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Fire Blight Susceptibility of 20 Diverse Pear *(Pyrus spp.)* Rootstock Breeding Parents

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Abstract

Fire blight is a bacterial disease caused by *Erwinia amylovora*, which can cause devastating losses to pear (*Pyrus* spp.) growers. Infections can lead to a reduction in fruit yield, the need to remove some or all scion tissues, and entire tree death. Rootstocks with lower fire blight susceptibility can confer some degree of tolerance to susceptible scions. Since most U.S. pear cultivars are susceptible to fire blight infection, breeding low-susceptibility rootstock cultivars can help decrease losses for the pear industry. The Washington State University (WSU) Pear Rootstock Breeding Program was established to develop *Pyrus* rootstocks, with target traits such as dwarfing, precocity, cold-hardiness and reduced fire blight susceptibility. This study evaluated fire blight response of 20 diverse accessions, as grafted scion tissue. Two greenhouse experiments were conducted in 2021 on up to 20 individuals per accession, which were arranged in a randomized complete block design with four blocks and five replicates. One actively growing shoot per tree was inoculated with *E. amylovora* strain 153n. Fire blight response was measured after disease progression stopped and was quantified as percent shoot length blighted (%SLB). Average accession responses ranged from 0.1 to 100 %SLB and were highly correlated between experiments (Pearson's $r = 0.83$, $P \leq 0.001$). Individuals in Experiment B had significantly higher severity of infection; however, the relative order of accession based on severity was consistent with that of Experiment A. In both experiments, nine accessions consistently exhibited low fire blight susceptibility (0.1 to 10.9 %SLB), while six accessions had high fire blight susceptibility (35.2 to 100 %SLB). Results from this study provide insights for 20 potential breeding parents in the WSU Pear Rootstock Breeding Program.

The Pacific Northwest (PNW) accounts for around 80% of U.S. pear (*Pyrus* spp.) production, which was valued at over $290 million in 2021 (USDA-ARS NASS, 2022). Pear orchards in the PNW typically use semi-dwarfing *Pyrus* rootstocks with only a few hundred trees per acre compared to thousands of trees per acre in high-density plantings (Elkins et al., 2012). Globally, pear producers typically use quince rootstocks to reduce scion vigor and facilitate high-density plantings; however, concerns about lack of cold-hardiness and potential graft incompatibility with pear scion cultivars have limited adoption of quince rootstocks in major pear-producing regions of the United States (Einhorn, 2021).

High-density planting systems allow for more uniform canopy structure and disease/pest management, thereby reducing labor and input costs while increasing production efficiency (Elkins et al., 2012). Transition to high-density pear planting systems has been limited due to the lack of dwarfing, precocious rootstocks that are suitable for the PNW (Elkins et al., 2012). Breeding for new pear rootstocks also targets traits such as low susceptibility to prevalent diseases and pests (Brewer and Volz, 2019; Guzman and Dhingra, 2019).

Fire blight, a bacterial disease caused by *Erwinia amylovora,* has a severe impact on rosaceous crops such as pear. Fire blight causes millions of dollars per year in damage due to loss of fruit production, removal and replacement of hundreds of acres of trees during extreme outbreaks, and labor required for scouting and removal of infected scion

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tissue (van der Zwet et al., 2012a). Rootstocks with low fire blight susceptibility can be re-grafted if an infected scion is removed, reducing losses due to tree replacement and establishment, and are critical for high-density planting systems where trees are in close contact (van der Zwet et al., 2012a).

Severity of fire blight can vary based on tissue type and maturity, tree vigor, environmental conditions, and virulence of *E. amylovora* strains (Billing, 2011; Norelli et al., 2003a; Norelli et al., 2003b; Schroth et al., 1974). Points of infection include pear blossoms, stomata in young shoots, and wounding to the scion and/or rootstock suckers (Schroth et al., 1974). Pear typically exhibits high levels of vigor which can facilitate bacterial spread throughout the tree (van der Zwet et al., 2012b).

Evaluation of fire blight response can be difficult due to varying symptoms, such as bacterial ooze, shoot cracking, shriveled necrotic lesions, and/or the characteristic shepherd's hook at the end of a shoot. Artificial inoculation in a greenhouse allows for standardization of bacterial strain(s), inoculum concentration, and inoculation method, such as cut-leaf shoot inoculation, as well as controlling of greenhouse environmental conditions (Norelli et al., 1988). While artificial inoculation may not fully replicate natural inoculation in an orchard setting, standardized inoculation helps minimize external factors when assessing germplasm for use as breeding parents (Peil et al., 2021; Pankova et al., 2023).

Low fire blight susceptibility is an important target in pear scion and rootstock breeding programs (Brewer et al., 2021; Brewer and Palmer, 2011; Musacchi et al., 2005; Peil et al., 2009, 2021). The Washington State University (WSU) Pear Rootstock Breeding Program (PRBP) was established in 2015 to develop pear rootstocks for the U.S. pear industry, and target traits such as conferred dwarfing, induced precocity, low disease susceptibility, and cold hardiness. The WSU PRBP *Pyrus* germplasm collection has a high level of diversity from wild relatives and interspecific hybrids. In this study, 20 diverse *Pyrus* accessions were evaluated to identify potential sources of reduced fire blight susceptibility. Data from this study can be used to help inform parental selection, which is particularly valuable in a crop that has a long generation time with an extended juvenile phase.

Materials and Methods

Seventeen accessions from the WSU *Pyrus* parental germplasm collection, along with 'Beurre d'Anjou' (referred to as 'Anjou'), 'Bartlett', and 'OH×F 87' as industry references, were evaluated in this study (Table 1). Dormant budwood of each accession was grafted onto actively growing 'OH×F 87' rootstocks, generating up to 20 clones per accession. Trees were grown in half-gallon bags in a greenhouse located at the WSU Tree Fruit Research and Extension Center (47°26'16.5"N 120°20'50"W). Six weeks after grafting, each tree was fertilized with 0.85 g of an 18N-7.8P-14.9K blend. Trees were divided into two experiments due to differential shoot growth rates, each of which was randomized into a complete block design, consisting of four blocks with five accession replicates per block. Secondary shoots from the graft stick were removed, leaving a single actively growing shoot. Adventitious rootstock shoots were also removed if present. The greenhouse was maintained with no supplemental light and maximum cooling for both experiments. Average temperatures recorded were ~21 °C (Experiment A) and ~24 °C (Experiment B), and recorded humidity levels were an average of ~85% (Experiment A) and ~75% (Experiment B).

Inoculum suspension was prepared with freeze-dried *E. amylovora* strain 153n according to the protocol described by Johnson et al. (2009). Inoculum suspension consisted of 0.01 M dibasic phosphate buffer, pH 7, with an inoculum concentration of 1×10^9 CFU/mL. Cut leaf inoculation was performed once per individual on an actively

Table 1. Species and reported susceptibility of 20 diverse *Pyrus* accessions.

Accession[z]	*Pyrus* species[y]	Reported susceptibility[x]
Anjou (Beurre d'Anjou)	*communis* L.	Moderate[t]
Bartlett	*communis* L.	High[t]
Farmingdale	*communis* L.	Low[t]
Mustafabey	*communis* L.	Moderate to high[u,v,w]
OH×F 333	*communis* L.	Low[t]
OH×F 87	*communis* L.	Low[s]
Old Home	*communis* L.	Low[t]
GE-2004-131	*communis* L. subsp. *caucasica* (Fed.) Browicz	Unknown
P-87	*communis* L. subsp. *pyraster* (L.) Ehrh.	Low[t]
Du Li	*betulifolia* Bunge	Unknown
OSU-2	*calleryana* Decne.	Low[t]
OSU-8	*calleryana* Decne.	Low[t]
P. salicifolia (hybrid) - Russia	*salicifolia* Pall.	Unknown
P. xerophila - Lawyer Nursery	*xerophila T.T.Yu*	Unknown
Hybrid 1	Interspecific; *dimorphophylla, fauriei*	Unknown
Hybrid 2	Interspecific; *betulifolia, calleryana, communis*	Unknown
Hybrid 3	Interspecific; *betulifolia, fauriei, spinosa*	Unknown
Hybrid 4	Interspecific; *elaeagrifolia, spinosa*	Unknown
Hybrid 5	Interspecific *calleryana; salicifolia; ussuriensis*	Unknown
Hybrid 6 (*P. betulifolia*-1 × P-79)	Interspecific; *betulifolia* (1), *communis* (P-79)	Low[t]

[z] Budwood was collected from 17 accessions in the WSU *Pyrus* parental germplasm collection, along with 'Bartlett', 'Anjou', and 'OH×F 87'.
[y] Ten species are represented overall, either as individual accession or within the background of an interspecific hybrid.
[x] Previously documented susceptibility is included when possible.
[w] Aysan et al., 1999
[v] Çitir and Mirik, 1999
[u] Demir and Gündogdu, 1993
[t] USDA-ARS NCGR, 2017
[s] Postman et al., 2013

growing shoot, preferably ≥ 10 cm in length. Scissors were dipped in inoculum suspension prior to bisecting the middle of two unfurling apical leaves (Norelli et al., 1988; Kostick et al., 2019; Zurn et al., 2020). Five replicates (individual trees) of each accession were inoculated per block; however, some blocks had fewer than five replicates due to graft failure. Inoculations were performed on 31 May 2021 (Experiment A) and 2 June 2021 (Experiment B). Experiment B was performed in the previously described manner, using freshly-prepared inoculum at the same concentration of the same freeze-dried *Ea*153n stock.

Shoots were assessed for response to fire blight after disease progression had stopped, beginning around six weeks post-inoculation (Kostick et al., 2019). Length of each necrotic response was measured and overlapping responses were measured as a continuous length to avoid double counting (Harshman et al., 2017). Total length of the shoot was recorded and percent shoot length blighted (%SLB) was calculated by dividing the sum of necrotic response lengths by total shoot length and multiplying by 100 (Kostick et al., 2019).

One-way analysis of variance (ANOVA) was performed to determine significant differences between experiments and among accessions. As Experiment A and Experiment B were determined to be significantly different, they were subsequently analyzed separately. Pearson's correlation of average %SLB between Experiment A and Experiment B was also calculated. Significant differences among accession %SLB were determined using Tukey's Honest Significant Difference (HSD). The unbalanced accession replicates due to graft failure necessitated the use of a linear mixed effects (LME) model with a restricted maximum likelihood (REML) fit. Block and replicate were calculated as random effects, while accession as a fixed effect. In addition, statistical differences of each accession's average fire blight incidence (number of individuals with any visible response out of the total number of individuals) were calculated using ANOVA and Tukey's HSD. All statistical analyses were performed using R 4.1.2 (R Core Team, 2022) and RStudio (RStudio Team, 2020), along with software packages 'agricolae' for ANOVA and Tukey's HSD (de Mendiburu, 2019), and 'lmerTest' for LME models (Kuznetsova et al., 2017).

Results

Overall fire blight responses (%SLB) differed significantly between Experiment A and B (ANOVA, $P \leq 0.05$; LME model, $P \leq 0.01$); however, the correlation of accessions' average %SLB was 0.83 (Pearson; $P \leq 0.001$), indicating overall consistency between the experiments (Fig. 1). Within each experiment, block and replicate did not have significant effects on %SLB ($P \geq 0.05$). Variability at the accession level was determined to be significant for %SLB based on an ANOVA and LME models (Experiment A, $P \leq 0.001$; Experiment B, $P \leq 0.001$).

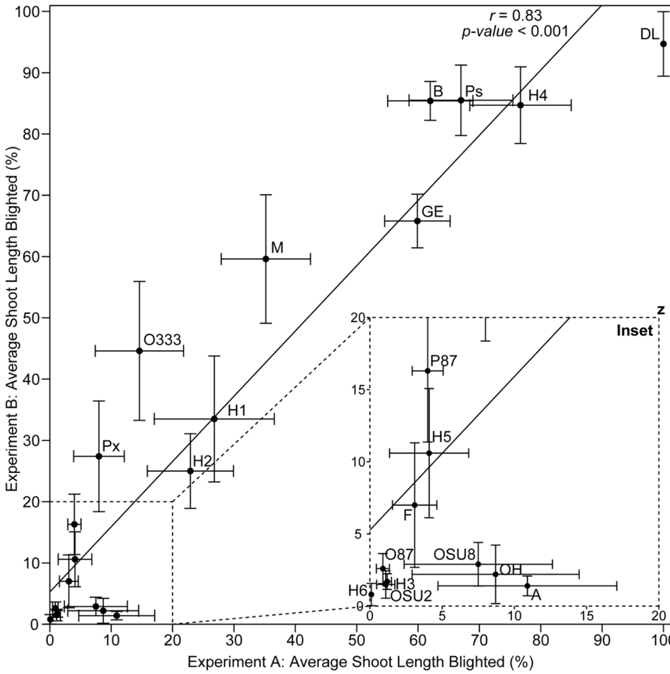
Accessions were assigned to mean separation groups through Tukey HSD tests (significance level < 0.05) for %SLB (Table 2). Five groups were designated within Experiment A, and eight groups within Experiment B. While significant differences were identified for %SLB (i.e., severity), there was no significant difference determined for percent incidence by accession across the two experiments.

Fire blight severity and incidence responses varied among accessions. For example, 'Hybrid 6' had a low severity response with %SLB of 0.1% and 0.8%, maximum %SLB of 1.4% and 15.4%, and a low incidence (5%) in both experiments (Table 2). 'Hybrid 3' had low severity for both average %SLB (A: 1.2%, B: 1.7%) and maximum %SLB (A: 3.9%, B: 7.6%); however, the incidence was 55% and 50% for Experiment A and Experiment B respectively. Accessions with lower %SLB and high incidence include 'Hybrid 5' (%SLB A: 4.1%, B: 10.6%; and incidence A: 63.6%, B: 44.4%), and 'P-87' (%SLB A: 4.0%, B: 16.3%; and incidence A: 64.7%, B: 89.5%). Moderate incidence with high severity was observed for several accessions, including 'Hybrid 1' and 'OH×F 333' that had Max %SLB of 100% in both experiments, with 40% incidence for 'Hybrid 1' in both experiments and respective incidences of 35% and 72% for 'OH×F 333'.

'Du Li' exhibited the highest susceptibility in both experiments. In Experiment A, it had 100% incidence and 100% severity for average %SLB, representing total shoot death for all individuals. In Experiment B, it had a 94.7% incidence with an average of 94.7 %SLB, representing 19 out of 20 individuals that had total shoot death.

Six accessions consistently showed the highest levels of susceptibility in both experiments ('Du Li', 'Hybrid 4', '*P. salicifolia* (hybrid) - Russia', 'Bartlett', 'GE-2004-131', 'Mustafabey'; Fig. 1 and Table 2), with high fire blight incidence (85-100%) and average %SLB ranging from 35% to 100%. Each had individuals where the fire blight infection resulted in total shoot death (i.e., maximum %SLB = 100%).

Nine accessions were identified with lower susceptibility in both experiments: 'Hybrid 6', 'OSU-2', 'OH×F 87', 'Hybrid 3', 'Old Home', 'Anjou', 'OSU-8', 'Farmingdale', 'Hybrid 5'; (Fig. 1 and Table 2). These accessions had an average %SLB ranging from 0 to 11, and an average fire blight incidence of 5% to 65%. Within all individuals from these nine accessions, a single 'Anjou' replicate had total shoot death in Experiment A and



Code	Accession ID
A	Anjou (Beurre d'Anjou)
B	Bartlett
DL	Du Li
F	Farmingdale
GE	GE-2004-131
H1	Hybrid 1
H2	Hybrid 2
H3	Hybrid 3
H4	Hybrid 4
H5	Hybrid 5
H6	Hybrid 6 (*P. betulifolia*-1 × P-79)
M	Mustafabey
O333	OH×F 333
O87	OH×F 87
OH	Old Home
OSU2	OSU-2
OSU8	OSU-8
P87	P-87
Ps	*P. salicifolia* (hybrid) - Russia
Px	*P. xerophila* - Lawyer Nursery

Fig. 1. Correlation between Experiments A and B for average fire blight response of 20 diverse *Pyrus* accessions (Pearson's $r = 0.83$, $P \leq 0.001$). Enlarged inset for accessions with low susceptibility.

none of the individuals had total shoot death in Experiment B. 'Hybrid 6' had the lowest average %SLB (Experiment A: 0.1; Experiment B: 0.8) and lowest incidence (5% for both Experiment A and B). The 5% fire blight incidence represented only 1 out of 20 individuals that displayed necrotic response.

Fire blight lesions varied among accessions with some being more prone to cracking responses (Fig. 2A) or shriveled necrotic tissue (Fig. 2C), while other accessions tended to have responses that were necrotic and cracking (Fig. 2B) (data not shown).

Discussion

Low fire blight susceptibility is an important trait for parental selection in the WSU Pear Rootstock Breeding Program. Up to 20 replicates of 20 diverse *Pyrus* accessions were evaluated for fire blight susceptibility as scions grafted on 'OH×F 87' rootstocks in two consecutive greenhouse experiments. Results from the second experiment validated those of the first.

Growth and environmental variability likely contributed to varying disease severity between experiments. Slightly warmer and less humid conditions in Experiment B were more conducive to bacterial and shoot growth, resulting in increased severity. Despite these differences, disease incidence was consistent between the two experiments, and the high correlation of disease severity (i.e., %SLB) illustrate repeatability of relative accession response (Fig. 1 and Table 2).

Our results agreed with previously reported susceptibility levels for ten of the eleven accessions; 'Anjou' had lower fire blight susceptibility in this experiment compared with previous reports (Table 1) (USDA-ARS NCGR, 2017). This could be due to differences in bacterial strain, orchard/greenhouse conditions

Fig. 2. Examples of observed pear fire blight responses: A – cracking; B – necrotic and cracking; C – shriveled necrotic.

or rootstock combinations, which have been reported to impact fire blight susceptibility in other accessions (Aleksandrova et al., 2020; Cabrefiga and Montesinos, 2005).

Three low susceptibility accessions were consistent with published literature: 'Old Home', 'Farmingdale', and 'OH×F 87' (Postman et al., 2013). 'OH×F 333' exhibited lower susceptibility in Experiment A, with a moderate susceptibility in Experiment B, both of which are consistent with previous reports of low and moderate susceptibility (Aleksandrova et al., 2020). Accessions 'P-87', 'OSU-2', 'OSU-8', and 'Hybrid 6' also displayed low susceptibility according to National Clonal Germplasm Repository information (USDA-ARS NCGR, 2017).

Two of the most susceptible accessions were 'Bartlett', which is consistent with previous reports (USDA-ARS NCGR, 2017), and 'Mustafabey', which has previously been reported as having moderate to high susceptibility (Aysan et al., 1999; Çitir and Mirik, 1999; Demir and Gündogdu, 1993). 'Mustafabey' was one of the six most susceptible accessions in this study, with Max %SLB of 100 in both experiments and incidences of 100% (Experiment A) and 85% (Experiment B). Average %SLB were more moderate in comparison to the other highly susceptible accessions, with 35.2% and 59.6% respectively compared to average %SLBs ranging from 59.9-100%.

No reports were found for susceptibility levels for the other nine accessions. Of these, four had high susceptibility in this study ('GE-2004-131', 'Du Li', '*P. salicifolia* (hybrid) - Russia', 'Hybrid 4'), three exhibited moderate susceptibility ('*P. xerophila* - Lawyer Nursery', 'Hybrid 1', 'Hybrid 2'), and two displayed low susceptibility ('Hybrid 3', 'Hybrid 5').

Table 2. Severity and incidence of fire blight response in 20 diverse *Pyrus* accessions.

Accession[z]	Experiment A MS groups[y]	Ave. %SLB[x]	Max %SLB[v]	% Incid.[u]	Num. indiv.[t]	Experiment B MS groups	Ave. %SLB	Max %SLB	% Incid.	Num. indiv.
Du Li	a	100.0	100.0	100.0	19	a	94.7	100.0	94.7	19
Hybrid 4	ab	76.7	100.0	95.0	20	ab	84.7	100.0	100.0	18
*P. salicifolia (*hybrid*)* - Russia	b	67.0	100.0	90.0	20	ab	85.5	100.0	95.0	20
Bartlett	b	62.0	100.0	100.0	20	abc	85.4	100.0	100.0	20
Mustafabey	bc	35.2	100.0	100.0	19	bcd	59.6	100.0	85.0	20
GE-2004-131	bc	59.9	100.0	100.0	20	bcde	65.8	100.0	100.0	20
OH×F 333[B]						cdef	44.6	100.0	72.2	18
Hybrid 1	cd	26.8	100.0	40.0	20	defg	33.5	100.0	40.0	20
Hybrid 2	cd	22.9	100.0	60.0	20	defg	25.0	100.0	68.4	19
OH×F 333[A]	de	14.6	100.0	35.0	20					
P-87	de	4.0	12.0	64.7	17	defg	16.3	76.7	89.5	19
P. xerophila – Lawyer Nursery	de	8.0	71.5	45.0	20	efg	27.4	100.0	60.0	20
Hybrid 5	de	4.1	30.7	63.6	11	fgh	10.6	31.7	44.4	9
Farmingdale	de	3.1	19.4	31.3	16	gh	7.0	63.8	33.3	15
OSU-8	de	7.5	86.9	29.4	17	gh	2.9	21.2	37.5	16
Anjou (Beurre d'Anjou)	de	10.9	100.0	30.0	20	gh	1.4	9.5	26.3	19
Old Home	de	8.7	87.7	20.0	20	gh	2.2	40.6	20.0	20
Hybrid 3	de	1.2	3.9	55.0	20	gh	1.7	7.6	50.0	20
OH×F 87	de	0.9	6.7	25.0	20	gh	2.6	16.7	50.0	20
OSU-2	de	1.1	10.6	20.0	20	gh	1.5	12.5	23.5	17
Hybrid 6 (*P. betulifolia*-1 × P-79)	e	0.1	1.4	5.0	20	h	0.8	15.4	5.0	20

[z] Accessions were ordered within their respective mean separation groups for percent shoot length blighted (%SLB) for easy comparison between experiments. Accession 'OH×F 333' was unable to be aligned across experiments and is designated with an 'A' or 'B' superscript to indicate the respective experiment.

[y] Mean separation groups within experiments were determined using an analysis of variance and Tukey's Honest Significant Difference test for %SLB.

[x] Average of individuals' %SLB within accession, calculated by dividing shoot length blighted by total shoot length, multiplied by 100.

[v] Maximum %SLB represents the most severe response for an individual within each accession

[u] %Incidence is calculated using the number of trees with fire blight response divided by total number of individuals per accession, multiplied by 100.

[t] Number of individuals inoculated per accession.

Ideally, a rootstock would have both low disease severity and incidence, such as 'Hybrid 6' (Table 2). 'Hybrid 3' maintained low severity (%SLB and Max %SLB), but had moderate incidence. A rootstock that sustains mild infections has a greater chance of surviving and can be re-grafted if necessary. However, high incidence of rootstock infection can potentially lead to an increased number of susceptible scions at risk for bacterial transmission (Santander et al., 2020). 'Hybrid 5' and 'P-87' had lower %SLB with a moderate Max %SLB and moderate incidence of infection. When an accession has moderate incidence with high severity, fewer trees may be infected, although the infection is more likely to lead to tree loss.

In summary, this fire blight study provides comparative data for potential breeding parents evaluated with the same strain and similar greenhouse growing conditions. Six accessions were identified as highly susceptible, and new information is reported for nine accessions with previously unknown fire blight responses. Accessions were identified in this study that had comparable or lower susceptibility than the industry standard 'OH×F 87'. The nine accessions that exhibited consistent lower susceptibility could be candidates for parents in future rootstock breeding crosses.

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