EFFECTS OF *XYLELLA FASTIDIOSA* GROUP, ALMOND CULTIVAR, AND CLIMATE ON THE ESTABLISHMENT AND PERSISTENCE OF INFECTIONS CAUSING ALMOND LEAF SCORCH

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ABSTRACT

Xylella fastidiosa (*Xf*) must survive multiple winters in an almond tree to reach sufficient populations for vector acquisition and economic impact in the orchard. The effects of cold temperatures on the establishment and persistence of *Xf* infections were measured in field and greenhouse-grown almond trees, with multiple *Xf* strains, and almond leaf scorch (ALS)-resistant and susceptible tree varieties. Potted trees infected with *Xf* were over wintered outside, or in cold rooms at 1.7ºC or 7ºC, for one, two, or four months. Cold exposure time negatively influenced potted tree recovery from *Xf* infection, while cold intensity did not: 21% of trees recovered after one month, 13% after two months, and 7% after four months. In the field, trees at UC Davis (UCD) and Intermountain Research and Extension Center in Tulelake (IRC) inoculated with either grape or almond-strain *Xf* had similar initial infection rates and bacterial populations, but ALS symptoms were much more severe at UCD, especially in 'Peerless' trees. At UCD, 10% of trees with almond-strain, and 78% of trees inoculated with grape-strain *Xf* were infected. Both strains initially infected trees at equal rates at IRC (64% almond, 40% grape). Winter conditions killed all *Xf* infections at IRC and all but one at UCD. These results partially support the hypothesis that almond-strain *Xf* is common in northern California almond orchards because almond strains initially infect trees at low rates, but survive the winter more frequently than grape strains to cause persistent ALS. Grape and almond strains initially infected field-grown trees similar to previously-reported infection rates. However, no almond infections and only one grape *Xf* infection over wintered in the field, even when the winter was mild. Previous studies found that almost all almond strain infections survived the winter, and 88 to 42% of grape-strain infections over wintered in field grown trees. Field inoculations at UCD, and potted tree inoculations were repeated in 2007, to get more data on initial infection establishment and over wintering rates.

OBJECTIVES

1. Compare establishment and over wintering of grape and almond strain *Xf* in susceptible and resistant almond cultivars.
2. Determine the effects of cold treatment on the over winter survival of *Xf* in almonds.

RESULTS

Controlled temperature study

Five months after inoculation with ALS 6 *Xf*, 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 *Xf*, 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 *Xf* after one month cold treatment, compared to 4 of 30 after two months, and 2 of 30 after four months **(Figure 1A)**. There were not significant differences in the number of recovered trees regardless of temperature or time ($\chi 2$ with pairwise comparisons).

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 *Xf*-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 *Xf* infected tree. Symptoms were worse in trees following four months of cold treatment, compared to trees with one and two months **(Figure 1B)**.

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). *Xf* 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 x 10^6 CFU/g. Trees in the 1.7ºC and 7ºC treatments were subjected to temperatures below the growth threshold of *Xf* for the entire period: 769 hours for one month, 1441 hours for two months, and 2905 hours for four months. Outside, trees were below 7ºC for 262 hours in the one month treatment, 557 hours in the two month treatment, and 673 hours for the four month treatments. Only trees kept outside were subjected to sub-freezing temperatures, for 10 hours, both in the two and four month treatments.

Field sites

In 2005, two to four months after inoculation, *Xf* 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; $\chi2 = 0.17$; $P > 0.05$; df =1; Figure 4), nor in median *Xf* populations in infected trees (4.0 x 10^6 colony-forming units per gram of petiole tissue at UCD; 1.2 x 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 2A).**

Grape strain *Xf* infected trees more frequently than almond *Xf* at UCD but not at IRC **(Figure 2B)**. 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 ($\chi2$ with Yates' correction = 38.71; $P < 0.001$; df = 1). At IRC, both almond and grape strains of *Xf* infected trees with the same frequency (16 of 39 inoculated with almond; 24 of 40 inoculated with grape strain; $\chi2 = 2.84$; $P > 0.05$; df = 1). Grape and almond strains reached similar titers in infected plants, median 3.48 x 10^6 CFU/g for almond strain *Xf*, and 5.71 x 10^6 CFU/g for grape strain, both sites combined. All *Xf* recovered from inoculated trees matched the type initially inoculated; there was no *Xf* 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 *Xf* in grapevines in extremely cold climates (Purcell 1980). No *Xf* was recovered from trees at IRC in 2006. At UCD, *Xf* 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 7ºC between inoculation and rating in 2006 (1070 over winter), including 44 hours below 0ºC (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 0ºC at IRC.

DISCUSSION

One hypothesis explaining the prevalence of almond-strain *Xf* in northern California almond orchards (Hendson 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 *Xf* 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 *Xf* infections over wintered in field plots, although almond-strain *Xf* over wintered in potted plants. Almeida 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 *Xf* infections over winter more frequently in susceptible 'Peerless' compared to 'Butte' since all but one *Xf* infections died over winter. The one surviving infection was in susceptible 'Peerless'. Repeated inoculations at UCD in 2007 will provide more data to test this hypothesis. The effect of cold on *Xf* survival was previously investigated in grapes but not in almonds. Exposure to very cold over wintering temperatures can eliminate *Xf* infections but also results in significant plant mortality (Purcell 1980). Potted almond trees in the controlled study exposed to four-month dormancy had more symptomatic leaves than trees dormant for one or two months. This is in contrast to previous studies in grapes (Feil and Purcell 2001), where *Xf* populations decreased 320-fold in only 18 days at 5ºC (41ºF). A second replication of the cold-chamber experiment is ongoing to test the hypothesis that threshold temperatures to kill almond-strain *Xf* are lower than those needed to kill grape *Xf* in almond trees.

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FUNDING AGENCIES

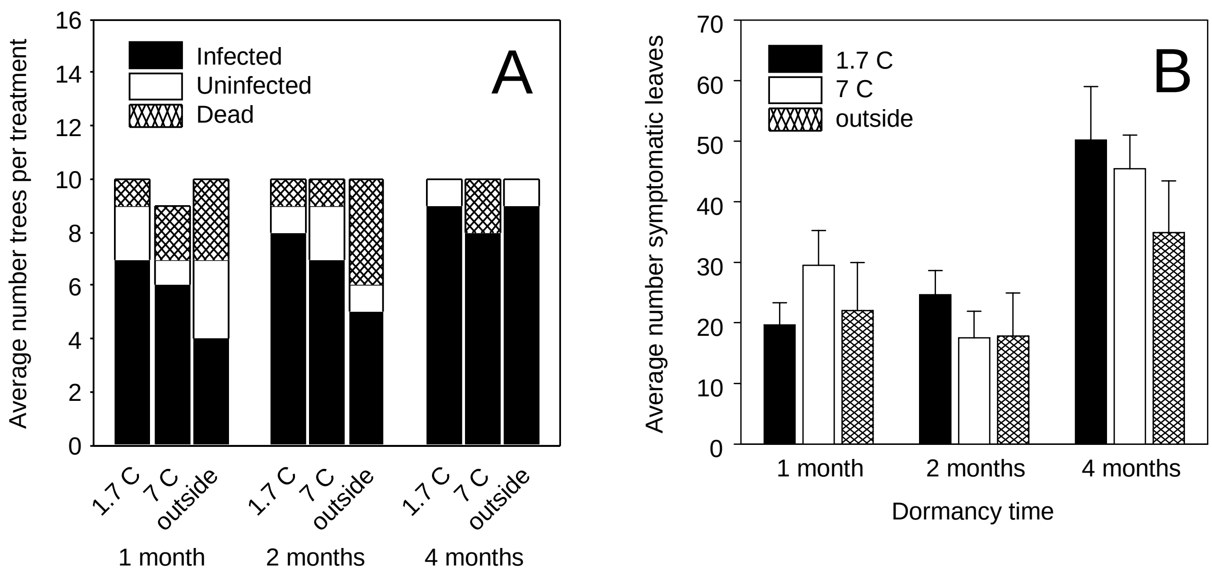

Figure 1. A) *Xf* infections and **B)** Almond Leaf Scorch symptoms in previously-infected potted almond trees after one, two or four months of dormancy at 1.7ºC, 7ºC, or outside in Parlier, CA.

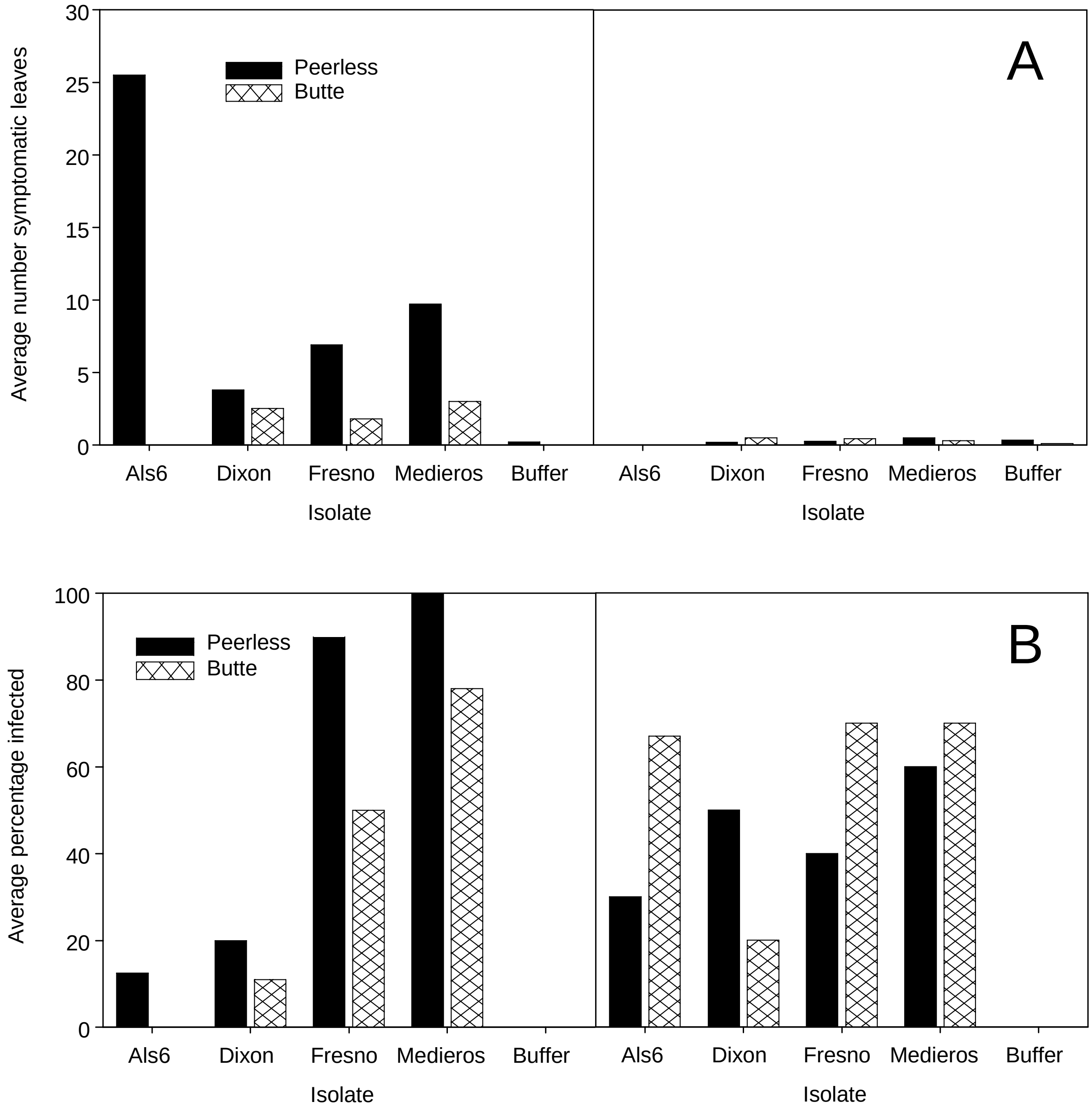

Figure 2. A) Almond leaf scorch symptoms and **B)** *Xf*-infections in 'Butte' and 'Peerless' almond trees at Davis (left) and Tulelake (right) field sites two to four months after inoculation.