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Screening *Cotoneaster* for resistance to fire blight by artificial inoculation

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Abstract. Cotoneaster is a genus of ornamental landscape plants commonly affected by fire blight. Fire blight is a disease caused by the bacterial pathogen, *Erwinia amylovora* (Burrill) Winslow et al. that attacks a wide range of taxa in the apple subfamily (Maloideae; Rosaceae). To assess susceptibility of species and identify potential sources of resistance, we inoculated 52 taxa of *Cotoneaster* with *E. amylovora*. Disease severity was scored by percent shoot necrosis (lesion length/ total shoot length). Disease screenings were conducted over two years and varying levels of susceptibility were observed. Some taxa were highly susceptible to fire blight and the disease resulted in whole plant mortality (*C. rhytidophyllus* Rehder & E.H. Wilson, *C. rugosus* E. Pritzel ex Diels, and *C. wardii* W.W. Smith). Other taxa repeatedly exhibited moderate to high levels of disease resistance (*C. arbusculus* G. Klotz, *C. chungtinensis* (T.T. Yu) J. Fryer & B. Hylmö, *C. delsianus* E. Pritzel var. *delsianus*, *C. sikangensis* Flinck & B. Hylmö, *C. simonsii* Baker, and *C. splendens* Flinck & Hylmö). Ongoing studies are being conducted to determine if taxa with high levels of resistance under artificial inoculation will exhibit high levels of resistance in the field under natural disease pressure. Identifying sources of disease resistance will

be useful for breeding programs to increase tolerance of these landscape plants with desirable horticultural characteristics to fire blight.

Cotoneaster is a diverse genus of over 400 species ranging in form from groundcovers to trees. The center of diversity for the genus is in Tibet and the Himalayas, but species are native across continental Asia, North Africa, and Europe (Dickoré and Kasperk, 2010; Fryer and Hylmö, 2009; Bartish et al., 2001). *Cotoneaster* is commonly used in managed landscapes as durable, low maintenance ornamental shrubs where they are valued for multi-season interest for flowers, foliage, and fruiting characteristics. Although *Cotoneaster* generally is robust and easy to cultivate, many taxa are susceptible to the bacterial disease fire blight caused by *Erwinia amylovora*. Disease susceptibility limits the potential of the genus in landscape applications. For example, in some areas of Europe, cotoneasters have been banned as landscape ornamentals due to susceptibility to fire blight and concern that diseased *Cotoneaster* plants in the landscape may provide an inoculum source for disease in pear and apple orchards (B. Duffy, personal communication).

Erwinia amylovora is native to the United States and has a host range limited within members of Rosaceae, most commonly affecting members of the apple sub-family (Maloideae) (van der Zwet and Beer, 1999). The disease can especially be problematic in areas where warm temperatures, rain, and humid conditions favor bacterial growth and disease development, such as the south, east and Midwest regions of the United States, as well as areas in Europe where the pathogen was introduced. The pathogen overwinters in woody host tissues where it replicates through the spring and summer producing a bacterial exudate, that provides a source of inoculum for infection within and among nearby plants (van der Zwet and Beer, 1999). The

bacteria are dispersed primarily through rain, wind, and visiting insects. The pathogen enters the plant through wounds or natural openings; flowers are particularly vulnerable. Infected shoots first appear water soaked and then develop a scorched appearance, often with a characteristic shepherd's crook. When bark of infected plants is removed, discolored vascular tissue may be seen. Fire blight will quickly kill entire plants if the disease progresses to the crown. In addition to threatening the health of the plant, the necrotic tissue is unsightly and devalues nursery and landscape plants. Fire blight is managed through labor-intensive sanitation or prophylactic applications of copper or antibiotics, where permitted (van der Zwet and Beer, 1999). Host resistance, as a first line of defense in an integrated pest management plan, could greatly reduce the need for chemical inputs and allow for more extensive planting of resistant taxa.

Screening for fire blight resistance in ornamentals such as *Malus* Tourn ex L, *Pyrus* L, *Pyracantha* M. Roem., and *Cydonia* Mill. has demonstrated a wide range of susceptibility within these genera (Bell et al., 2004; Lespinasse and Aldwinckle, 2000; van der Zwet and Beer, 1999; Bouma, 1990). Screening for fire blight resistance in *Cotoneaster* has been relatively limited with only a portion of the genus screened. Previous screenings of *Cotoneaster* germplasm has resulted in the release of cultivars with fire blight resistance including *C. salicifolius* Franchet 'October Glory' and 'Willeki', *C. henryanus* (C.K. Schneider) Rehder & E.H. Wilson 'Corina' (Fryer and Hylmö, 2009), and *C. dammeri* C.K. Schneider 'Eicholz' (Bellenot-Kapusta et al., 2002), 'Holsteins Resi', and 'Thiensen' (Losing, 1992). Persiel and Zeller (1978;

1981; 1990) showed there was varying resistance within sexual populations of diploid species, which has allowed for selection of resistant taxa.

Many species of *Cotoneaster* are reported to be apomictic clones (Dickoré and Kasperek, 2010; Fryer and Hylmö, 2009) and currently there are no known selections for fire blight resistance from these species. In addition, most reports on fire blight resistance in *Cotoneaster* have been observational studies under landscape conditions without testing by artificial inoculation (Zeller, 1979; Jorgensen and Jensen, 1978; Davis and Peterson, 1976; Hodgkin and Fletcher, 1965; New Jersey Agricultural Experimental Station, 1932). Disease occurrence often varies between years and environments; therefore, results from observational surveys under low disease pressure may be inconclusive. Examination of disease development in *Cotoneaster* under controlled artificial inoculation may clarify the list of resistant taxa. Our objective was to evaluate susceptibility of a collection of *Cotoneaster* following inoculation with a virulent strain of fire blight under glasshouse conditions.

Materials and Methods

Plant material and culture conditions

The germplasm was obtained mostly as open pollinated seed from Index Seminum from a range of botanic gardens and institutions across the world. In addition, a few plants were obtained from commercial Oregon nurseries (*C. hortizontalis* Decaisne 'Variegatus', *C.* ×*suecicus* G. Klotz 'Coral Beauty', and *C. thymifolius* Baker). All taxa were grown in above ground containers and served as

donor plants from which cuttings were collected. Rooted cuttings were transferred to 1.65 L containers (Gage Dura-Pot, Lake Oswego, OR) with a custom substrate composed of 1 pumice: 2 peat: 7 douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] bark with standard nursery amendments (Rexius, Eugene, OR) and liquid-fed weekly (Jack's Professional 20N - 8.74P - 16.6K with micronutrients; J.R. Peters Laboratory, Allentown, PA), and watered by hand, as needed. Aphids and other pests were managed with M-pede® (Gowan Company, Yuma, AZ). Plants were maintained in a glasshouse with day/night set temperatures of 25/16 °C to encourage growth and then inoculated when average shoot length was approximately 30 cm. All plants were vigorously growing at the time of inoculation. This study took place over two consecutive years.

A total of 52 taxa were screened in this study. In year 1 (2011), 31 taxa were screened (Table 1). In year 2 (2012), 35 taxa were screened (Table 2). Fourteen of the taxa evaluated in 2011 were screened again to assess consistency between both years of the study.

In both years, plants were arranged in a randomized complete block design (RCBD) with 3 blocks and 3 plants per taxa per block. Also included in each block was a negative control plant on which two leaves were bisected with scissors that were dipped in sterile deionized water to confirm that observed lesions were not due to a wound response.

Inoculation and disease severity rating

139 The pathogen strain for this study was *E. amylovora* strain Ea153. Strain
140 Ea153 was isolated from diseased *Malus* ×*domestica* Borkh. 'Gala' apple trees in
141 eastern Oregon and its pathogenicity has been demonstrated in numerous field trials
142 (Stockwell et al., 2011). The pathogen was cultured in 200 mL Kings medium B broth
143 in a 1 L flask for 48 h at 27 °C on a rotary shaker (200 RPM). After 48 h, cells were
144 pelleted by centrifugation (3,220 *g*, 10 min) and mixed with powdered skim milk
145 [38% (w/w)]. The bacterial suspension was lyophilized, ground to a fine powder, and
146 frozen at -80 °C. Titer was calculated by dilution plating. Bacteria were re-suspended
147 in sterile deionized water to a concentration of 10^9 CFU/mL in 2011 and concentration
148 was reduced to 10^7CFU/mL in 2012 due to a calculation error.
149 In year 1, plants were inoculated on 3 March 2011 and final lesion length
150 measurements were taken on 24 May 2011. In year 2, plants were inoculated on 27
151 Feb. 2012 and final measurements were taken on 23 April 2012. Glasshouse
152 temperatures were recorded during both years (Fig. 1). Plants were inoculated by
153 bisecting the two youngest leaves on one shoot of each plant with scissors dipped in
154 the bacterial suspension. The necrotic lesion length was measured weekly for eight
155 weeks post-inoculation. The percent shoot necrosis, our measure of disease severity,
156 was calculated as the final length of the lesion divided by the entire shoot length and
157 expressed as an average percentage of the three plants within each block. The
158 pathogen was re-isolated on Kings medium B from lesions eight weeks after
159 inoculation to confirm the presence of the bacterium as the causal agent for disease.
160 Shavings were collected from two symptomatic taxa and one negative control in each

block for a total of six plants with lesions and three negative controls. Shavings from each lesion margin were placed in 10 mM phosphate buffer and spread onto solidified Kings medium B amended with 50 μg/mL cycloheximide **(**Sigma-Aldrich, St. Louis,MO) to discourage growth of fungi and yeasts. Plates were incubated at room temperature for 48 to 72 h, at which time the characteristic domed, white, mucoid colonies were visible. Lateral-flow immunoassay strips specific to *E. amylovora* were used to confirm the identity of isolated colonies as the pathogen (Braun-Kiewnick et al., 2011; BIOREBA, Reinach, Switzerland).

Data analysis

Disease severity data were analyzed within year by ANOVA (SAS Version 9.2, SAS Institute Inc., Carey, NC). The full model in ANOVA was used to test for species by year interaction and investigate disease ratings of checks between years. Area under disease progress curves (AUDPC) were calculated for species of interest and used to compare disease progression within and between years (Shaner and Finney, 1977; R Development Core Team, 2008) using ANOVA (JMP® Pro version 10.0.2, SAS Institute Inc., Carey, NC). Where appropriate, means were separated using Tukey's Honestly Significant Difference (HSD; $P = 0.05$). Plants demonstrating no disease incidence, as measured as the area under the disease progress curve (AUDPC) in either 2011 or 2012, were excluded from the analysis and considered potentially resistant.

In an attempt to clarify how our results correspond to the literature, disease sensitivity scores were also assigned to species in this study and to species covered in the literature. Disease sensitivity scores were assigned on a three-point scale; 2 points were given for species noted as generally being susceptible to fire blight or to have greater than 10% shoot necrosis, 1 point was assigned when the taxon was described as having some resistance or between 5% and 10% shoot necrosis, and 0 points for species that were identified as resistant or highly resistant by the reporting authors and less than 5% shoot necrosis in our study. An average score of sensitivity was also calculated by adding the sensitivity scores across the papers reporting for the species and dividing by the number of reporting papers (Table 3).

Results

Disease severity on taxa screened in year 1

In 2011, the disease severity on inoculated plants ranged from 0% shoot necrosis to total plant death (≥100% shoot necrosis). Control plants showed no symptoms of stem necrosis following leaf bisection with scissors dipped in sterile deionized water. The ≥100% disease severity rating indicates that the disease symptoms extended beyond the inoculated shoot and into the crown of the plant and also confirms the virulence of strain Ea153 on *Cotoneaster* species. Plants of three of the species tested (*C. rhytidophyllus*, *C. rugosus*, and *C. wardii*) were highly susceptible and were killed by the pathogen.

Some species showed no symptoms and other species appeared to show levels of quantitative resistance, whereby only a portion of the shoot was necrotic (Table 1). Mean separation of species did not discern between species exhibiting 0 to 11% shoot necrosis, meaning that plants with 11% shoot necrosis were not statistically different from plants that expressed no symptoms. This range was too wide to be applicable for our cultivar selection program; therefore, a tolerance threshold of 5% shoot necrosis was used to identify highly resistant plants. Six of the 30 taxa screened in the first year showed no shoot necrosis; these were *C. arbusculus*, *C. atropurpureus* Flinck & B. Hylmö, *C. delsianus*, *C. franchetii* Bois, *C. sikangensis*, and *C. splendens*. In addition, four other species, *C. atrovirens* J. Fryer & B. Hylmö, *C. chungtinensis*, *C. simsonii* Baker, and *C. sternianus* (Turrill) Boom, had under 5% shoot necrosis. The ten species identified as resistant in 2011 were screened again in 2012 to confirm disease resistance, with the exception of *C. atropurpureus* and *C. franchetii*, which had failed to yield enough asexual propagules for repeated testing.

Disease severity on taxa screened in year 2

In 2012, disease symptoms did not appear to be as severe as in the previous year; disease severity at the end of the study ranged from 0 to 62% (Table 2). Statistically, there was no difference among species with 0 to 6% shoot necrosis. All of the species that were identified as highly resistant in 2011 (≤ 5% disease severity; Table 1) that were repeated in 2012, also appeared highly resistant in the 2012 screening (Table 2). Additional taxa that were only tested in the second year and

scored as highly resistant included *C. acutifolius* Turczaninov, *C. boisianus* G. Klotz, *C. bullatus* Bois, *C. daliensis* J. Fryer & B. Hylmö, *C. fastigiatus* J. Fryer & B. Hylmö, *C. hypocarpus* J. Fryer & B. Hylmö, *C. lidjiangensis* G. Klotz, *C. milkedandaensis* J. Fryer & B. Hylmö, *C. pannosus* Franchet, and *C.* ×*suecicus* 'Coral Beauty'.

Discussion

In this study, we identified several taxa of *Cotoneaster* with resistance to fire blight using a tolerance threshold of 5% to identify highly resistant taxa, as only low levels of damage are acceptable in the nursery industry and in landscapes where aesthetics are of high importance. To our knowledge, this study is the first to rate fire blight susceptibility after artificial inoculation with the pathogen in 27 taxa of *Cotoneaster*. Twelve of these species showed no symptoms of fire blight inoculation. Additionally, *C. arbusculus*, *C. sikangensis*, and *C. splendens* were screened in both 2011 and 2012 and expressed no symptoms in either year, suggesting a high level of resistance.

Disease severity was also examined in species that expressed symptoms by calculating area under disease progress curve (AUDPC) for species that were not susceptible (Table 4), unique to each year (Table 5) and for those screened in both years (Table 6). Year, species, and their interaction proved to be statistically significant ($P<0.001$) when we compared the disease symptoms between the two years. Effects from environmental variables which are difficult to control, even in a

247 glasshouse environment likely were driving forces for differences in disease pressure
248 between years. Smith and Pusey (2010) discuss the impact that temperature has on the
249 growth rate of the bacteria but the interaction is more complex than only the impact on
250 the pathogen. In our study, the primary effect would have been on the rate of growth
251 of the pathogen and plant growth rate; those plants that are growing faster presumably
252 would have been more susceptible to infection. Although year had a significant
253 interaction with disease response, we observed consistent trends between years for
254 many species and *C. frigidus* Wallich ex Lindley, *C. henryanus*, and *C. salicifolius*
255 were the only species for which there were statistical differences between years for
256 AUDPC (Table 6).

257 In general, results obtained in this study were positively correlated with those
258 previously reported. *Cotoneaster nitens* Rehder & E.H. Wilson was susceptible in our
259 study (Table 1) and reported as susceptible by others including Lecomte and Cadic
260 (1993), Davis and Peterson (1976), and Thomas and Ark (1934) (Table 3). Some
261 conflicts in susceptibility ratings of species of *Cotoneaster* within the literature and
262 from our results were observed. For example, *C. simsonii* exhibited only low levels of
263 disease severity in our study (0.43% shoot necrosis in 2011 and 0.69% in 2012; Tables
264 1 and 2), whereas this species was rated as susceptible by Lecomte and Cadic (1993),
265 and by Zeller (1979) but also was identified as resistant by the New Jersey
266 Agricultural Experiment Station (1932), and Thomas and Thomas (1931) (Table 3).
267 Many variables may be responsible for these conflicts; for example, there may be
268 variation in susceptibility among selections of the species that were tested in Europe in

269 the 1970s and 1990s and those tested in the US in the 1930s. Additionally, some of
270 the reported results were from ratings after artificial inoculation (e.g. Bellenot-Kapusta
271 et al., 2002; Persiel and Zeller, 1981) and others from natural infection observations
272 (e.g. Jorgensen and Jensen, 1978; New Jersey Agricultural Experiment Station, 1932).
273 In studies where artificial inoculation was used to determine susceptibility to fire
274 blight, inoculum concentration ranged from 10^6 CFU/mL to 10^9 CFU/mL, or in some
275 cases was not specified. Bellenot-Kapusta et al. (2002) inoculated using titers at
276 concentrations ranging from 10^6 CFU/mL to 10^8 CFU/mL. No correlation of
277 inoculation titer to disease susceptibility was reported, but plant vigor was suspected
278 to influence the disease severity (Bellenot-Kapusta et al., 2002). We cannot rule out
279 that titer was not responsible for differences between years but their findings provide
280 support that other factors are involved. Plant vigor and growth may be correlated to
281 temperature and environmental factors, which may also influence the pathogenicity of
282 fire blight. In our study there were many different species used between years, which
283 may have different inherent growth rates in addition to having levels of resistance.
284 Mean shoot length at the time of inoculation in our study was 30 cm; however, some
285 species are large and very fast growing while others are extremely small and only
286 grow a few centimeters per year. Inherent growth rate differences combined with the
287 fact that temperatures were different between years, which can also affect the growth
288 rate of the pathogen, and it is not surprising that we observed differences between
289 years.

We did not investigate the mechanisms of resistance influencing lesion length in our study. We observed, however, that some species exhibited an apparent hypersensitive response in inoculated leaves, though we did not perform microscopic analysis. On inoculated leaves of *C. chungtinensis* and *C. delsianus* var. *delsianus*, the leaves were necrotic at the inoculation site and then rapidly shriveled and abscised before disease symptoms progressed into the stem. Nonetheless, leaf abscission was not observed with all resistant species. Many resistant species, such as *C. arbusculus* and *C. splendens*, maintained healthy green leaves following inoculation with little or no necrosis observed even at the bisection site.

Maas Geesteranus and Heyting (1978) proposed that under natural insect vectoring and disease pressure small or pubescent-leaved species of *Cotoneaster* exhibit less disease, possibly because the leaf morphology is less hospitable for insect vectors of the pathogen. We did not observe this trend with artificially inoculated plants, as some large fleshy-leaved species (*C. arbusculus* and *C. bullatus*) were resistant to fire blight and some small hairy-leaved species (*C. buxifolius* Wallich ex Lindley and *C. thymifolius*) were susceptible. From a breeding perspective, resistance by vector avoidance would only provide low levels of security in nursery production. In nurseries, the plants are regularly handled, sheared, and kept under overhead irrigation. Nursery production practices may provide wounds and promote environments that could contribute to an outbreak of fire blight independent of wounding and vectoring by insects. Therefore, breeding to increase resistance based

311 on vector avoidance may reduce the incidence of disease in landscapes but would not
312 be an effective mechanism to control disease in production nurseries.

313 We observed a range of susceptibility in *Cotoneaster* species to fire blight and
314 identified several taxa that showed consistent resistance. Artificial inoculation in a
315 glasshouse proved to offer an effective means to evaluate resistance to fire blight.
316 Future work will continue to evaluate additional species for resistance, as well as
317 investigating heritability of resistance among species and interspecific hybrids.
318 Evaluation of susceptibility through floral inoculation may also yield useful
319 information, as pollinators are often a vectoring agent of the disease in the landscape
320 and outbreaks of fire blight in fruit orchard systems are most severe when flowers are
321 infected (van der Zwet and Beer, 1999).

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Table 1. Percent shoot necrosis of *Cotoneaster* taxa inoculated with *Erwinia amylovora* strain Ea153 (10^9 CFU/mL) in 2011 with a foliar bisection assay

Species	Accession	% Shoot necrosis[z]
C. rhytidophyllus	09-0020	106.71[y]
C. wardii	09-0026	102.61[y]
C. rugosus	09-0021	102.24[y]
C. braydii E.C. Nelson & J. Fryer	09-0076	87.19
C. salificolius var. *floccosus*	09-0022	85.66
C. glabratus Rehder& E.H. Wilson	09-0016	79.46
C. applanatus E. Pritzel	09-0067	79.30
C. nitens	09-0052	68.81
C. cinerascens (Rehder)Flinck & B. Hylmö	09-0083	65.02
C. cashmeriensis G. Klotz	09-0080	62.56
C. buxifolius	09-0077	60.49
C. frigidus	09-0045	59.34
C. amoenus E.H. Wilson	09-0066	49.82
C. henryanus	09-0017	47.57
C. turbinatus	10-0096	43.70
C.divarcatus Rehder & E.H. Wilson	10-0089	32.66
C. bacillaris Wallich ex Lindley	09-0073	27.39
C. zabelii C.K. Schneider	09-0027	15.78
C. hebephyllus Diels	10-0091	15.39

C. cochleatus	09-0085	14.09
C. congestus Baker	10-0088	9.72
C. sternianus	09-0025	2.56
C. atrovirens	09-0072	2.02
C. simsonii	09-0023	0.43
C. chungtinensis	09-0082	0.35
C. franchetii	09-0015	0.00
C. arbusculus	09-0068	0.00
C. atropurpureus	09-0071	0.00
C. dielsianus var. *dielsianus*	09-0013	0.00
C. sikangensis	10-0095	0.00
C. splendens	09-0024	0.00

[z]Mean % shoot necrosis from fire blight, Tukey's HSD value 0.1060 ($P<0.05$).

[y]Shoot necrosis >100% signifies that the symptoms of fire blight extended beyond the inoculated shoot towards the crown and resulted in plant death.

Table 2. Percent shoot necrosis of *Cotoneaster* taxa inoculated with *Erwinia amylovora* strain Ea153 (10^7 CFU/mL) in 2012 with a foliar bisection assay

Species	Accession	% Shoot necrosis[z]
C. rubens W.W. Smith	10-0016	62.17
C. shansiensis J. Fryer & B.Hylmö	10-0017	54.11
C. horizontalis 'Variegatus'	10-0123	53.55
C. thymifolius	10-0122	23.78
C. vilmorinianus G. Klotz	11-0010	23.62
C. racemiflorus (Desfontaines) Booth ex Bosse	10-0154	21.00
C. vandelaarii J. Fryer & B. Hylmö	10-0139	18.72
C. genitanus Hurus	10-0132	15.24
C. astrophorus J. Fryer & B.Hylmö	10-0127	14.80
C. cochleatus	09-0085	12.47
C. lomaheunensis (Syn. *C. poluninii* G.Klotz)	10-0136	7.06
C. procumbens G. Klotz	10-0137	6.22
C. acutifolius	10-0126	4.67
C. henryanus[x]	09-0017	3.86
C. congestus[x]	10-0088	3.47
C. lidjiangensis	10-0135	1.75
C. ×*suecicus* 'Coral Beauty'	10-0166	1.68
C. dielsianus var. *dielsianus*[y]	09-0013	1.51
C. sternianus	09-0025	1.34

451	*C. divarcatus*[x]	10-0089	0.77
452	*C. simsonii*[y]	09-0023	0.69
453	*C. arbusculus*[y]	09-0068	0.00
454	*C. atrovirens*	09-0072	0.00
455	*C. boisianus*	09-0047	0.00
456	*C. bullatus*	09-0012	0.00
457	*C. chungtinensis*[y]	09-0082	0.00
458	*C. daliensis*	10-0129	0.00
459	*C. fastigiatus*	10-0013	0.00
460	*C. frigidus*[x]	09-0045	0.00
461	*C. hypocarpus*	10-0133	0.00
462	*C. milkedandaensis*	10-0174	0.00
463	*C. pannosus*	09-0046	0.00
464	*C. salicifolius* var. *floccosus*[x]	09-0022	0.00
465	*C. sikangensis*[y]	10-0095	0.00
466	*C. splendens*[y]	09-0024	0.00

467 [z]Mean % shoot necrosis from fire blight, Tukey's HSD value 0.0597 ($P<0.05$).

468 [y]Taxa rated highly resistant from both 2011 and 2012 disease screenings.

469 [x]Taxa scored as sensitive in year 2011disease screenings.

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Table 3. Comparison of results from current study on resistance of *Cotoneaster* taxa to fire blight to reports in the literature

	Mean score[z]	This study 2012[y]	This study 2011[y]	Lecomte and Cadic 1993[xy]	Zeller 1979[x]	Jorgensen and Jensen 1978[x]	Davis and Peterson 1976[x]	Hodgkin and Fletcher 1965[x]	Thomas and Ark 1934[y]	NJ disease 1932[x]	Thomas and Thomas 1931[xy]	Smith 1930[y]
C. amoenus	2	-	2	2	-	-	-	-	-	-	-	-
C. acutifolius	1.3	0	-	-	2	-	2	-	1	-	-	-
C. bullatus	1.3	0	-	1	2	-	-	-	2	-	-	-
C. buxifolius	2	-	2	-	-	-	-	-	2	-	-	-
C. congestus	0.8	0	2	1	-	-	-	-	-	-	-	-
C. dielsianus	0.6	0	0	2	2	0	0	-	1	0	0	-
C. divaricatus	1.4	0	2	2	-	1	2	-	-	-	-	-
C. franchetii	0.7	-	0	2	-	-	0	-	-	0	0	-
C. frigidus	1.2	0	2	2	2	-	-	-	-	-	0	-
C. glabratus	1.7	-	2	2	-	-	-	-	1	-	-	-
C. hebephyllus	2	-	2	2	-	-	-	-	-	-	-	-
C. henryanus	1.5	0	2	-	2	-	-	-	-	-	-	-
C. horizontalis	1.7	2	-	2	2	0	2	-	-	-	2	-

C. nitens	1.8	-	2	2	-	-	1	-	2	-	-	-
C. pannosus	1.5	0	-	-	-	-	-	-	-	2	2	2
C. racemiflorus	2	2	-	2	-	-	-	-	-	-	-	-
C. rhytidophyllus	2	-	2	-	2	-	-	-	-	-	-	-
C. salicifolius	1.8	0	2	2	2	2	-	2	-	2	2	-
C. sikangensis	0.7	0	0	2	-	-	-	-	-	-	-	-
C. simonsii	0.7	0	0	2	2	-	-	-	-	0	0	-
C. sternianus	1.3	0	2	2	-	-	-	-	-	-	-	-
C. thymifolius	2	2	-	2	-	-	-	-	-	-	-	-
C. wardii	2		2	-	-	2	-	-	-	-	-	-
C. ×suecicus												
‘Coral Beauty’	1	0	-	2	-	-	-	-	-	-	-	-
C. zabelii	1.5	-	2	2	-	-	0	-	2	-	-	-

[z]Average disease score from this study and the literature; 2 indicates taxa rated as susceptible, 1 indicates some level of resistance observed, 0 rated as resistant or no disease symptoms observed.

[y]Data from plants that were artificially inoculated.

[x]Data from non-inoculated observational studies that relied on natural infection.

Table 4. Potentially resistant *Cotoneaster* taxa that had no disease symptoms as measured as the area under the disease progress curve (AUDPC) when inoculated with *Erwinia amylovora* Ea153 under controlled conditions in 2011 (10^9 CFU/mL) or 2012 (10^7 CFU/mL).

Year	Taxon	Accession
2011	*C. arbusculus*[z]	09-0068
	C. atropurpureus	09-0071
	C. delsianus var. *delsianus*	09-0013
	C. sikangensis[z]	10-0095
	C. splendens[z]	09-0024
2012	*C. arbusculus*[z]	09-0068
	C. atrovirens	09-0072
	C. boisianus	09-0047
	C. bullatus	09-0012
	C. chungtinensis	09-0082
	C. daliensis	10-0129
	C. fastigiatus	10-0013
	C. frigidus	09-0045
	C. milkdedandaensis	10-0174
	C. pannosus	09-0046
	C. salicifolius var. *floccosus*	09-0022

532	*C. sikangensis*[z]	10-0095
533	*C. splendens*[z]	09-0024

534 [z] Species was screened in both years 2011 and 2012

Table 5. Disease incidence as measured as the area under the disease progress curve (AUDPC) for *Cotoneaster* taxa evaluated for fire blight susceptibility in either 2011 or 2012 but not in both years.

Year	Species	Accession	AUDPC
2011[z]	*C. rhytidophyllus*	09-0020	1.0671
	C. wardii	09-0026	1.0261
	C. rugosus	09-0021	1.0224
	C. braydi	09-0076	0.8719
	C. glabratus	09-0016	0.7946
	C. applanatus	09-0067	0.793
	C. nitens	09-0052	0.6881
	C. cinerascens	09-0083	0.6502
	C. cashmiriensis	09-0080	0.6256
	C. buxifolius	09-0077	0.6049
	C. amoenus	09-0066	0.4982
	C. turbinatus	10-0096	0.437
	C. bacillaris	09-0073	0.2739
	C. zabelii	09-0027	0.1578
	C. hebephyllus	10-0091	0.1539
2012[y]	*C. rubens*	10-0016	0.6217
	C. shansiensis	10-0017	0.5411

557	*C. horizontalis* ‘Variegatus’	10-0123	0.5355
558	*C. thymifolius*	10-0122	0.2378
559	*C. vilmorinianus*	11-0010	0.2362
560	*C. racemiflorus*	10-0154	0.21
561	*C. vandelaarii*	10-0139	0.1872
562	*C. genitianus*	10-0132	0.1524
563	*C. astrophoros*	10-0127	0.148
564	*C. lomahuensis*	10-0136	0.0706
565	*C. procumbens*	10-0137	0.0622
566	*C. acutifolius*	10-0126	0.0467
567	*C. lidjiangensis*	10-0135	0.0175
568	*C.* ×*suecicus* ‘Coral Beauty’	10-0166	0.0168

569 [z]HSD value of 0.4341 for year 2011 at $p<0.0001$

570 [y]HSD value of 0.301 for year 2012 at $p<0.0001$

Table 6. Simple effects (species x year) for disease incidence as measured as the area under the disease progress curve (AUDPC) for individual *Cotoneaster* taxa evaluated for fire blight susceptibility in both 2011 and in 2012

Species	Accession	2011[z]	2012	p - value
C. atrovirens	09-0072	0.0202	0.0000	0.3322
C. chungtiensis	09-0082	0.0035	0.0000	0.3322
C. cochleatus	09-0085	0.1409	0.1247	0.8833
C. congestus	10-0088	0.0972	0.0347	0.3497
C. dielsianus	09-0013	0.0000	0.0151	0.1501
C. divaricatus	10-0089	0.3266	0.0077	0.0563
C. frigidus	09-0045	0.5934	0.0000	<0.0001
C. henryanus	09-0017	0.4757	0.0386	0.0051
C. salicifolius	09-0022	0.8566	0.0000	<0.0001
C. simsonsii	09-0023	0.0043	0.0069	0.7475
C. sternianus	09-0025	0.0256	0.0134	0.6570

[z]HSD value of 0.0527 at significant difference within column in 2011, $p<0.0001$. No significant differences were observed among species in 2012.

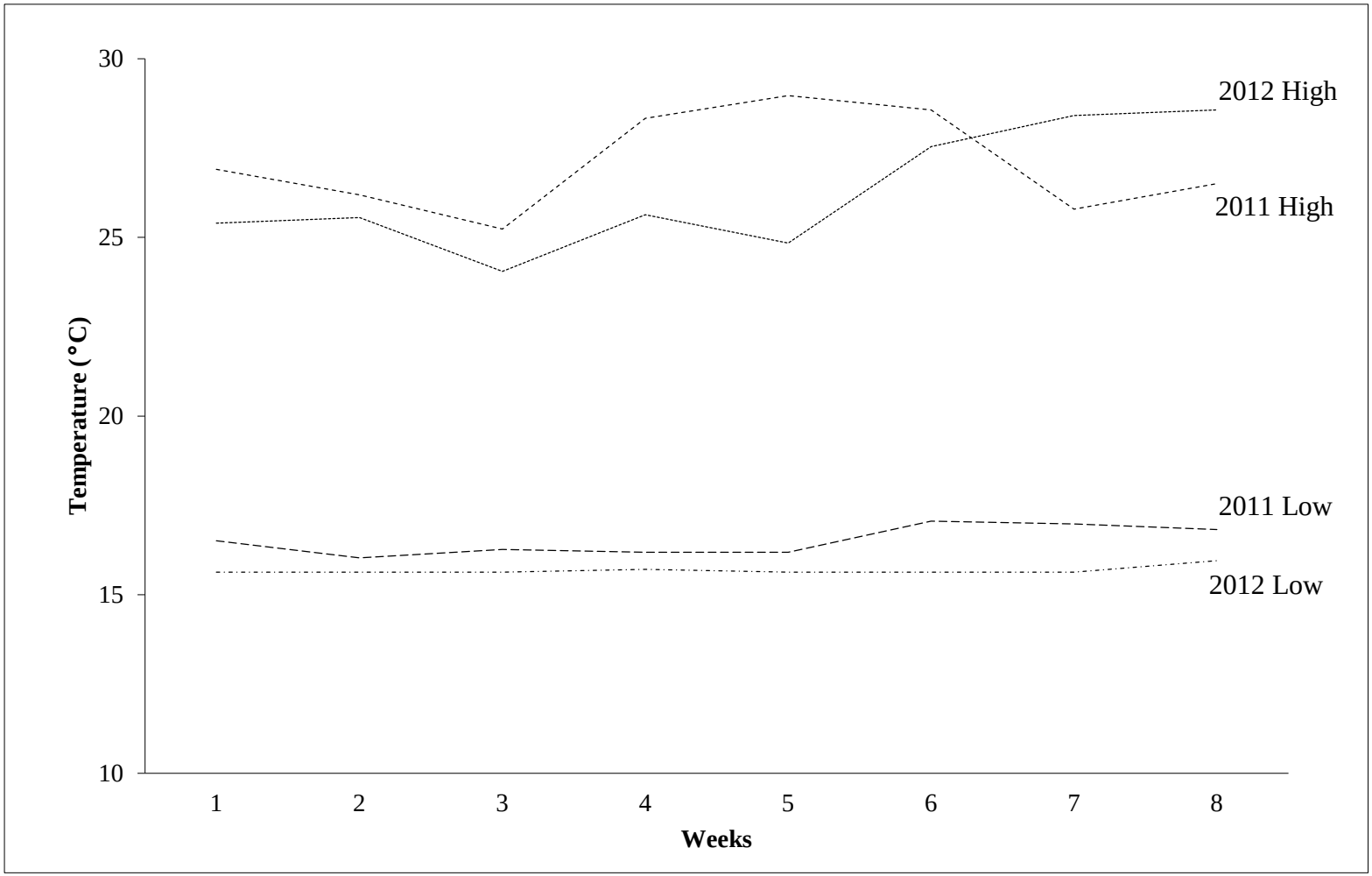

Figure 1. High and low temperatures recorded for the duration of both eight week disease screening trials during 2011 and 2012 in the glasshouse where plants were grown