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American elm cultivars: Variation in compartmentalization of infection by *Ophiostoma novo-ulmi* and its effects on hydraulic conductivity

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Summary

Five cultivars and two populations of wild-type seedlings of American elm (*Ulmus americana*), 3 and 4 years old, were examined for differences in their abilities to compartmentalize and resist infection by artificially inoculating with *Ophiostoma novo-ulmi*. Morphological characteristics of tree defence, often referred to as the compartmentalization of decay in trees model, were used as a conceptual framework, with particular emphasis on the limiting of tangential spread of infection within the xylem and barriers that limit spread outwards to cells formed after infection. To investigate the change in functional xylem over time, 3-year-old trees were assessed at multiple time points following inoculation for hydraulic conductivity. Three and four-year-old cut trees were placed in 0.1% w/v safranin O for 18 to 24 hr to indicate functional xylem. Transverse sections of the stained stems were used to calculate per cent of sap-conducting xylem area and the per cent of circumference conducting of first formed cells and later formed cells. At each collection time, trees were assessed for disease severity on a 1–12 scale, based on the percentage of permanent wilt in the crown. There was considerable variation between cultivars in disease severity and their capacity to localize and resist infection. "Prairie Expedition," which had the lowest disease severity rating in 2015 and the second lowest in 2016, consistently limited the spread of infection into newly formed xylem and had functional xylem around the entire circumference of the stem at 90 days post-inoculation. "Valley Forge" in 2016 had the lowest overall disease severity rating and was the only cultivar to consistently limit the tangential spread of infection within extant xylem. This research identifies key characteristics that some cultivars have to resist and limit infection and provides new information that can be used in disease screening programmes to evaluate other cultivars and older plant material.

1 | INTRODUCTION

Despite extensive research on Dutch elm disease (DED), it still remains unclear what mechanisms allow certain elm genotypes to be resistant while others are susceptible. Currently, multiple American elm (*Ulmus americana* L.) cultivars are available with varying levels of putative resistance to DED (Townsend, Bentz, & Douglass, 2005; Townsend, Bentz, & Johnson, 1995). Although cultivars are available, there has been a lack of research to explore potential differences in host responses of these cultivars to infection/inoculation by *Ophiostoma novo-ulmi* Brasier. Determining similarities and differences among these genotypes in how they respond to the pathogen could help influence how specific cultivars are used in both breeding programmes and the landscape.

An important question, in regard to resistance to DED, is how do different genotypes minimize the spread of the pathogen throughout the tree? One of the proposed mechanisms is compartmentalization of the pathogen within the tree (Bonsen, Scheffer, & Elgersma, 1985; Ouellette & Rioux, 1992; Rioux & Ouellette, 1991; Shigo & Tippett,

1981; Tippett & Shigo, 1981). Compartmentalization involves the tree "walling off" the infection through a two-part process, reaction zone formation and barrier zone formation (Shigo, 1984; Shortle & Dudzik, 2012). These walls may function as sealants that limit the spread of aeration and drying and fungal ingress (Boddy, 1991). In previous studies on elm, scientists have found similarities to the compartmentalization of decay in trees (CODIT) model, by Shigo and Marx (1977), when examining how trees respond to infection (Bonsen et al., 1985; Ouellette & Rioux, 1992; Rioux & Ouellette, 1991; Shigo & Tippett, 1981; Tippett & Shigo, 1981). Scientists have effectively used the CODIT model in studying other vascular wilts, such as oak wilt and Fusarium wilt (Baayen, Ouellette, & Rioux, 1996; Tainter & Fraedrich, 1986). The CODIT model is described as four walls that help restrict the spread of infection (Shigo & Marx, 1977). Walls 1–3 and wall 4 of the CODIT model are considered separate parts and can be equated to the reaction zones and barrier zones of compartmentalization, respectively (Pearce, 1996; Shigo, 1984). Cultivars in this study were examined to determine differences in their effectiveness in using walls 3 and 4 of the CODIT model to limit spread of the infection. The wall 3 reaction zone is formed by ray parenchyma cells and can reduce the tangential spread of infection around the circumference. Wall 4 is the only wall formed after wounding and is known as a barrier zone (Shigo & Marx, 1977). In *U. americana*, barrier zones are composed of parenchyma cells and fibres (Rioux & Ouellette, 1991). Barrier zones can limit the spread of infection into xylem cells formed after infection (Shigo, 1984; Shigo & Marx, 1977).

The use of aqueous stains to indicate conductive xylem elements has been used to study multiple vascular pathogen/plant interactions (Edwards, Pascoe, & Salib, 2007; Inch & Ploetz, 2012; Takahashi, Matsushita, & Hogetsu, 2010). Aqueous stains, such as safranin O or acid fuchsin, move through the sap-conducting xylem by applying a pressure gradient. After stain infiltration, stems are cut and photographed. A common approach to analysing sap-conducting xylem area is through the use of thresholding and calculation of the per cent of xylem area that is conducting water (Edwards et al., 2007; Joseph, Kelsey, & Thies, 1998; Murata, Matsuda, Yamada, & Ito, 2009; Murata, Yamada, Matsuda, & Ito, 2007). Although commonly used, there are some limitations to this method of analysis. Per cent of sap-conducting xylem area does not indicate whether earlywood or latewood vessels are still conducting or where the conducting xylem vessels are relative to the inoculation site. Although more time-consuming, determining the per cent of the circumference that is conducting helps to give more insight to how the pathogen is affecting the entire stem. Additionally, this allows for a quantitative assessment of the effectiveness of genotypes to minimize the spread of the infection both tangentially and outward into newly formed cells.

Although *U. americana* cultivars with varying levels of resistance to DED are commercially available, there has not been a comprehensive study published that examines the ability of different cultivars to compartmentalize infection by *O. novo-ulmi*. Therefore, the objectives of this study were to (i) determine whether differences exist between selected cultivars of *U. americana*, with varying levels of resistance to DED, in their ability to compartmentalize infection by *O. novo-ulmi*, (ii) quantitatively assess differences in compartmentalization among cultivars and (iii) investigate the change in functioning xylem over time.

2 | MATERIALS AND METHODS

2.1 | Plant material

Five cultivars of *U. americana* were used in this study, including "Brandon," "New Harmony," "Prairie Expedition," "Princeton" and "Valley Forge." All of the cultivars, except for "Brandon," which was grafted onto a wild-type rootstock, were grown from stem cuttings. Two populations of wild-type *U. americana* seedlings were also used. They were collected from Tennessee (wild-type [US]) and Ontario, Canada (wild-type [CA]). Trees were planted in a nursery field at the University of Minnesota, St. Paul campus, during the summer of 2014. During the growing season, the trees were watered as needed and received 4.9 ml of Osmocote® Plus (15-9-12) (Everris NA Inc., Dublin, OH, USA) every three months. In the 2015 trial, trees inoculated were 3 years old, while those inoculated in 2016 were 4 years old.

2.2 | Inoculum preparation

An isolate of *O. novo-ulmi* with known pathogenicity was collected from Minnesota and used for inoculations. Cultures were grown on selective media for *Ophiostoma* described by Harrington (1981) for 10 days. Three 0.5-cm^2 pieces of colonized media were added to 100 ml of liquid media consisting of 20 g dextrose, 2 g L-asparagine, 1.5 g KH_2PO_4, 1 g $MgSO_4 \times 7\ H_2O$, 0.00015 g $FeCL_3 \times 6\ H_2O$, 0.0001 g $ZnSO_4$, 0.00018 g $MnCl_2 \times 4\ H_2O$, 0.001 g thiamine HCl, 0.001 g pyridoxal, 1 L distilled water (Stennes, 1981). Cultures were allowed to grow for 3 days at room temperature while on a shaker at 150 rpm. The concentration of the spore suspension was then determined using a hemacytometer and adjusted to 1×10^6 spores/ml.

2.3 | Inoculation

Trees used in the 2015 trial were inoculated on May 28 (43 days after budbreak), while trees in the 2016 trial were inoculated on May 26 (40 days after budbreak). In 2015, 18 trees of each cultivar were inoculated with *O. novo-ulmi*. Additional trees for each cultivar, depending on availability, were inoculated to more accurately determine disease severity. These additional trees were not used to examine functional xylem or compartmentalization. In 2016, five trees of each cultivar were inoculated with *O. novo-ulmi*, except for "Valley Forge," which had four, due to limited plant material. One-half metre above the ground, a 4-mm-deep hole was made using a power drill with a 2.38-mm-diameter bit. Twenty-five microlitres of a spore suspension (1×10^6 spores/ml) was injected into the hole using a micropipette, and the wound was subsequently wrapped with Parafilm M® (Bemis Co., Inc., Neenah, WI, USA) to avoid desiccation. As controls, 18 trees of each cultivar in 2015 and one tree of each cultivar in 2016 were mock-inoculated with 25 µl of sterile water instead of inoculum.

2.4 | Disease ratings

Trees were assessed for symptoms at 5, 10, 15, 20, 40 and 90 days post-inoculation (DPI). Disease ratings were based on the percentage of the crown exhibiting permanent wilt. Ratings were made on a 1–12 ordinal scale: 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%.

2.5 | Hydraulic conductivity measurements and dye ascent

During the 2015 trial, trees were harvested at 5, 10, 15, 20, 40 and 90 DPI. At each time point, three inoculated and three mock-inoculated trees were harvested for each cultivar. Due to a harvesting complication at 90 DPI, one mock-inoculated "New Harmony" was not included in analysis for hydraulic conductivity, per cent of sap-conducting xylem area or per cent of circumference conducting. Within 24 hr before harvesting, silicone was applied to the inoculation wound. Main stems were bent over into a shallow tub and cut underwater to avoid cavitation of xylem vessels approximately 30 cm below the inoculation point and kept in water. Stems were then transported to a glasshouse where they were recut underwater to 25 cm below the inoculation point. Cut stems were then placed in a 10 mM KCl with 0.1% w/v safranin O, which had been filtered through a 0.22-um Durapore® membrane GV filter (Merck Millipore Ltd., Carrigtwohill, Ireland) and vacuumed for 1 hr at 71 KPa. Eighteen to 24 hr later, after the dye was visible in the leaves, the stems were recut underwater for hydraulic conductance measurements. A 15-cm segment was cut, 7.5 cm below and 7.5 cm above the wound. The segment was then assessed for hydraulic conductivity using an apparatus described in Venturas, MacKinnon, Jacobsen, and Pratt (2015), which was based on Sperry, Donnelly, and Tyree (1988). Briefly, tubing was clamped to the anterior end of the segment with a pressure head of 0.0018 MPa. In previous trials, it was determined a pressure head of 0.0018 MPa was small enough to prevent clearing of pre-existing cavitations within the stem (G. L. Beier, unpublished data). The posterior end of the sample was attached to tubing, which led to a scale to measure the output. A 10 mM KCl solution, vacuumed and filtered as described above, was run through the stem for 5–7 min. Data were collected using LabVIEW (National Instruments Co., Austin, TX, USA), and Excel® (Microsoft® Co., Redmond, WA, USA) was used to calculate the flow rate through the stem. Conductivity considering path length (K_h) was calculated using the following formula: $K_h = (J^*L)/\Delta P$, where J is the flow rate in kg H_2O s^{-1}, L is the length of the stem in metres, and ΔP is the pressure gradient in MPa. To determine stem area-specific hydraulic conductivity (K_s), the following formula was used $K_s = K_h$/xylem area, where the xylem area in m^2 was determined as follows. At the posterior end of the segment and 2.5 cm away from the posterior end, cross sections were made using a razor blade or band saw and the freshly cut surface was recut with a microtome blade. Sections were allowed to dry at room temperature for 3–4 days. Cross sections were scanned at 2,400 DPI using an Epson Perfection V37 flatbed scanner (Epson America, Inc., Long Beach, CA, USA). The area of the xylem was determined by removing the pith and bark in ImageJ (Schneider, Rasband, & Eliceiri, 2012). The smaller of the two areas was used to calculate K_s.

For the 2016 trial, five trees were inoculated and one tree was mock-inoculated for each cultivar. In the previous year, one of the "Valley Forge" replicates was damaged and only four trees were inoculated. Trees were harvested at 90 DPI. Due to the large diameter of stems in 2016, trees were cut predawn and then placed in water. Stems were cut 15 cm above the wound and placed in the 10 mM KCl with 0.1% w/v Safranin O solution as described above. It was not feasible to perform hydraulic conductivity experiments on the stems harvested in 2016 because stems were too large.

2.6 | Per cent of sap-conducting xylem area assessment

In 2015, a cross section of each tree (after being placed in the 10 mM KCl with 0.1% w/v Safranin O solution for 18–24 hr) was made 10 cm above the inoculation point for each harvest time and 30 cm at 90 DPI. In 2016, cross sections were made 30 cm above the inoculation point. Cross sections were made and scanned as described above. Using Adobe Photoshop™ (Adobe Systems Inc., San Jose, CA, USA), the pith and bark were manually removed using the lasso tool. The most recent annual ring was separated from previous rings using the lasso tool. The sap-conducting xylem in the most recent annual ring, which was red due to the safranin O staining, was measured in ImageJ (Schneider et al., 2012) using the thresholding tool. To determine the per cent of sap-conducting xylem area, the entire annual ring area was measured by adjusting the thresholding parameters.

2.7 | Per cent of circumference conducting assessment

Cross sections obtained as described above were assessed for per cent of circumference conducting. The xylem of the most recent annual ring was split into two groups, first formed cells and later formed cells. First formed cells were considered to be the first row of large cells formed. For first formed cells, an area was considered to be non-conducting if three or more cells were non-conducting in a row. All remaining xylem was considered later formed cells. To determine the per cent of circumference conducting, an angle was made with the vertex centred in the pith in ImageJ (Schneider et al., 2012). All angles of conducting xylem were added together and divided by 360 to determine the per cent circumference conducting (Figure 1).

2.8 | Statistical analysis

Analysis was performed using the statistical package R version 3.2.2 (R Development Core Team, Vienna, Austria). Because disease severity was measured on an ordinal scale, data were analysed with the nonparametric Kruskal–Wallis rank-sum test followed

by Dunn's multiple comparisons test. Data on per cent of sap-conducting xylem area, per cent of circumference conducting and hydraulic conductivity were often not normally distributed so they were analysed using the nonparametric Kruskal–Wallis rank-sum test followed by Dunn's multiple comparisons test. For multiple comparison tests, the Benjamini and Hochberg (1995) *p*-value adjustment was used. To examine relationships between variables, Spearman's correlation was used.

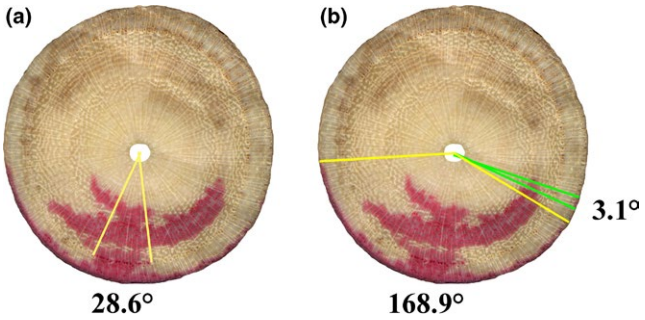

FIGURE 1 Demonstration on how per cent of circumference conducting is calculated for first formed cells (a) and later formed cells (b) in the current annual ring. An angle with the vertex in the centre of the pith is extended to the conducting area (stained red) of the first formed cells, which are considered the first row of large cells formed. This process is repeated for the later formed cells, which is all the remaining xylem not included in the first formed cells. If the conducting area is not continuous, as in image (b), multiple angles are used. All angles are added together and divided by 360 to determine the per cent of circumference conducting

3 | RESULTS

3.1 | Disease severity assessment

There was considerable variation in mean disease severity rating among cultivars for both 2015 and 2016. Generally, disease progression was more rapid in 2015 compared with 2016 (Table 1). In the 2015 inoculation, "Prairie Expedition" had the lowest mean disease severity rating of 5.7 at 90 DPI. There was a statistically significant difference between "Prairie Expedition" and "New Harmony," "Princeton" and wild-type (CA). All replicates for both "Princeton" and "New Harmony" had a disease severity rating of 12 (100% permanent wilt of crown) at 90 DPI. In 2016, "Valley Forge" had a mean disease severity rating of 3.3 at 90 DPI. This was the lowest 90 DPI mean disease severity rating for either year. Every cultivar, except "Brandon," had a lower mean disease severity rating in 2016 than in 2015 at 90 DPI. The ranks of the cultivars changed considerably from the 2015 to 2016 inoculation season for mean disease severity (Table 1). "Brandon" went from 2nd lowest disease severity rating in 2015 to the highest in 2016 at 90 DPI. None of the mock-inoculated trees showed wilt symptoms in either year.

3.2 | Hydraulic conductivity

Although initial stem area-specific conductivity (K_s) varied at 5 DPI for the different cultivars, in inoculated treatments, all cultivars showed a general trend of decreasing mean K_s from 5 to 40 DPI (Figure 2). There was no flow in samples measured at 40 and 90 DPI, for both "New Harmony" and wild-type (US). At 90 DPI,

TABLE 1 Disease severity ratings (1-12 scale) of *Ulmus americana* cultivars at multiple time points following inoculation with *Ophiostoma novo-ulmi* in 2015 and 2016

		Days Post-Inoculation (DPI)			
		Disease Severity (mean ± se)			
Year	Cultivar	10 DPI	20 DPI	40 DPI	90 DPI[1]
2015	'Prairie Expedition'	2.3 ± 0.3 (n=30)	6.6 ± 0.5 (n=21)	6.5 ± 0.6 (n=15)	5.7 ± 1.0 (n=9) a
	'Brandon'	1.3 ± 0.1 (n=30)	4.1 ± 0.6 (n=21)	6.1 ± 0.7 (n=15)	6.9 ± 0.8 (n=9) a
	'Valley Forge'	1.1 ± 0.1 (n=30)	5.4 ± 0.3 (n=21)	6.9 ± 0.3 (n=15)	8.7 ± 0.9 (n=9) ab
	Wild Type (US)	2.0 ± 0.3 (n=27)	7.4 ± 0.7 (n=18)	8.7 ± 0.8 (n=12)	9.3 ± 0.8 (n=6) abc
	Wild Type (CA)	4.2 ± 0.5 (n=30)	9.4 ± 0.4 (n=21)	10.5 ± 0.5 (n=15)	10.7 ± 0.7 (n=9) bc
	'New Harmony'	1.9 ± 0.3 (n=18)	8.0 ± 0.5 (n=12)	11.3 ± 0.4 (n=9)	12.0 ± 0 (n=6) bc
	'Princeton'	2.0 ± 0.2 (n=30)	8.4 ± 0.3 (n=21)	10.5 ± 0.4 (n=15)	12.0 ± 0 (n=9) c
2016	'Valley Forge'	1.0 ± 0 (n=4)	3.3 ± 0.5 (n=4)	3.5 ± 0.3 (n=4)	3.3 ± 0.3 (n=4) a
	'Prairie Expedition'	1.2 ± 0.2 (n=5)	5.6 ± 1.2 (n=5)	5.6 ± 0.9 (n=5)	5.6 ± 0.9 (n=5) ab
	'Princeton'	1.0 ± 0 (n=5)	4.0 ± 0.3 (n=5)	5.6 ± 0.7 (n=5)	6.2 ± 1.5 (n=5) abc
	Wild Type (US)	1.0 ± 0 (n=5)	2.4 ± 0.7 (n=5)	6.4 ± 1.0 (n=5)	6.8 ± 1.2 (n=5) abc
	Wild Type (CA)	1.2 ± 0.2 (n=5)	7.2 ± 1.4 (n=5)	7.8 ± 1.2 (n=5)	9.6 ± 1.0 (n=5) bc
	'New Harmony'	1.0 ± 0 (n=5)	3.8 ± 0.2 (n=5)	9.2 ± 0.7 (n=5)	11.2 ± 0.8 (n=5) c
	'Brandon'	1.0 ± 0 (n=5)	5.6 ± 0.7 (n=5)	9.8 ± 0.6 (n=5)	11.8 ± 0.2 (n=5) c

[1]Groups containing the same letter in the same year are not significantly different at 90 DPI according to Dunn's multiple comparison test with a Benjamini and Hochberg *p*-value adjustment (α=0.05).

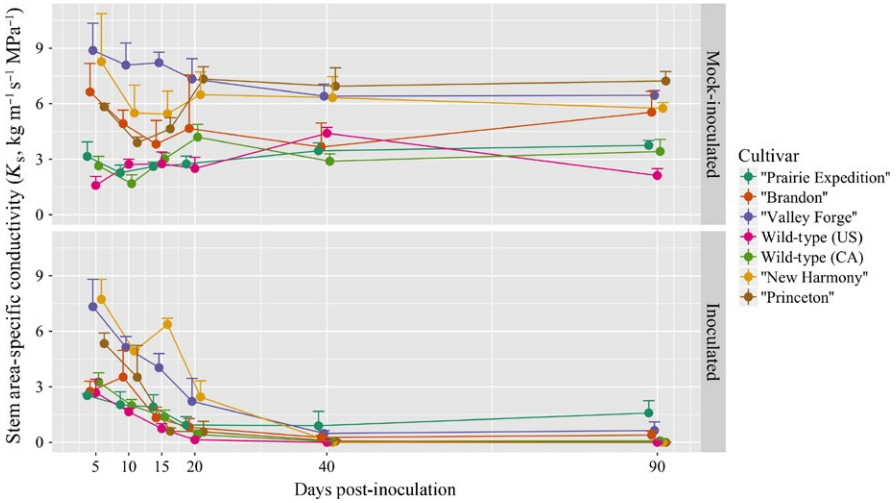

FIGURE 2 Stem area-specific conductivity (K_s, kg m^{-1} s^{-1} MPa^{-1}) in cultivars of *Ulmus americana* mock-inoculated (top) or inoculated with *Ophiostoma novo-ulmi* (bottom) over time for the 2015 trial. Cultivars are listed from lowest mean disease severity rating at 90 days post-inoculation (DPI) in 2015 (top) to highest (bottom). Each circle represents the mean, and the bar represents one standard error (*SE*) of the mean

"Princeton" had a K_s value of 0 kg m^{-1} s^{-1} MPa^{-1}; however, from 40 to 90 DPI, the remaining four cultivars showed an increase in mean K_s. Notably, "Prairie Expedition" increased in mean K_s from 0.90 to 1.59 kg m^{-1} s^{-1} MPa^{-1}, which was only 57.6% less than that of mock-inoculated controls at 90 DPI. "Valley Forge," which had the next highest K_s value at 90 DPI, exhibited a 90% reduction in K_s when compared to mock-inoculated controls. At 90 DPI, the only statistically significant difference for K_s was between "Prairie Expedition" and "New Harmony."

3.3 | Per cent of sap-conducting xylem area

At 10 cm above the inoculation point, all cultivars displayed a decrease of over 50% in their per cent of sap-conducting xylem area from 5 to 20 DPI (Figure 3). The most extreme decrease was observed in the wild-type (US), which saw a decline from 91.1% to 0.5%. By 40 DPI, every cultivar had a mean per cent of sap-conducting xylem area <7%, except for "Prairie Expedition," which had 22.7%. The three cultivars with the lowest disease severity rating at 90 DPI, "Prairie Expedition," "Brandon" and "Valley Forge," had their lowest mean per cent of sap-conducting xylem area at 40 DPI and then increased at 90 DPI. For the mock-inoculated trees, the lowest mean per cent of sap-conducting xylem area for any cultivar at any time point was 82.5%.

When examining 30 cm above the inoculation point at 90 DPI, the cultivar with the lowest disease severity rating had the highest per cent of sap-conducting xylem area for both 2015 and 2016 (Table 1, Figure 4). In 2015, the effect of cultivar on per cent of sap-conducting xylem area was not found to be statistically significant ($p = .12$). However, the effect of cultivar in 2016 was found to be statistically significant ($p < .001$). In 2016, "Valley Forge" had a mean per cent of sap-conducting xylem area of 83.7%, which was 60% higher than the next closest cultivar, "Prairie Expedition," at 23.4%. The lowest mean per cent of sap-conducting xylem area were observed in "Brandon" and "New Harmony," which had 0.2% and 1.7%, respectively. There was a strong to very strong negative correlation between per cent of sap-conducting xylem area and disease severity rating in 2015 and 2016 (r_s = −.74 and −.93, respectively) (Table 2).

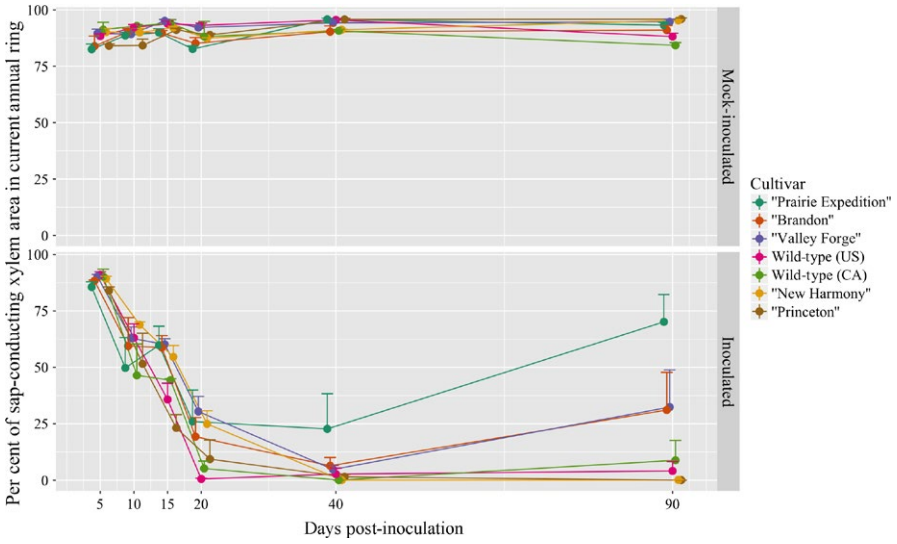

FIGURE 3 Per cent of sap-conducting xylem area in the current annual ring in cultivars of *Ulmus americana* mock-inoculated (top) or inoculated with *Ophiostoma novo-ulmi* (bottom) over time for the 2015 trial. Cultivars are listed from lowest mean disease severity rating at 90 days post-inoculation (DPI) in 2015 (top) to highest (bottom). Each circle represents the mean, and the bar represents one standard error (*SE*) of the mean. Measurements were taken 10 cm above the inoculation site

3.4 | Per cent of circumference conducting

For inoculated trees in 2015, at 10 cm above the inoculation site, all cultivars displayed a similar trend in the decrease of their per cent of circumference conducting of first formed cells over time (Figure 5). The cultivar with the highest per cent of circumference conducting at 90 DPI was "Prairie Expedition," with 12.3% (Figure 5). At 90 DPI, the lowest mean per cent of circumference conducting of first formed cells was 87% for the mock-inoculated controls. There was much more variation in per cent of circumference conducting of later formed cells between the cultivars, especially at 90 DPI (Figure 5). "Prairie Expedition" and "Brandon," which had the two lowest 90 DPI mean disease severity ratings (Table 1), had their lowest per cent of circumference conducting of later formed cells at 20 DPI and then increased at subsequent time points. At 20 DPI, "Prairie Expedition" had <40% of its circumference with conducting later formed cells, but by 90 DPI, all trees examined had 100% (Figure 7). All mock-inoculated controls were at 100% circumference conducting of later formed cells at 90 DPI.

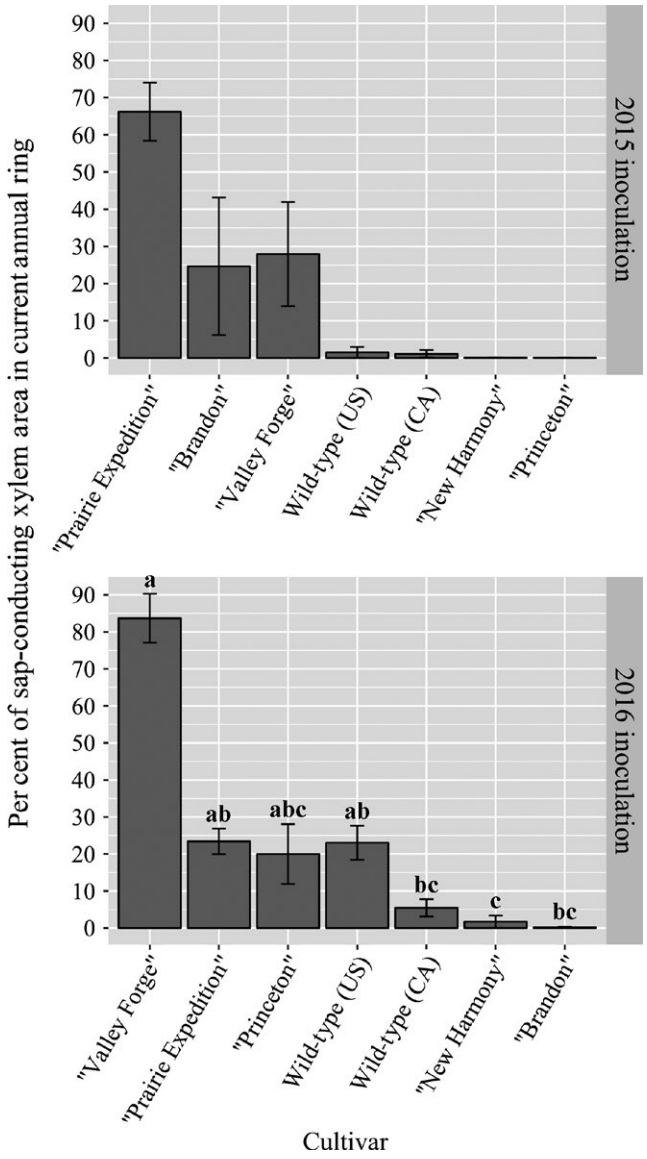

FIGURE 4 Per cent of sap-conducting xylem area in the current annual ring in cultivars of *Ulmus americana* inoculated with *Ophiostoma novo-ulmi* at 90 days post-inoculation in 2015 (top) and 2016 (bottom). For each year, cultivars are listed from lowest mean disease severity rating at 90 days post-inoculation (DPI) (left) to highest (right). Bars represent the mean ± standard error (*SE*) of the mean. Measurements were taken 30 cm above the inoculation site. For 2015, the effect of cultivar on per cent of sap-conducting xylem area was not found to be statistically significant according to the Kruskal–Wallis rank-sum test (α = 0.05). Cultivars sharing the same lower case letter are not significantly different according to Dunn's multiple comparison test with a Benjamini and Hochberg *p*-value adjustment (α = 0.05)

At 30 cm above the inoculation site at 90 DPI, all cultivars in the 2015 trial had <5% circumference conducting of first formed cells (Figure 6). The effect of cultivar on per cent circumference conducing was not found to be statistically significant ($p = .61$). In 2016, however, "Valley Forge" had a mean of 57% circumference conducting of first formed cells (Figure 8a), while the next closest was "Princeton" at 10% (Figure 6). The difference between "Valley Forge" and "Brandon," "New Harmony," and wild-type (US) was statistically significant. In 2015, mock-inoculated controls for all cultivars except wild-type (CA) had means over 95% for per cent circumference conducting of first formed cells. The wild-type (CA) had 66.6%, and this mainly resulted from the influence of one of the replicates having only 9.0%. All mock-inoculated controls in 2016 had over 91% circumference conducting of first formed cells, except for "Brandon," which had 78.7%.

The effect of cultivar on per cent circumference conducting in later formed cells in 2015 was not statistically significant ($p = .06$), but was significant in 2016 ($p = .001$). There was considerable variation among the cultivars in their abilities to maintain functional xylem in the later formed cells (Figures 6 and 8). "Prairie Expedition" had the highest mean per cent of circumference conducting for both 2015 and 2016 at 100%. In 2016, both "Valley Forge" and wild-type (US) also had a mean per cent of circumference conducting above 95%. There was a significant difference between the group of three cultivars with the highest mean per cent circumference conducting of later formed cells and the two lowest cultivars, "Brandon" and "New Harmony" (Figure 6). All mock-inoculated controls in both years had 100% circumference conducting of later formed cells. There was a very strong negative correlation between per cent of circumference conducting of later formed cells and disease severity in 2015 and 2016 (r_s −0.91 and −0.80, respectively) (Table 2).

4 | DISCUSSION

Using artificial inoculation with *O. novo-ulmi* in cultivars and wild-type populations, we found significant differences in susceptibility among *U. americana* genotypes, providing clear evidence for variation in disease resistance. Previous studies have shown that *U. americana* genotypes differ in their susceptibility to DED (Townsend et al., 1995, 2005), and our work confirms these findings. We further show evidence for compartmentalization of infection by *O. novo-ulmi* in some genotypes but not others, providing a mechanistic explanation for differences in susceptibility.

TABLE 2 Matrix of Spearman's correlation coefficients among variables measured in inoculated *Ulmus americana* cultivars at 90 days post-inoculation for 2015 and 2016

2015						
	DSR	**Area Cond.**	**FFC Cond.**	**LFC Cond.**	**FFC & LFC Avg.**	**Ks**
DSR	1.00					
Area Cond.	−0.74 *	1.00				
FFC Cond.	−0.60 *	0.56 *	1.00			
LFC Cond.	−0.91 *	0.89 *	0.57 *	1.00		
FFC & LFC Avg.	−0.92 *	0.88 *	0.68 *	0.99 *	1.00	
Ks	−0.93 *	0.78 *	0.65 *	0.92 *	0.93 *	1.00
2016						
	DSR	**Area Cond.**	**FFC Cond.**	**LFC Cond.**	**FFC & LFC Avg.**	
DSR	1.00					
Area Cond.	−0.93 *	1.00				
FFC Cond.	−0.55 *	0.54 *	1.00			
LFC Cond.	−0.80 *	0.82 *	0.45 *	1.00		
FFC & LFC Avg.	−0.85 *	0.86 *	0.81 *	0.84 *	1.00	

Variables are: disease severity rating (DSR); percent of sap-conducting xylem area in the current annual ring (Area Cond.); percent of circumference conducting of first formed cells (FFC Cond.) and later formed cells (LFC Cond.) in the current annual ring at 30 cm above the inoculation point; average percent of circumference conducting of first formed cells and later formed cells (FFC & LFC Avg.); stem area-specific conductivity (Ks, kg m^{-1} s^{-1} MPa^{-1}). Twenty-one plants were used in 2015 and 34 were used in 2016. Correlations followed with an * were found to be statistically significant (α=0.05).

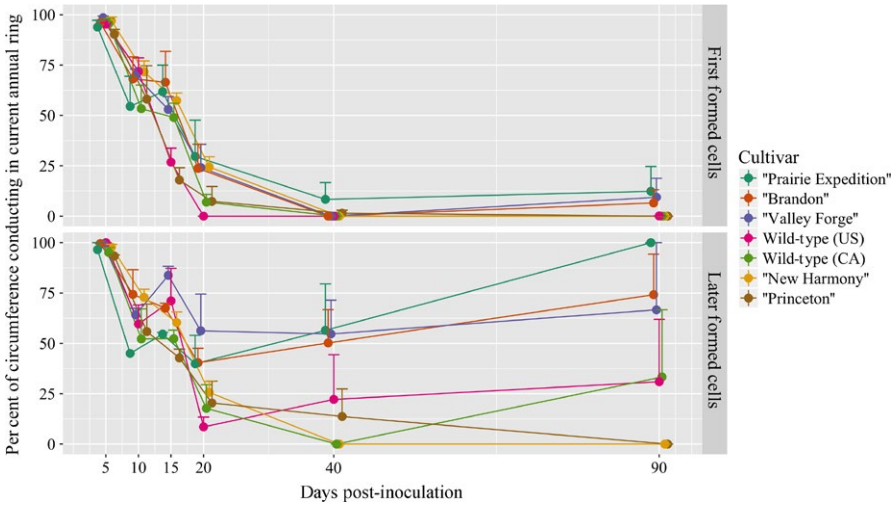

FIGURE 5 Per cent of circumference conducting in the current annual ring for first formed cells (top) and later formed cells (bottom) in cultivars of *Ulmus americana* inoculated with *Ophiostoma novo-ulmi* over time for the 2015 trial. Cultivars are listed from lowest mean disease severity rating at 90 days post-inoculation (DPI) in 2015 (top) to highest (bottom). Each circle represents the mean, and the bar represents one standard error (*SE*) of the mean. For 5, 10 and 15 DPI, some samples did not have later formed cells around the entire circumference of the stem and therefore were not included in analysis of later formed cells. Measurements were taken 10 cm above the inoculation site

Variation in disease severity between trials can be a challenge for scientists screening *Ulmus* genotypes for resistance to DED. Townsend et al. (2005) reported that "Valley Forge" and "Princeton" had 0% foliar symptoms and "New Harmony" had a mean of 4% foliar symptoms at 4 weeks post-inoculation, which is considerably lower than those found in the present study at 20 DPI, particularly in the 2015 trial (Table 1). A potential explanation for the difference in foliar symptoms is the age and size of the plant material examined. Townsend et al. (2005) used 9-year-old plant material in their study. In an earlier study by Townsend et al. (1995) where 3-year-old plant material was evaluated, "Princeton" and "New Harmony" had mean foliar symptoms of 54% and 74% at 4 weeks post-inoculation, respectively, when inoculated in mid-May. The difference in disease susceptibility of trees at different ages and sizes may have impacted the disease severity levels observed in 2015 and 2016. If the pathogen is moving at a static rate around the circumference of the stem, trees with larger diameters would have a longer time to form barrier zones before the stem loses all conductivity. Additionally, the percentage of the entire

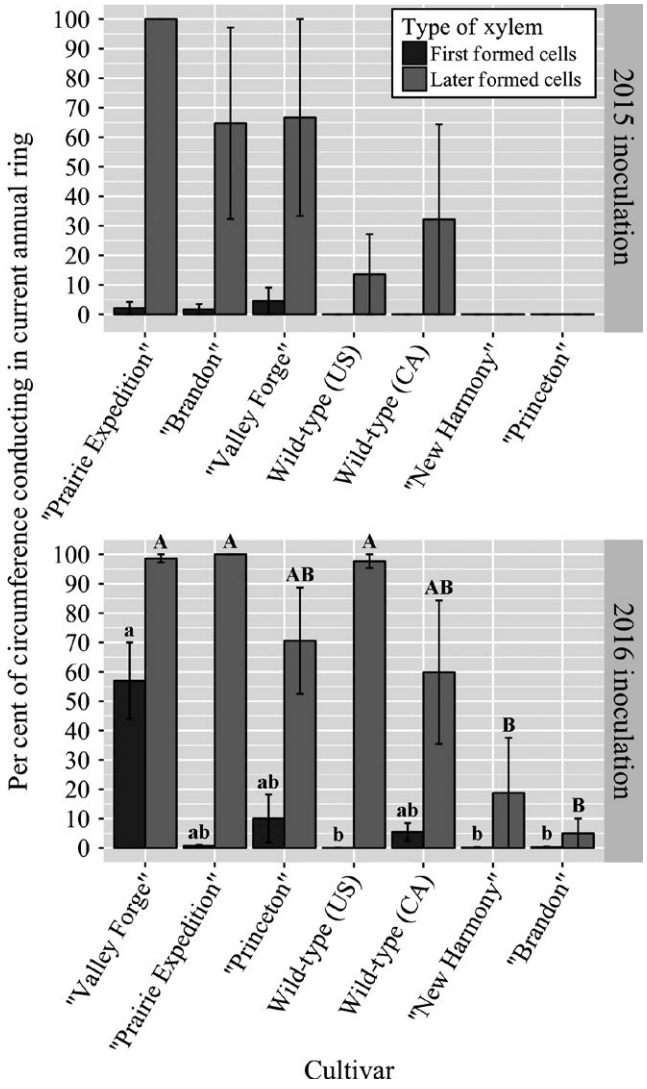

FIGURE 6 Per cent of circumference conducting in the current annual ring for first formed cells (dark grey) and later formed cells (light grey) in cultivars of *Ulmus americana* inoculated with *Ophiostoma novo-ulmi* at 90 days post-inoculation in 2015 (top) and 2016 (bottom). For each year, cultivars are listed from lowest mean disease severity rating at 90 days post-inoculation (DPI) (left) to highest (right). Bars represent the mean ± standard error (*SE*) of the mean. First formed cells were considered the first row of large cells formed in the annual ring. All other xylem was considered later formed cells. Measurements were taken 30 cm above the inoculation site. For 2015, the effect of cultivar on first formed cells and later formed cells was not found to be statistically significant according to the Kruskal–Wallis rank-sum test ($\alpha = 0.05$). Cultivars sharing the same lower case letter for first formed cells or upper case letter for later formed cells are not significantly different according to Dunn's multiple comparison test with a Benjamini and Hochberg *p*-value adjustment ($\alpha = 0.05$)

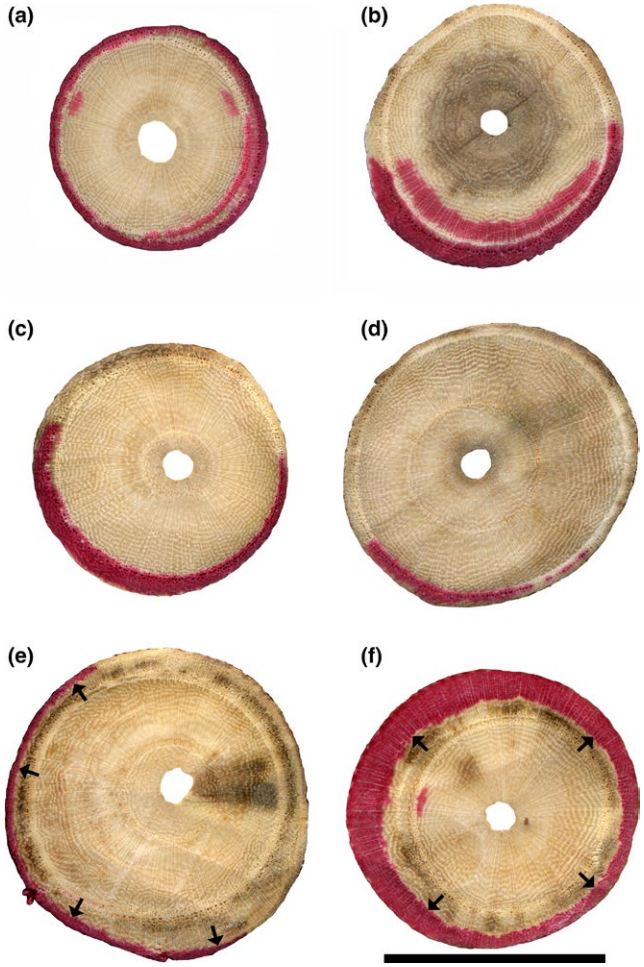

FIGURE 7 Representative transverse sections of *Ulmus americana* "Prairie Expedition" inoculated with *Ophiostoma novo-ulmi* displaying change in conduction over time (scale bar = 1 cm). Samples were collected during the 2015 trial. Transverse sections were made 10 cm above the inoculation site. Sections were oriented so the side of inoculation is at the top. Trees were stained with safranin O to indicate conducting (stained red) and non-conducting (not stained) areas of xylem. Black arrows represent areas likely to be barrier zones separating necrotic tissue from healthy conducting tissue. (a) 5 days post-inoculation (DPI); (b) 10 DPI; (c) 15 DPI; (d) 20 DPI; (e) 40 DPI, limited spread into the newly formed xylem on the left side of the section suggesting a barrier zone has formed; (f) 90 DPI, maintained conductive xylem around the entire circumference of the stem suggesting a barrier zone has limited spread into newly formed xylem

stem affected would be less in larger stems. All cultivars except for "Brandon" had lower disease severity ratings in 2016 compared with 2015. A potential explanation for the relatively low disease rating score in 2015 for "Brandon" could be stress related. When the trees were received from the production nursery, it was observed that many large roots had been cut during the harvest process. Additionally, it was found "Brandon" had the lowest mean growth in control trees at 40 DPI in 2015, as measured by the width of the most recent annual ring (G. L. Beier, unpublished data). A study by Ouellet and Pomerleau (1965) on 1-year-old *U. americana* seedlings, found smaller, less vigorous seedlings had fewer plants display disease symptoms following inoculation compared with the vigorous, larger plants.

Wild-type seedlings of American elm are sometimes used as susceptible controls in DED screening trials (Townsend et al., 1995, 2005). Unexpectedly in our study, in both years, the two groups of wild-type seedlings performed better than "New Harmony." These seedlings

were collected from seed sources in Tennessee, United States and Ontario, Canada, and had not been previously tested for resistance to DED. Large surviving elms, unknown to the collectors, may have some levels of resistance to DED. Using clones with known susceptibility in future studies would be advisable.

Trees suffering from DED primarily decline due to xylem dysfunction (MacHardy & Beckman, 1973). The effect of DED on a tree's hydraulic conductivity can be quite rapid. A study by Urban and Dvorak (2014) on *Ulmus glabra* Huds. found a significant reduction in sap flow at 10 DPI in inoculated trees compared with control trees and by 16 DPI all sap flow had stopped. In resistant genotypes, the effects can be much less severe. When examining the specific leaf hydraulic conductivity of resistant genotypes of *Ulmus minor* Mill. at 21 DPI, Li et al. (2016) found no significant difference between the inoculated and mock-inoculated treatments. However, in susceptible genotypes, the mean specific leaf hydraulic conductivity of controls was over two times greater than that of the inoculation treatment. Resistant genotypes did show an increase in the per cent of leaves wilting from 21 to 30 DPI, but the latest time point examined for hydraulic conductivity was 21 DPI, so it is unknown whether there would have been a significant difference between inoculated and mock-inoculated trees at later time points. Additionally, without further time points, it is unclear whether the susceptible genotypes would have performed similar to "Prairie Expedition," where conductivity increased later in the season as newly formed xylem, not invaded by the pathogen, was produced.

Cultivars examined in this study varied considerably in their ability to limit the spread of *O. novo-ulmi* into xylem formed after wounding (Figure 8). Wall 4 of the CODIT model involves barrier zones formed after wounding, which can limit the spread of pathogens into newly formed xylem tissue (Shigo & Marx, 1977). Previous studies have shown barrier zones may form in infected elms (Banfield, 1968; Buisman, 1935; Rioux & Ouellette, 1991; Tippett & Shigo, 1981). Although barrier zones may form, they do not always limit spread of the infection. "Prairie Expedition" was the only cultivar to have 100% circumference conducting of later formed cells for all samples at 90 DPI for both years suggesting a barrier zone was formed. However, it is possible that the limiting of fungal spread was not from barrier zones, but from other factors, such as phytoalexins or pathogenesis-related proteins. If barrier zones were formed, having 100% circumference conducting of later formed cells does not confirm barrier zones were continuous around the circumference of the stem. Rioux and Ouellette (1991) found barrier zones in branches of artificially inoculated *U. americana* were often discontinuous.

The timing of barrier zone formation may be critical in limiting the spread of infection. Currently, cultivars are being examined to determine whether differences exist in the timing of barrier zone formation. In the 2015 trial, the per cent of circumference of later formed cells decreased until day 20 and then increased at 40 and 90 DPI for "Prairie Expedition" and "Brandon," which had the lowest disease severity rating at 90 DPI. These results suggest a barrier zone, and new conducting tissue is formed sometime between 20 and 40 DPI (Figure 7). A study by Rioux and Ouellette (1991) on *U. americana* inoculated with *O. ulmi* found when barrier zones formed in small branches and annual shoots they were first detectable at 22 DPI with the average time to barrier zone formation at 30 DPI. Additionally, Bonsen et al. (1985) noted barrier zones started forming in *Ulmus* × *hollandica* Mill. twigs between 20 and 40 DPI.

Wall 3 reaction zones have been implicated in the limitation of tangential pathogen spread in non-host species inoculated with *O. ulmi* (Ouellette & Rioux, 1992; Rioux & Ouellette, 1989). Reducing the tangential spread allows for more xylem to remain functional as well as potentially reducing the energy demands by not requiring barrier zones to be formed around the entire circumference of the stem. Effective wall 3 reaction zones have been less frequently documented in *U. americana* compared with barrier zones. When Rioux and Ouellette (1991) examined *U. americana* branches infected with *O. ulmi*, they noted that reaction zones limiting tangential spread were

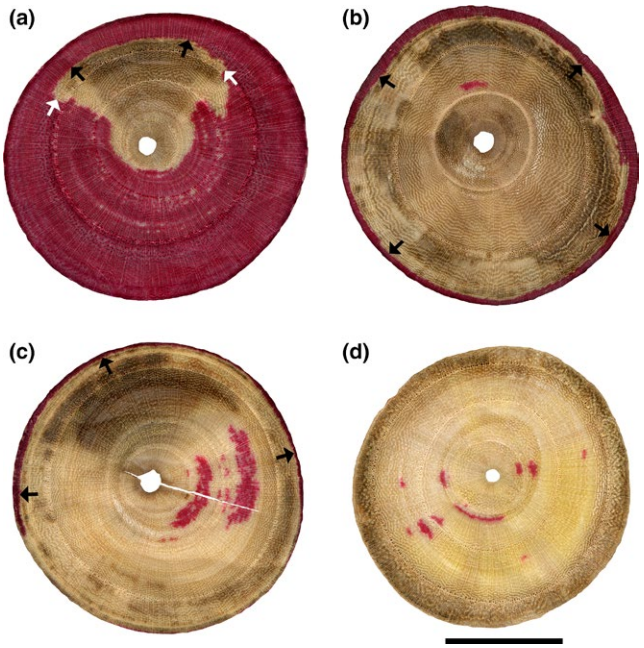

FIGURE 8 Representative transverse sections of *Ulmus americana* cultivars inoculated with *Ophiostoma novo-ulmi* at 90 days post-inoculation (scale bar = 1 cm). Samples were collected during the 2016 trial. Transverse sections were made 30 cm above the inoculation site. Sections were oriented so the side of inoculation is at the top. Trees were stained with safranin O to indicate conducting (stained red) and non-conducting (not stained) areas of xylem. Black arrows represent areas likely to be barrier zones separating necrotic tissue from healthy conducting tissue. White arrows represent areas limiting infection likely through a wall 3 reaction zone. (a) "Valley Forge," limited tangential spread and spread into newly formed xylem suggesting both an effective wall 3 reaction zone and barrier zone; (b) "Prairie Expedition," failed to limit tangential spread, but successfully limited spread into newly formed xylem suggesting an ineffective wall 3 reaction zone and effective barrier zone; (c) "Princeton," failed to limit tangential spread, but partially limited spread into newly formed xylem suggesting an ineffective wall 3 reaction zone and partially effective barrier zone; (d) "New Harmony," failed to limit tangential spread and spread into newly formed xylem suggesting an ineffective wall 3 reaction zone and an ineffective or no barrier zone

rarely observed. A potential explanation for the lack of effectiveness of the wall 3 reaction zone is that groups of ray parenchyma, which form the wall 3 reaction zone, are not continuous throughout the stem (Shigo & Marx, 1977). Additionally, in a recent review on the role of parenchyma in plant defences by Morris, Brodersen, Scharze, and Jansen (2016), the authors suggest an effective barrier zone may come at the cost of a weak reaction zone. During the 2015 study, no cultivars exhibited a wall 3 reaction zone capable of significantly reducing pathogen spread tangentially around the stem as evident by the low per cent of circumference conducting of first formed cells at 90 DPI (Figure 6). "Valley Forge" was the only cultivar in 2016 to consistently limit the tangential spread of the pathogen (see, e.g., Figure 8a).

A major objective of this study was to compare the effectiveness of calculating per cent of circumference conducting for first formed and later formed cells compared with traditional thresholding to calculate per cent of sap-conducting xylem area. In addition to gaining more insight into the ability of genotypes to limit tangential spread and spread outward into newly formed cells, there was higher correlation between disease severity and average per cent of circumference conducting of first formed and later formed cells compared to per cent of sap-conducting xylem area in 2015, but not in 2016 (Table 2). When comparing these methods, only the most recent annual ring was used. A study on *U. americana* by Ellmore and Ewers (1986) found the outermost annual ring accounted for 92% of the total hydraulic conductivity in stems. Per cent of circumference conducting for first formed and later formed cells is a potentially useful tool in examining other pathosystems, where compartmentalization may be observed.

Although differences in ability to compartmentalize infection help explain why some cultivars are more resistant to DED, other factors, such as anatomical characteristics, may also play a role in determining whether a cultivar is resistant or susceptible. Scientists have long noted that resistant elms tend to have smaller vessels than susceptible elms (Elgersma, 1970; McNabb, Heybroek, & Macdonald, 1970; Pope, 1943; Sinclair, Zahand, & Melching, 1975; Solla & Gil, 2002; Venturas, Lopez, Martin, Gasco, & Gil, 2014). Additionally, it has been found that susceptible genotypes have longer vessels than resistant genotypes (Elgersma, 1970; Venturas et al., 2014). However, a study by Martin, Solla, Ruiz-Villar, and Gil (2013) on *Ulmus minor* found vessel length did not explain differences in susceptibility of genotypes. The timing of the transition from earlywood to latewood may also affect resistance. Numerous studies have found that late season inoculations result in less symptom development compared with inoculation during peak susceptibility (Pomerleau, 1965; Smalley, 1963; Smalley & Kais, 1966; Smalley & Lester, 1983; Takai & Kondo, 1979), yet it remains unclear whether this reduction in disease severity is directly related to the change in wood anatomy from earlywood to latewood. Studies are currently underway to examine cultivars used in this study to determine whether differences exist in anatomical characteristics that may further explain differences in resistance.

Based on these findings, young *U. americana* cultivars differ in how they compartmentalize infection with *O. novo-ulmi*. Although cultivars may allow the pathogen to spread tangentially around the stem at a similar rate, as was the case in 2015, cultivars with the lowest disease severity restricted the spread of infection into newly formed xylem, as evident by having a high percentage of circumference conducting of later formed cells. In 2016, "Valley Forge," in addition to restricting the spread into newly formed xylem, was the only cultivar that consistently limited the tangential spread. Not surprisingly, "Valley Forge" had the lowest mean disease severity rating of any cultivar. This combination of restricting the pathogen may explain why it has performed so well in DED screening trials compared with other American elm genotypes (Townsend et al., 1995). As genotypes vary in their ability to restrict infection from spreading, selections that are being inoculated and screened for DED resistance by breeders should be evaluated for their capacity to limit tangential and radial outward spread. As evident by the standard error (*SE*) of the means in per cent of circumference conducting of later formed cells (Figure 6), some cultivars lacked consistency in limiting outward spread to cells formed after wounding. Histological assessments are currently underway to examine the anatomical and histological characteristics of these samples to determine why some failed and others were successful. When selecting plant material for breeding programmes, genotypes able to consistently limit the spread of the pathogen would be recommended over those which are inconsistent. Methods described in this study should enable breeders to more effectively evaluate the abilities of different genotypes to compartmentalize infection by *O. novo-ulmi*.

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REFERENCES

Baayen, R. P., Ouellette, G. B., & Rioux, D. (1996). Compartmentalization of decay in carnations resistant to *Fusarium oxysporum* f. sp. *dianthi*. *Phytopathology*, *86*, 1018–1031.

Banfield, W. M. (1968). Dutch elm disease recurrence and recovery in American elm. *Massachusetts Agricultural Experiment Station Bulletin*, *568*, 60pp.

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 Statistical Methodology*, *57*, 289–300.

Boddy, L. (1991). Microenvironmental aspects of xylem defenses to wood decay fungi. In R. A. Blanchette, & R. A. Biggs (Eds.), *Defense mechanisms of woody plants against fungi* (pp. 96–127). New York, NY: Springer-Verlag, Berlin Heidelberg.

Bonsen, K. J. M., Scheffer, R. J., & Elgersma, D. M. (1985). Barrier zone formation as a resistance mechanism of elms to Dutch elm disease. *IAWA Bulletin*, *6*, 71–77.

Buisman, C. (1935). The anatomy of wood of elms infected with *Graphium ulmi*. *Plantenzickten*, *41*, 104–120.

Edwards, J., Pascoe, I. G., & Salib, S. (2007). Impairment of grapevine xylem function by *Phaeomoniella chlamydospora* infection is due to more than physical blockage of vessels with 'goo'. *Phytopathologia Mediterranea*, *46*, 87–90.

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.

Ellmore, G. S., & Ewers, F. W. (1986). Fluid flow in the outermost xylem increment of a ring-porous tree, *Ulmus Americana*. *American Journal of Botany*, *73*, 1771–1774.

Harrington, T. C. (1981). Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. *Mycologia*, *73*, 1123–1129.

Inch, S. A., & Ploetz, R. C. (2012). Impact of laurel wilt, caused by *Raffaelea lauricola*, on xylem function in avocado, *Persea americana*. *Forest Pathology*, *42*, 239–245.

Joseph, G., Kelsey, R. G., & Thies, W. G. (1998). Hydraulic conductivity in roots of ponderosa pine infected with black-stain (*Leptographium wageneri*) or annosus (*Heterobasidion annosum*) root disease. *Tree Physiology*, *18*, 333–339.

Li, M., Lopez, R., Venturas, M., Martin, J. A., Dominguez, J., Gordaliza, G. G., … Rodriguez-Calcerrada, J. (2016). Physiological and biochemical differences among *Ulmus minor* genotypes showing a gradient of resistance to Dutch elm disease. *Forest Pathology*, *46*, 215–228.

MacHardy, W. E., & Beckman, C. H. (1973). Water relations in American elm infected with *Ceratocystis ulmi*. *Phytopathology*, *63*, 98–103.

Martin, 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.

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

Morris, H., Brodersen, C., Scharze, F. W. M. R., & Jansen, S. (2016). The parenchyma of secondary xylem and its critical role in tree defense against fungal decay in relation to the CODIT model. *Frontiers in Plant Science*, *7*, 1665.

Murata, M., Matsuda, Y., Yamada, T., & Ito, S. (2009). Differential spread of discoloured and non-conductive sapwood among four Fagaceae species inoculated with *Raffaelea quercivora*. *Forest Pathology*, *39*, 192–199.

Murata, M., Yamada, T., Matsuda, Y., & Ito, S. (2007). Discoloured and non-conductive sapwood among six Fagaceae species inoculated with *Raffaelea quercivora*. *Forest Pathology*, *37*, 73–79.

Ouellet, C. E., & Pomerleau, R. (1965). Recherches sur la résistance de l'orme d'Amerique au *Ceratocystis ulmi*. *Canadian Journal of Botany*, *43*, 85–96.

Ouellette, G. B., & Rioux, D. (1992). Anatomical and physiological aspects of resistance to Dutch elm disease. In R. A. Blanchette, & R. A. Biggs (Eds.), *Defense mechanisms of woody plants against fungi* (pp. 257–301). New York, NY: Springer-Verlag, Berlin Heidelberg.

Pearce, R. B. (1996). Antimicrobial defences in the wood of living trees. *New Phytologist*, *132*, 203–233.

Pomerleau, R. (1965). The period of susceptibility of *Ulmus americana* to *Ceratocystis ulmi* under conditions prevailing in Quebec. *Canadian Journal of Botany*, *43*, 787–792.

Pope, S. A. (1943). *Some studies on the Dutch elm disease and the causal organism*. PhD Thesis. Ithaca, NY: Cornell University.

Rioux, D., & Ouellette, G. B. (1989). Light microscope observations of histological changes induced by *Ophiostoma ulmi* in various nonhost trees and shrubs. *Canadian Journal of Botany*, *67*, 2335–2351.

Rioux, D., & Ouellette, G. B. (1991). Barrier zone formation in host and nonhost trees inoculated with *Ophiostoma ulmi*. I. Anatomy and histochemistry. *Canadian Journal of Botany*, *69*, 2055–2073.

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

Shigo, A. L. (1984). Compartmentalization: A conceptual framework for understanding how trees grow and defend themselves. *Annual Review of Phytopathology*, *22*, 189–214.

Shigo, A. L., & Marx, H. (1977). *Compartmentalization of decay in trees*. Agric. Info. Bull. No. 405. Washington, DC: U.S. Department of Agriculture, Forest Service. 73 pp.

Shigo, A. L., & Tippett, J. T. (1981). Compartmentalization of American elm tissues infected by *Ceratocystis ulmi*. *Plant Disease*, *65*, 715–718.

Shortle, W. C., & Dudzik, K. R. (2012). *Wood decay in living and dead trees: A pictorial overview*. Gen. Tech. Rep. NRS-97. Newtown Square, PA: USDA Forest Service Northern Research Station.

Sinclair, W. A., Zahand, J. P., & Melching, J. B. (1975). Anatomical marker for resistance of *Ulmus americana* to *Ceratocystis ulmi*. *Phytopathology*, *65*, 349–352.

Smalley, E. B. (1963). Seasonal fluctuations in susceptibility of young elm seedlings to Dutch elm disease. *Phytopathology*, *53*, 846–853.

Smalley, E. B., & Kais, A. G. (1966). Seasonal variations in the resistance of various elm species to Dutch elm disease. In H. D. Gerhold, R. E. McDermott, E. J. Schreiner, & J. A. Winieski (Eds.), *Breeding pest-resistant trees* (pp. 279–292). London, UK: Pergamon Press Ltd.

Smalley, E. B., & Lester, D. T. (1983). 'Regal' elm. *HortScience*, *18*, 960–961.

Solla, A., & Gil, L. (2002). Xylem vessel diameter as a factor in resistance of *Ulmus minor* to *Ophiostoma novo-ulmi*. *Forest Pathology*, *32*, 123–134.

Sperry, J. S., Donnelly, J. R., & Tyree, M. T. (1988). A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell and Environment*, *11*, 35–40.

Stennes, M. A. (1981). Thiabendazole hypophosphite and carbendazim phosphate as systemic fungicides for practical Dutch elm disease control. M.S. thesis. University of Minnesota, St. Paul, MN.

Tainter, F. H., & Fraedrich, S. W. (1986). Compartmentalization of *Ceratocystis fagacearum* in turkey oak in South Carolina. *Phytopathology*, *76*, 698–701.

Takahashi, Y., Matsushita, N., & Hogetsu, T. (2010). Spatial distribution of *Raffaelea quercivora* in xylem of naturally infested and inoculated oak trees. *Phytopathology*, *100*, 747–755.

Takai, S., & Kondo, E. S. (1979). Seasonal development of Dutch elm disease on white elms in central Ontario, Canada. I. Following wound inoculation. *Canadian Journal of Botany*, *57*, 341–352.

Tippett, J. T., & Shigo, A. L. (1981). Barrier zone formation: A mechanism of tree defense against vascular pathogens. *IAWA Bulletin*, *2*, 163–168.

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.

Townsend, A. M., Bentz, S. E., & Johnson, G. R. (1995). Variation in response of selected American elm clones to *Ophiostoma ulmi*. *Journal of Environment Horticulture*, *13*, 126–128.

Urban, J., & Dvorak, M. (2014). Sap flow-based quantitative indication of progression of Dutch elm disease after inoculation with *Ophiostoma novo-ulmi*. *Trees*, *28*, 1599–1605.

Venturas, M., Lopez, R., Martin, J. A., Gasco, 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.

Venturas, M. D., MacKinnon, E. D., Jacobsen, A. L., & Pratt, R. B. (2015). Excising stem samples under water at native tension does not induce xylem cavitation. *Plant, Cell and Environment*, *38*, 1060–1068.

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