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ORIGINAL ARTICLE

Xylem characteristics in *Ulmus americana* cultivars and their potential use as a preliminary screening method for Dutch elm disease resistance

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Abstract

Traditional screening of American elm (*Ulmus americana*) for resistance to Dutch elm disease (DED) often requires many years between initial propagation of trees and inoculation of older trees in the field. Previously published studies have found an association between smaller vessel diameters and increased resistance to DED, but further validation was needed to determine whether it could provide a rapid screening method to identify candidate trees for further testing. This investigation examined xylem characteristics in main stems of three-year-old trees for five cultivars and two wild-type seedling populations of *U. americana* with varying levels of resistance to DED. Cultivars with low disease severity ratings tended to have smaller vessel diameters and higher vessel densities than cultivars with high disease severity ratings. Xylem characteristics were also assessed in branches and main stems of larger plant material. Data suggest that the use of main stems is preferential to branches when evaluating large trees, as main stems provided more resolution in differentiating between the genotypes. Results from this study indicate that there is potential for the use of xylem characteristics, such as vessel diameter and vessel density, for selecting trees with putative resistance. However, caution should be used due to the potential effects of the environment, such as the effect of water availability and its impacts on xylem development.

1 | INTRODUCTION

There is great interest throughout much of the north temperate region of the world to breed and select elms for resistance to Dutch elm disease (DED) (Warren, 2000; Mittempergher & Santini, 2004; Slavicek et al., 2009; Knight et al., 2012; Martín et al., 2015; Pecori et al., 2017; Martín et al., 2019). Many researchers use disease severity ratings after inoculation with *Ophiostoma novo-ulmi* Brasier to determine whether a plant is potentially resistant (Guries & Smalley, 1990; Solla et al., 2000; Mittempergher & Santini, 2004; Pecori et al., 2017). Inoculations can be done in the field, greenhouse or growth chamber. Before inoculations are performed, the plants must be propagated to achieve an adequate number of replications for testing. Vegetatively propagating elms can be quite costly and challenging because of variation in success rates due to differences in genotypes, collection times and hormone treatments (Kreiser et al., 2016). Plants are often not inoculated until the third or (preferably) fourth year after propagating because inoculated young trees may fail to develop foliar symptoms compared with older trees (Solla et al., 2005). Maintaining plants for several years before inoculation can be expensive, in regard to both labour and space. Since plant breeding and selection for resistance rely on screening a large

number of plants to find the few that may have resistance, it would be advantageous to eliminate non-resistant plants in preliminary screening methods so more plants could be tested with the same amount of resources.

Another method of screening plants for resistance is through the use of morphological and anatomical characteristics that correlate with resistance. Elgersma (1970) and McNabb et al. (1970) found that resistant species/hybrids of elms had smaller vessel diameters than susceptible species/hybrids. A later study by Sinclair et al. (1975a) determined that xylem vessels in putative resistant *Ulmus americana* L. were generally smaller than those of susceptible American elms. They developed a vessel diameter index (VDI) to aid in screening elms for resistance to DED. The vessel diameter index was defined as 'mean percentage of vessels of diameter ≥50 μm among vessels intersected by arbitrary radii through rings 2 and 3 in elm branches' (Sinclair et al., 1975a). Some of the results presented by Sinclair et al. (1975a) were supported by a later study by Solla and Gil (2002a) on *Ulmus minor* Mill. Although Solla and Gil (2002a) did not find a relationship between the mean vessel diameters in the second annual ring, there was a correlation between mean vessel diameter and resistance in the third annual ring. Morphological/anatomical characteristics that correlate with disease resistance have been identified in other plants, such as Norway maple (*Acer platanoides* L.), peanut (*Arachis hypogaea* L.) and grape (*Vitis* sp.) (Godoy et al., 1985; Gökbayrak et al., 2010; Pouzoulet et al., 2017; Wittberg, 1983).

Although the possibility of preliminary screening by observations of xylem characteristics has been suggested previously, this method does not appear to be utilized by researchers working on breeding and selecting resistant American elms. A potential explanation for the underutilization of this method may be due to concern over the variability in xylem characteristics. Factors such as the age of the plant material (Leal et al., 2011; Martín et al., 2013; Solla et al., 2005), the amount of moisture available (Arend & Fromm, 2007; Lovisolo & Schubert, 1998; Pita et al., 2018; Solla & Gil, 2002b) and sun exposure (Lemoine et al., 2002) have all been shown to affect xylem development in tree species. Another potential explanation for its limited use is the lack of experimental testing with American elm cultivars that have been confirmed to be resistant to DED. Since multiple cultivars of American elm with varying levels of resistance to DED have been released in the last three decades (Townsend et al., 1995, 2005), sufficient plant material is now available to examine the practicality of using anatomical characteristics for preliminary screening for resistance. The objectives of this study were to (a) determine whether differences exist in xylem characteristics of American elm cultivars with varying levels of resistance to DED; (b) examine whether any xylem characteristics were correlated with disease susceptibility; (c) determine whether larger plant material showed the same trends for xylem characteristics as younger plant material; and (d) examine whether differences in xylem characteristics between cultivars are consistent from year to year.

2 | MATERIAL AND METHODS

2.1 | Plant material

To examine xylem characteristics in trees of different sizes, three groups of trees were assessed (Table 1). Tree size was referred to as small (1–3 cm diameter at 0.5 m), medium (5–10 cm dbh) and large (10–19 cm dbh). The small tree group was composed of five commercially available cultivars of *Ulmus americana*: 'Brandon', 'New Harmony', 'Prairie Expedition', 'Princeton' and 'Valley Forge'. Cultivars were acquired from commercial nurseries, where they were grown from stem cuttings, except for 'Brandon', which was grafted onto *U. americana* wild-type rootstock. Additionally, two groups of wild-type elm seedlings were utilized. These seedlings were sourced from Ontario,

TABLE 1 Summary of the experimental design used in this study, including tree location, cultivars assessed, replicates per cultivar, stem size, tissue assessed, annual ring assessed and growth year(s) assessed

	Tree size		
	Small	**Medium**	**Large**
Location	Falcon heights, MN	Saint Paul and Chaska, MN	Chaska, MN
Cultivars assessed[y]	BR, NH, PE, PR, VF, WTCA, WTUS	NH, PE, PR, VF	PR, VF
Replicates per cultivar	6 except for NH, which had 3	3	3
Stem size	1–3 cm diameter at 0.5 m	5–10 cm dbh	10–19 cm dbh
Tissue assessed	Main stem	Branches	Branches and main stem
Annual ring assessed[a]	Second	Third	Third for branches; NA for main stem
Growth year(s) assessed	2015	2014	2014 and 2015

Abbreviations: BR, Brandon; NH, New Harmony; PE, Prairie Expedition; PR, Princeton; VF, Valley Forge; WTCA, wild type (CA); WTUS, wild type (US).

[a]The annual ring that begins with the pith was not counted as an annual ring.

Canada (wild type (CA)) and Tennessee (wild type (US)). Trees were planted during the summer of 2014 in the nursery fields at the University of Minnesota, St. Paul, MN, and were watered as needed. In 2015, trees were 3 years old. 'New Harmony', 'Prairie Expedition', 'Princeton' and 'Valley Forge' were the medium-sized trees assessed. For large-sized trees, only 'Princeton' and 'Valley Forge' were examined due to limited availability of larger plant material of these cultivars. All medium and large trees were within 25 miles of the University of Minnesota, St. Paul campus (Table 1).

2.2 | Inoculations and disease ratings

To evaluate resistance to DED, an inoculation trial in the field was undertaken. Only the small trees were examined for resistance to DED. On 28 May 2015, trees were either inoculated with *Ophiostoma novo-ulmi* (trees used for determining disease susceptibility) or mock-inoculated with sterile water (trees used for determining xylem characteristics). The strain of *Ophiostoma novo-ulmi* used was isolated from an infected *Ulmus americana* in Minnesota in 2014, and it was later determined to be subspecies *americana* by Hessenauer et al. (2020). The method of inoculation is described fully in Beier et al. (2017). Briefly, a 4-mm deep hole was made with a 2.38-mm drill bit at 0.5 m above the soil line. Trees were subsequently inoculated with 25 µl of a spore suspension (1 x 10^6 spores/ml) or sterile water, and the wound was wrapped with Parafilm M® (Bemis Co., Inc.). For each cultivar, 6–9 trees were inoculated with *O. novo-ulmi* and 3–6 were mock-inoculated with sterile water depending on plant availability. Trees inoculated with *O. novo-ulmi* were assessed 90 days post-inoculation (DPI) on a 12-point scale ranging between 1 = 0%, 2 = 1%–9%, 3 = 10%–19%, 4 = 20%–29%, 5 = 30%–39%, 6 = 40%–49%, 7 = 50%–59%, 8 = 60%–69%, 9 = 70%–79%, 10 = 80%–89%, 11 = 90%–99% and 12 = 100%.

2.3 | Sample collection

For small trees, samples were collected from three-year-old mock-inoculated trees on July 7, 2015 (40 days after mock inoculation). This time period was selected because there was a clear distinction between disease severity of the most susceptible and most resistant cultivars by that time. For each cultivar, six trees were processed, except for 'New Harmony', which had three, due to a lack of plant material. A portion of stem was collected approximately 0.60 m above the ground. Samples were then placed in a −20°C freezer until sectioned. For medium trees, two branches with at least three annual rings were collected from different areas of the lower crown. From each branch, a segment was collected from the centre of the portion of the branch with three annual growth rings (not including the annual ring that begins with the pith). The year of growth analysed was 2014. The process for large trees was the same as for medium trees; however, two portions of the branch were processed. The third annual ring of 2014 and 2015 year's growth was analysed. Additionally, two increment cores per tree were collected from opposite sides of the tree, and the annual growth rings from 2014 and 2015 were assessed. Samples were then placed in a −20°C freezer until processed.

2.4 | Histology

For main stems of the small trees, four pieces (approximately 2 mm wide) spaced 90° apart with a random starting point were cut from the stem segment using a high-profile microtome blade. For branches of medium and large trees, three pieces (approximately 1 mm wide) spaced 120° degrees apart with a random starting point were used. Pieces were soaked in 100% TFM™ tissue freezing medium (Electron Microscopy Sciences, Hatfield, PA) for approximately 16 hr. An IEC Minotome® cryostat (International Equipment Co.) at −20°C was used to cut 15- to 30-µm-thick transverse sections. Due to the large size and brittleness of the increment cores, cores were free-hand-sectioned. After sectioning, the sections were cleared with water and subsequently stained with 0.1% safranin O (dye content ≥85%) (Sigma-Aldrich®) (w/v) solution for 20 s. After removing excess safranin O with a paper towel, sections were mounted in water by placing a drop of DI water onto the surface of the sample and then subsequently covering the sample with a coverslip. Sections were allowed to air-dry at room conditions for 24 hr. Images were taken at 40× using a Nikon Eclipse E600 microscope (Nikon Instruments Inc.) with a Nikon Digital Camera DXM 1200F (Nikon Instruments Inc.).

2.5 | Xylem analysis

Many samples were larger than the field of view for the low magnification of the microscope objective and field of view for the digital documenting system, so multiple images of the same section were stitched together in Photoshop™ (Adobe Systems Inc.) using the photomerge feature. Focus stacking was performed as needed in Photoshop™. A 500-µm-wide area of the xylem following the same ray parenchymal cells of the annual ring of interest was analysed. This area included earlywood and latewood vessels. Due to the large size of the core samples and the likely importance of vessels formed early in the season, only the first 2,000 µm of the annual ring was analysed. Vessel elements were manually traced or selected using the magic wand in Photoshop™. Images were subsequently analysed using the thresholding feature in ImageJ (Schneider et al., 2012) to generate a mask of vessel elements with a *D* (equivalent circle diameter) ≥15 µm. The masked images were then analysed using ROXAS 3.0 (von Arx et al., 2013). Jansen et al. (2009) found that the total intervessel cell wall thickness (mean ± *SD*) in *Ulmus americana* was 2.946 ± 0.665 µm; therefore, the double cell wall thickness was set to 4 µm to ensure most connected vessels would be included when analysed for vessel aggregation. Multiple variables were measured including the following: equivalent circle diameter (*D*) in µm, which is the diameter of a circle having the same lumen area (walls excluded) as the measured

TABLE 2 Xylem characteristics in the most recent annual ring of main stems from small-sized (1–3 cm diameter at 0.5 m ht.)

	Cultivar[1,2]							
Variable[3]	'Prairie Expedition'	'Brandon'	'Valley Forge'	Wild type (US)	Wild type (CA)	'New Harmony'	'Princeton'	r_s[4]
DSR[5]	5.7 ± 1.0 a	6.9 ± 0.8 a	8.7 ± 0.9 ab	9.3 ± 0.8 abc	10.7 ± 0.7 bc	12.0 ± 0 bc	12.0 ± 0 c	1.00
D	32.6 ± 0.5 d	34.8 ± 1.9 cd	32.2 ± 1.1 d	43.5 ± 1.7 a	36.9 ± 1 bc	40.1 ± 0.8 ab	44.3 ± 0.8 a	0.76 *
D_{MAX}[6]	115.9 ± 3.7 bc	109.9 ± 4.5 c	140.1 ± 2.1 a	105.3 ± 3 c	113.9 ± 5.5 c	138.2 ± 1.5 ab	125.5 ± 3.1 abc	0.27
VDI	7.2 ± 0.8 c	18.3 ± 2.9 b	9.2 ± 2.2 c	38.5 ± 4.2 a	17.7 ± 1.6 b	23.8 ± 3 b	35.2 ± 1.8 a	0.63
V_D	246.3 ± 16.7 a	197.7 ± 28.1 a	212.4 ± 19.8 a	101.6 ± 9.3 b	120.7 ± 12.7 b	125.7 ± 6 b	97.5 ± 5.6 b	−0.77 *
V_G	3.22 ± 0.17 a	2.17 ± 0.16 c	2.72 ± 0.07 b	1.67 ± 0.06 d	1.97 ± 0.05 cd	2.19 ± 0.1 c	1.82 ± 0.06 cd	−0.52
V_S	14.08 ± 1.08 e	26.00 ± 3.79 bc	16.91 ± 0.78 de	36.41 ± 2.43 a	27.49 ± 1.3 bc	23.26 ± 1.81 cd	32.08 ± 1.28 ab	0.52
V_M	4.81 ± 0.26 a	3.43 ± 0.18 c	4.13 ± 0.13 b	2.64 ± 0.08 e	3.04 ± 0.1 cde	3.38 ± 0.11 cd	2.92 ± 0.1 de	−0.68

Note: Ulmus americana cultivars and wild-type seedlings harvested on 7 July 2015 and their relationship to mean disease severity ratings of trees inoculated with *Ophiostoma novo-ulmi*.

[1]Except for the variable DSR, means ± *SE* of four sections from each of six trees, except for 'New Harmony', which had three trees.

[2]Except for the variables DSR and D_{MAX}, means containing the same letter within a row are not significantly different according to Fisher's LSD multiple comparisons test with a Benjamini and Hochberg *p*-value adjustment ($\alpha = 0.05$).

[3]Variables are DSR, disease severity rating at 90 DPI on a 12-point ordinal scale; *D*, equivalent circle diameter (diameter of the circle having the same area as the measured vessel); D_{MAX}, maximum vessel diameter; *VDI*, mean percentage of vessels with a diameter ≥50 μm; *VD*, vessel density (number of vessels per mm^2); V_G, vessel grouping index (mean number of vessels per group, solitary vessels are also considered a group); V_S, vessel solitary fraction (ratio of solitary vessels to all vessels); V_M, mean group size of non-solitary vessels.

[4]Spearman's correlation coefficient between DSR (disease severity rating) and variables using group means. Correlation coefficients followed by an * were found to be statistically significant ($\alpha = 0.05$).

[5]DSR was assessed on 6–9 trees inoculated with *Ophiostoma novo-ulmi* for each cultivar. Trees used for disease severity ratings were not assessed for xylem characteristics. DSR was analysed using non-parametric analysis due to violations of the assumptions of ANOVA. Groups with the same letter in the same row were not statistically significant according to Dunn's multiple comparison with a Benjamini and Hochberg *p*-value adjustment ($\alpha = 0.05$).

[6]D_{MAX} was analysed using non-parametric analysis due to violations of the assumptions of ANOVA. Groups with the same letter in the same row were not statistically significant according to Dunn's multiple comparison with a Benjamini and Hochberg *p*-value adjustment ($\alpha = 0.05$).

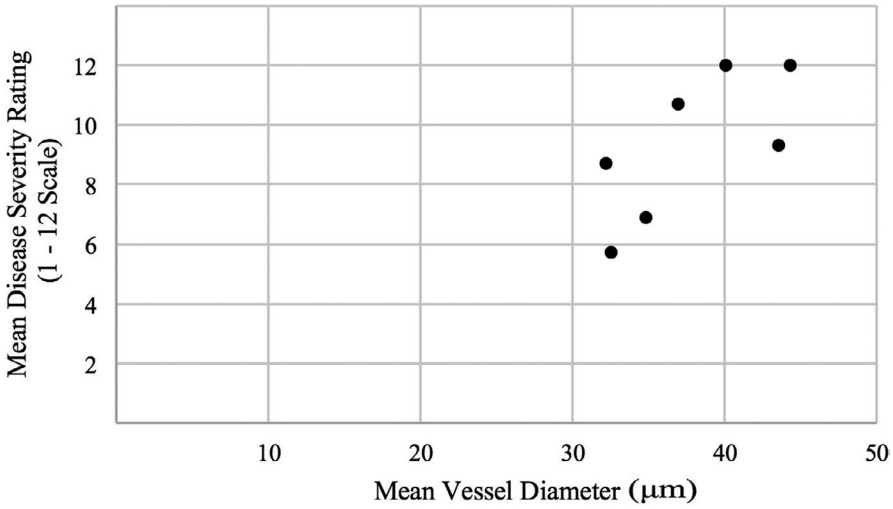

FIGURE 1 Relationship between mean disease severity rating at 90 DPI and mean vessel diameter for *Ulmus americana* cultivars ($r_s = 0.76$, $p = 0.049$)

vessel;maximum equivalent circle diameter (D_{MAX}) in μm; vessel diameter index (*VDI*), modified from Sinclair et al. (1975a), the percentage of vessels with *D* greater than 50 μm; vessel density (V_D) in vessels/mm^2; vessel grouping index (V_G), mean number of vessels per group, where solitary vessels are also considered a group (Carlquist, 2001); vessel solitary fraction (V_S), per cent of solitary vessels to all vessels (von Arx et al., 2013); and mean group size (V_M), mean group size of non-solitary vessels (von Arx et al., 2013).

2.6 | Statistical analysis

Statistical analysis was performed using the statistical package R version 3.2.2 (R Development Core Team). For small- and medium-sized trees, xylem characteristics were analysed using one-way ANOVA ($\alpha = 0.05$). Fisher's LSD test was used as a multiple means comparison test with a Benjamini and Hochberg (1995) *p*-value adjustment ($\alpha = 0.05$). Some of the data lacked a normal distribution or homoscedasticity as evident by the results of the Shapiro–Wilk normality test and Levene's test for homogeneity of variance and could not be transformed to normality. Therefore, data violating the assumptions of ANOVA were analysed using the Kruskal–Wallis test followed by Dunn's multiple comparison test with a Benjamini and Hochberg (1995) *p*-value adjustment. For xylem measurements of small-sized trees, a grand mean was calculated for each cultivar using four stem sections for each of the six trees, $n = 6$ (except for 'New Harmony', which had three trees, $n = 3$). For branches of medium-sized trees, the grand mean was calculated for each cultivar using three stem sections from each of the two branches for each of the three trees, $n = 3$. For analysis of branches and main stems of large-sized trees, Student's *t* tests were used to compare 'Valley Forge' with 'Princeton' for both 2014 and 2015. To compare each cultivar between 2014 and 2015, paired Student's *t* tests were used. If data violated the assumptions of the Student's *t* test and could not be transformed to normality, the data were not analysed. Non-parametric analysis of these data was not feasible due to the small sample sizes. For branches in large trees, the grand mean was calculated for each cultivar using three sections from each of the two branches for each of the three trees, $n = 3$, while for main stems, the grand mean for each cultivar was calculated using the first 2000 μm of two increment core sections from each of the three trees, $n = 3$. To examine the relationship between disease severity and xylem variables measured, Spearman's rank–order correlation was used since disease severity was measured on an ordinal scale. Since disease severity was measured on separate individuals, group means were used for determining correlation between vessel diameters within genotypes. To determine the correlation between vessel diameter between small- and medium-sized trees, Pearson's product–moment correlation was used, utilizing group means.

3 | RESULTS

3.1 | Vessel diameter

When examining main stems of small trees, mean vessel diameter (*D*) varied considerably among the cultivars (Table 2). There was a strong positive correlation between mean disease severity rating (DSR) and mean *D* ($r_s = 0.76$, $p = 0.049$) (Figure 1). The three cultivars with the lowest mean DSR also had the lowest mean *D*. There was a statistically significant difference between those and the two cultivars with the highest DSR, 'New Harmony' and 'Princeton' ($p < 0.05$). 'Prairie Expedition', which had the lowest average DSR (5.7), had the second lowest mean *D* (32.6 μm). 'Valley Forge', which had the third lowest DSR, had the smallest mean *D* (32.2 μm). Despite having the lowest mean *D*, 'Valley Forge' had the highest mean maximum vessel diameter (D_{max}) at 140.1 μm and was significantly different from all other cultivars except, 'New Harmony' and 'Princeton' ($p < 0.05$).

Branches of medium-sized trees showed a similar trend to that of the stems of small trees. The four cultivars examined had the same rank for mean *D* for both small-sized trees and medium-sized trees (Tables 1 and 2). 'Valley Forge' had the smallest mean *D* at 25.7 μm, while 'Princeton' had the largest at 33.3 μm. There was a significant difference in mean *D* between 'Valley Forge' and 'Prairie Expedition' when compared to 'New Harmony' and 'Princeton' ($p < 0.05$). All

cultivars had a smaller mean *D* in the branches of medium-sized trees compared with main stems of small trees (Tables 1 and 2). There was a very strong positive correlation between mean vessel *D* of cultivars in main stems of small trees and that of branches of medium trees ($r = 0.97$, $p = 0.03$) (data not shown).

The difference between mean vessel *D* for 'Princeton' and 'Valley Forge' was the smallest in branches of large trees (Tables 1, 2 and 3). Vessels of 'Valley Forge' had a smaller mean *D* than 'Princeton' in both 2014 and 2015, but the difference in means was only found to be statistically significant in 2014 (Table 4). In 2014, the difference in means was 3.1 μm, while in 2015, it was only 0.3 μm. There was a statistically significant difference for mean *D* between 2014 and 2015 for 'Valley Forge', but not for 'Princeton' ($p < 0.05$). In both years, 'Valley Forge' had a larger mean D_{MAX} than 'Princeton' and the difference in 2014 was found to be statistically significant.

When analysing the first 2,000 μm of annual rings from increment core sections of main stems in large trees, the difference between the two cultivars was considerably larger than that of branches (Table 4). In 2014, 'Valley Forge' had a mean *D* of 43.5 μm, while 'Princeton' had a mean of 51.0 μm ($p < 0.05$). Similar to the results found in branches, the difference between the cultivars was far less in 2015, with a difference in means of only 2.3 μm ($p > 0.05$). There was a significant decrease in mean *D* for 'Princeton' from 2014 to 2015, but not for 'Valley Forge' ($p < 0.05$).

3.2 | Vessel distribution

In main stems of small trees, 'Prairie Expedition', 'Brandon' and 'Valley Forge' had higher percentages of vessels in the 15–25 μm class compared with the remaining cultivars (Figure 2). For both 'Brandon' and 'Valley Forge', the largest proportion of vessels were in the 15–25 μm class, while 'Prairie Expedition' had the largest proportion of vessels in the 25–35 μm class (Figure 2). 'Princeton' had highest mean percentage of vessels for the >85 μm diameter class (Figure 2). For the vessel diameter index (*VDI*), which is the percentage of vessels over 50 μm, there was a statistically significant difference between 'Prairie Expedition', 'Brandon' and 'Valley Forge', when compared to 'Princeton' ($p < 0.05$) (Table 2).

In branches of medium-sized trees, 'Valley Forge' had the largest percentage of vessels in the 15–25 μm class at 67%, while 'Princeton' had the lowest at less than 40% (Figure 3). The four cultivars were very similar in the percentage of vessels in all vessel diameter classes above 55 μm (Figure 3). 'Princeton' and 'New Harmony' had a considerably larger percentage of vessels in the 35–45 μm and 45–55 μm diameter classes compared with 'Prairie Expedition' and 'Valley Forge' (Figure 3). When examining cultivars for *VDI*, there was a significant difference between 'Prairie Expedition' and 'Valley Forge' compared with 'New Harmony' and 'Princeton' ($p < 0.05$; Table 3).

For the branches of large 'Valley Forge' and 'Princeton' trees, over 80% of the total vessels were in the two smallest vessel diameter classes, 15–25 μm and 25–35 μm, for both 2014 and 2015 (Figure 4). The remaining vessel diameter classes all had means for the proportion of vessels at less than 5%, except for 'Valley Forge' in 2015 for the >85 μm diameter class at 6% (Figure 4). In main stems for 2014, 'Valley Forge' had higher percentages of vessels in the diameter classes less than 65 μm and 'Princeton' had higher percentages for the classes greater than 65 μm (Figure 5). In the 2015 growth ring, the two cultivars showed much smaller differences in percentages for the 15–25 μm and 25–35 μm vessel diameter classes

TABLE 3 Xylem characteristics in the third annual ring of branches of medium-sized (5–10 cm dbh) *Ulmus americana* cultivars for 2014

Variable[2]	Cultivar[1]			
	'Prairie Expedition'	'Valley Forge'	'New Harmony'	'Princeton'
D	27.9 ± 0.6 b	25.7 ± 1.1 b	31.8 ± 0.2 a	33.3 ± 1 a
D_{MAX}	93.9 ± 3.7 a	108.7 ± 8.0 a	97.5 ± 5.3 a	110.0 ± 5.3 a
VDI	5.9 ± 1.2 b	4.6 ± 0.5 b	11.6 ± 1.4 a	12.7 ± 1.5 a
V_D	244.5 ± 11.5 b	286.0 ± 12.1 a	177.1 ± 1.9 c	160.1 ± 12.1 c
V_G[3]	2.81 ± 0.11 a	2.88 ± 0.37 a	2.25 ± 0.06 a	2.38 ± 0.05 a
V_S	18.27 ± 1.27 a	18.01 ± 2.69 a	21.67 ± 1.35 a	21.27 ± 0.79 a
V_M	4.48 ± 0.09 a	4.28 ± 0.56 a	3.43 ± 0.04 a	3.60 ± 0.04 a

[1]Means ± *SE* of three sections per two branches from each of three trees. Except for the variable V_G, means containing the same letter within a row are not significantly different according to Fisher's LSD multiple comparisons test with a Benjamini and Hochberg *p*-value adjustment ($\alpha = 0.05$).

[2]Variables are *D*, equivalent circle diameter (diameter of the circle having the same area as the measured vessel); D_{MAX}, maximum vessel diameter; *VDI*, mean percentage of vessels with a diameter ≥50 μm; *VD*, vessel density (number of vessels per mm^2); V_G, vessel grouping index (mean number of vessels per group, solitary vessels are also considered a group); V_S, vessel solitary fraction (ratio of solitary vessels to all vessels); V_M, mean group size of non-solitary vessels.

[3]V_G was analysed using non-parametric analysis due to violations of the assumptions of ANOVA. Groups with the same letter in the same row were not statistically significant according to Dunn's multiple comparison with a Benjamini and Hochberg *p*-value adjustment ($\alpha = 0.05$).

TABLE 4 Xylem characteristics in branches (third annual ring) and main stems of large-sized (10 – 19 cm dbh) *Ulmus americana* 'Valley Forge' and 'Princeton' for 2014 and 2015

		Year					
		2014		2015		Difference Between Years[3]	
		Cultivar[1,2]					
	Variable[4]	'Valley Forge'	'Princeton'	'Valley Forge'	'Princeton'	'Valley Forge'	'Princeton'
Branches	*D*	25.4 ± 0.9 a	28.5 ± 0.3 b	28.6 ± 0.5 a	28.9 ± 1.0 a	*	NS
	D_{MAX}	101.8 ± 2.5 a	88.0 ± 1.1 b	104.0 ± 6.4	94.0 ± 1.2	NS	NA
	VDI	6.9 ± 1.8	9.5 ± 0.3	11.5 ± 1.3 a	12.0 ± 1.4 a	NA	NS
	V_D	278.4 ± 10.3 a	248.3 ± 9.2 a	279.9 ± 17.7 a	245.7 ± 14.4 a	NS	NS
	V_G	2.62 ± 0.13 a	2.25 ± 0.07 a	2.31 ± 0.18	1.91 ± 0.10	NS	NA
	V_S	19.55 ± 1.06 a	21.41 ± 1.39 a	25.06 ± 2.37 a	29.40 ± 2.00 a	NS	*
	V_M	4.03 ± 0.27 a	3.34 ± 0.13 a	3.74 ± 0.38	3.00 ± 0.16	NS	NA
Main Stems	*D*	43.5 ± 0.7 a	51.0 ± 1.2 b	42.1 ± 1.5 a	44.4 ± 1.2 a	NS	*
	D_{MAX}	214.0 ± 7.7 a	183.5 ± 8.8 a	201.5 ± 9.7 a	188.2 ± 9.5 a	NS	NS
	VDI	24.3 ± 2.0	33.9 ± 0.9	20.1 ± 2.2	24.4 ± 1.8	NA	NA
	V_D	128.8 ± 3.5 a	82.3 ± 8.9 b	147.2 ± 17.7 a	113.5 ± 4.0 a	NS	NS
	V_G	2.18 ± 0.15 a	1.66 ± 0.08 b	2.19 ± 0.21 a	1.99 ± 0.09 a	NS	NS
	V_S	29.80 ± 3.91 a	41.47 ± 3.80 a	31.33 ± 4.21 a	34.87 ± 2.73 a	NS	NS
	V_M	4.00 ± 0.08	3.09 ± 0.06	4.34 ± 0.35 a	3.90 ± 0.19 a	NS	NA

[1]For branches: means ± *SE* of three sections per two branches from each of three trees. For main stems: means ± *SE* of the first 2,000 μm of two increment core sections from each of three trees. Means containing the same letter in the same year within a row are not significantly different according to Student's *t* test ($\alpha = 0.05$).

[2]Means without a letter were not analysed due to violations of the assumption of normality, which could not be transformed.

[3]The difference in means from 2014 and 2015 for both cultivars were compared using a paired Student's *t* test: *$p < .05$; NS = $p > .05$; NA = Analysis was not performed due to violations of the assumption of normality.

[4]Variables are *D*, equivalent circle diameter (diameter of the circle having the same area as the measured vessel); D_{MAX}, maximum vessel diameter; *VDI*, mean percentage of vessels with a diameter ≥50 μm; *VD*, vessel density (number of vessels per mm^2); V_G, vessel grouping index (mean number of vessels per group, solitary vessels are also considered a group); V_S, vessel solitary fraction (ratio of solitary vessels to all vessels); V_M, mean group size of non-solitary vessels.

compared with 2014 (Figure 5). For both branches and main stems, 'Valley Forge' had smaller mean *VDI* in both 2014 and 2015 compared with 'Princeton', but due to normality issues, significance testing could only be performed in the branches of 2015, and it was not found to be statistically significant ($p > 0.05$; Table 4). Differences in *VDI* between the cultivars were much smaller in 2015 compared with 2014 (Table 4).

3.3 | Vessel aggregation

Vessel density (V_D) had the strongest correlation with DSR of the variables measured ($r_s = -0.77$, $p = 0.04$). Cultivars with lower DSR tended to have higher V_D than those with higher DSR (Table 2). In main stems of small trees, 'Prairie Expedition' had the highest mean vessel grouping index (V_G) (3.22). The difference in mean V_G between 'Prairie Expedition' and all other cultivars was found to be statistically significant ($p < 0.05$). Additionally, 'Prairie Expedition' had the highest mean group size (V_M) and V_D (Table 2). 'Valley Forge' was the second highest for V_G, V_M and V_D (Table 2). Interestingly, the wild type (US), which was ranked fourth for DSR, had the lowest V_G and V_M and the second lowest V_D (Table 2).

When examining branches of medium-sized trees, there was a significant difference between 'Prairie Expedition' and 'Valley Forge', when compared to 'New Harmony' and 'Princeton' for V_D ($p < 0.05$) (Table 3). However, none of the differences for V_G, V_M and V_S were found to be statistically significant among the cultivars (Table 3).

In branches and main stems of large trees, 'Valley Forge' had a higher mean V_D than 'Princeton' for both 2014 and 2015, but the only difference in vessel density between the two cultivars found to be statistically significant was for main stems in 2014 ($p < 0.05$; Table 4). Similar to previous trends in other sized plant material, 'Valley Forge' had higher mean V_G and V_M and a lower V_S than 'Princeton' in 2014 and 2015 for both branches and main stems; however, the only difference that was found to be significant was for V_G in main stems in 2014 ($p < 0.05$) (Tables 1, 2 and 3).

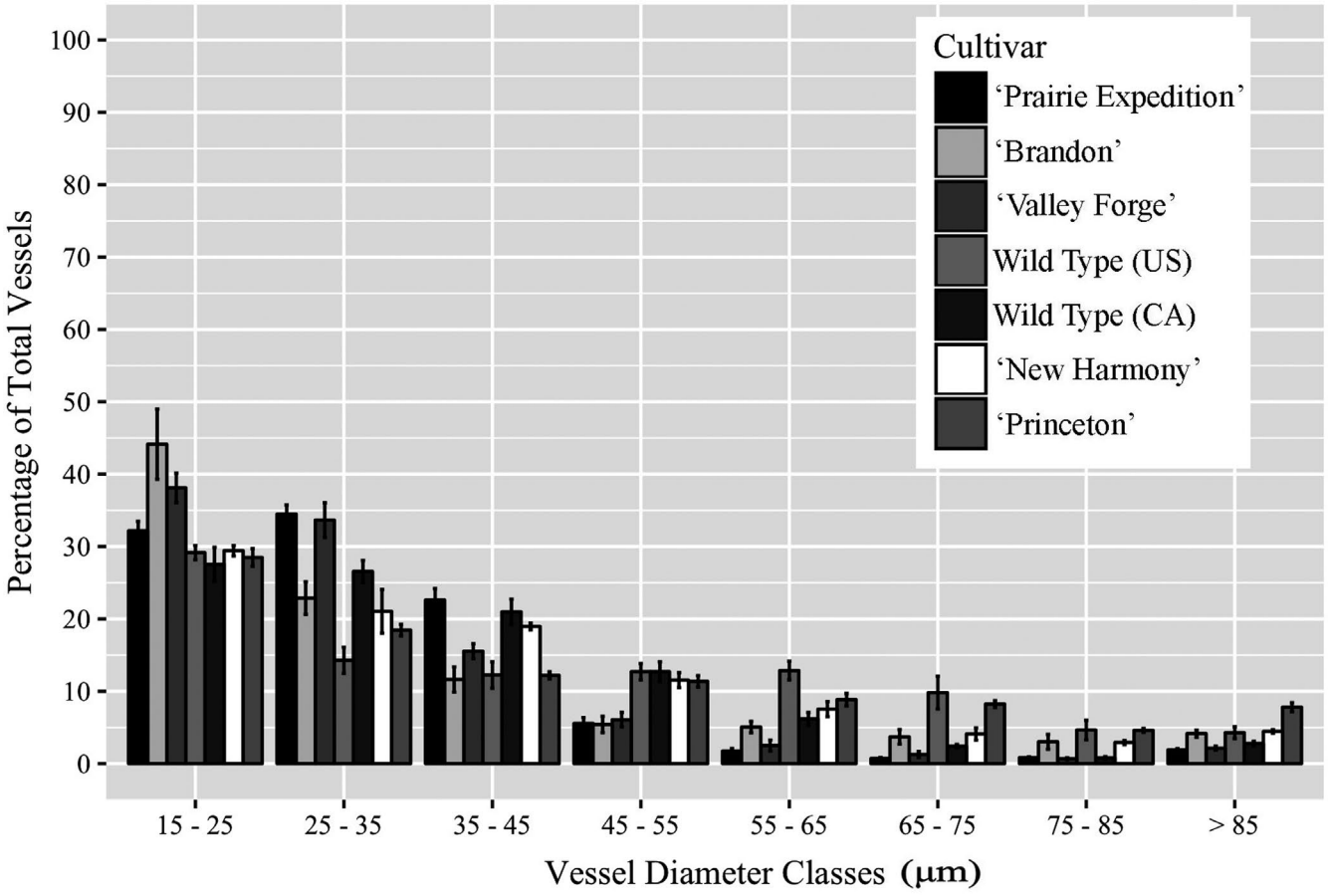

FIGURE 2 Distribution of vessels in the most recent annual ring of main stems of small-sized (1–3 cm diameter at 0.5 m ht.) *Ulmus americana* cultivars and wild-type seedlings harvested on 7 July 2015. Bars represent the mean ± *SE* of four sections from each of six trees, except for 'New Harmony', which had three trees

4 | DISCUSSION

American elm cultivars exhibited a variety of differences in xylem characteristics (Table 2). These differences may help explain some of the variability in resistance to Dutch elm disease that these trees appear to have. Since vessel density and vessel diameter showed strong correlations with disease severity (Table 2), there may be opportunities for using these characteristics in preliminary screening procedures. Findings from this study support those of previous authors that resistant elm genotypes generally have smaller vessel diameters/lumens compared with susceptible genotypes (Elgersma, 1970; McNabb et al., 1970; Pita et al., 2018; Sinclair et al., 1975a; Solla & Gil, 2002a). However, the relationship between vessel diameter and disease resistance is not perfect as evident by the correlation ($r_s = 0.76$, $p = 0.049$) (Table 2; Figure 1). There are potential risks associated with using small vessel diameters as a preliminary screening method, such as including susceptible genotypes with small vessels and more importantly, excluding resistant genotypes with large vessels. Sinclair et al. (1975a) acknowledged the risk of type II errors when using *VDI* (the per cent of vessels over 50 μm) for selecting for resistance. It is important that breeders weigh this risk before using this type of selection method. There have been many other disease resistance mechanisms associated with resistance, and those should not be discounted. If only genotypes with small vessel diameters are moved on in breeding programmes, some of those other resistance mechanisms, not related to xylem characteristics, may be lost.

Although much of the focus on preliminary screening methods has been on vessel diameter (Sinclair et al., 1975a; Solla & Gil, 2002a), the strongest correlation found in our study between the xylem characteristics examined and mean disease severity rating was for vessel density ($r = -0.77$, $p = 0.04$; Table 2). A previous study on *Ulmus minor* supports this finding on the relationship between vessel density and disease severity rating (Venturas et al., 2014). These investigators found offspring of a resistant-by-resistant cross had significantly higher vessel densities and lower wilt percentages than the offspring of a susceptible-by-susceptible cross. However, in a separate study on *Ulmus minor*, Martín et al. (2009) found that the susceptible group had greater mean vessel density in the earlywood compared with the resistant group, but the difference in the latewood was not significant. Additionally, Pita et al. (2018) found that under low watering conditions one of the two susceptible cultivars of *Ulmus minor* examined had significantly greater vessel density than the two resistant cultivars. Vessel density has been associated with resistance to vascular wilts in other woody plants as well. When

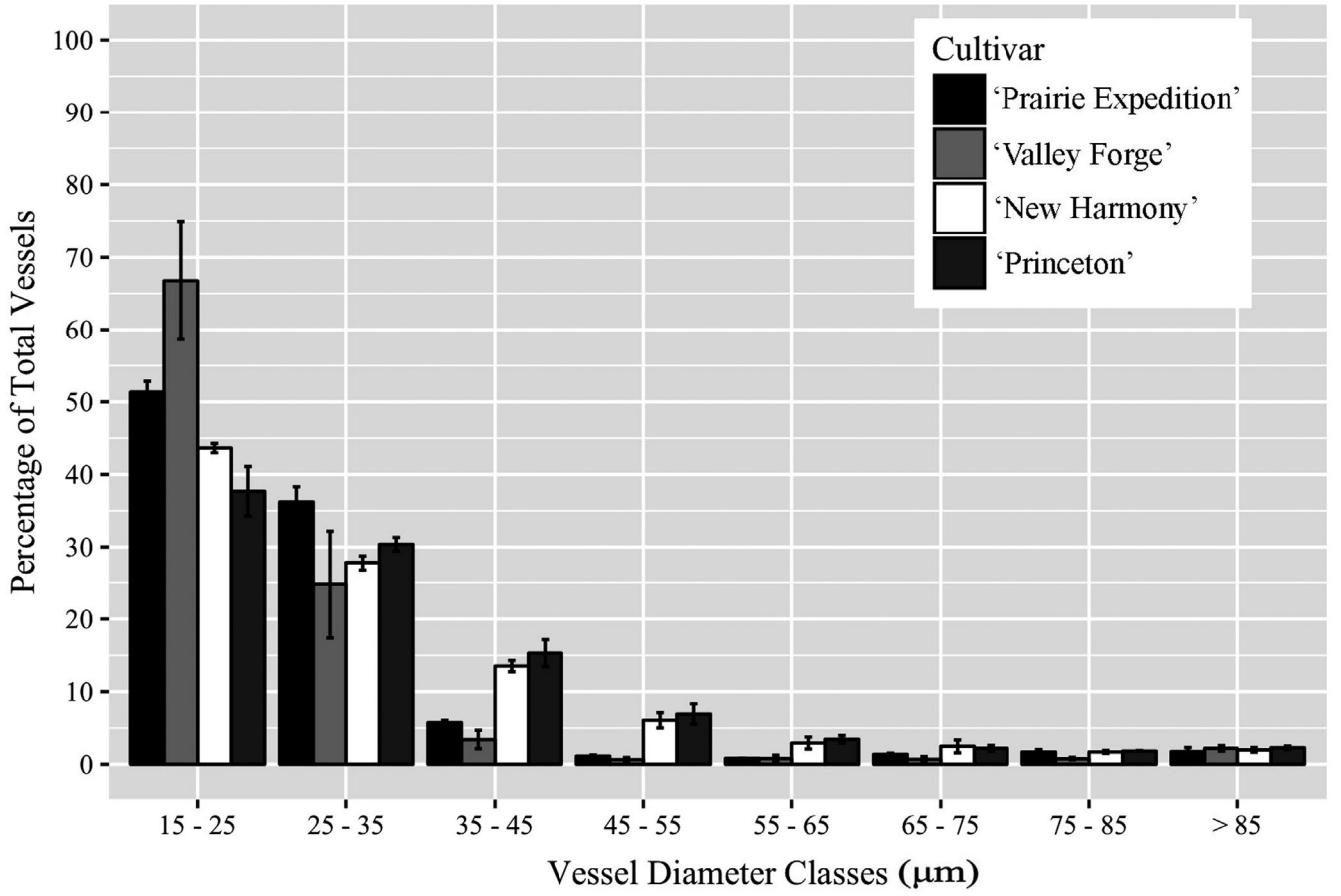

FIGURE 3 Distribution of vessels in the third annual ring of branches of medium-sized (5–10 cm dbh) *Ulmus americana* cultivars. Bars represent the mean ± *SE* of three sections per two branches from each of three trees. The year of growth analysed was 2014

examining anatomical characteristics in Norway maple (*Acer platanoides*), Wittberg (1983) found genotypes resistant to *Verticillium* wilt had significantly greater mean vessel densities in stems compared with susceptible genotypes. Using vessel density may prove to be a more useful preliminary screening method than assessing vessel diameter. In addition to having a slightly stronger correlation for the trees examined (Table 2), vessel density is easier and quicker to measure than vessel diameter. A concern, which should be considered before using vessel density as a preliminary screening method for selecting potentially resistant trees, is the variability within a genotype, which is evident by the large standard error of the mean (Table 4).

A major potential limitation to the use of xylem characteristics as preliminary screening methods for resistance to Dutch elm disease is variability due to environmental conditions. In branches and increment cores of main stems of large 'Valley Forge' and 'Princeton' trees, there were no statistically significant differences between the cultivars for vessel diameter or vessel density in 2015; however, in 2014, most of the differences were found to be statistically significant (Table 4). A potential explanation for the difference between years is the amount of precipitation that occurred in those years. Based on local weather data from the National Oceanic and Atmospheric Administration, from April to June there were 578 mm of precipitation in 2014 and only 275 mm in 2015. In a study on *Ulmus minor*, Pita et al. (2018) found that water stress resulted in a significant effect on average vessel lumen area. Additionally, it has been shown in other woody species that water availability can impact xylem development. In poplar (*Populus nigra* L. x *P. maximowiczii* Henry), trees receiving an early summer drought had vessels with significantly smaller cross-sectional areas and higher vessel densities compared with well-watered controls (Arend & Fromm, 2007). Additionally, a study by Lovisolo and Schubert (1998) found *Vitis vinifera* L. grown under water stress had smaller mean vessel diameters. These fluctuations in xylem characteristics are problematic, since it is unclear how each genotype will react to such conditions.

If xylem characteristics, such as vessel diameter and vessel density, are associated with resistance, there are potential opportunities to modify them. The use of graft combinations could potentially reduce the size of vessel diameters. Certain rootstocks of *Vitis vinifera* were shown to cause significant changes in the size of vessel diameters in scions (Santarosa et al., 2016). However, grafting onto different rootstocks, such as dwarfing rootstocks, does not always result in a significant change in vessel diameter for the scion (Tombesi et al., 2010). Another method to alter xylem characteristics is by limiting water availability. Although it is not possible to limit natural precipitation events, supplemental watering can be

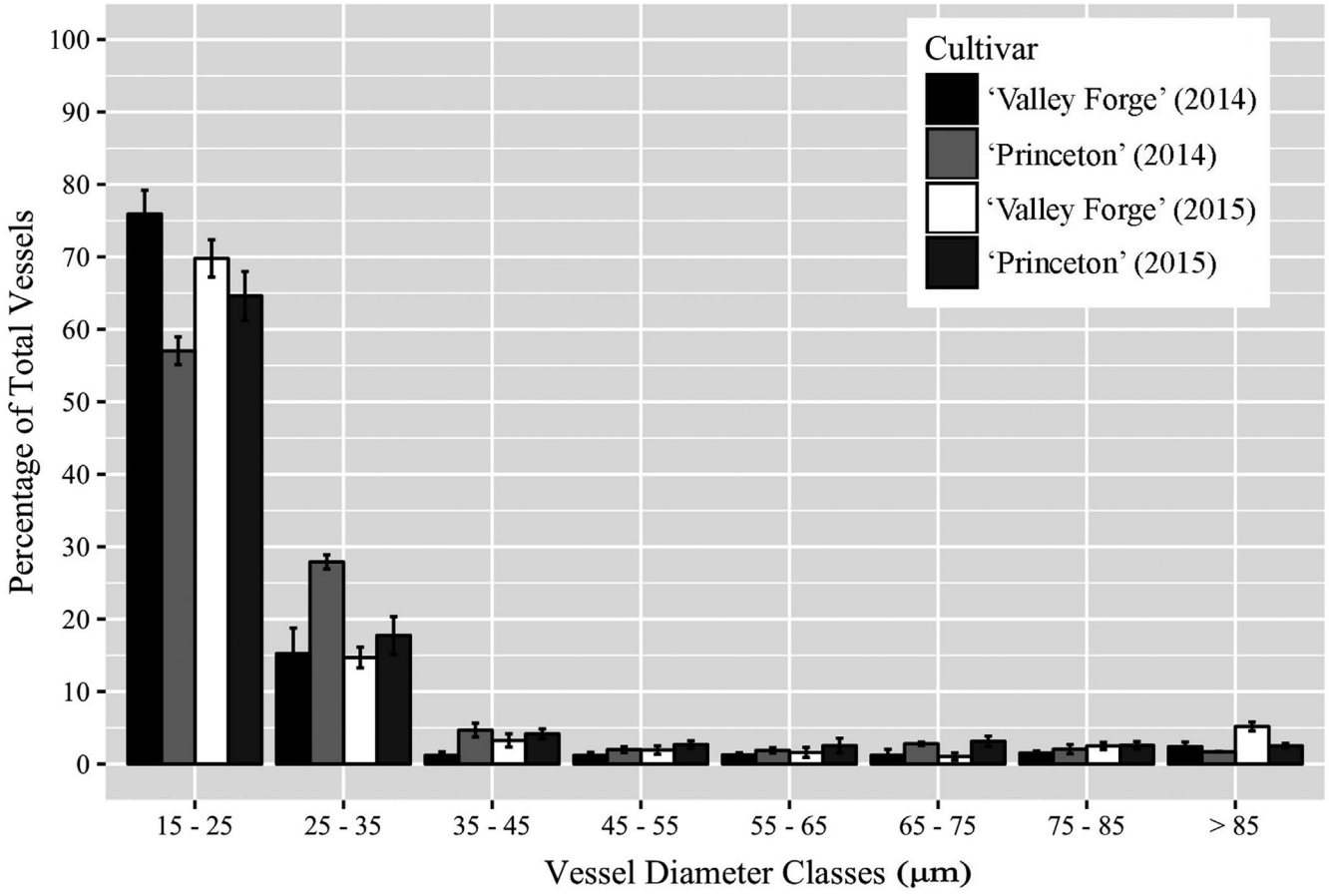

FIGURE 4 Distribution of vessels in the third annual ring of branches of large-sized (10–19 cm dbh) *Ulmus americana* 'Valley Forge' and 'Princeton'. Bars represent the mean ± *SE* of three sections per two branches from each of three trees. The years of growth analysed were 2014 and 2015

adjusted. As described previously, water stress can result in reduced vessel diameters (Lovisolo & Schubert, 1998) and increased vessel density in other woody plant species (Arend & Fromm, 2007). Solla and Gil (2002b) demonstrated in *Ulmus minor* that trees subjected to light watering prior to inoculation and also light watering 15 days following inoculation and then subsequent heavy watering displayed significantly less symptom expression when compared to heavy watering followed by light watering. Additionally, they found that vessels formed during the initial watering treatment were significantly smaller in the trees under light watering when compared to those under the heavy watering (Solla & Gil, 2002b). Plant growth regulators have also been shown to alter xylem growth in trees (Beckman, 1958; Digby & Wareing, 1966; Sorce et al., 2013; Venn et al., 1968; Yamamoto et al., 1987). Specifically, in American elm, Beckman (1958) found that the application of 4,5-MTMA reduced the sapwood thickness and reduced the incidence of wilt symptoms when inoculated with *Ophiostoma ulmi*. A later study by Yamamoto et al. (1987) on American elm seedlings found the application of an ethylene-releasing compound (ethrel) caused a significant reduction in vessel diameter. Although the repeated application of plant growth regulators to trees is likely not feasible, other more permanent options may exist. After inserting indoleacetic acid biosynthesis genes into hybrid aspen (*Populus tremula* L. x *Populus tremuloides* Michx.), Tuominen et al. (1995) found certain transformed trees displayed significantly higher vessel densities and smaller vessel diameters compared with the wild-type control. While there are methods to potentially modify xylem characteristics, testing with American elm is required to determine their practicality and usefulness.

Unfortunately, there has been little testing to determine specifically how xylem characteristics may be adding to resistance. The prevailing hypothesis is that smaller vessels are able to be filled more rapidly with gums, gels and/or tyloses, which are tree defence compounds, than in larger vessels (Elgersma, 1970; McNabb et al., 1970; Sinclair et al., 1975b; Solla & Gil, 2002a). A recent study by Pouzoulet et al. (2019) in *Vitis* provided evidence that smaller vessel lumens occluded faster than larger vessel lumens. Restricting movement in the smaller vessels faster would theoretically slow the spread of the pathogen. Additionally, it is postulated that larger vessels require more material to occlude them, which would reduce the amount of energy for defence elsewhere in the tree (Pouzoulet et al., 2014). A potential explanation for why higher vessel densities are associated with resistance is the pathogen would need to move through greater numbers of cell walls and break down more pit membranes when moving tangentially and radially around the stem. Another factor that may be affecting movement of the pathogen is pit morphology characteristics. A study by Martín et al. (2009) found that resistant

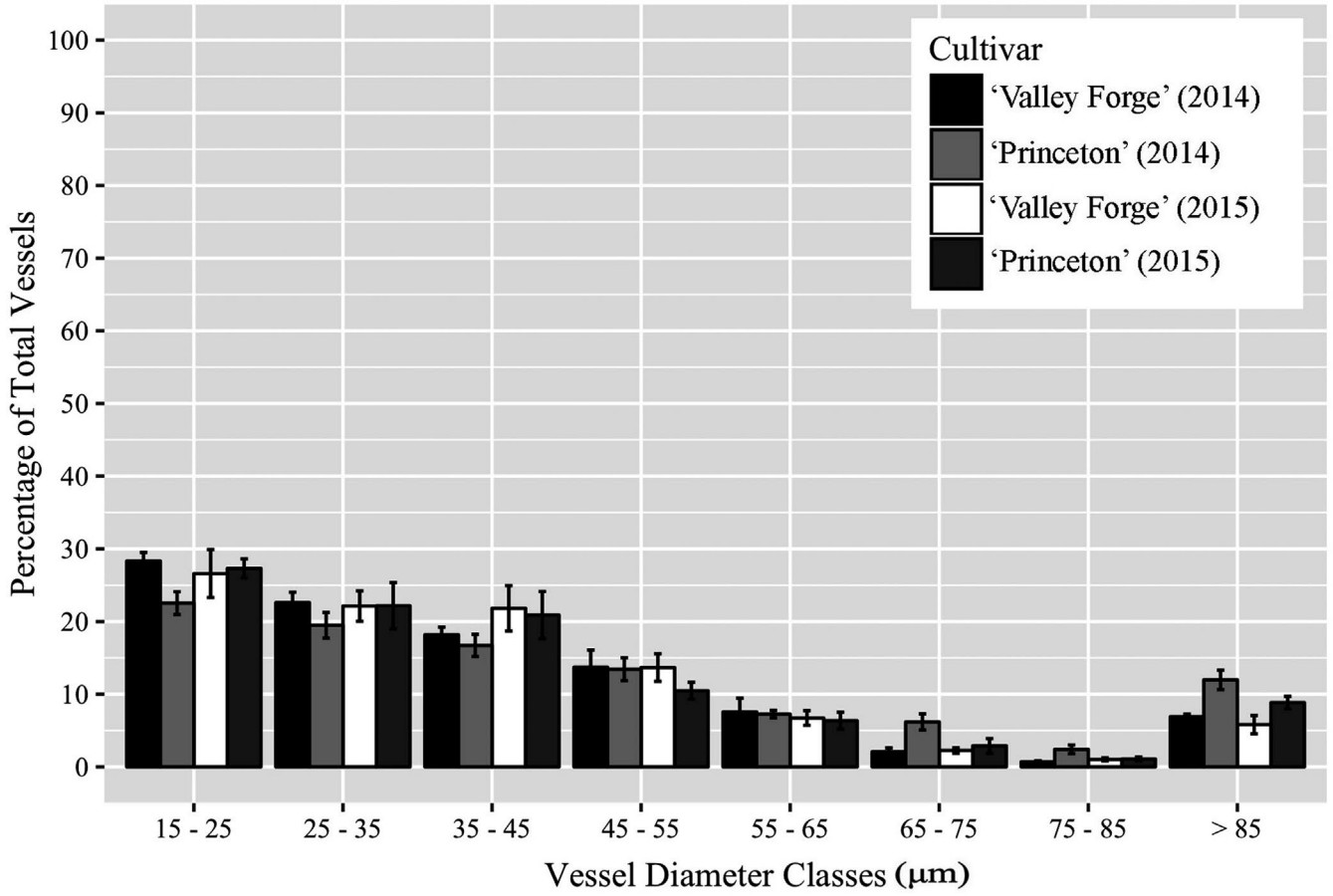

FIGURE 5 Distribution of vessels in the main stem of large-sized (10–19 cm dbh) *Ulmus americana* 'Valley Forge' and 'Princeton'. Bars represent the mean ± *SE* of two increment core sections from each of three trees. The first 2,000 μm of the annual ring was analysed for 2014 and 2015

Ulmus minor clones had significantly smaller mean horizontal diameter of the pit aperture, aperture area and abundance of pits compared with susceptible *Ulmus minor*.

Although specific xylem characteristics may play a role in the resistance of American elms to DED, many other factors are likely involved. Host responses to infection are seemingly critical in limiting the extent of pathogen spread. Previous studies have demonstrated that there are significant differences in the abilities of cultivars to compartmentalize infection (Beier & Blanchette, 2018; Beier et al., 2017). Additionally, differences in the amount of phenolic compounds produced, as measured by autofluorescence with UV excitation, have been observed (Beier & Blanchette, 2018). The speed at which tyloses are produced, which are outgrowths of the parenchyma cells into the vessel lumen, may also be contributing to resistance. When examining *Ulmus* x *hollandica* Mill., Elgersma (1973) found significantly more tyloses formed at 3 and 5 DPI in a resistant cultivar compared with a susceptible cultivar. Differences in production of pathogenesis-related (PR) proteins may further explain variability in resistance among cultivars. Following inoculation, 'Valley Forge' has shown significantly higher expression of PR5b, which is a thaumatin-like protein with known antifungal properties (Velazhahan et al., 1999), compared with a susceptible control (Sherif et al., 2016). An effective early oxidative burst has also been implicated in resistance to DED. Using an in vitro plant culture system, Martín, et al. (2019) found that the tolerant *Ulmus minor* clone examined had a significantly higher lipid peroxidation at 1 day post-inoculation than the susceptible clone. Additionally, they found that plantlets of the tolerant clone had significantly greater apical growth and total chlorophyll at 21 days post-inoculation compared with the susceptible clone (Martín, et al., 2019). They proposed that these may have potential as early screening methods, but only one resistant and one susceptible clone were examined (Martín, et al., 2019). A major advantage of in vitro screening methods is the reduction in time between propagation and screening.

Information gained from this study supports the potential use of xylem characteristics as a preliminary screening method for selecting elms for resistance to DED. Vessel diameter and vessel density appear to be the most promising xylary characteristics, as they displayed the strongest correlations with disease susceptibility (Table 2). Generally, the cultivars with low disease susceptibility had smaller vessel diameters and higher vessel densities than those cultivars with high disease susceptibility (Table 2). While the use of vessel diameter has been most frequently cited as a potential screening method (Sinclair et al., 1975a; Solla & Gil, 2002a), vessel density may

also be useful. If vessel density or diameter is to be used, based on findings from this study, using the first 2,000 μm of the annual ring from increment core sections may be advisable over branches, which was suggested by Sinclair et al. (1975a). Differences in means between the two cultivars in 2014 for vessel density and vessel diameter were considerably larger in main stems compared with branches (Table 4). Additionally, when examining the large 'Valley Forge' and 'Princeton' trees used for the 2014 growing season, the difference in vessel density was not significant when assessing branches; however, in increment cores from the main stem, the difference was found to be significant (Table 4). However, one explanation for why the branches may not have been as useful as the main stems for differentiating the cultivars is ontogeny. In a study by Solla et al. (2005), they found most of the *Ulmus minor* clones that they examined did not reach their maximum earlywood vessel size until year 4. While this has not been examined in *Ulmus americana*, it is possible that older branches may have been better at differentiating between the cultivars. Another potential drawback to the use of branches is variation in xylem due to its position in the tree. It has been shown in American beech (*Fagus sylvatica* L.) that the amount of sunlight a branch receives can significantly affect its vessel diameter size and vessel density (Lemoine et al., 2002). Since the main stem of elms is generally straight, tension wood, which forms in response to gravity, will occur more frequently in branches formed at an angle than in the straight trunk of the tree. The formation of tension wood can result in significantly altered xylem characteristics (Jourez et al., 2001; Yamamoto & Kozlowski, 1987). Collection of branch material can also be problematic in mature elms, where specialized climbing equipment or a lift truck may be required to reach the crown.

Although larger vessel diameters and lower vessel densities were associated with greater disease susceptibility, further testing of other plant material is warranted before this could be implemented into screening programmes. Ideally, as selection programmes repeatedly test clones from candidate trees, increment core samples should be collected. Correlations should be determined for disease severity ratings from repeated years of testing and xylem characteristics, mainly vessel density and vessel diameter. To help account for the potential variation in the xylem caused by environmental factors and juvenile growth habits, multiple annual rings should be assessed. Since the trees compared in these studies were grown under very similar growing conditions, it is important to determine whether trees growing in different environments can be effectively compared. Although there are concerns to consider when using xylem characteristics, such as vessel diameter and vessel density, to screen trees, this has potential to be a useful procedure that can be used along with other criteria, such as ability to compartmentalize infection (Beier & Blanchette, 2018), to help select genotypes for further testing.

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PEER REVIEW

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REFERENCES

Arend, M., & Fromm, J. (2007). Seasonal change in the drought response of wood cell development in poplar. *Tree Physiology*, *27*, 985–992. https://doi.org/10.1093/treephys/27.7.985

Beckman, C. H. (1958). Growth inhibition as a mechanism in Dutch elm disease therapy. *Phytopathology*, *48*, 172–176.

Beier, G. L., & Blanchette, R. A. (2018). Defence response in the xylem of *Ulmus americana* cultivars after inoculation with *Ophiostoma novo-ulmi*. *Forest Pathology*, *48*, e12453. https://doi.org/10.1111/efp.12453

Beier, G. L., Held, B. W., Giblin, C. P., Cavender-Bares, J., & Blanchette, R. A. (2017). American elm cultivars: Variation in compartmentalization of infection by *Ophiostoma novo-ulmi* and its effects on hydraulic conductivity. *Forest Pathology*, *47*, e12369. https://doi.org/10.1111/efp.12369

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Carlquist, S. (2001). *Comparative Wood Anatomy – Systematic, Ecological, and Evolutionary Aspects of Dicotyledon Wood*, 2nd ed. Springer Verlag Berlin Heidelberg.

Digby, J., & Wareing, P. F. (1966). The effect of applied growth hormones on cambial division and the differentiation of cambial derivatives. *Annals of Botany*, *30*, 539–548. https://doi.org/10.1093/oxfordjournals.aob.a084095

Elgersma, D. M. (1970). Length and diameter of xylem vessels as factors in resistance of elms to *Ceratocystis ulmi*. *Netherlands Journal of Plant Pathology*, *76*, 179–182. https://doi.org/10.1007/BF01974328

Elgersma, D. M. (1973). Tylose formation in elms after inoculation with *Ceratocystis ulmi*, a possible resistance mechanism. *Netherlands Journal of Plant Pathology*, *79*, 218–220. https://doi.org/10.1007/BF01974237

Godoy, R., Smith, O. D., Taber, R. A., & Pettit, R. E. (1985). Anatomical traits associated with pod rot resistance in peanut. *Peanut Science*, *12*, 77–82. https://doi.org/10.3146/pnut.12.2.0008

Gökbayrak, Z., Özer, C., & Söylemezoglu, G. (2010). Use of morphological markers to identify foliar disease resistance in grapevine. *The Journal of Animal & Plant Sciences*, *20*, 243–247.

Guries, R. P., & Smalley, E. B. (1990). *Selecting and testing elms: The Wisconsin elm breeding program*. The Morton Arboretum. In Proceedings of the Seventh Conference of the Metropolitan Tree Improvement Alliance

Hessenauer, P., Fijarczyk, A., Martin, H., Prunier, J., Charron, G., Chapuis, J., Bernier, L., Tanguay, P., Hamelin, R. C., & Landry, C. R. (2020).

Hybridization and introgression drive genome evolution of Dutch elm disease pathogens. *Nature Ecology & Evolution*, *4*, 626–638. https://doi.org/10.1038/s41559-020-1133-6

Jansen, S., Choat, B., & Pletsers, A. (2009). Morphological variation of intervessel pit membranes and implications to xylem function in angiosperms. *American Journal of Botany*, *96*, 409–419. https://doi.org/10.3732/ajb.0800248

Jourez, B., Riboux, A., & Leclercq, A. (2001). Anatomical characteristics of tension wood and opposite wood in young inclined stems of poplar (*Populus euramericana* cv 'Ghoy'). *IAWA Journal*, *22*, 133–157. https://doi.org/10.1163/22941932-90000274

Knight, K. S., Slavicek, J. M., Kappler, R., Pisarczyk, E., Wiggin, B., & Menard, K. (2012). Using Dutch elm disease-tolerant elm to restore floodplains impacted by emerald ash borer. In R. A. Sniezko, A. D. Yanchuk, J. T. Kliejunas, K. M. Palmieri, J. M. Alexander, & S. J. Frankel, (Eds.). *Proceedings of the Fourth International Workshop on genetics of host-parasite interactions in forestry: disease and insect resistance in forest trees*, (317–323). Gen. Tech. Rep. PSW-GTR-240.

Kreiser, M., Giblin, C., Murphy, R., Fiesel, P., Braun, L., Johnson, G., Wyse, D., & Cohen, J. D. (2016). Conversion of Indole-3-Butyric Acid to Indole-3-Acetic Acid in shoot tissue of hazelnut (*Corylus*) and elm (*Ulmus*). *Journal of Plant Growth Regulation*, *35*, 710–721. https://doi.org/10.1007/s00344-016-9574-5

Leal, S., Sousa, V. B., Knapic, S., Louzada, J. L., & Pereira, H. (2011). Vessel size and number are contributors to define wood density in cork oak. *European Journal of Forest Resources*, *130*, 1023–1029. https://doi.org/10.1007/s10342-011-0487-3

Lemoine, D., Jacquemin, S., & Granier, A. (2002). Beech (*Fagus sylvatica* L.) branches show acclimation of xylem anatomy and hydraulic properties to increased light after thinning. *Annals of Forest Science*, *59*, 761–766. https://doi.org/10.1051/forest:2002062

Lovisolo, C., & Schubert, A. (1998). Effects of water stress on vessel size and xylem hydraulic conductivity in *Vitis vinifera* L. *Journal of Experimental Botany*, *49*, 693–700. https://doi.org/10.1093/jxb/49.321.693

Martín, J. A., Sobrino-Plata, J., Coira, B., Medel, D., Collada, C., & Gil, L. (2019). Growth resilience and oxidative burst control as tolerance factors to *Ophiostoma novo-ulmi* in *Ulmus minor*. *Tree Physiology*, *39*, 1512–1524. https://doi.org/10.1093/treephys/tpz067

Martín, J. A., Sobrino-Plata, J., Rodriguez-Calcerrada, J., Collada, C., & Gil, L. (2019). Breeding and scientific advances in the fight against Dutch elm disease: Will they allow the use of elms in forest restoration? *New Forests*, *50*, 183–215. https://doi.org/10.1007/s11056-018-9645-5

Martín, J. A., Solla, A., Esteban, L. G., de Palacios, P., & Gil, L. (2009). Bordered pit and ray morphology involvement in elm resistance to *Ophiostoma novo-ulmi*. *Canadian Journal of Forest Resources*, *39*, 420–429. https://doi.org/10.1139/X08-183

Martín, J. A., Solla, A., Ruiz-Villar, M., & Gil, L. (2013). Vessel length and conductivity of *Ulmus* branches: Ontogenetic changes and relation to resistance to Dutch elm disease. *Trees*, *27*, 1239–1248. https://doi.org/10.1007/s00468-013-0872-2

Martín, J. A., Solla, A., Venturas, M., Collada, C., Domínguez, J., Miranda, E., Fuentes, P., Burón, M., Iglesias, S., & Gil, L. (2015). Seven *Ulmus minor* clones tolerant to *Ophiostoma novo-ulmi* registered as forest reproductive material in Spain. *iForest*, *8*, 172–180. https://doi.org/10.3832/ifor1224-008

McNabb, H. S. Jr, Heybroek, H. M., & Macdonald, W. L. (1970). Anatomical factors in resistance to Dutch elm disease. *Netherlands Journal of Plant Pathology*, *76*, 196–204. https://doi.org/10.1007/BF01974331

Mittempergher, L., & Santini, A. (2004). The history of elm breeding. *Investigación Agraria. Sistemas Y Recursos Forestales*, *13*, 161–177.

Pecori, F., Ghelardini, L., Luchi, N., Pepori, A. L., & Santini, A. (2017). Lights and shadows of a possible strategy to cope with alien and destructive forest pathogens: The example of breeding for resistance to Dutch elm disease in Italy. *Baltic Forestry*, *23*, 255–263.

Pita, P., Rodríguez-Calcerrada, J., Medel, D., & Gil, L. (2018). Further insights into the components of resistance to *Ophiostoma novo-ulmi* in *Ulmus minor*: Hydraulic conductance, stomatal sensitivity and bark dehydration. *Tree Physiology*, *38*, 253–263. https://doi.org/10.1093/treephys/tpx123

Pouzoulet, J., Pivovaroff, A. L., Santiago, L. S., & Rolshausen, P. E. (2014). Can vessel dimension explain tolerance toward fungal vascular wilt diseases in woody plants? Lessons from Dutch elm disease and esca disease in grapevine. *Frontiers in Plant Science*, *5*, 253. https://doi.org/10.3389/fpls.2014.00253

Pouzoulet, J., Scudiero, E., Schiavon, M., & Rolshausen, P. E. (2017). Xylem vessel diameter affects the compartmentalization of the vascular pathogen *Phaeomoniella chlamydospora* in grapevine. *Frontiers in Plant Science*, *8*, 1442. https://doi.org/10.3389/fpls.2017.01442

Pouzoulet, J., Scudiero, E., Schiavon, M., Santiago, L. S., & Rolshausen, P. E. (2019). Modeling of xylem vessel occlusion in grapevine. *Tree Physiology*, *39*, 1438–1445. https://doi.org/10.1093/treephys/tpz036

Santarosa, E., Dutra de Souza, P. V., de Araujo Mariath, J. E., & Lourosa, G. V.(2016). Physiological interactions between rootstock-scion: Effects on xylem vessels in cabernet sauvignon and merlot grapevines. *American Journal of Enology and Viticulture*, *67*, 65–76. https://doi.org/10.5344/ajev.2015.15003

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years to image analysis. *Nature Methods*, *9*, 671–675. https://doi.org/10.1038/nmeth.2089

Sherif, S. M., Shukla, M. R., Murch, S. J., Bernier, L., & Saxena, P. K. (2016). Simultaneous induction of jasmonic acid and disease-responsive genes signifies tolerance of American elm to Dutch elm disease. *Scientific Reports*, *6*, 21934. https://doi.org/10.1038/srep21934

Sinclair, W. A., Zahand, J. P., & Melching, J. B. (1975a). Anatomical marker for resistance of *Ulmus americana* to *Ceratocystis ulmi*. *Phytopathology*, *65*, 349–352. https://doi.org/10.1094/Phyto-65-349

Sinclair, W. A., Zahand, J. P., & Melching, J. B. (1975b). Localization of infection in American elms resistant to *Ceratocystis ulmi*. *Phytopathology*, *65*, 129–133. https://doi.org/10.1094/Phyto-65-129

Slavicek, J. M., Baggett, K., & Swanson, G. (2009). Restoration of the American elm on the Chippewa National Forest through generation of Dutch elm disease tolerant, cold-hardy, and site-adapted trees. In K. A. McManus, & K. W. Gottschalk, (Eds.), *Proceedings of the 20th U.S. Department of Agriculture interagency research forum on invasive species*, (95). Gen. Tech. Rep. NRS-P-51.

Solla, A., Buron, M., Iglesias, S., & Gil, L. (2000). Spanish Program for the Conservation and Breeding of Elms Against DED. In C.Dunn (Eds.). *The Elms*, (295–303). New York, NY: Springer Science + Business Media, LLC. https://doi.org/10.1007/978-1-4615-4507-1_19

Solla, A., & Gil, L. (2002a). Xylem vessel diameter as a factor in resistance of *Ulmus minor* to *Ophiostoma novo-ulmi*. *Forest Pathology*, *32*, 123–134. https://doi.org/10.1046/j.1439-0329.2002.00274.x

Solla, A., & Gil, L. (2002b). Influence of water stress on Dutch elm disease symptoms in *Ulmus minor*. *Canadian Journal of Botany*, *80*, 810–817. https://doi.org/10.1139/b02-067

Solla, A., Martín, J. A., Ouellette, G. B., & Gil, L. (2005). Influence of plant age on symptom development in *Ulmus minor* following inoculation by *Ophiostoma novo-ulmi*. *Plant Disease*, *89*, 1035–1040. https://doi.org/10.1094/PD-89-1035

Sorce, C., Giovannelli, A., Sebastiani, L., & Anfodillo, T. (2013). Hormonal signals involved in the regulation of cambial activity, xylogenesis and vessel patterning in trees. *Plant Cell Reports*, *32*, 885–898. https://doi.org/10.1007/s00299-013-1431-4

Tombesi, S., Johnson, R. S., Day, K. R., & DeJong, T. M. (2010). Interactions between rootstock, inter-stem and scion xylem vessel characteristics

of peach trees growing on rootstocks with contrasting size-controlling characteristics. *AoB PLANTS*, *2010*, plq013. https://doi.org/10.1093/aobpla/plq013

Townsend, A. M., Bentz, S. E., & Douglass, L. W. (2005). Evaluation of 19 American elm clones for tolerance to Dutch elm disease. *Journal of Environmental Horticulture*, *23*, 21–24. https://doi.org/10.24266/0738-2898-23.1.21

Townsend, A. M., Bentz, S. E., & Johnson, G. R. (1995). Variation in response of selected American elm clones to *Ophiostoma ulmi*. *Journal of Environmental Horticulture*, *13*, 126–128. https://doi.org/10.24266/0738-2898-13.3.126

Tuominen, H., Sitbon, F., Jacobson, C., Sandberg, G., Olsson, O., & Sunberg, B. (1995). Altered growth and wood characteristics in transgenic hybrid aspen expressing *Agrobacterium tumefaciens* T-DNA indoleacetic acid-biosynthetic genes. *Plant Physiology*, *109*, 1179–1189. https://doi.org/10.1104/pp.109.4.1179

Velazhahan, R., Datta, S. K., & Muthukrishnan, S. (1999). The PR-5 family: Thaumatin-like proteins in plants. In S. K. Datta, & S. Muthukrishnan (Eds.), *Pathogenesis-related proteins in plants* (pp. 107–129). CRC Press.

Venn, K. O., Nair, V. M. G., & Kuntz, J. E. (1968). Effects of TCPA on oak sapwood formation and the incidence and development of oak wilt. *Phytopathology*, *58*, 1071.

Venturas, M., López, R., Martín, J. A., Gascó, A., & Gil, L. (2014). Heritability of *Ulmus minor* resistance to Dutch elm disease and its relationship to vessel size, but not to xylem vulnerability to drought. *Plant Pathology*, *63*, 500–509. https://doi.org/10.1111/ppa.12115

von Arx, G., Kueffer, C., & Fonti, P. (2013). Quantifying plasticity in vessel grouping – added value from the image analysis tool ROXAS. *IAWA Journal*, *34*, 433–445. https://doi.org/10.1163/22941932-00000035

Warren, K. (2000). The return of the elm: Status of elms in the nursery industry. In C. P. Dunn (Ed.), *The elms: Breeding, conservation and disease management* (pp. 341–348). Kluwer Academic Publications.

Wittberg, R. A. (1983). *Vascular anatomy as a selection criterion for Verticillium wilt resistance in Norway maple (*Acer platanoides*)*. : In Proceedings of the North Central Tree Improvement Conference.

Yamamoto, F., Angeles, G., & Kozlowski, T. T. (1987). Effect of ethrel on stem anatomy of *Ulmus americana* seedlings. *IAWA Bulletin*, *8*, 3–9. https://doi.org/10.1163/22941932-900010

Yamamoto, F., & Kozlowski, T. T. (1987). Effects of flooding, tilting of stems, and ethrel application on growth, stem anatomy, and ethylene production of *Acer platanoides* seedlings. *Scandinavian Journal of Forest Research*, *2*, 141–156. https://doi.org/10.1080/02827588709382453

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