equal rates at IRC (64% almond, 40% grape). Winter conditions killed all *X. fastidiosa* infections at IRC and all but one at UCD. One explanation for the prevalence of almond-strain *X. fastidiosa* in northern California almond orchards is that almond strains initially infect trees at low rates, but survive the winter more frequently than grape strains to cause persistent ALS. Data from this study supported half this hypothesis, with almond strains initially infecting 11 to15% of field-grown trees, similar to the 21 to 33% infection rate previously reported. Grape *X. fastidiosa* isolates infected 79% of inoculated trees at UCD in this study, and 64 and 75% in field trials in Davis and Parlier. In this study, no almond infections and only one grape *X. fastidiosa* infection over wintered in field plots, even at UCD where the winter was mild, with only 44 hours below freezing. Previous researchers found that almost all infections by almond strains survived the winter and 88-42% of grape-strain infections over wintered in field grown trees in Parlier, CA. We repeated field inoculations in 2007 at UCD, to get more data on initial infection establishment and over wintering rates.

Objectives:

Data from this project addresses several questions about ALS epidemiology:

1. What proportion of new ALS infections are cured during dormancy, and does winter severity influence ALS severity in the following summer?
2. Does the strain of *X. fastidiosa* present (almond or grape) in infected almond trees influence the likelihood that ALS will develop in an orchard?
3. Is grape type *X. fastidiosa* (for example, from an adjacent vineyard with Pierce's disease) likely to spread to healthy almond orchards?

Answers to these questions will help growers assess the risk posed to their orchards by *X. fastidiosa* and better manage ALS if it occurs. Specific study objectives were 1) to compare the establishment and over wintering of grape and almond strain *Xylella fastidiosa* in susceptible and resistant almond cultivars, and 2) to determine the effects of cold treatment on the over winter survival of *Xylella fastidiosa* in almonds.

Materials and Methods:

Field trials: One hundred bare-root almond trees, 50 'Peerless' and 50 'Butte', both on Nemaguard rootstock (Dave Wilson Nursery, Hickman, CA) were planted 10 February at Armstrong Farm at University of California, in Davis, CA (UCD), and 5 May 2005 the Intermountain Research and Extension Center in Tulelake, CA (IRC). Trees were drip irrigated at UCD and sprinkler irrigated at IRC, trunks painted to prevent sunburn, and at UCD, encased with plastic "grow tubes" to prevent rodent damage. Trees were planted in a randomized complete block design with a split plot (almond cultivars) in each block. There were ten replicates of each treatment combination (*X. fastidiosa* isolate x almond cultivar). Isolates were randomly assigned to trees. Trees were inoculated with one isolate of *X. fastidiosa*: Fresno-ALS, Dixon, ALS 6, or Medeiros **(table 1)**. All isolates of *X. fastidiosa* were isolated from infected plants in Solano, Fresno, or San Joaquin counties, and were pathogenic in recent greenhouse tests. Trees were inoculated when emerging shoots were at least 6 mm in diameter, 3 May (UCD) or 1 July 2005 (IRC). Inoculum was prepared in the field from two-week-old cultures of *X. fastidiosa* on solid

media. Each isolate was inoculated into 3 in 1 stem per plant with a 10 µl drop of bacteria suspended in buffer (approximately 10,000,000 bacteria/ ml). The drops were placed on a green, growing shoot and probed with a #2 insect pin until they were drawn into the stem. Inoculation sites marked with permanent metal tags and paint.

Table 1: Inoculation treatments of *X. fastidiosa in* field-grown trees at UCD and IRC.

X. fastidiosa isolate	Group[a]/ sub-species[b]	Originally isolated from
ALS6	almond – 2 / *multiplex*	almond
Dixon	almond – 1 / *multiplex*	almond
Fresno-ALS	grape / *piercei*	almond
Medieros	grape / *piercei*	grape
Buffer	-	-

[a] Reference: Hendson et al. 2000; [b] Schunzel 2006.

X. fastidiosa infections were assessed on 17 August and 20 September 2005 at UCD; and on 28 August, 26 September, and 23 October 2006 at UCD. At IRC trees were inspected on 6 September 2005 and 5 September 2006. At UCD, repeated measures of symptom severity and *X. fastidiosa* populations were averaged between sampling dates. The number of scorched leaves on the inoculated stem was counted to determine infection severity, and two or more almond petioles distal to the inoculation site were cultured from each tree to determine *X. fastidiosa* presence and population (Hill and Purcell 1995). *X. fastidiosa* strains were distinguished based on their growth on two different media, PD3 and PWG (Davis et al. 1981, Davis et al. 1983, Almeida and Purcell 2003). *X. fastidiosa* colonies were lysed at 95°C and the crude DNA extract used in PCR reaction with Taq Master Mix (Qiagen, Hercules, CA) and RST31 and 33 primers (Minsavage 1994). Amplified DNA was digested with *Rsa1* restriction enzyme, which selectively cut the DNA of almond *X. fastidiosa* isolates into two pieces of 233kb and 500kb, but left amplified DNA of grape *X. fastidiosa* intact (Hendson 2001).

Inoculations were repeated at UCD in May 2007. Initial infection establishment will be assessed in August and September 2007, and over wintering infections measured in fall 2008, as previously described. Over wintering temperatures were recorded with a data logger at UCD (Onset Computer Corp., Bourne, MA), and at IRC by a CIMIS weather station 20 meters from the field site.

Controlled temperature study: In the first replication, 125 potted 'Peerless' trees on Nemaguard rootstock were inoculated with the ALS 6 isolate of *X. fastidiosa*, an almond strain of *X. fastidiosa* in the *multiplex* subspecies. Thirty trees were inoculated with buffer alone, in the same manner as for the field plots. Trees were kept in a greenhouse at 19.5°C +/- 5°C at Kearny Agricultural Center in Parlier, CA, in 7.57L pots. Trees were fertilized with 20-20-20 fertilizer at the label rate, and exposed to natural lighting and day length. In November 2005, 90 *X. fastidiosa*-infected trees, and 27 buffer-inoculated

trees with were moved to screen cages outside to go dormant. In January 2006, trees were divided randomly between treatments. Thirty *X. fastidiosa*-infected trees and 9 buffer-inoculated trees remained outside, or were placed in cold rooms of 1.7°C or 7°C. Ten infected and 3 uninfected trees were removed from each cold treatment after 1, 2 and 4 months, and allowed to break bud in the greenhouse. These intervals were reflective of dormancy periods in previous studies with almonds and grapevines (1 month; Almeida and Purcell 2003c, Feil and Purcell 2001), typical dormancy in the central valley (2 months; going fully dormant in December and flowering in February) and an extreme treatment for abnormally long dormancy (4 months). Temperature recorders (Onset Computer Corp., Bourne, MA) were placed in each cold room, in the screen cage outside, and in the greenhouse. Plants were tested for *X. fastidiosa* on 24 October 2005 and 11 September 2006, as previously described for field-grown trees. Additional symptom data were recorded on 17 August 2006.

In the second replication, 300 'Peerless' trees on Hansen rootstock were inoculated with *X. fastidiosa*, 150 with Fresno-ALS, a PD strain, and 150 with ALS6, an ALS strain, in July 2007. Thirty trees were left as uninoculated controls. Plants were grown in the greenhouse with natural lighting, in 7.58L pots with Supersoil. Trees were fertilized with dilute 20-20-20 fertilizer and Osmocote slow release fertilizer at the label rate. In November 2007 their disease symptoms will be rated and *X. fastidiosa* infections and populations confirmed as previously described. Equal numbers of infected trees from each isolate will be randomly assigned to the following treatments in **Table 2.**

Table 2: Treatments for second cold-chamber experiment. Each *X. fastidiosa* treatment (Fresno-ALS: grape strain, ALS6: almond strain, or uninoculated) will have the following temperature and time exposures. There will be 10 replicates for each treatment combination, or 270 trees total.

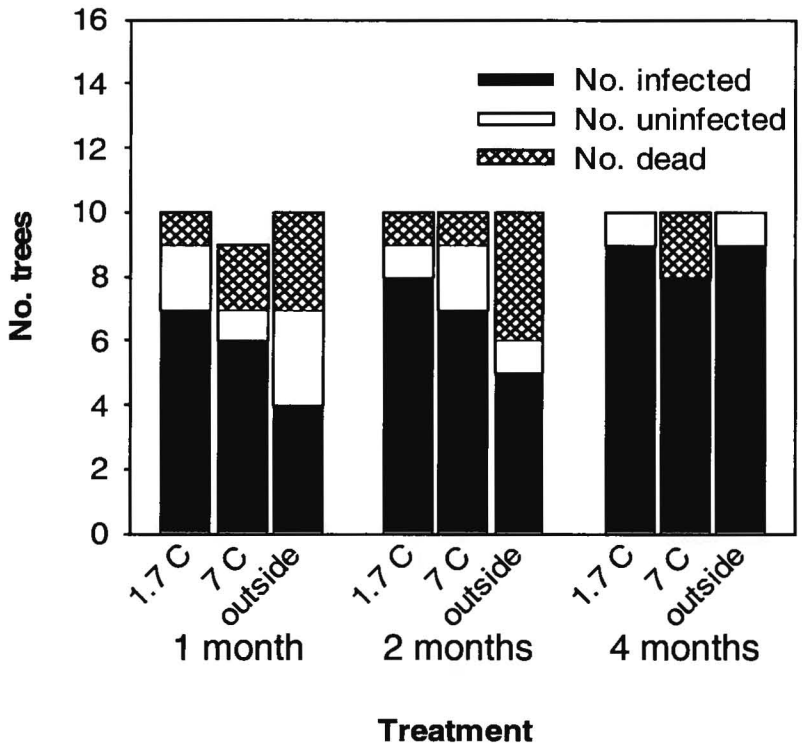
Temperature Treatment	Base Temperature	Exposure	Cold Temperature	Exposure (hrs & days)
1	1.7°C (34°F)	Dec - Feb	-4°C (25°F)	5 h / 5 d
2	1.7°C (34°F)	Dec - Feb	-4°C (25°F)	5 h / 10 d
3	1.7°C (34°F)	Dec - Feb	-4°C (25°F)	5 h / 15 d
4	1.7°C (34°F)	Dec - Feb	-4°C (25°F)	24 h / 5 d
5	1.7°C (34°F)	Dec - Feb	-6.7°C (20°F)	5 h / 5 d
6	1.7°C (34°F)	Dec - Feb	-6.7°C (20°F)	5 h / 10 d
7	1.7°C (34°F)	Dec - Feb	-6.7°C (20°F)	5 h / 15 d
8	1.7°C (34°F)	Dec - Feb	-6.7°C (20°F)	24 h / 5 d
9	1.7°C (34°F)	Dec - Feb	-	-

Results and Discussion:

***Controlled temperature study*:** Five months after inoculation with ALS 6 *X. fastidiosa*, 72% of inoculated trees (89 of 125) developed almond leaf scorch, averaging 12.8 (SE =1.25) symptomatic leaves per infected tree, and a median population of 4.4 x 10^6 CFU/g. None of the buffer-inoculated trees were infected with *X. fastidiosa*, and they averaged 0.3 scorched or yellowed leaves per tree.

Fourteen of 89 infected trees died during or after cold treatment, compared to 1 of 27 buffer-inoculated trees ($\chi 2$ with Yates' correction = 3.17; $P > 0.05$; df = 1). Six of 29 trees were negative for *X. fastidiosa* after one month cold treatment, compared to 4 of 30 after two months, and 2 of 30 after four months **(figure 1)**. There were not significant differences in the number of recovered trees regardless of temperature or time ($\chi 2$ with pairwise comparisons).

Figure 1: *X. fastidiosa* infections in previously-infected potted almond trees after 1, 2 or 4 months of dormancy at 1.7°C, 7°C, or outside in Parlier, CA.



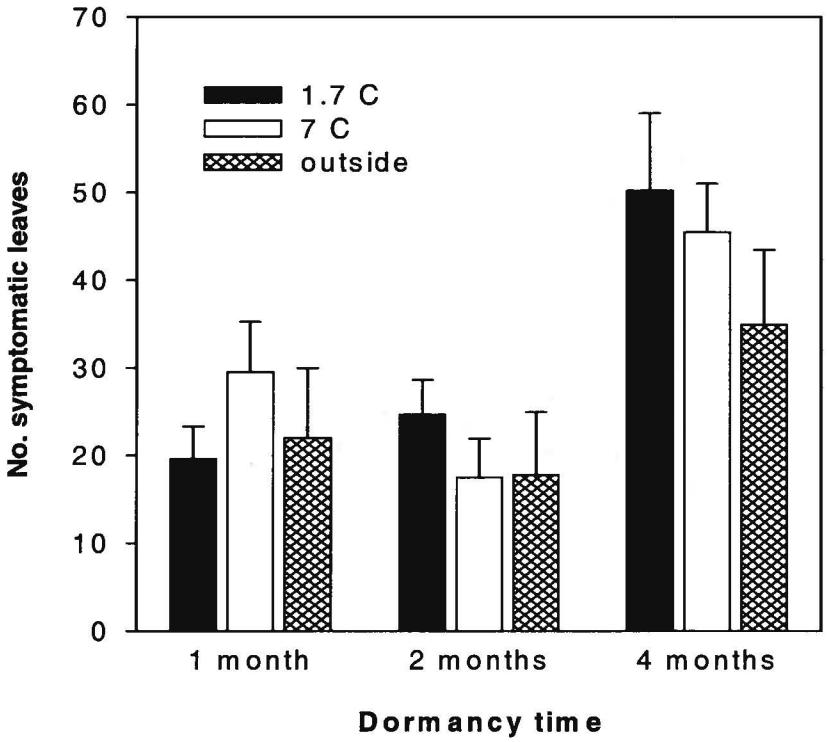
ALS symptoms were twice as severe after cold treatment as before. In 2005, buffer-inoculated trees averaged 0.26 scorched or yellowed leaves per tree (SE = 0.25), compared to 12.8 symptomatic leaves per *X. fastidiosa*-infected tree (SE = 1.23). In 2006, there were an average of 1.29 (SE = 0.42) scorched and yellowed leaves per buffer inoculated tree and 30.24 (SE=2.57) symptomatic leaves per *X. fastidiosa* infected tree. Symptoms were worse in trees following four months of cold treatment, compared to trees with one and two months **(figure 2)**. The time of dormancy, but not

the temperature, influenced symptom severity (2-way ANOVA, Standard Least Squares: Time $P = 0.0002$, df =2; Temperature $P = 0.593$, df =2; Temperature*Time $P = 0.39$, df = 4; root mean square error = 17.65).

X. fastidiosa populations in infected trees were similar across all treatments (2-way ANOVA, Time $P = 0.74$; Temperature $P = 0.19$; Time*Temperature Interaction $P = 0.39$) with a median population of 2.82×10^6 CFU/g.

Trees in the 1.7°C and 7°C treatments were subjected to temperatures below the growth threshold of *X. fastidiosa* for the entire period: 769 hours for 1 month, 1441 hours for 2 months, and 2905 hours for 4 months. Outside, trees were below 7°C for 262 hours in the 1 month treatment, 557 hours in the 2 month treatment, and 673 hours for the 4 month treatments. Only trees kept outside were subjected to sub-freezing temperatures, for 10 hours, both in the 2 and 4 month treatments.

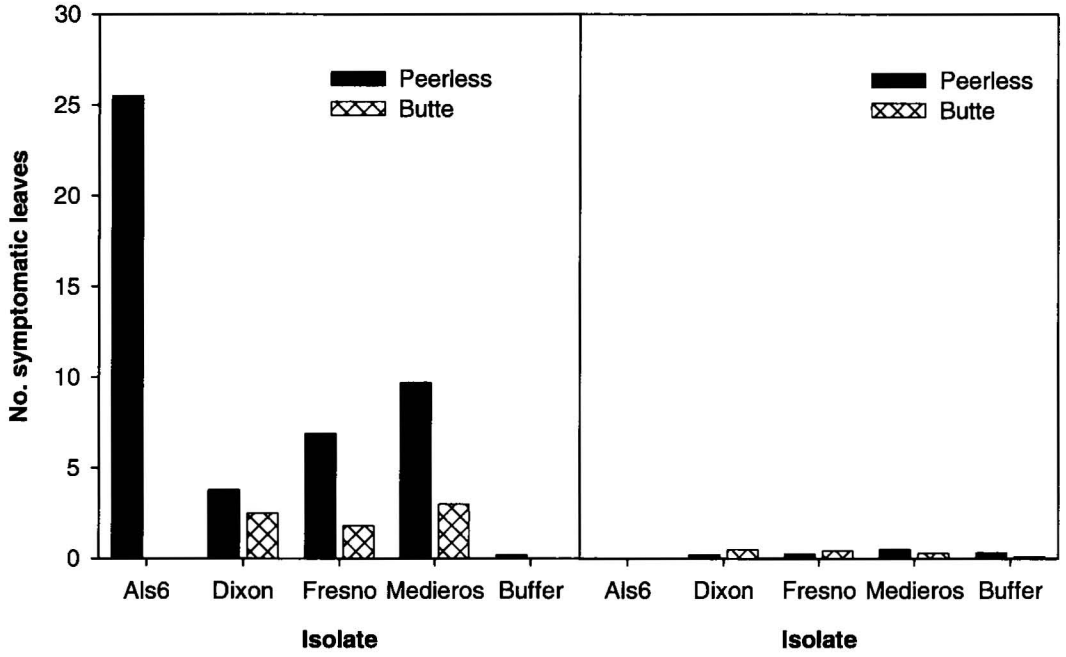
Figure 2: Almond Leaf Scorch symptoms in *X. fastidiosa* inoculated almond trees following 1, 2, and 4 months of cold treatment at 1.7°C, 7°C, or outside at Parlier, CA.



Field sites: In 2005, two to four months after inoculation, *X. fastidiosa* was recovered from 49% of bacteria-inoculated trees (76 of 155), and from none of the buffer-inoculated trees. There was no difference in the proportion of infected trees at UCD or at IRC (36 of 76 at UCD; 40 of 79 at IRC; $\chi 2 = 0.17$; $P > 0.05$; df =1; figure 4), nor in median *X. fastidiosa* populations in infected trees (4.0×10^6 colony-forming units per gram of petiole tissue at UCD; 1.2×10^7 CFU/g at IRC; t-test with $\log_{10}$-transformed

data; $P = 0.18$; df = 75). However, disease symptoms were more severe at UCD, especially in 'Peerless' trees, averaging 8.8 (SE = 2.1) symptomatic leaves per infected tree, compared to 2.3 leaves per infected 'Butte' tree (SE = 0.7; $P = 0.02$; two-sample t-test; df = 35). At IRC, symptoms were negligible, as both varieties averaged 0.3 scorched leaves per infected tree (SE = 0.1; $P = 0.76$; two-sample t-test; df = 39). Background yellowing and scorching in uninfected trees was 0.11 leaves per tree (SE = 0.08) at UCD and 0.21 (SE = 0.1) leaves per tree at IRC **(Figure 3).**

Figure 3: Almond leaf scorch symptoms in *X. fastidiosa*-infected 'Butte' and 'Peerless' almond trees at Davis (left) and Tulelake (right) field sites 2-4 months after inoculation.



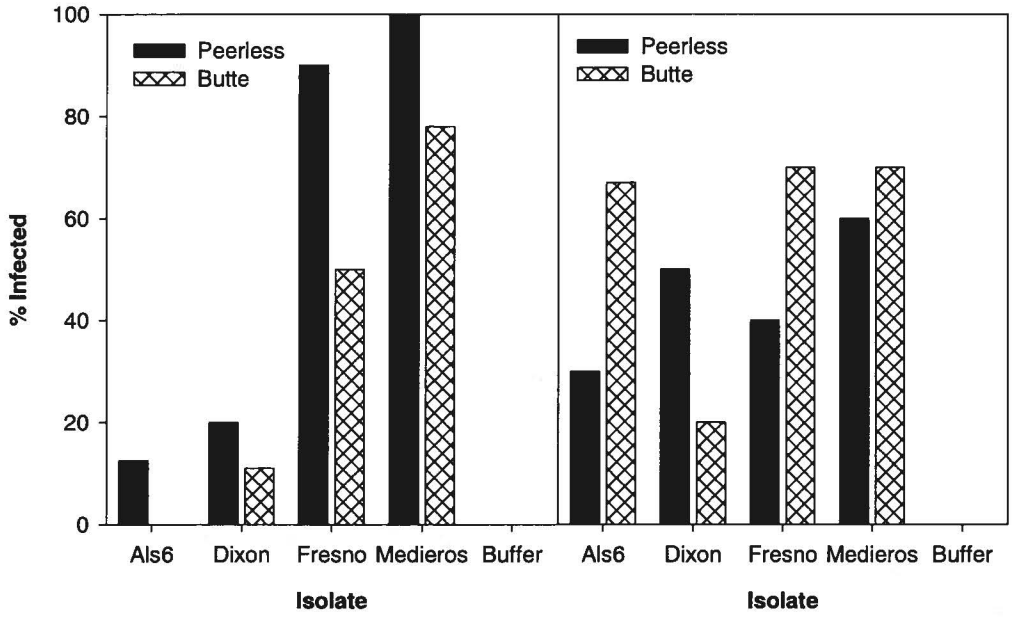
Grape strain X. *fastidiosa* infected trees more frequently than almond strain *X. fastidiosa* at UCD but not at IRC **(figure 4)**. At UCD, 4 of 37 trees inoculated with Dixon or ALS-6 developed infections, compared to 32 of 39 trees with Fresno-ALS or Medeiros ($\chi 2$ with Yates' correction = 38.71; $P < 0.001$; df = 1). At IRC, both almond and grape strains of *X. fastidiosa* infected trees with the same frequency (16 of 39 inoculated with almond; 24 of 40 inoculated with grape strain; $\chi 2 = 2.84$; $P > 0.05$; df = 1). Grape and almond strains reached similar titers in infected plants, median 3.48×10^6 CFU/g for almond strain *X. fastidiosa*, and 5.71×10^6 CFU/g for grape strain, both sites combined. All *X. fastidiosa* recovered from inoculated trees matched the type initially inoculated; there was no *X. fastidiosa* movement between trees at either field site.

Over the winter, two trees died at UCD, and 62 trees or inoculated branches died at IRC. Surviving trees (previously infected in 2005) at IRC were evenly distributed among PD, ALS and buffer isolate treatments, with 6 buffer-inoculated, 3 ALS 6-inoculated, 4 Dixon-inoculated, 4 Fresno-inoculated, and 6 Medeiros-inoculated trees surviving. While mortality was high, similar losses were seen in previous studies examining the over

winter survival of *X. fastidiosa* in grapevines in extremely cold climates (Purcell 1980b). No *X. fastidiosa* was recovered from trees at IRC in 2006. At UCD, *X. fastidiosa* was recovered from only one previously-infected tree, the Medieros isolate in a 'Peerless' tree. At both sites, there was negligible leaf scorch and chlorosis in uninfected trees. 'Butte' trees at IRC were beginning to senesce at the time of assessment.

Trees at UCD were subjected to 1076 hours of temperatures below 7°C between inoculation and rating in 2006 (1070 over winter), including 44 hours below 0°C. (An average of 2928 hours elapsed between inoculation and rating in 2005, and 12,223 hours in 2006). Trees at IRC received four times as much cold, 4659 hours over winter between inoculation in July 2005 and rating for disease in September 2006 (4600 over winter) of 10,343 total hours. Trees spent 1852 hours below 0°C at IRC.

Figure 4: *X. fastidiosa*-infections in 'Butte' and 'Peerless' almond trees at Davis (left) and Tulelake (right) field sites 2-4 months after inoculation.



One hypothesis explaining the prevalence of almond-strain *X. fastidiosa* in northern California almond orchards (Henderson 2001, Shapland 2006) is that almond strains initially infect trees at low rates, but survive the winter more frequently than grape strains to cause persistent disease (Almeida and Purcell 2003). Data from this study supported half this hypothesis, since almond strains initially infected 11 to15% of trees, similar to the 21 to 33% infection rate previously reported (Almeida and Purcell 2003). Grape *X. fastidiosa* isolates infected 79% of inoculated trees at UCD in this study, and 64 and 75% in field trials in Davis and Parlier. In this study, no almond infections and only one grape strain *X. fastidiosa* infections over wintered in field plots, although almond-strain *X. fastidiosa* over wintered in potted plants. Almieda and Purcell (2003) found that almost all infections with almond strains survived the winter and 88-42% of

grape-strain infections over wintered, with field grown trees in Parlier, CA, a similar climate to UCD.

Since only one infection survived the winter in the field plots, there is so far no data to support the hypothesis that *X. fastidiosa* infections over winter more frequently in susceptible 'Peerless' compared to 'Butte' since all but one *X. fastidiosa* infections died over winter. The one surviving infection was in susceptible 'Peerless'. The repeated inoculations at UCD in 2007will provide more data to test this hypothesis.
The effect of cold on *X. fastidiosa* survival was previously investigated in grapes but not in almonds. Potted almond trees in the controlled over wintering study, exposed to a 4-month dormancy, had greater numbers of symptomatic leaves than trees dormant for one or two months. This is in contrast to previous research in grapes (Feil and Purcell 2001), where *X. fastidiosa* populations decreased 320-fold in only 18 days at 5°C (41°F). A second replication of the cold-chamber experiment is planned to test the hypothesis that threshold temperatures to kill almond-strain *X. fastidiosa* are lower than those needed to kill grape *X. fastidiosa* in almond trees.

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Recent Publications:

Daane, K. M., Kirkpatrick, B, and Wistrom, C. 2006. Effects of group, cultivar, and climate on the establishment and persistence of *Xylella fastidiosa* infections causing almond leaf scorch, pp. 139-142. *In* M. A. Tariq et al. [eds.], *Proceedings, 2006 Pierce's Disease Research Symposium*. Copeland Printing, Sacramento, CA.

Shapland, E.B., Daane, K.M., Yokota, G.Y., Wistrom, C., Connell, J.H., Duncan, R.A., and Vivieros, M.A. 2006. Ground vegetation survey for *Xylella fastidiosa* in California almond orchards. Plant Disease 90: 905-909.

Two additional peer-reviewed publications are planned.

Daane, K. M., and Wistrom, C. 2006. POSTER: Occurrence and movement of almond leaf scorch in almonds. *91st Pacific Branch Entomological Society of America*. Mar. 2007. Portland, OR.