Screening *Cotoneaster* sp. for Resistance to Fire Blight Using Foliar Inoculation with Two Strains of *Erwinia amylovora*

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Abstract. The genus *Cotoneaster* is composed of ≈400 species with a wide variety of growth habits and forms. These hardy landscape shrubs used to be commonplace because of their low maintenance and landscape functionality. However, the interest in and sales of cotoneaster have decreased for a variety of reasons, with the greatest being its susceptibility to a bacterial disease fire blight caused by *Erwinia amylovora*. The resistances of 15 different genotypes of *Cotoneaster* to a wild-type strain of *Erwinia amylovora* (Ea153) and a strain LA635 that has a natural mutation in *avrRpt2* that encodes for a type III secretion effector were tested separately by inoculating leaves. Fire blight resistance was assessed by calculating the percent shoot necrosis (PSN) [$\text{PSN} = 100 \times (\text{lesion length} \div \text{total branch length})$] at 6 to 8 weeks after inoculation. Across all experiments, *Cotoneaster* genotypes H2011-01-002 and *C. ×suecicus* ‘Emerald Sprite’ consistently had the lowest PSN values when inoculated with either strain. *Cotoneaster ×suecicus* ‘Emerald Beauty’ was significantly more resistant to Ea153 than to LA635, whereas *C. splendens* was significantly more susceptible to Ea153 than to LA635.

Cotoneaster is a genus of hardy, ornamental shrubs in the Maloideae of Rosaceae that is composed of ≈400 species that are widely distributed throughout the northern hemisphere (Dickoré and Kasperk, 2010; Fryer and Hylmö, 2009). The genus is divided into two subgenera: *Chaenopetalum*, which blooms all at

once with white spreading petals, and subgenus *Cotoneaster*, which blooms successively over a long period of time with cup-like pink flowers (Fryer and Hylmö, 2009). The subgenera are divided further into series based on botanical characteristics and geographic origins (Fryer and Hylmö, 2009).

Cotoneasters are ideal urban plants because of their ability to tolerate pollution, grow in poor soils, and withstand harsh pruning. Many species have multi-season interest in the form of autumn color or a dense, consistent green foliage and fruits that persist through the winter. The size, color, and texture of leaves vary widely among species and cultivars. The fruit are mostly bright orange–red, but yellow and black-fruited species exist. The fruits attract wildlife, including birds such as robins and waxwings, which have spread cotoneasters from cultivation into the wild. Cotoneasters are easy to grow and are commonly used as foundation plants, as hedges, in parking lots, or in a mass planting along roadsides. Although cotoneasters often are attractive and utilitarian for landscaping, most available cultivars are susceptible to the bacterial plant disease fire blight caused by *Erwinia amylovora*.

Bacterial cells that overwinter in fire blight cankers likely serve as primary inoculum in

the spring (Khan et al., 2012). Stem cankers produce bacterial ooze that is then disseminated by insects, wind, and rain to flowers or wounds (Vogt et al., 2013; Wöhner et al., 2018). After tissue invasion, the pathogen produces effector proteins that cause necrosis and can eventually kill the plant (Oh and Beer, 2005).

Fire blight periodically causes heavy losses for apple (*Malus ×domestica*) and pear (*Pyrus communis*) production. The estimated annual expenses for fire blight management and tree losses in the United States are approximately US\$100 million and upward of US\$9 million in Switzerland, thus illustrating why fire blight is one of the most economically devastating diseases in pome fruit production worldwide (Norelli et al., 2003; Vogt et al., 2013). Fire blight also affects landscape plants such as *Cotoneaster*, which has led to substantial decrease in sales. Copper and streptomycin are the only registered chemicals for fire blight control on *Cotoneaster* (Vanneste, 2000). In some regions, streptomycin-resistant strains of the pathogen are present; furthermore, in many countries, streptomycin is not permitted for fire blight control on *Cotoneaster* (McGhee et al., 2011; Peil et al., 2019; Vanneste, 2000; Vogt et al., 2013). With few materials for disease control, host plant resistance would be the most effective way to control the disease (Norelli et al., 2003). Crossing sensitive plant species with those that show resistance can be an effective method of introducing resistance genes and potentially creating new cultivars. Without new, disease-resistant cultivars, fire blight management on *Cotoneaster* will remain challenging.

The cotoneasters currently being planted as ornamentals are not representative of the whole genus, as illustrated by Dirr (2009), who listed only 14 species, thus omitting the major diversity of the genus. Neither subgenus is underrepresented among cultivated material, and there are relatively popular plants from both, including *C. apiculatus* and *C. horizontalis* from subgenus *Cotoneaster* and *C. dammeri* and *C. ×suecicus* ‘Coral Beauty’ from subgenus *Chaenopetalum*. Ongoing research by our group has assessed resistance among species to fire blight in inoculated greenhouse trials (Rothleutner et al., 2014). Resistant taxa were crossed with species of horticultural interest to develop new cultivars with increased resistance to the bacterial disease. The Oregon State University Ornamental Plant Breeding Laboratory has developed a number of experimental *Cotoneaster*, including genotypes H2011-01-002, H2011-02-001, H2011-02-005, and H2017-005-01, that have a wide variety of foliar and floral characteristics and growth habits. Two of these genotypes, H2011-02-001 and H2011-02-005, exhibited tolerance to fire blight in pathogen-inoculated greenhouse experiments and were released as the cultivars Emerald Sprite PP31,719 and Emerald Beauty PP32,308, respectively, by the Oregon Agricultural Experiment Station.

Breeding plants for disease resistance often becomes a multistep process as more

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germplasm sources carrying desirable traits are identified and/or if pathogens evolve and adapt to overcome resistance in plants. This latter case has been observed in an apple breeding program focused on fire blight resistance. Wild apple species have been explored as sources of resistance to fire blight (Aldwinkle and Preczewski, 1979; Peil et al., 2007, 2009; van der Zwet and Keil, 1979; Vogt et al., 2013), with one of importance being *Malus × robusta* 5 (Mr5) (Peil et al., 2007). Mr5 has a major quantitative trait locus (QTL) for resistance to fire blight on linkage group 3 (Peil et al., 2007, 2009, 2011). Wöhner et al. (2014) reported that there were major resistance genes on linkage group 3 of Mr5 and suggested that there is likely a gene-for-gene relationship in the Mr5–*E. amylovora* pathosystem (Emeriewen et al., 2019; Fahrenttrapp et al., 2013; Wöhner et al., 2014, 2018). This resistance has been of particular interest to breeders and has been used to introduce fire blight resistance to many newly released apple cultivars (Durel et al., 2009). Unfortunately, Vogt et al. (2013) found that strains of *E. amylovora* lacking the type III secretion effector AvrRpt2 or harboring *avrRpt2* with a mutation were able to overcome the resistance of Mr5. The mutation in *avrRpt2*, among strains able to cause disease on Mr5, was a naturally occurring single-nucleotide polymorphism (SNP) that resulted in the substitution of cysteine with serine at codon 156 (Emeriewen et al., 2019; Smits et al., 2014; Vogt et al., 2013; Wöhner et al., 2014).

The objectives of this study were to 1) evaluate fire blight resistance of *Cotoneaster* genotypes in repeated experiments under controlled conditions of growth chambers and 2) evaluate the severity of fire blight on *Cotoneaster* genotypes inoculated with a wild-type strain of the pathogen compared with a pathogen with an *avrRpt2*^{C156S} mutation.

Materials and Methods

Plant material. *Cotoneaster* species, cultivars, and hybrids were screened for resistance to fire blight in five experiments (Table 1). Plants for resistance testing were clonally propagated from stem cuttings. For growth chamber experiments, plants were grown in 10-cm square pots in a potting mix with 2 perlite : 1 peat (by volume). For the greenhouse experiment, plants were potted in unaged douglas fir bark (*Pseudotsuga menziesii*; Lane Forest Products, Eugene, OR) in 2.5-L containers. Plants were hand-watered as needed and fertilized weekly using liquid soluble fertilizer 20N–8.74P–16.6K (Jack’s Professional General Purpose; J.R. Peters Laboratory, Allentown, PA). All plants were actively growing at the time of all experiments.

Bacterial strains. Two strains of *Erwinia amylovora* were used to assess fire blight resistance. A wild-type strain of the pathogen Ea153 was isolated from fire blight infection on ‘Gala’ apple in Oregon. Ea153 has been used in field trials and is sensitive to streptomycin (Johnson et al., 1993; Stockwell et al.,

1998). Pathogen strain LA635 has the *avrRpt2*^{C156S} mutation and causes fire blight disease on *Malus × robusta* 5 (Wöhner et al., 2018). LA635 is resistant to streptomycin because of the commonly detected *rpsL*^{K43R} mutation; the mutations in *rpsL* and *avrRpt2* were confirmed by a genome sequence analysis (Smits et al., 2014). The strains were stored at –80 °C in nutrient broth (Difco Laboratories, Franklin Lakes, NJ) amended with 15% glycerol until use.

Preparation of freeze-dried inoculum. Strain Ea153 was grown on *Pseudomonas* agar F (Difco Laboratories) amended with cycloheximide (50 µg/mL) (hereafter referred to as PAF). Strain LA635 was grown on *Pseudomonas* agar F amended with cycloheximide (50 µg/mL) and streptomycin (100 µg/mL) (hereafter referred to as PAF-Sm). After 5 d, bacterial lawns were harvested, mixed with a skim milk cryoprotectant (Stockwell et al., 1998), frozen at –80 °C, and lyophilized with a freeze dryer (FreeZone 6 L; Labconco Co., Kansas City, MO). Freeze-dried bacterial preparations were stored in sealed tubes at –80 °C. The titer of the stored freeze-dried cell preparations of each strain was checked before each experiment by rehydrating weighed samples of the freeze-dried bacteria and dilution plating on PAF and PAF-Sm. Colonies were counted after 3 d and converted to colony-forming units (cfu) per milligram.

Inoculation using leaf bisection and data collection. Plants were inoculated with Ea153 or LA635 at a concentration of 10⁸ cfu/mL in 2018, and at a concentration of 10⁹ cfu/mL in 2019. Inoculations were performed using a foliar assay and bisecting the two youngest leaves with a pair of scissors dipped in inoculum before each cut. Control plants were cut with scissors dipped in sterile water. Stems were wrapped with tape just below the inoculation site. Disease severity was measured using the method of Bellenot-Kapusta et al. (2002) because plant habit and branch lengths vary considerably across the *Cotoneaster*

taxa. Every week for 8 weeks, the lesion length and total length of the inoculated branch were measured (in mm). The PSN was calculated using the following equation:

$$PSN = \left[100 \left(\frac{\text{lesion length}}{\text{total branch length}} \right) \right]$$

The area under the disease progress curve (AUDPC) was calculated for taxa in 2019 growth chamber experiments and used to compare disease progression between strains for each genotype using R (R CoreTeam, 2019). Figures were produced using the package Agricolae (Mendiburu, 2019). The AUDPC was calculated using the following function (Paraschivu and Cotuna, 2013):

$$AUDPC = \sum_i^{N-1} \left[\left\{ \frac{(Y_i + Y_{i+1})}{2} \right\} (t_{i+1} - t_i) \right]$$

where Y_i is disease severity on the i th date; t_i is the i th day; N is the total number of observations or dates when fire blight lesion length was recorded (Paraschivu and Cotuna, 2013). The AUDPC was the sum of the area under the curve formed by weekly means over the course of the 8 weeks of observation. At 8 weeks after inoculation, the lesion border was harvested from randomly selected plants with fire blight symptoms, including one representative of each taxon. The tissue was diced, suspended in 2 mL of 10 mM phosphate buffer (pH 7.0), and incubated at room temperature for 1 h; vortexing was performed occasionally. Dilutions of the suspensions were spread on PAF, PAF-Sm, and the semi-selective medium for *E. amylovora* (referred to as CCT) (Ishimaru and Klos, 1984).

Experimental design. Growth chamber experiments were conducted using Percival model LED-30HL1 (Percival Scientific Inc., Perry, IA) growth chambers with an IntelluUltra real-time controller. The growth chambers had a woodless design, programmable temperature, light-emitting diode (LED) lighting, and relative humidity. Growth chambers

Table 1. Experiments screening *Cotoneaster* taxa for fire blight resistance.

Taxon	Accession	Expt. ^z				
		1	2	3	4	5
<i>C. acutifolius</i>	10-0126		X			X
<i>C. apiculatus</i>	16-0027	X	X	X	X	X
<i>C. daliensis</i>	10-0129		X			X
<i>C. dammeri</i>	19-0036			X	X	X
<i>C. dammeri</i> × <i>C. apiculatus</i>	H2017-005-01	X	X	X	X	X
<i>C. dielsianus</i>	09-0013		X			X
<i>C. frigidus</i>	11-0065		X			X
<i>C. salicifolius</i> var. <i>floccosus</i>	09-0022		X			X
<i>C. sikangensis</i>	11-0057		X			X
<i>C. simonsii</i>	09-0023		X			X
<i>C. splendens</i>	09-0024	X	X	X	X	X
<i>C. ×suecicus</i> ‘Coral Beauty’	17-0006	X	X	X	X	X
<i>C. ×suecicus</i> ‘Coral Beauty’ × <i>C. splendens</i>	H2011-01-002	X	X	X	X	X
<i>C. ×suecicus</i> ‘Emerald Sprite’ PP31,719	H2011-02-001	X	X	X	X	X
<i>C. ×suecicus</i> ‘Emerald Beauty’ PP32,308	H2011-02-005	X	X	X	X	X

^zExpts. 1 to 4 were conducted in growth chambers and Expt. 5 was conducted in a glasshouse. Plants were inoculated with wild-type strain *Erwinia amylovora* Ea153 in Expts. 2, 4, and 5. Plants in Expts. 1 and 3 were inoculated with *E. amylovora* strain LA635, which has a single nucleotide mutation (C156S) in *avrRpt2*, which encodes for a type III secretion effector. Expts. 1 and 2 were conducted in 2018 and Expts. 3 to 5 were conducted in 2019.

were programmed for all four experiments with a ramping cool white LED light for a 16-h photoperiod (8 h of 50% light and 8 h of 100% light), with daytime temperatures of 25 °C (± 0.5 °C), nighttime temperatures of 20 °C (± 0.5 °C), and constant relative humidity of 70% to 80% (± 10 %). The glasshouse conditions were set at temperatures of 24 °C day/17 °C night with a 14-h photoperiod for experiments supplied by 400-W high-pressure sodium lamps.

All experiments were arranged in a randomized complete block design. In growth chamber experiments, each chamber represented a block. In Expts. 1, 3, and 4, there were four blocks and in Expt. 2, there were three blocks. In all five experiments, each block had three subsamples (plants) per genotype and a water-inoculated control. In each growth chamber experiment, one shoot was inoculated. In the glasshouse experiment, the three blocks were spatially separated and located distal to the cooling fans. Three shoots were inoculated on each plant and used to calculate the mean percent shoot necrosis for each genotype. In all experiments, the total shoot length and lesion length on inoculated shoots were measured in millimeters weekly for 2 months.

Statistical analysis. Data were analyzed using an analysis of variance (ANOVA) and PROC GLM (SAS version 9.4; SAS Institute Inc., Cary, NC) to generate a general linear model ANOVA. Initially, the full model that included treatment (control that was inoculated with water) was analyzed. The treatment was then removed, and the model was analyzed using block and taxa as explanatory variables. When appropriate, means were separated using Tukey's honestly significant difference ($\alpha < 0.05$). Because of the prevalence of 0% PSN datapoints, the data violated the assumption of normality as assessed using PROC UNIVARIATE (SAS version 9.4; SAS Institute Inc.). Specifically, tests for normality yielded the following results: Expt. 1, $W = 0.78$, $P < 0.0001$; Expt. 2, $W = 0.62$, $P < 0.0001$; Expt. 3, $W = 0.91$, $P = 0.01$; Expt. 4, $W = 0.9$, $P = 0.01$; and Expt. 5, $W = 0.90$, $P = 0.001$. Transformation did not improve normality; therefore, we used a mixed model analysis. Additionally, the normality plots deviated from normality solely because of the presence of numerous data points of 0; otherwise, they fit a normal distribution.

Results

Wild-type and *avrRpt2* mutant of *E. amylovora* caused disease on *Cotoneaster*. Symptoms of fire blight were observed on susceptible *Cotoneaster* genotypes in each experiment. The wild-type strain of *E. amylovora*, Ea153, was reisolated consistently from lesion borders on plants inoculated with that isolate (Fig. 1). The morphology of colonies recovered from Ea153-inoculated plants was typical of *E. amylovora* on CCT and PAF, and no colonies were observed on PAF-Sm. Similarly, LA635 was reisolated from plants inoculated with the *avrRpt2* mutant. Colony morphology

Fig. 1. Fire blight symptoms in *Cotoneaster*. (A) Fire blight causing necrosis of stems below the inoculation site (marked by red tape) showing the common shepherd's crook symptom. (B) Necrosis of vascular tissue in the lesion border of *Cotoneaster dammeri*.

of bacteria recovered from LA635-inoculated plants were typical of *E. amylovora* on CCT, PAF, and PAF-Sm.

Variable susceptibility among genotypes observed for both the wild-type and *avrRpt2* mutant of *E. amylovora* during 2018 growth chamber experiments. In Expt. 1, the PSN of plants inoculated with LA635 ranged from 0% to 21% (Table 2). Using the full model, genotype ($P < 0.0001$) and treatment ($P = 0.0003$) were significant, but block was not ($P = 0.2373$). When the treatment was removed, genotype ($P < 0.0001$) remained significant but block was not ($P = 0.2084$). Three genotypes, *C. splendens* (09-0024), H2011-01-002, and 'Emerald Sprite', had less than 5% shoot necrosis, whereas *Cotoneaster* $\times$ *suecicus*

'Coral Beauty' exhibited the highest PSN (21%). Some plants appeared to exhibit quantitative resistance between these extremes; small lesions were observed but there was limited spread.

In Expt. 2, the PSN of plants inoculated with wild-type Ea153 ranged from 0 to 26%. Genotype ($P = 0.05$) and treatment ($P = 0.0136$) were significant, but block was not ($P = 0.8825$). When the treatment was removed, the model was not significant at $\alpha = 0.05$ (0.0579); therefore, mean separation was not possible, and the results are presented and discussed in relative terms such as means $\pm$ SE but not statistical differences. There were large variations among plants within blocks, including 0% and 66% PSN within block 1 for

Table 2. Percent shoot necrosis of *Cotoneaster* genotypes inoculated in growth chambers with an *avrRpt2* mutant of *Erwinia amylovora* strain LA635 (Expt. 1) or a wild-type strain Ea153 (Expt. 2), both at a concentration of 10^8 cfu/mL, in 2018 with a foliar bisection assay.

Taxon	Accession	Percent shoot necrosis ²
2018 — Inoculated with LA635 — Expt. 1		
<i>C. $\times$ suecicus</i> 'Coral Beauty' $\times$ <i>C. splendens</i>	H2011-01-002	0 a
<i>C. $\times$ suecicus</i> 'Emerald Sprite' PP31,719	H2011-02-001	0.5 a
<i>C. splendens</i>	09-0024	1 a
<i>C. apiculatus</i>	16-0027	7 ab
<i>C. dammeri</i> $\times$ <i>C. apiculatus</i>	H2017-005-01	8 ab
<i>C. $\times$ suecicus</i> 'Emerald Beauty' PP32,308	H2011-02-005	14 bc
<i>C. $\times$ suecicus</i> 'Coral Beauty'	17-0006	21 c
2018 — Inoculated with Ea153 — Expt. 2 ³		
<i>C. daliensis</i>	10-0129	0
<i>C. sikangensis</i>	11-0057	0
<i>C. $\times$ suecicus</i> 'Coral Beauty' $\times$ <i>C. splendens</i>	H2011-01-002	0
<i>C. $\times$ suecicus</i> 'Emerald Sprite' PP31,719	H2011-02-001	0
<i>C. dielsianus</i>	09-0013	1 $\pm$ 0.6
<i>C. $\times$ suecicus</i> 'Emerald Beauty' PP32,308	H2011-02-005	4 $\pm$ 4.2
<i>C. $\times$ suecicus</i> 'Coral Beauty'	17-0006	4 $\pm$ 2.4
<i>C. apiculatus</i>	16-0027	6 $\pm$ 4.3
<i>C. splendens</i>	09-0024	6 $\pm$ 2.2
<i>C. simonsii</i>	09-0023	8 $\pm$ 6.8
<i>C. dammeri</i> $\times$ <i>C. apiculatus</i>	H2017-005-01	13 $\pm$ 8.7
<i>C. salicifolius</i> var. <i>floccosus</i>	09-0022	13 $\pm$ 13.2
<i>C. frigidus</i>	11-0065	20 $\pm$ 13.0
<i>C. acutifolius</i>	10-0126	26 $\pm$ 9.3

²Mean percent shoot necrosis from fire blight from 12 plants used for Expt. 1 and 9 plants used for Expt. 2. Means followed by different letters within the year significantly differed based on Tukey's honestly significant difference test ($\alpha < 0.05$), with a minimum significant difference value of 12% shoot necrosis in Expt. 1.

³Model not significant ($P = 0.0579$).

Means $\pm$ SE of the mean.

C. acutifolius and 0% and 100% PSN within block 2 of *C. frigidus* (data not shown). *Cotoneaster acutifolius* (10-0126) had the highest PSN (26%). Seven different genotypes had less than 5% shoot necrosis: *C. daliensis* (10-0129); *C. dielsianus* (09-0013); *C. sikangensis* (11-0057); *C. ×suecicus* ‘Coral Beauty’; H2011-01-002; ‘Emerald Sprite’; and ‘Emerald Beauty’ (Table 2). In both experiments, plants inoculated with two different strains of *E. amylovora*, H2011-01-002 showed complete resistance, with a PSN of 0%. Control plants showed no symptoms of stem necrosis after leaf bisection with scissors dipped in sterile deionized water.

Most genotypes exhibited similar responses to the wild-type and *avrRpt2* mutant of *E. amylovora* during 2019 growth chamber experiments. In Expts. 3 and 4, the severity of symptoms ranged from 3% to 59% (Table 3). Genotype and treatment were both significant ($P < 0.0001$), but block was not ($P > 0.1$). Taxa remained highly significant in both experiments with the treatment removed ($P < 0.0001$), but block was still not significant in Expt. 3 ($P = 0.9196$) and Expt. 4 ($P = 0.8818$). Only two genotypes exhibited different responses to the two different bacterial strains: *C. splendens* (09-0024) and ‘Emerald Beauty’. *Cotoneaster splendens* (09-0024) was resistant to LA635, with only 4% shoot necrosis; this species was sensitive to the wild-type strain Ea153, with 33% shoot necrosis. ‘Emerald Beauty’ exhibited greater resistance to the wild-type strain Ea153, with

5% shoot necrosis, and exhibited sensitivity to LA635, with 36% shoot necrosis. H2011-01-002 had the lowest PSN in Expts. 3 and 4, with 3% shoot necrosis when inoculated with LA635 and 3% shoot necrosis when inoculated with the wild-type strain Ea153. ‘Emerald Sprite’ showed relatively high rates of resistance to both strains, with 8% shoot necrosis when inoculated with LA635 and 3% shoot necrosis when inoculated with the wild-type strain Ea153. *Cotoneaster dammeri* (19-0036) in Expts. 3 and 4 was significantly less resistant than all other genotypes tested, with the highest PSN in both experiments. The H2017-005-01 hybrid, a *C. dammeri* (19-0036) progeny, had a significantly lower PSN than the *C. dammeri* (19-0036) parent, but the PSN was significantly greater than those of five genotypes inoculated with LA635 and four other genotypes inoculated with Ea153 (Table 3). Control plants showed no symptoms of stem necrosis after leaf bisection with scissors dipped in sterile deionized water. There appeared to be a difference in the progression of disease symptoms of the two strains of the pathogen during the experiment for ‘Emerald Beauty’ and *C. splendens* (09-0024) but not for genotype H2017-005-01, which had a similar AUDPC in both experiments (Table 4; Fig. 2).

Genotypes ranged from asymptomatic to nearly complete mortality when inoculated with wild-type *E. amylovora* in a glasshouse during 2019. In Expt. 5, there was a range of sensitivity to fire blight among the genotypes

ranging from very high levels of resistance (0% PSN) to almost complete mortality (97% PSN; Table 5). Genotype ($P < 0.0001$), treatment ($P < 0.0001$), and block were significant ($P = 0.043$). With the treatment removed from the model, taxa ($P < 0.0001$) and block (0.0001) remained significant. *Cotoneaster sikangensis* (11-0057) and ‘Emerald Beauty’ were asymptomatic after inoculation (Table 5; Figs. 3 and 4). Genotypes H2011-01-002 and ‘Emerald Sprite’ exhibited relatively high resistance, with 4% and 3% shoot necrosis, respectively (Table 5; Figs. 3 and 4). Several taxa showed high susceptibility, with more than 50% shoot necrosis, including *C. acutifolius* (10-0126), *C. daliensis* (10-0129), *C. dammeri* (19-0036), *C. dielsianus* (09-0013), *C. salicifolius* var. *floccosus* (09-0022), and *C. ×suecicus* ‘Coral Beauty’. Genotype H2017-05-001 showed a PSN of 49%, which was intermediate of its two parents, *C. dammeri* (19-0036; 73%) and *C. apiculatus* (16-0027; 43%). *Cotoneaster splendens* (09-0024) exhibited 11% shoot necrosis, which was greater than that of its progeny H2011-01-002 (4%). Control plants showed no symptoms of stem necrosis after leaf bisection with scissors dipped in sterile deionized water.

Discussion

In this series of experiments, we characterized the resistance of 15 genotypes of *Cotoneaster* to *Erwinia amylovora*. This is the third study performed at Oregon State University to rate disease resistance among *Cotoneaster* genotypes in the program; however, it is the first to examine sensitivity to an *avrRpt2* mutant strain of this bacterial pathogen. This study was a continuation to determine the level of resistance of these genotypes to the same strain tested before (Rothleitner et al., 2014) and an *avrRpt2* mutant strain that overcame resistance in the prominent wild apple rootstock Mr5.

Trees and shrubs under “natural” field conditions are typically infected by *E. amylovora* through flowers. Even though artificial shoot inoculation through leaf bisection represents a different path of infection, it is a proven method of studying host plant resistance to fire blight (Harshman et al., 2017; Peil et al., 2019; Persiel and Zeller, 1981). As reported by Rothleitner et al. (2014), a threshold tolerance of 5% can be used as a selection tool for breeding purposes because of the relatively low threshold for damage in ornamental plants. However, even 5% necrosis may be unacceptable, depending on specific situations. The titer of our inoculum used in this study varied from 10^8 cfu/mL in 2018 to 10^9 cfu/mL in 2019, but disease was observed during both years. These inocula concentrations are within the range of concentrations used by other studies (10^6 – 10^9 cfu/mL) (Bellenot-Kapusta et al., 2002; Persiel and Zeller, 1978). The relatively small size of *Cotoneaster* makes it a good plant to use in growth chambers, where we can have consistent environmental conditions, which is

Table 3. Percent shoot necrosis of *Cotoneaster* genotypes inoculated in growth chambers with an *avrRpt2* mutant of *Erwinia amylovora* (strain LA635) (Expt. 3) or wild-type pathogen Ea153 (Expt. 4), with both strains at a concentration of 10^9 cfu/mL, with a foliar bisection assay in 2019.

Taxon	Accession	Percent shoot necrosis ^z	
		Ea153	LA 635
<i>C. ×suecicus</i> ‘Emerald Sprite’ PP31,719	H2011-02-001	3 a	8 a
<i>C. ×suecicus</i> ‘Coral Beauty’ × <i>C. splendens</i>	H2011-01-002	3 a	3 a
<i>C. ×suecicus</i> ‘Emerald Beauty’ PP32,308	H2011-02-005	5 a	36 bc
<i>C. ×suecicus</i> ‘Coral Beauty’	17-0006	13 ab	9 a
<i>C. apiculatus</i>	16-0027	25 bc	18 ab
<i>C. dammeri</i> × <i>C. apiculatus</i>	H2017-005-01	30 bc	32 bc
<i>C. splendens</i>	09-0024	33 c	4 a
<i>C. dammeri</i>	19-0036	59 d	49 c

^zMean percent shoot necrosis from fire blight on 12 plants. Means followed by different letters within the column significantly differed based on Tukey’s honestly significant difference test ($\alpha < 0.05$), with minimum significant difference values of 18% shoot necrosis for LA635 and 18% shoot necrosis for Ea153.

Table 4. Area under the disease progress curve (AUDPC) for *Cotoneaster* genotypes evaluated for fire blight resistance to a wild-type strain (Ea153) or an *avrRpt2* mutant (LA635) of *Erwinia amylovora*.

Taxon	Accession	AUDPC ^z	
		Ea153	LA635
<i>C. ×suecicus</i> ‘Emerald Sprite’ PP31,719	H2011-02-001	117	353
<i>C. ×suecicus</i> ‘Coral Beauty’ × <i>C. splendens</i>	H2011-01-002	139	115
<i>C. ×suecicus</i> ‘Emerald Beauty’ PP32,308	H2011-02-005	248	1232
<i>C. ×suecicus</i> ‘Coral Beauty’	17-0006	545	396
<i>C. apiculatus</i>	16-0027	977	806
<i>C. dammeri</i> × <i>C. apiculatus</i>	H2017-005-01	1129	1435
<i>C. splendens</i>	09-0024	1368	137
<i>C. dammeri</i>	19-0036	2309	2185

^zAUDPC values were calculated from weekly measurements of lesion and stem lengths over the course of 8 weeks. AUDPC values were calculated with R.

Fig. 2. Area under the disease progress curve (AUDPC) of three different genotypes from the experiments performed in growth chambers (Expts. 3 and 4). ‘Emerald Beauty’ PP32,308 (H2011-02-005) and *C. splendens* displayed different percent shoot necrosis when inoculated with the wild-type strain Ea153 or the *avrRpt2* mutant LA635. Genotype H2017-005-01 was susceptible to both strains of the pathogen.

an improvement over some previous studies involving environmental conditions that likely impacted results (Rothleutner et al., 2014).

According to our results collected in the greenhouse experiment, the pattern of fire blight sensitivity was similar to that reported

by Lecomte and Cadic (1993) for *C. apiculatus*, *C. dammeri*, *C. dielsianus*, *C. salicifolius* var. *floccosus*, and *C. ×suecicus* ‘Coral Beauty’. There often is a high degree of variability in resistance within species between years on the same plants, and this was clear according to our results as well as to those of others (Persiel and Zeller, 1978; Rothleutner et al., 2014), thus justifying this series of five experiments over the course of years and in two environments. For example, Rothleutner et al. (2014) reported that *C. salicifolius* var. *floccosus* was highly susceptible during one year but asymptomatic during the next year. This could have been because of a variety of reasons, such as inoculation titer, temperature of the greenhouses, or plant placement in the greenhouses. The confounding results also could have occurred because of sexual and apomictic reproduction from seed (Persiel and Zeller, 1981, 1990); however, Rothleutner et al. (2014) used clonal plants produced from stem cuttings in all experiments. Our results apply only to the particular genotypes (accessions) for species used in the study and should not be considered species-wide levels of resistance. Furthermore, the rather dramatic variation seen within a single block of Expt. 2, which was conducted in a growth chamber, illustrates the sometimes inexplicable variation when dealing with the complex interaction of plants and pathogens.

Apple breeders have incorporated fire blight-resistant wild apple material with sensitive cultivars as a way to develop resistant cultivars. The resistance found in Mr5 has been studied, and a major QTL has been found on linkage group 3. There is a gene-for-gene interaction in the host–pathogen system of Mr5 and *E. amylovora* whereby a SNP in the apple and a SNP in the pathogen allow an *avrRpt2* mutant strain of the pathogen to overcome the resistance that the genotype once offered (Emeriewen et al., 2019; Vogt et al., 2013; Wöhner et al., 2018). The same strain of the pathogen used by Wöhner et al. (2018) was used in this project to determine if *Cotoneaster* genotypes resistant to the wild-type pathogen are able to maintain resistance against naturally occurring *avrRpt2* mutant strains of the pathogen (Smits et al., 2014). However, because we have already observed a mutant strain overcome what was previously a reliable source of resistance in apple, future work involving breeding cotoneaster and other rosaceous crops should attempt to pyramid resistance genes to prevent or at least slow current and future mutant strains overcoming single sources of resistance.

There were only two genotypes that had different PSN when inoculated with the wild-type and *avrRpt2* mutant. ‘Emerald Beauty’ exhibited high resistance to the wild-type strain Ea153 and increased disease severity with the *avrRpt2* mutant LA635. The interesting result was that *C. splendens* (09-0024), in 2019, exhibited high resistance to LA635, but it was susceptible to wild-type Ea153. The disease progressed rapidly through the stem during the first few weeks after inoculation with Ea153 or, conversely, was stalled for the

Table 5. Percent shoot necrosis of *Cotoneaster* genotypes inoculated in greenhouse with Ea153, a wild-type strain of *Erwinia amylovora*, at 10^9 cfu/mL in 2019 with a foliar bisection assay (Expt. 5).

Taxon	Accession	Percent shoot necrosis ^z
<i>C. sikangensis</i>	11-0057	0 a
<i>C. ×suecicus</i> ‘Emerald Beauty’ PP32,308	H2011-02-005	0 a
<i>C. ×suecicus</i> ‘Emerald Sprite’ PP31,719	H2011-02-001	3 ab
<i>C. ×suecicus</i> ‘Coral Beauty’ × <i>C. splendens</i>	H2011-01-002	4 ab
<i>C. splendens</i>	09-0024	11 abc
<i>C. frigidus</i>	11-0065	20 bc
<i>C. simonsii</i>	09-0023	24 c
<i>C. apiculatus</i>	16-0027	43 d
<i>C. dammeri</i> × <i>C. apiculatus</i>	H2017-005-01	49 d
<i>C. ×suecicus</i> ‘Coral Beauty’	17-0006	58 de
<i>C. dielsianus</i>	09-0013	59 de
<i>C. daliensis</i>	10-0129	66 ef
<i>C. dammeri</i>	19-0036	73 ef
<i>C. acutifolius</i>	10-0126	79 gh
<i>C. salicifolius</i> var. <i>floccosus</i>	09-0022	97 h

^zMean percent shoot necrosis from fire blight on nine plants. Means followed by different letters significantly differed based on Tukey’s honestly significant difference test ($\alpha < 0.05$), with a least significant difference value of 17% shoot necrosis.

Fig. 3. *Cotoneaster* genotypes H2011-01-002, ‘Emerald Sprite’ PP31,719, and ‘Emerald Beauty’ PP32,308 at 8 weeks after inoculation with wild-type strain Ea153 in the glasshouse. The inoculation site is marked with red tape (Expt. 5).

Fig. 4. ‘Emerald Beauty’ PP32,308 and *Cotoneaster* ×*suecicus* ‘Coral Beauty’ 8 weeks after inoculation with wild-type strain Ea153 in the glasshouse. The inoculation site is marked with red tape (Expt. 5).

first 2 weeks after inoculation with LA635 (Fig. 2). These results suggest that the different genotypes may possess different mechanisms for disease resistance. In all experiments, *C. dammeri* (19-0036) was more susceptible to fire blight than all other genotypes tested, indicating that it is a poor parent to use in crosses. This was shown through one of its progeny, H2017-005-01, which had more than 30% shoot necrosis, suggesting that susceptibility was inherited. *Cotoneasters*, however, are largely apomictic tetraploids; therefore, diploids that produce sexual progeny such as *C. dammeri* often are used out of necessity, regardless of susceptibility. Diploid cultivars Emerald Sprite USPP31,719 and Emerald Beauty PP32,308 will be useful for future breeding because of their increased resistance to fire blight compared with *C. dammeri* (19-0036) and ‘Coral Beauty’.

Our study found a varying range of susceptibility of *Cotoneaster* to the wild-type and a mutant strain of the fire blight pathogen. Several genotypes were observed to have consistently high levels of resistance to both wild-type and mutant strains of the pathogen, which bodes well for releasing more reliably resistant cultivars. This study and previous fire blight disease resistance studies have provided insight regarding potential sources of resistance to develop fire blight-resistant cultivars of *Cotoneaster*.

Literature Cited

- Aldwinkle, H.S. and J.L. Preczewski. 1979. Reaction of terminal shoots of apple cultivars to invasion by *Erwinia amylovora*. *Phytopathology* 66:1439–1444, doi: <https://doi.org/10.1094/Phyto-66-1439>.
- Bellenot-Kapusta, V., R. Chartier, M.N. Brisset, and J.P. Paulin. 2002. Selection of a genotype of *Cotoneaster* with a high level of resistance to fire blight. *Acta Hort.* 590:385–387, doi: <https://doi.org/10.17660/ActaHortic.2002.590.59>.
- Dickoré, W.B. and G. Kasperk. 2010. Species of *Cotoneaster* (Rosaceae, Maloideae) indigenous to, naturalising or commonly cultivated in Central Europe. *Willdenowia* 40:13–45, doi: <https://doi.org/10.3372/wi.40.40102>.
- Dirr, M.A. 2009. *Manual of woody landscape plants: Their identification, ornamental characteristics, culture propagation and uses*. 6th ed. Stipes Publishing, Champaign, IL.
- Durel, C., C. Denancé, and M. Brisset. 2009. Two distinct major QTL for resistance to fire blight co-localize on linkage group 12 in apple genotypes ‘Evereste’ and *Malus floribunda* clone 821. *Genome* 52:139–147, doi: <https://doi.org/10.1139/G08-111>.
- Emeriewen, O.F., T. Wöhner, H. Flachowsky, and A. Peil. 2019. *Malus* hosts-*Erwinia amylovora* interactions: Strain pathogenicity and resistance mechanisms. *Front. Plant Sci.*, doi: <https://doi.org/10.3389/fpls.2019.00551>.
- Fahrentrapp, J., G. Brogini, A. Peil, and M. Kellerhals. 2013. A candidate gene of *Malus ×robusta* 5 for breeding towards fire blight resistance. *Acta Hort.* 976:573–575, doi: <https://doi.org/10.17660/ActaHortic.2013.976.82>.
- Fryer, J. and B. Hylmö. 2009. *Cotoneasters: A comprehensive guide to shrubs for flowers, fruit and foliage*. Timber Press, Portland, OR.
- Harshman, J.M., K.M. Evans, H. Allen, R. Potts, J. Flamenco, H.S. Adwinkle, M.E. Wisniewski, and J.L. Norelli. 2017. Fire blight resistance in

- wild accessions of *Malus sieversii*. Plant Dis. 101:1738–1745, doi: <https://doi.org/10.1094/PDIS-01-17-0077-RE>.
- Ishimaru, C. and E.J. Klos. 1984. New medium for detecting *Erwinia amylovora* and its use in epidemiological studies. Phytopathology 74:1342–1345, doi: <https://doi.org/10.1094/Phyto-74-1342>.
- Johnson, K.B., V.O. Stockwell, D.M. Burgett, D. Sugar, and J.E. Loper. 1993. Dispersal of *Erwinia amylovora* and *Pseudomonas fluorescens* by honey bees from hives to apple and pear blossoms. Phytopathology 83:995–1002, doi: <https://doi.org/10.1094/Phyto-83-478>.
- Khan, M.A., Y. Zhao, and S.S. Korban. 2012. Molecular mechanisms of pathogenesis and resistance to the bacterial pathogen *Erwinia amylovora*, causal agent of fire blight disease in Rosaceae. Plant Mol. Biol. Rpt. 30:247–260, doi: <https://doi.org/10.1007/s11105-011-0334-1>.
- Lecomte, P. and A. Cadic. 1993. Further results on shoot susceptibility of *Cotoneaster* to fire blight. Acta Hort. 338:407–412, doi: <https://doi.org/10.17660/ActaHortic.1993.338.67>.
- McGhee, G.C., J. Guasco, L.M. Bellomo, S.E. Blumer-Schuette, W.W. Shane, A. Irish-Brown, and G.W. Sundin. 2011. Genetic analysis of streptomycin-resistant (SmR) strains of *Erwinia amylovora* suggests that dissemination of two genotypes is responsible for the current distribution of SmR *E. amylovora* in Michigan. Phytopathology 101:182–191, doi: <https://doi.org/10.1094/PHYTO-04-10-0127>.
- Mendiburu, F. 2019. Agricolae: Statistical procedures for agricultural research. National Engineering University (UNI), Lima, Peru. <<https://CRAN.R-project.org/package=agricolae>>.
- Norelli, J.N., A.L. Jones, and H.S. Aldwinke. 2003. Fire blight management in the twenty-first century: Using new technologies that enhance host resistance in apple. Plant Dis. 87:756–765, doi: <https://doi.org/10.1094/PDIS.2003.87.7.756>.
- Oh, C. and S.V. Beer. 2005. Molecular genetics of *Erwinia amylovora* involved in the development of fire blight. FEMS Microbiol. Lett. 253: 185–192, doi: <https://doi.org/10.1016/j.femsle.2005.09.051>.
- Paraschivu, M. and O. Cotuna. 2013. The use of the area under the disease progress curve (AUDPC) to assess the epidemics of *Septoria tritici* in winter wheat. Res. J. Agr. Sci. 45: 193–201.
- Persiel, F. and W. Zeller. 1978. Differences in susceptibility to fire blight, *Erwinia amylovora* (Burr.) Winslow et al., in non apomictal species, varieties and oecotypes of *Cotoneaster*. Acta Hort. 86:45–50, doi: <https://doi.org/10.17660/ActaHortic.1978.86.7>.
- Persiel, F. and W. Zeller. 1981. Some progress in breeding *Cotoneaster* for resistance to fireblight, *Erwinia amylovora* (Burr.) Winslow et al. Acta Hort. 117:83–88, doi: <https://doi.org/10.17660/ActaHortic.1981.117.13>.
- Persiel, F. and W. Zeller. 1990. Breeding upright growing types of *Cotoneaster* for resistance to fire-blight, *Erwinia amylovora* (Burr.) Winslow et al. Acta Hort. 273, 297–302, doi: <https://doi.org/10.17660/ActaHortic.1990.273.43>.
- Peil, A., T. Garcia-Liberos, K. Richter, F.C. Trognitz, M.V. Hanke, and H. Flachowsky. 2007. Strong evidence for a fire blight resistance gene of *Malus × robusta* located on linkage group 3. Plant Breed. 126:470–475, doi: <https://doi.org/10.1111/j.1439-0523.2007.01408.x>.
- Peil, A., V.G.M. Bus, K. Geider, K. Richter, H. Flachowsky, and M.V. Hanke. 2009. Improvement of fire blight resistance in apple and pear. Intl. J. Plant Breed. 3:1–27.
- Peil, A., H. Flachowsky, M.V. Hanke, K. Richter, and J. Rode. 2011. Inoculation of *Malus × robusta* 5 progeny with a strain breaking resistance to fire blight reveals a minor QTL on LG5. Acta Hort. 869:357–362, doi: <https://doi.org/10.17660/ActaHortic.2011.896.49>.
- Peil, A., C. Hübert, A. Wensing, A. Horner, O.F. Emeriewen, K. Richter, T.W. Wöhner, D. Chagne, C. Orellana-Torres, M. Saeed, M. Troggo, E. Stefani, S.E. Gardiner, M.V. Hanke, H. Flachowsky, and V.G.M. Bus. 2019. Mapping of fire blight resistance in *Malus × robusta* 5 flowers following artificial inoculation. BMC Plant Biol. 19:532, doi: <https://doi.org/10.1186/s12870-019-2154-7>.
- R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <<https://www.R-project.org/>>.
- Rothleutner, J.J., R.N. Contreras, V.O. Stockwell, and J.S. Owen. 2014. Screening *Cotoneaster* for resistance to fire blight by artificial inoculation. HortScience 49:1480–1485, doi: <https://doi.org/10.21273/HORTSCI.49.12.1480>.
- Smits, T.H.M., V.M. Guerrero-Prieto, G. Hernández-Escarcega, J. Blom, A. Goesmann, F. Rezzonico, B. Duffy, and V.O. Stockwell. 2014. Whole-genome sequencing of *Erwinia amylovora* strains from Mexico detects single nucleotide polymorphisms in *rpsL* conferring streptomycin resistance and in the *avrRpt2* effector altering host interactions. Genome Announc. 2:e01229-13, doi: <https://doi.org/10.1128/genomeA.01229-13>.
- Stockwell, V.O., K.B. Johnson, and J.E. Loper. 1998. Establishment of bacterial antagonists of *Erwinia amylovora* on pear and apple blossoms as influenced by inoculum preparation. Phytopathology 88:506–513, doi: <https://doi.org/10.1094/PHYTO.1998.88.6.506>.
- van der Zwet, T. and H. Keil. 1979. Fire blight: A bacterial disease of rosaceous plants. USDA Handbook 510. <<https://naldc.nal.usda.gov/download/CAT79714375/PDF>>.
- Vanneste, J.L. 2000. Fire blight: The disease and its causative agent, *Erwinia amylovora*. CABI Publishing, New York, NY.
- Vogt, I., T.W. Wöhner, K. Richter, H. Flachowsky, G.W. Sundin, A. Wensing, E.A. Savory, K. Geider, B. Day, M. Hanke, and A. Peil. 2013. Gene-for-gene relationship in the host–pathogen system *Malus × robusta* 5–*Erwinia amylovora*. New Phytol. 197:1262–1275, doi: <https://doi.org/10.1111/nph.12094>.
- Wöhner, T.W., H. Flachowsky, K. Richter, T. Garcia-Liberos, F. Trognitz, M.V. Hanke, and A. Peil. 2014. QTL mapping of fire blight resistance in *Malus × robusta* 5 after inoculation with different strains of *Erwinia amylovora*. Mol. Breed. 34:217–230, doi: <https://doi.org/10.1007/s11032-014-0031-5>.
- Wöhner, T.W., K. Richter, G.W. Sundin, Y. Zhao, V.O. Stockwell, J. Sellmann, H. Flachowsky, M.V. Hanke, and A. Peil. 2018. Inoculation of *Malus* genotypes with a set of *Erwinia amylovora* strains indicates a gene-for-gene relationship between the effector gene *eop1* and both *Malus floribunda* 821 and *Malus* ‘Evereste’. Plant Pathol. 67:938–947, doi: <https://doi.org/10.1111/ppa.12784>.