Susceptibility to *Xylella fastidiosa* in a First-generation Hybrid from a non-traditional Peach–Almond Cross

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Abstract. To facilitate development of *Prunus* L. rootstocks with desirable agronomic traits, domesticated peach (*Prunus persica*) and almond (*P. dulcis*) were crossed with wild almond relatives. This work reports that a hybrid from a *P. webbii* × *P. persica* cv. Harrow Blood cross is susceptible to almond leaf scorch disease (ALSD). ALSD is caused by the fastidious, xylem-limited bacterium *Xylella fastidiosa*. The *P. webbii* × ‘Harrow Blood’ hybrid, along with its parents, was inoculated with two ALSD-inducing strains (*X. fastidiosa* subsp. *fastidiosa* strain M23 and subsp. *multiplex* strain Dixon). Both *X. fastidiosa* strains grew to high titer in the susceptible *P. webbii* parent and in the interspecific hybrid; defoliation was also observed. As expected, ‘Harrow Blood’ did not exhibit defoliation symptoms or support growth of *X. fastidiosa*. This result contrasts with earlier work demonstrating that a *P. persica* × *P. dulcis* hybrid is not a suitable host for *X. fastidiosa* subsp. *fastidiosa* M23. It appears that the genetic basis of resistance/susceptibility differs between a *P. persica* × *P. dulcis* cross and the *P. webbii* × *P. persica* cross reported here. Understanding the degree of susceptibility to *X. fastidiosa* in complex hybrids of subgenus *Amygdalus* should be an important part of rootstock development.

Selection of the most appropriate rootstock is a critical variable for maximizing almond production. To this end, development of novel rootstocks is a key component of many *Prunus* breeding programs. ‘Nemaguard’, a seed-propagated rootstock with resistance to *Meloidogyne incognita* and *M. javanica* and wide graft compatibility, has been a standard in California orchards since its introduction (Weinberger, 1959). Today, rootstock breeding efforts focus on developing molecular markers for important horticultural traits. To this end, numerous large segregating populations were created to provide a means of linking molecular markers to specific phenotypic categories. *Prunus persica* (L.) Batsch cv. Harrow Blood, a cold-hardy but root knot nematode-susceptible rootstock, was hybridized with root knot

nematode-resistant ‘Okinawa’ peach for the further development of an F2 population segregating for root knot nematode resistance (Gillen and Bliss, 2005). Several F2 trees from this population were later selected and released as clonal rootstocks for stone fruit and almonds with scion vigor-controlling abilities (Bliss et al., 2011, 2012a, 2012b, 2013). ‘Harrow Blood’ also was hybridized with an undomesticated almond [*Prunus webbii* (Spach) Vierhapper] to generate a diverse F2 population segregating for floral, vegetative, and fruiting characteristics. Use of almond species other than *Prunus dulcis* (Mill.) D.A. Webb for rootstock development broadens the genetic base and introduces diversity beyond that present in standard peach × almond crosses (Kester and Gradziel, 1996). This large *P. webbii* × ‘Harrow Blood’ population is being studied broadly relative to parental characters, including phenology of the trees during bloom and fruit ripening, tree vigor, morphological characters, and reaction to inoculation with *Xylella fastidiosa*.

Xylella fastidiosa is a fastidious, Gram-negative gamma-proteobacterium that is a xylem-limited phytopathogen (Wells et al., 1987). *X. fastidiosa* causes a variety of leaf scorching and scalding diseases including ALSD and Pierce’s disease (PD) of grapevine. *X. fastidiosa* multiplies in the plant, cutting off free flow of water and nutrients in infected xylem vessels. As the disease progresses, leaves turn yellow and die (scorching), fruit withers, and eventually the whole plant dies. Although as a species, *X. fastidiosa* can infect a wide range of host species, individual strains tend to be much

more limited in host range (Schaad et al., 2004). Host range and genomic differences have been used to divide *X. fastidiosa* into four subspecies: *fastidiosa*, *multiplex*, *pauca*, and *sandyi* (Schaad et al., 2004; Schuenzel et al., 2005). Subspecies *pauca* strains are found in South America and generally infect either coffee or citrus but not both (Almeida et al., 2008). PD is caused by subsp. *fastidiosa* strains, which also are capable of causing ALS (Chen et al., 2005). Oleander leaf scorch is caused by strains of subsp. *sandyi* (Purcell et al., 1999). Subsp. *multiplex* appears to be the only subspecies native to the United States (Nunney et al., 2010) and has been isolated from a wide variety of species, most notably tree species such as olive, almond, peach, plum, oak, elm, pecan, and mulberry (Hernandez-Martinez et al., 2006; Krugner et al., 2014; Melanson et al., 2012; Parker et al., 2012; Schaad et al., 2004; Schuenzel et al., 2005). An interspecific homologous recombination has been shown to occur among various *X. fastidiosa* strains and likely has enabled a recent shift of subsp. *multiplex* to blueberry (Nunney et al., 2014).

ALSD has been present in California for more than 60 years (Almond Board of California, 2004). Nut yields have been shown to be significantly reduced for infected trees, although some almond cultivars are more susceptible to yield reduction than others (Sisterson et al., 2008). In contrast, peach does not appear to be susceptible to *multiplex* or *fastidiosa* strains that cause ALS (Mircetich et al., 1976; Wells et al., 1981). Peach is susceptible to phony peach strains of *X. fastidiosa* (Hopkins et al., 1973), which are not currently present in California orchards. Hence, peach rootstocks would not be expected to contribute to increasing the frequency of ALS. However, because peach × almond (*P. persica* × *P. dulcis*) hybrids are being increasingly used as rootstocks in California almond orchards, susceptibility of such hybrids are of concern. One peach × almond hybrid has been shown to resist infection by *X. fastidiosa* subsp. *fastidiosa* strain M23 (Ledbetter and Rogers, 2009). In addition to these standard peach × almond hybrids, wild almond (*Prunus webbii*) has been used as a source of genetic diversity in *Prunus* rootstock breeding efforts (Ledbetter et al., 2009). Similar to sweet almond, *P. webbii* has been shown to be susceptible to *X. fastidiosa* subsp. *fastidiosa* strain M23; however, it was more readily cured of infection by the mild winter chilling experienced in central California (Ledbetter et al., 2009). The present study examines peach (*Prunus persica* cv. Harrow Blood), wild almond (*P. webbii*), and their interspecific hybrid for susceptibility to *X. fastidiosa* subsp. *fastidiosa* strain M23 and subsp. *multiplex* strain Dixon.

Materials and Methods

Plant materials. Mature trees of peach (*Prunus persica* cv. Harrow Blood), *P. webbii*, and an F1 hybrid (P66) created from them (*P. webbii* × ‘Harrow Blood’) were available

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in the Parlier research orchard to supply clonal scion materials for propagation. Dormant 1-year-old 'Nemaguard' peach rootstock seedlings were sourced locally (Dave Wilson Nursery, Reedley, CA) and potted in 15.6-L pots filled with # 4 Sunshine Mix (SunGro, Seba Beach, Alberta, Canada), providing sufficient volume for root growth and development during the growing season. Approximately 3 weeks after planting (mid-February), dormant scions were cleft-grafted to potted rootstocks and allowed to heal under ambient environmental conditions. In each growing season, 48 seedling 'Nemaguard' rootstocks were grafted with each of the three clonal scions to provide sufficient replicates for *Xylella fastidiosa*-inoculated trees and non-inoculated controls. As bud swell became evident on dormant scions, grafted trees were moved to a climate-controlled greenhouse. Growing trees were trained to a single shoot arising from the grafted scion. Tree maintenance during the growing season consisted of weekly irrigation, application of crop care products, and fertilization as needed to maintain an optimum growth environment. Replicate experiments were conducted in the 2012 and 2013 growing seasons; similar results were obtained both years. Data shown are from 2013.

***X. fastidiosa* inoculations.** *X. fastidiosa* subsp. *fastidiosa* strain M23 and subsp. *multi-plex* strain Dixon were used for all inoculations. M23 was originally isolated from an ALSD-affected almond tree in Kern County, CA (Chen et al., 2005); Dixon was originally isolated from an ALSD-affected almond tree in Solano County, CA (Hendson et al., 2001). Bacteria were grown on periwinkle wilt agar as previously described (Davis et al., 1981). After 10 to 12 d growth at 28 °C, colonies were scraped from plates, washed three times in sterile H₂O, and resuspended at an OD₆₀₀ = 0.2. Each scion was inoculated with either M23 or Dixon by placing a 10-μL drop of bacterial suspension ≈5 to 10 cm above the graft union and piercing the stem under the drop with a needle; a second 10-μL drop was placed on the opposite side of the stem and pierced for a total of 20 μL inoculum per replicate. Eight to 18 replicates per genotype were inoculated with each bacterial strain and a similar number was set aside as non-inoculated controls.

Plant sampling. Approximately 24 weeks after inoculation, symptom expression was assessed and samples were harvested to determine presence of *X. fastidiosa*. First, the distance between the graft union and the position of the lowest leaf remaining on the scion was measured. Second, two petioles were harvested from positions ≈30 cm above the graft union; for replicates exhibiting defoliation, the lowest two remaining petioles were taken. Finally, a 2-cm section of stem was harvested from a position 30 cm above the graft union. One *P. webbii* replicate only grew 25 cm above the graft union; so, for this seedling, petioles and the 2-cm stem segment were harvested at ≈20 cm above the graft union.

***X. fastidiosa* quantitation.** *X. fastidiosa* titer in plant tissues (both petioles and stems) was measured by quantitative real-time polymerase chain reaction (qRT-PCR) using a Step One Plus RT-PCR machine (Applied Biosystems, Foster City, CA) as previously described (Ledbetter and Rogers, 2009). Data were compiled and analyzed in Excel (Version 2010; Microsoft Corporation, Redmond, WA) using the *t* test (two sample assuming unequal variances) and analysis of variance (single factor) functions as found in the Excel Analysis ToolPak Add-In.

Results

Defoliation. ALSD symptoms on sweet almond (*P. dulcis*) can be expected to first appear as leaves with dead, brown edges and adjacent chlorotic areas (scorching) located mainly on the basal portion of the scion above the inoculation site. *P. webbii*, the susceptible parent, displayed very limited scorching on lower leaves starting 2 to 3 months after inoculation. At that point, *P. webbii* also began to defoliate, starting at the base of the scion. As the growing season progressed, the defoliation continued; classic scorching symptoms were observed on only a couple leaves on any of the 22 inoculated *P. webbii* seedlings (11 inoculated with each *X. fastidiosa* strain). The P66 hybrid displayed a similar defoliation and lack of scorching. At no point in the growing season was either scorching or defoliation observed on the resistant parent, 'Harrow Blood', or on any of the uninoculated controls.

At the conclusion of the experiment, 24 weeks after inoculation, significant defoliation was observed in inoculated *P. webbii* and hybrid P66 plants, as shown in Figure 1. Position of the lowest leaves remaining varied from 10 to 60 cm above the graft union for *P. webbii* plants and 10 to 55 cm for P66 plants. P66 plants inoculated with M23 exhibited significantly less defoliation than did *P. webbii* plants inoculated with M23 (22.2 cm vs. 30.5 cm; *t* test with unequal variance; *P* < 0.03). Defoliation did not differ between *P. webbii* and P66 plants inoculated with Dixon.

***X. fastidiosa* titer.** Because of the defoliation observed on many *P. webbii* and P66 plants, it was not possible to sample petioles at 30 cm above the graft union on most *P. webbii* and P66 plants. If no leaves were present 30 cm above the graft union, the lowest two securely attached petioles were sampled. *X. fastidiosa* was only detected in petioles from five of 11 *P. webbii* plants inoculated with M23 and petioles from two of 11 *P. webbii* plants inoculated with Dixon (data not shown). No *X. fastidiosa* was detected in P66 hybrid petioles. Petioles were harvested from 30 cm above the graft union on 'Harrow Blood'; no *X. fastidiosa* was detected in any 'Harrow Blood' petioles nor in any uninoculated control petioles.

To improve and standardize detection of *X. fastidiosa*, 2-cm stem segments were harvested from all plants for DNA extraction and

qRT-PCR. As shown in Figure 2, *X. fastidiosa* was detected in stems from all 22 inoculated *P. webbii* plants (11 with M23 and 11 with Dixon) and in 20 of 36 P66 hybrid plants (14 of 18 with M23 and six of 18 with Dixon). Titers of both M23 and Dixon were significantly lower in P66 than in *P. webbii*. Like with petioles, no *X. fastidiosa* was detected in any 'Harrow Blood' stem segments or segments from uninoculated controls.

Discussion

This study examined potential host suitability of several *Prunus* genotypes using two different virulent *X. fastidiosa* strains (M23, subsp. *fastidiosa* and Dixon, subsp. *multi-plex*). Both strains were originally isolated from ALSD-affected almond trees in the San Joaquin Valley of California and have shown significant virulence on almond in previous studies (Krugner et al., 2014; Ledbetter et al., 2009; Ledbetter and Rogers, 2009). Because both subspecies are commonly present in almond orchards, testing susceptibility with both gives a more complete picture of the potential for disease development. Our previous greenhouse studies with M23 have achieved close to 100% ALSD symptom development and disease initiation using similar growth regimes and inoculation procedures (Ledbetter et al., 2009; Ledbetter and Rogers, 2009).

As a result of the tendency of *P. webbii* to defoliate after inoculation, *X. fastidiosa* detection by qRT-PCR was more robust and reproducible when stem segments, rather than petioles, were sampled. We hypothesize that as soon as a low threshold of *X. fastidiosa* is present in an individual *P. webbii* petiole, petiole senescens and the leaf falls off. This hypothesis explains the lack of detectable *X. fastidiosa* in most *P. webbii* petioles and in all P66 hybrid petioles sampled. The qRT-PCR assay has a lower limit of detection of ≈200 *X. fastidiosa* genomes per petiole (E.E. Rogers, unpublished data). Two hundred bacterial cells spread over a whole petiole might not be expected to cause defoliation. However, if the *X. fastidiosa* was concentrated in the base of the petiole, adjacent to the stem, bacterial titers might very well be high enough to block xylem elements, causing localized senescence and leaf loss. Our hypothesis also explains the lack of classic scorching symptoms on *P. webbii* and P66 hybrid leaves; individual leaves fall off long before *X. fastidiosa* titers are high enough to cause scorch symptom development.

There was no correlation between titer of *X. fastidiosa* and position of the lowest leaf in any *Prunus* genotype tested (data not shown). This includes the F1 hybrid P66 where *X. fastidiosa* was not detected in 16 of 36 individuals (two of 18 for M23 and 12 of 18 for Dixon). Some P66 individuals with undetectable *X. fastidiosa* showed significant defoliation, whereas other individuals had high titers but little defoliation. Additional experimentation is needed to explain the obviously complex relationship between

Fig. 1. Defoliation observed in inoculated seedlings. Average distance between graft union and position of lowest securely attached leaf was measured in centimeters. HB = *P. persica* cv. Harrow Blood; PW = *P. webbii*; P66 = *P. webbii* × ‘Harrow Blood’ hybrid. Error bars indicate sd; letters indicate significant differences among averages ($P < 0.03$ for all comparisons).

Fig. 2. Titer of *X. fastidiosa* genomes per 2-cm stem sample. Average titer was measured by quantitative real-time polymerase chain reaction. Numbers below the x-axis are number of plants with detectable *X. fastidiosa* over total number of plants inoculated. Xf = *X. fastidiosa*; HB = *P. persica* cv. Harrow Blood; PW = *P. webbii*; P66 = *P. webbii* × ‘Harrow Blood’ hybrid. Error bars indicate sd. Horizontal lines above bars indicate significant differences between averages (** $P < 0.01$; *** $P < 0.001$).

bacterial titer and defoliation. The intermediate susceptibility to *X. fastidiosa* demonstrated by the P66 hybrid is consistent with a codominant mode of inheritance for this trait.

As expected, this study demonstrated that peach parent ‘Harrow Blood’ was resistant to both almond strains of *X. fastidiosa* with inoculated plants remaining asymptomatic and bacterial populations below the limit of detection. Susceptibility of *P. webbii* to M23 was confirmed and expanded to include strain Dixon (Ledbetter et al., 2009). The interspecific hybrid, P66, was shown to be almost as susceptible as the *P. webbii* parent, indicating susceptibility follows a dominant mode of

inheritance in this cross. This contrasts with our previous finding that a *P. persica* × *P. dulcis* interspecific hybrid was not a suitable host for M23 (Ledbetter and Rogers, 2009); therefore, it appears that resistance is dominant in the *P. persica* × *P. dulcis* cross. There is precedence for differences in the genetic basis of resistance/susceptibility: approximately one-third of *Citrus* interspecific hybrids (‘Murcott’ tangor-resistant × ‘Pera’ sweet orange-susceptible) are susceptible to Citrus leprosis virus; the remaining two-thirds are resistant (Bastianel et al., 2006). Additionally, ‘Murcott’ tangor is resistant and ‘Pera’ sweet orange is susceptible to Citrus variegated chlorosis caused by *X. fastidiosa*. All

‘Murcott’ × ‘Pera’ interspecific hybrids tested have shown detectable levels of *X. fastidiosa* and many also developed disease symptoms (Coletta-Filho et al., 2007). Systematic inoculation and screening of F2 siblings from both the *P. webbii* × ‘Harrow Blood’ and the *P. persica* × *P. dulcis* crosses will provide more detailed information on the inheritance of *X. fastidiosa* susceptibility in *Prunus* rootstock germplasm.

This study raises serious implications for *Prunus* rootstock breeding with interspecific hybrids. Both popularity and availability of new “peach–almond” rootstocks have increased in the last decade as has nursery advertisement for the horticultural benefits derived from using these new hybrids. Specific classes of interspecific hybrids such as peach–almond hybrids must not be considered uniform with regard to their general or specific reactions to phytopathogens or horticultural qualities. *X. fastidiosa* transmission from infected *Prunus* rootstocks to scions has been previously observed (Latham et al., 1980). Use of rootstocks susceptible to *X. fastidiosa* allows another potential route of entry for *X. fastidiosa* and subsequently ALSD and its associated yield loss in commercial almond plantings. Our results here indicate that any *Prunus* hybrid of potential agronomic importance should be tested on an individual basis to determine its degree of susceptibility to *X. fastidiosa*.

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