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

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ABSTRACT
Almonds are one of the most widely-grown crops that can host *Xylella fastidiosa (Xf)*, so it is useful to assess the potential for almonds to serve as a source of *Xf* infections in grapes, and to explain why *Xf* dispersal between almond orchards and vineyards is uncommon. We are comparing infection establishment and survival at two field sites and in a controlled test with potted plants, varying three factors that may influence almond leaf scorch in almonds: cultivar, genetics of the pathogenic bacteria, and winter severity. In spring and summer 2005, we inoculated grape type and two almond types of *Xf* into highly susceptible 'Peerless' and less-susceptible 'Butte' almond trees. After vector inoculation, *Xf* must survive multiple winters in an almond tree to reach sufficient populations for sharpshooter acquisition and economic impact disease levels. Therefore, field sites were selected with moderate and severe winter temperatures. We also initiated a controlled dormancy test with potted plants and cold storage rooms at Kearny Agricultural Center, Parlier, California. Almond trees in the field were inoculated with buffer or *Xf* belonging to the grape type or two almond types, and will be held at different chill temperatures for varying lengths of time.

INTRODUCTION
Because almonds are one of the most widely-grown crops that can host *Xf* in the Central Valley, they might serve as a source of *Xf* infections in grapes, although for unknown reasons *Xf* dispersal between almond orchards and vineyards is uncommon (A. Purcell – *unpublished data*). Almond leaf scorch (ALS) is caused when *Xf* multiplies extensively within the xylem of infected trees, eventually severely limiting nut production (Davis et al. 1980). The disease was first formally described in 1974, though outbreaks occurred in Los Angeles and Contra Costa counties in the 1950's (Moller et al. 1974). Symptoms of ALS are similar to Pierce's disease (PD) in grapes and include leaves with marginal necrosis and chlorosis and the lack of terminal growth. Initial infections spread slowly and often occur only on one branch, but after a few years are easily visible on the entire tree (Almeida and Purcell 2003c), reducing almond productivity (Mircetich et al. 1976, Moller et al. 1974). In both grapes and almonds, *Xf* multiplies to high populations (1,000,000 bacteria per gram of plant tissue) and is acquired and transmitted by insect vectors (Almeida and Purcell 2003a, Almeida and Purcell 2003c, Purcell 1980a). In laboratory tests, *Xf* was transmitted to almonds by 5 species of xylem-feeding insects, including the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Almeida and Purcell 2003b, 2003c).

In previous studies, almond cultivars varied greatly in their susceptibility to ALS, with some developing extensive leaf scorch, and others showing little disease. *Xf* inoculations made from May through July had the best odds of surviving the following winter. (B. Kirkpatrick – *unpublished data*). We compared *Xf* infection establishment and survival in two cultivars, highly susceptible 'Peerless' and less-susceptible 'Butte,' both on Nemaguard rootstock.

The genetic type of *Xf* may also impact almond susceptibility, and will certainly impact formation of PD. Three genetic types of *Xf* have been identified from almond trees. One type was identical to *Xf* from PD-infected grapevines. The other two genetic types were unique to almonds (Hendson et al. 2001). The three types were distinguished by growth on selective media and DNA digestion with restriction enzymes (Almeida and Purcell 2003c). Recent cross-inoculation studies in the greenhouse showed that the genetic type influenced the ability of the bacteria to over winter in grapes or almonds, as almond types died in grapes and grape types died in almonds (Almeida and Purcell 2003c). For this reason, we also used different genetic types of *Xf* in our field trials.

After vector inoculation, *Xf* must survive multiple winters in an almond tree to reach sufficient populations for sharpshooter acquisition and economic impact disease levels. Growth chamber and field studies with grapevines showed that the degree of plant dormancy, as well as severe cold, affected the over winter survival of *Xf* (Feil and Purcell 2001, Purcell 1980b). To date, there is no information available on the effects of winter dormancy on *Xf* infections in almonds. Growth chamber and

field studies with grapevines showed that the degree of plant dormancy, as well as severe cold, affected the over winter survival of *Xf* infections. To date, there is no information available on winter dormancy effects on *Xf* infections in almonds.

We are comparing infection establishment and survival at two field sites and in a controlled test with potted plants, varying three factors that may influence almond leaf scorch in almonds: cultivar, genetics of the pathogenic bacteria, and winter severity. Therefore, field sites were selected with moderate and severe winter temperatures (Armstrong Farm at University of California, Davis, and Intermountain Research and Extension Center at Tulelake, California, respectively) in order to study treatment impact under different winter temperatures. A controlled dormancy severity test with potted plants and growth chambers was also started at Kearny Agricultural Center, near Parlier, California.

OBJECTIVES

1. Compare the establishment and multi-year persistence of *Xf* isolates belonging to three ALS genetic groups in almond cultivars with either low or high susceptibility to almond leaf scorch.
2. Compare effects of winter severity and the degree of plant dormancy on the infection rate, symptom severity, and titer of *Xf* in inoculated almonds.
3. Use collected data on almond leaf scorch development to determine if almond orchards may serve as a reservoir of *Xf*.

RESULTS

Field trials

One hundred bare-root almond trees, fifty of each cultivar, were planted in spring 2005 at two different field sites: Armstrong Farm at University of California, Davis, (hereafter referred to as UCD), and Intermountain Research and Extension Center, Tulelake, CA (hereafter referred to as IRC). Trees were planted in a complete randomized block design with a split plot (almond cultivars) in each block. There are ten replicates of each treatment combination (*Xf* isolate x almond cultivar). Trees are drip irrigated at UCD and sprinkler irrigated at IRC.

The almonds trees were inoculated with different genetic types of *Xf.* In our study, each tree was inoculated with one of five treatments: Fresno-ALS (isolated from almonds but genetically similar to *Xf* that causes PD in grapes; PD-*Xf*), Dixon (ALS-*Xf* type 1) and ALS 6 (ALS-*Xf* type 2), Medeiros (from grapes), or buffer control. All isolates of *Xf* were isolated from infected plants in Solano, Fresno, or San Joaquin Counties, and were pathogenic in recent greenhouse tests. Inoculations were done in early May (UCD) and early July (IRC) when the young shoots were at least 6 mm in diameter. Inoculum was prepared in the field from two week old cultures *Xf* grown on solid media. Each isolate was mechanically inoculated into 3 or 4 sites in 1 stem per plant by placing a 10 µL drop of bacteria suspended in sodium-citrate-phosphate buffer (approximately 10,000,000 bacteria/ mL). The drop was placed on a green, growing shoot and probed with a #2 insect pin until it was drawn into the stem. Inoculation sites were marked with permanent metal tags and paint.

Leaves immediately adjacent to the inoculation sites were tested for *Xf* in fall 2005 to see if inoculations were successful. The severity of infection was rated by the number of scorched leaves on the inoculated stem. Almond petioles from each tree were cultured to determine *Xf* infection and population. Subsequent strain identification of *Xf* was accomplished by re-streaking growing bacteria on two different artificial media, PD3 and PWG (Davis et al 1983, Davis et al 1980, Hill and Purcell 1995). All types of *Xf* grow on PWG, while ALS-*Xf* type 2 and PD types grow on PD3 as well. ALS-*Xf* type 1 does not (Almeida and Purcell 2003c). To separate ALS and PD isolates, polymerase chain reaction (PCR) was used to amplify DNA from the bacteria, and DNA was digested with *Rsa 1,* a restriction enzyme that cuts the DNA of ALS-*Xf* isolates into two pieces, but does not cut the DNA of PD-*Xf* (Almeida and Purcell 2003c). We will be able to know what infections over wintered by summer 2006, and compare infection establishment, bacterial titer, and rate of disease development in field-grown almond trees. Trees will also be evaluated for the presence of *Xf* in 2007 and 2008.

A preliminary screening found that almond leaf scorch symptoms were much more severe at the UCD site, especially in 'Peerless' trees, with an average of 4.6 scorched leaves per tree, compared to 0.8 in 'Butte.' Both cultivars at IRC had no scorched leaves, an average of 0.2 and 0.1 leaf per tree for 'Butte' and 'Peerless,' respectively. However, there was no difference in the proportion of infected trees at UCD (32 of 78 infected at UCD, 41 of 96 infected at IRC; Chi-square $P > 0.05$), nor in the median populations of *Xf* present in inoculated trees at UCD (6.2×10^6 CFU/g) or IRC (1.3×10^7; $\log_{10}$-transformed; $P = 0.26$). The difference in symptoms may have two explanations: i) trees at UCD were tested for *Xf* 3.5 mos after inoculation and had longer to develop symptoms, compared to trees at IRC, which were tested 2 months after inoculation; or ii) the infected trees were under more moisture stress at UCD, which led to the development of disease symptoms.

There were not large differences between infection percentage (41% of 'Butte,' 38% of 'Peerless'; Chi-Square $P>0.05$), or *Xf* population (2×10^6CFU/g for 'Peerless' and 9×10^6CFU/g for 'Butte'; $\log_{10}$-transformed; $P = 0.11$) for the two cultivars. 'Peerless' had much fewer scorched leaves than 'Butte' at UCD, but not at IRC, as discussed in the previous paragraph.

One significant difference was the infection percentage of the various isolates, as grape strain *Xf* was more frequently recovered from inoculated trees than either almond strain. Fresno and Medeiros were recovered from 64 and 77% of trees, respectively, whereas ALS6 and Dixon were recovered from 27 and 28% of trees. Leaf scorch symptoms were more severe

in trees inoculated with grape-type isolates Fresno and Medeiros (an avg. of 2.8 and 3.2 scorched leaves/tree), compared to almond isolates Dixon and ALS6 (0.3 and 0.9 scorched leaves/tree), and background leaf scorch in buffer-inoculated trees (0.1/ tree).

Bacterial populations in trees infected with grape and almond isolates were similar, even though infection percentage and symptom severity was greater in grape isolates of *Xf*. Median populations of *Xf* in infected trees were: 6.2 x 10^6 CFU/g (ALS6), 2.8 x 10^6 CFU/g (Dixon), 5.5 x 10^6 CFU/g (Fresno), 2.4 x 10^7 CFU/g (Medeiros), and 0 CFU/g (buffer). Bacterial populations were high even in only a few trees in the treatment were infected with *Xf*, as in ALS6 inoculated plants at UCD. In the future, ArcSin transformation may be necessary with infection data, and log10 transformation may be necessary to analyze population data. We will use ANOVA where applicable to detect differences in infection percentage and bacterial populations between cultivars and bacterial isolates.

Glasshouse and Growth Chamber trial

An additional experiment was initiated to examine the effect of over wintering temperature in the survival of *Xf* infections in controlled environments. We inoculated 155 potted two-year-old 'Peerless' almond trees in spring 2005. One hundred twenty five trees were inoculated with the ALS 6 isolate of *Xf* and 30 with buffer alone, in the same manner as for the field plots. Trees were kept in the greenhouse at Kearny Agricultural Center (Parlier, CA) and were tested for infection in fall 2005. Only trees positive for *Xf* will be used for the rest of the experiment (108 infected trees total, 27 buffer-inoculated). Trees will be allowed to go dormant in screen cages outside.

In December 2005, plants will be divided equally between treatments. One-third will remain outside in the field, 1/3 will be kept at 7°C (45°F), and 1/3 at 1.7°C (35°F). *Xf* dies at these temperatures in grapevines (Almeida and Purcell 2003c, Feil and Purcell 2001). Trees will be removed from each cold treatment at intervals of 1, 2 and 4 months, and allowed to break bud in the greenhouse. These intervals are reflective of dormancy periods used in previous studies with almonds and grapevines (1 mo.; Almeida and Purcell 2003c, Feil and Purcell 2001), typical dormancy in the Central Valley (2 mos.; going fully dormant in December and flowering in February) and an extreme treatment for abnormally long dormancy (4 months). Plants will be kept the greenhouse until they develop almond leaf scorch symptoms, and then sampled via culture as previously described.

CONCLUSIONS

As this is the first year of a three-year study, with the over wintering portion of the treatment yet to be applied, it would be premature to draw conclusions from our data at this time. The effect of overwintering conditions on *Xf* infections will be determined by culturing in summer 2006. Inoculations for plants where *Xf* was not recovered will be repeated in May 2006, and isolations will be repeated in August and September 2006 and 2007. These preliminary results were collected at UCD and the IRC in August and September 2005. Further samples remain to be taken from UC Davis and Kearny Agric. Center.

REFERENCES

Almeida, R.P.P. and A.H. Purcell.. 2003a. *Homalodisca coagulata* transmission of *Xylella fastidiosa* to almond. Plant Disease 87: 1255-1259.

Almeida, R.P.P. and A.H. Purcell. 2003b. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata*. J. Econ. Entomol. 96: 264-271.

Almeida, R.P.P. and A.H. Purcell. 2003c. Biological traits of *Xylella fastidiosa* strains from grapes and almonds. Appl. Environ. Microbiol. 69: 7447-7452.

Davis, M.J., B.C. Raju, R.H. Brlansky, R.F. Lee, L.W. Timmer, R.C. Norris and R.E. McCoy. 1983. Periwinkle wilt bacterium: Axenic culture, pathogenicity, and relationships to other gram-negative, xylem-inhabiting bacteria. Phytopathology 73: 1510-1515.

Davis, M.J., S.V. Thomson and A.H. Purcell. 1980. Etiological role of the xylem-limited bacterium causing Pierce's disease in almond leaf scorch. Phytopathology 70: 472-475.

Feil, H. and A.H. Purcell. 2001. Temperature-dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. Plant Disease 85: 1230-1234.

Hendson, M., A.H. Purcell, D. Chen, C. Smart, M. Guilhabert and B. Kirkpatrick. 2001. Genetic diversity of Pierce's disease strains and other pathotypes of *Xylella fastidiosa*. Appl. Environ. Microbiol. 67: 895-903.

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

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

Moller, W.J., R.R. Sanborn, S.M. Mircetich, H.E. Williams and J.A. Beutel. 1974. A newly recognized and serious leaf scorch disease of almond. Plant Dis. Rep.58: 99-101.

Purcell, A.H. 1980a. Almond leaf scorch: Leafhopper and spittlebug vectors. J. Econ. Entomology 73: 834-83.

Purcell, A.H. 1980b. Environmental therapy for Pierce's disease of grapevines. Plant Disease 64: 388-390.

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