

The Effects of Propagation Techniques on Cell Wall Chemistry and Wood Anatomy in Micropropagated and Grafted Plants of the Dutch Elm Hybrid ‘Dodoens’

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ABSTRACT. Determination of wood anatomy traits and the chemical attributes of plant cell walls is of great importance for the evaluation of both the effects of hybridization and the results of breeding strategies within the genus *Ulmus*, because these are both aimed at an enhanced tolerance to dutch elm disease (caused by *Ophiostoma ulmi* and *O. novo-ulmi*) and to the improvement of trees having desired mechanical properties. The objective of this study was to determine whether the routinely applied vegetative propagation techniques of in vitro micropropagation or grafting would result in any change to lignin monomer composition and content, macromolecular traits of cellulose, neutral sugar composition, or the vascular and fiber anatomy traits in the stems of the dutch elm hybrid cultivar Dodoens (i.e., open-pollinated *Ulmus glabra* ‘Exoniensis’ × *U. wallichiana* P39). Propagation techniques appeared to have no direct effect on lignin monomer composition. The differences in the relative proportion of guaiacyl units in lignin between the stock types were not significant, showing that no advantage could be attributed to either stock type toward an enhanced tolerance to dutch elm disease. The micropropagated plants reached significantly higher values for 13 traits (32.5%), primarily associated with the relative proportion of D-glucose and the macromolecular traits of cellulose to compensate for a lower content of holocellulose. The grafts reached higher values for 10 traits (25%), including the relative proportions of D-xylose, D-mannose, and D-galactose. The effect of the rootstock might contribute to different amounts of these cell wall substances in the grafts. The grafts also reached a higher lignin content, which may provide minor advantages in terms of mechanical and physical properties to the cell walls of this stock type. Similarities between the stock types were found for 17 traits (42.5%). Both stock types formed compact homogeneous clusters clearly separated from each other in the multivariate wood trait analysis.

Since 1918, when dutch elm disease (DED) emerged in Holland, a majority of both wild and street populations of *Ulmus* trees in Europe and North America have been destroyed. The ascomycetous fungi *Ophiostoma ulmi* and *O. novo-ulmi* were identified as the causative organisms of DED. The former

fungus was responsible for the first pandemic of the disease in the 1920s–40s, whereas the latter highly pathogenic and aggressive fungus was responsible for the advent of the second pandemic during the 1970s (Brasier, 1991; Smalley and Guries, 1993). A rapid emergence of hybrids between two subspecies of *O. novo-ulmi* has recently been reported, and it is likely that complex hybrid swarms are now expanding across the European continent (Brasier and Kirk, 2010). The initial elm breeding programs, launched in The Netherlands, were focused on the identification of resistant elms and emphasized the native European elms, especially *U. glabra* and *U. minor*. Asian elms, particularly *U. wallichiana* and *U. pumila*, proved to be a useful

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additional source of DED-resistance genes (Heybroek, 1983; Santini et al., 2008, 2010; Smalley and Guries, 1993). A series of new dutch elm hybrid cultivars were released in the 1960s and 1970s, and several hundred thousands grafted trees planted widely in Europe (Gerhold, 1970; Hiemstra et al., 2006) now show a varying degree of resistance to *O. novo-ulmi* isolates.

The use of tissue culture techniques assists in the propagation of high-quality plant material identified by tree breeding programs. In addition to elm breeding strategies, in vitro propagation methods may significantly contribute to the efforts of tree improvement through existing germplasm conservation, in vitro selection, and rapid distribution of new and improved cultivars (Biroščíková et al., 2004; Conde et al., 2008; Đurkovič et al., 2010; Harvengt et al., 2004; Shukla et al., 2012). Transgenic elms showing reduced DED symptoms have been regenerated (Gartland et al., 2005; Newhouse et al., 2007), and small-scale field tests of transgenic elms have been established (Merkle et al., 2007).

Determination of the chemical attributes of plant cell walls is of great importance for evaluating both the effects of hybridization and the results of plant breeding. The major chemical components of woody plant cell walls are the polysaccharide fractions (holocellulose) and lignin. Holocellulose is composed of cellulose and a complex mixture of polymers formed from simple saccharides known collectively as hemicelluloses. Hemicelluloses are typically characterized by the xylose, mannose, arabinose, and galactose contents. Microfibrils of cellulose constitute the reinforcing rods of the cell wall. Depending on the natural source, glucopyranose units ranging in number from 305 to 15,300 (Fengel and Wegener, 2003) are polymerized to form cellulose chains. This biopolymer imparts tensile strength to the wall to resist turgor pressure and to allow for growth habit. Lignins also impart strength to cell walls, facilitate water transport, and impede the degradation of cell wall polysaccharides, thus acting as a major line of defense against pathogens, insects, and other herbivores. The composition and crosslinking of lignin have significant impacts on the lignin degradability and susceptibility to kraft delignification (Chen et al., 2001; Grabber, 2005). One measure of dicotyledonous angiosperm lignin composition is the content ratio of guaiacyl (G) and syringyl (S) units. These aromatic units determine the type and number of crosslinks. The physical relationship between holocellulose and lignin affects many fiber characteristics. Variation in the chemical content of wood is generally influenced by the interaction of developmental, seasonal, and geographic conditions (Sewell et al., 2002). However, to the best of our knowledge, no studies have evaluated the effects of propagation techniques on cell wall chemistry in woody plants.

One of the key traits that seems to encapsulate, if not link, many of the plant functional traits is tissue density. In particular, for woody plants, the density of woody tissue appears to be central in many aspects of plant form, function, and diversity. Wood density describes the proportion of a stem that is tissue and cell walls (i.e., secondary xylem conduit walls) and the space within cell walls (i.e., secondary xylem conduit apertures) (Swenson and Enquist, 2007). In angiosperm tree species, vessel size (the transverse lumen area of individual vessels) and vessel density (number of vessels per transverse unit area) are the primary indicators of vascular strategy (Zanne and Falster, 2010).

Recently, we have found that G-rich lignin was involved in successful defense of infected elms against DED (Đurkovič et al., 2014). The previous study has revealed that the dutch elm hybrid cultivar Dodoens, a popular street tree, showed tolerance to the current prevalent strain of DED, *O. novo-ulmi* ssp. *americana* × *novo-ulmi* (Đurkovič et al., 2013). The objectives of this study were: 1) to determine whether routinely applied vegetative propagation techniques that maintain true types of elm genotypes (i.e., in vitro micropropagation vs. widely used grafting of past decades) would affect lignin content and lignin monomer composition in the stems of this dutch elm hybrid; and 2) to identify the differences between micropropagated plants and the grafts with respect to contents of cell wall components, the macromolecular traits of cellulose, neutral sugar composition, plus vessel and fiber anatomy traits, to reveal any mechanical advantages or compensations for either stock type.

Materials and Methods

PLANT MATERIAL, STUDY SITE, AND SAMPLING. The experiments were conducted on clonally micropropagated and grafted plants of the dutch elm hybrid cultivar Dodoens, which has shown tolerance to an aggressive isolate M3 of *O. novo-ulmi* ssp. *americana* × *novo-ulmi* (Đurkovič et al., 2013). The procedure of in vitro micropropagation through axillary and adventitious shoot formation on woody plant and DX culture media supplemented with 6-benzylaminopurine has been described in detail by Krajňáková and Longauer (1996). In this study, the authors also confirmed the genetic identity of micropropagated plants without an occurrence of somaclonal variation. The grafted plants were obtained through splice grafting, which consisted of joining a ‘Dodoens’ scion onto the stem of a DED-tolerant *U. pumila* ‘Pusztá’ rootstock to reduce the possibilities of DED transmission by root graft connections in the soil (Green et al., 1985). Grafting of scions onto rootstock was performed 2 cm upward from the root–stem junction, and the scions used for both grafting and in vitro culture establishment originated from identical donor plants. Three-year-old micropropagated and grafted plantlets of uniform size were selected and then planted in the experimental field plot at Banská Belá, Slovakia (lat. 48°28' N, long. 18°57' E, altitude 590 m). Proper care was taken during planting to avoid damage to the root system. The planting holes were dug with a spacing of 2 × 3 m. No post-planting treatments such as irrigation, fertilization, etc., were applied. According to the meteorological station at Arboretum Kysihýbel in Banská Štiavnica (altitude 540 m), located 3.6 km southwest of the study site, the climate of the area is characterized by a mean annual temperature of 7.7 °C and a mean annual precipitation of 831 mm. The study site soil, which has a silt loam texture, is identified as an Eutric Cambisol formed from the slope deposits of volcanic rocks (andesite and pyroclastic materials).

The sampling was done during the winter season after completion of the tenth growing season after planting. Wood discs 2 cm thick were sampled 20 cm above the tree base from the micropropagated plants and 20 cm above the graft union from the grafts; i.e., 22 cm above the soil level. The discs were symmetrical, visibly free of reaction wood, and did not contain knotwood. For each examined trait, measurements were performed on five randomly chosen plants per stock type, which were unaffected by DED.

PREPARATION AND DETERMINATION OF EXTRACTIVES. The bark was peeled off the wood samples, and the wood was frozen and

stored at $-18\text{ }^{\circ}\text{C}$ before chemical analyses. Separated wood was mechanically disintegrated to sawdust, and a fraction size of 0.5 to 1.0 mm was extracted in a Soxhlet apparatus with a mixture of ethanol and toluene (2:1) for 8 h according to the American Society for Testing and Materials (ASTM) International standard procedure D 1107-96.

DETERMINATIONS OF LIGNIN CONTENT AND LIGNIN MONOMER COMPOSITION. Lignin content was determined according to the U.S. Department of Energy, National Renewable Energy Laboratory analytical procedure (Sluiter et al., 2010). The samples were hydrolyzed in a two-stage process. In the first stage, 72% (w/w) H_2SO_4 at a temperature of $30\text{ }^{\circ}\text{C}$ was used for 2 h, and in the second stage, the samples were refluxed after dilution to 4% (w/w) H_2SO_4 for 4 h.

Nitrobenzene oxidation (NBO) was carried out using 2 M NaOH in 10-mL stainless steel vessels at a temperature of $180\text{ }^{\circ}\text{C}$ for 2 h (Đurkovič et al., 2012). An analysis of oxidation products was performed with a high-performance liquid chromatography (HPLC) system (1200; Agilent Technologies, Santa Clara, CA) equipped with Agilent ChemStation software. The isocratic HPLC was conducted on a $2.6\text{-}\mu\text{m}$, $100 \times 4.6\text{-mm}$ column (Kinetex C18; Phenomenex, Torrance, CA) coupled with a C18 SecurityGuard cartridge (Phenomenex). The column and detector temperatures were set at $35\text{ }^{\circ}\text{C}$. The mobile phase consisted of water:methanol:propionic acid (840:160:1), and the flow rate was $1.0\text{ mL}\cdot\text{min}^{-1}$. The syringyl-to-guaiacyl (S/G) ratio in lignin was calculated by the following formula: $\text{S/G} = (\text{syringaldehyde} + \text{syringic acid})/(\text{vanillin} + \text{vanillic acid})$.

DETERMINATIONS OF CELLULOSE CONTENT AND MACROMOLECULAR TRAITS OF CELLULOSE. Cellulose content was determined by the Seifert method (Seifert, 1956). A mixture of acetylacetone, dioxane, and hydrochloric acid (6:2:1.5) under reflux for 30 min was used for delignification of wood samples.

Molecular weight distribution analysis of the cellulose samples was performed after their conversion into cellulose tricarbanilates. Cellulose tricarbanilates were dissolved in tetrahydrofuran and filtered through a Puradisc 25 NYL filter (Whatman International, Maidstone, U.K.) with a pore size of $0.45\text{ }\mu\text{m}$. Size-exclusion chromatography was performed at $35\text{ }^{\circ}\text{C}$ with tetrahydrofuran at a flow rate of $1\text{ mL}\cdot\text{min}^{-1}$ on two PLgel, $10\text{-}\mu\text{m}$, $7.5 \times 300\text{-mm}$, MIXED-B columns (Agilent Technologies) preceded by a PLgel, $10\text{-}\mu\text{m}$, $7.5 \times 50\text{-mm}$, Guard-column (Agilent Technologies) as described by Kačik et al. (2009). Data acquisitions were carried out with ChemStation software (Agilent Technologies) and calculations were performed with the Clarity GPC module (DataApex, Prague, Czech Republic). Numerical outputs were obtained for M_n (number-average molecular weight) and M_w (weight-average molecular weight), and the values were recalculated to underivatized cellulose using a coefficient $k = 162\text{ g}\cdot\text{mol}^{-1}/519\text{ g}\cdot\text{mol}^{-1}$. Polydispersity index (PDI) of cellulose was calculated as the ratio M_w/M_n . Degree of polymerization (DP_w) values were calculated by dividing the molecular weight by the monomer equivalent weight of anhydroglucose ($\text{DP}_w = M_w/162$).

Fourier-transform infrared spectroscopy (FT-IR) spectra of wood powder samples were recorded on an FT-IR spectrometer equipped with a Smart iTR attenuated total reflectance sampling accessory (Nicolet iS10; Thermo Fisher Scientific, Waltham, MA). The spectra were acquired by accumulating 64 interferograms at a resolution of 4 cm^{-1} in an absorbance mode (A) at wave numbers from 4000 to 400 cm^{-1} . Spectral bands were measured using OMNIC 8.0 software (Thermo

Fisher Scientific). Crystallinity of cellulose indices were calculated for lateral order index [LOI (A_{1422}/A_{897})] according to O'Connor et al. (1958), and for total crystallinity index [TCI (A_{1371}/A_{2884})] according to Nelson and O'Connor (1964b).

DETERMINATIONS OF POLYSACCHARIDE CONTENT AND NEUTRAL SUGAR COMPOSITION. Total content of polysaccharides (i.e., holocellulose) was determined using the method of Wise et al. (1946). Qualitative and quantitative analyses of saccharides were carried out by HPLC according to the ASTM International standard procedure E 1758-01. The samples were hydrolyzed in a two-stage process. In the first stage, 72% (w/w) H_2SO_4 at a temperature of $30\text{ }^{\circ}\text{C}$ was used for 1 h, and in the second stage, the formed oligomers were hydrolyzed to monosaccharides after dilution to 4% (w/w) H_2SO_4 at a temperature of $121\text{ }^{\circ}\text{C}$ for 1 h. The analyses were performed with a HPLC chromatograph (1200; Agilent Technologies) equipped with an Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA) at a temperature of $80\text{ }^{\circ}\text{C}$ and a mobile phase flow rate of $0.6\text{ mL}\cdot\text{min}^{-1}$.

SCANNING ELECTRON MICROGRAPHY. Wood samples were immediately immersed in formaldehyde-ethanol-acetic acid fixative solution and stored overnight in a refrigerator at $4\text{ }^{\circ}\text{C}$. For scanning electron micrography (SEM), wood sections (transverse, radial, and tangential surfaces) were mounted on specimen stubs, sputter-coated with gold, and observed by high-vacuum SEM using a Tescan instrument (VEGA TS 5130; Tescan, Brno, Czech Republic) operating at 15 kV .

VESSEL AND FIBER TRAITS. Vascular characteristics were determined for the outer three growth rings using the NIS-Elements image analysis software (Laboratory Imaging, Prague, Czech Republic). Vessel lumen areas (A) and vessel densities per square millimeter of wood (N) were determined from each transverse stem section. Measurements of A and calculations of N were made on the population of vessels, which ranged from 217 to 280 vessels per square millimeter of wood. The additional indicators of vascular strategy such as vessel lumen fraction ($F = A \cdot N$) and the vessel size:number ratio ($S = A/N$) were also calculated (Zanne et al., 2010). Total theoretical relative conductivity (RC) per square millimeter was calculated as the sum of individual RCs divided by the area of secondary xylem section, whereas the individual RC was calculated according to Zimmermann (1983) as the fourth power of the equivalent circle diameter of vessel lumen.

For the fiber morphology determination, the phloem and cortex were peeled off and the remaining wood was macerated in a boiling solution containing hydrogen peroxide and acetic acid (1:1) according to the procedure described in Chaffey et al. (2002). Fiber length, fiber width, shape factor (ratio between projected length and real fiber length), and the proportion of fiber length classes (less than 0.20 mm , 0.20 to 0.49 mm , 0.50 to 0.99 mm , 1 to 3 mm) were determined with a fiber tester (L&W; Lorentzen & Wettre, Kista, Sweden). Measurements were performed on five replicates per stock type, and the number of fibers within each population of replicate ranged from 20,002 to 20,276 cells.

STATISTICAL ANALYSIS. Data showed a normal distribution and were therefore analyzed by Student's t test. In the case of five traits (S/G ratio in lignin, both absolute yields and relative proportions of L-arabinose, F , and fiber length class 0.50 to 0.99 mm), variance differences between the stock types were significant, and therefore a t test for unequal variances was

used. In the remaining cases, variance differences between the stock types were non-significant, and a *t* test for equal variances was used.

Multivariate associations among 30 wood traits were analyzed using a principal component analysis (PCA) to describe patterns of covariation among the content of main cell wall components, lignin monomer composition, macromolecular traits of cellulose, neutral sugar composition, vascular characteristics, and fiber traits.

Results and Discussion

CONTENTS OF CELL WALL COMPONENTS AND LIGNIN MONOMER COMPOSITION. Non-significant differences between the stock types were found for the contents of cellulose and extractives (Table 1). However, the grafts reached higher contents of both lignin and holocellulose than the micropropagated plants. This difference might perhaps be attributed to the effect of the rootstock. Gonçalves et al. (2006) documented that the concentration of several leaf metabolites such as starch, total phenolics, total chlorophyll, and total carotenoids was significantly influenced by the rootstock genotype in grafted *Prunus avium* trees. In addition, the effect of the rootstock was also reported for the content of quebrachitol (a cyclic polyol stereoisomer of inositol) in latex samples of grafted mature *Hevea brasiliensis* trees (do Nascimento et al., 2011). Higher lignin content found in the grafts is usually responsible for higher adhesion among mechanically active wood fibers (Mancera et al., 2012) plus higher strength, hydrophobicity, and rigidity to the secondary cell walls of water-conducting and supportive tissues (Bonawitz and Chapple, 2010). Higher lignin content also enhances energy storage properties of wood under dynamic loads (Mousavioun et al., 2013) and reduces the growth strain and creep behavior of wood (Wang et al., 2010).

Based on the analysis of NBO products (Table 2), the proportion of S and G units in lignin was balanced, and there was a non-significant difference in the S/G ratio between the stock types. This indicates that propagation techniques had no direct effect on lignin monomer composition; hence, no

advantage could be attributed to either stock type toward an enhanced tolerance to DED. In addition, the representation of *p*-hydroxyphenyl units in both stock types was almost negligible. In angiosperms, lignin macromolecular structure depends on the proportion of S and G aromatic units, which determine the type and number of crosslinks within the macromolecule as well as the reactivity of the lignin. Lignin rich in G units has relatively more carbon-carbon bonds than lignin rich in S units because the aromatic C-5 position of G units is free to make linkages. Nitrobenzene oxidation products originate from the corresponding phenylpropane units and their $\alpha(\beta)$ -O-4 alkyl-aryl ethers as a result of the oxidation of lignin in an alkaline environment. Thus, the S/G ratio provides information regarding the relative amounts of uncondensed guaiacyl- and syringyl-propane units constituting the original lignin. In addition, the yield of these aldehydes reflects the degree of condensation of the lignin, because these uncondensed structures are cleaved by the nitrobenzene oxidation leaving condensed lignins as the residue (Kačik et al., 2012). Recent studies revealed that the S/G ratio has a significant influence on crosslinking between lignin and other cell wall components, thus modifying the microscopic structure and topochemistry of the cell wall. We have found that only G-rich lignin was involved in successful defense of infected elms against *O. novo-ulmi* ssp. *americana* \times *novo-ulmi* (Đurkovič et al., 2014). Lignin rich in G units (Vane et al., 2006) as well as ferulate and monolignol metabolites (Lloyd et al., 2011) were also found to be major sources of plant resistance against white-rot and necrotrophic fungi, respectively.

MACROMOLECULAR TRAITS OF CELLULOSE AND NEUTRAL SUGAR COMPOSITION. The micropropagated plants reached higher values for the molecular size distribution traits such as M_w and PDI as well as for DP_w (Table 3). Owing to its high degree of polymerization, cellulose is considered to be primarily responsible for the tensile strength of wood. Therefore, reducing the length of the cellulose molecules (DP_w) would cause a reduction in macro-strength properties (Sweet and Winandy, 1999; Vizárová et al., 2012). From a mechanical point of view, a lower content of holocellulose in the micropropagated plants

Table 1. Contents of main cell wall components and extractives present in the wood of the dutch elm hybrid ‘Dodoens’.

Wood component	Stock type		<i>t</i> test (<i>P</i>)
	Micropropagated	Grafted	
Lignin [mean \pm SD (%)]	22.19 \pm 0.22	23.10 \pm 0.25	5.47 (0.0016)
Holocellulose [mean \pm SD (%)]	82.46 \pm 0.41	83.19 \pm 0.28	2.96 (0.0251)
Cellulose [mean \pm SD (%)]	35.21 \pm 0.41	35.22 \pm 0.39	0.02 (0.9866)
Extractives [mean \pm SD (%)]	3.47 \pm 0.11	3.62 \pm 0.13	1.72 (0.1371)

Table 2. Nitrobenzene oxidation (NBO) products present in the wood of the dutch elm hybrid ‘Dodoens’.

NBO product	Stock type		<i>t</i> test (<i>P</i>)
	Micropropagated	Grafted	
<i>p</i> -Hydroxybenzoic acid [mean \pm SD (%)]	0.06 \pm 0.00	0.05 \pm 0.01	4.28 (0.0052)
<i>p</i> -Hydroxybenzaldehyde [mean \pm SD (%)]	0.04 \pm 0.00	0.04 \pm 0.00	2.15 (0.0747)
Vanillic acid [mean \pm SD (%)]	0.29 \pm 0.03	0.28 \pm 0.02	0.81 (0.4482)
Vanillin [mean \pm SD (%)]	3.28 \pm 0.19	2.97 \pm 0.12	2.74 (0.0338)
Syringic acid [mean \pm SD (%)]	0.52 \pm 0.03	0.42 \pm 0.02	4.90 (0.0027)
Syringaldehyde [mean \pm SD (%)]	3.00 \pm 0.13	3.00 \pm 0.05	0.07 (0.9427)
S/G ratio (mean \pm SD) ^z	0.99 \pm 0.08	1.05 \pm 0.02	1.40 (0.2480)

^zSyringyl-to-guaiacyl ratio in lignin.

might be compensated for by a higher value of DP_w within the cellulose chain to maintain the tensile strength of the wood for this stock type. In addition, no differences were recorded for either crystallinity of cellulose indices. The ratio of IR band areas at 1422 and 897 cm^{-1} is referred to as LOI, which represents the ordered regions perpendicular to the chain direction. The absorption band at 1422 cm^{-1} represents CH_2 scissoring motion at C_6 (Nelson and O'Connor, 1964a, 1964b; Široký et al., 2010). The 897- cm^{-1} band indicates the vibrational mode involving C_1 and the four atoms attached to it, which is characteristic of β -anomers or β -linked glucose polymers (Nelson and O'Connor, 1964a, 1964b; Široký et al., 2010). The ratio of band areas at 1371 and 2884 cm^{-1} is known as TCI, which represents the overall degree of order in cellulose. The 1371- cm^{-1} band is associated with the bending mode of C-H (Nelson and O'Connor, 1964a, 1964b; Široký et al., 2010). The 2884- cm^{-1} band represents C-H and CH_2 stretching, which is unaffected by changes in crystallinity (Nelson and O'Connor, 1964a; Qiu et al., 2012).

The results for the absolute yields of saccharides and their relative proportions in the wood of the stock types are given in Table 4. Total yields were higher in the micropropagated plants. The most substantial difference between the stock types was found for the relative proportion of D-glucose (Glc), which was significantly higher in the micropropagated plants. The grafts reached higher relative proportions of D-xylose (Xyl), D-mannose (Man), and D-galactose (Gal). Non-significant differences between the stock types were found for the relative proportion

of L-arabinose (Ara). do Nascimento et al. (2011) reported different relative amounts of sucrose (a disaccharide composed of Glc and fructose) in latex samples of *H. brasiliensis* trees, which were grafted onto various rootstocks. This clearly indicates the effect of the rootstock on the relative amounts of saccharides in a scion, and it may be a major reason for the differences discovered between the stock types in this study.

VASCULAR TRAITS. Both stock types shared ring-porous wood. Wide vessels in earlywood were arranged at the growth ring boundary, whereas narrow vessels in latewood spread in wavy tangential bands (Fig. 1A and D). In wide earlywood vessels, perforation plates were simple, and helical thickenings were absent or rarely present. Vessel walls had alternately arranged intervessel pits, and vessel-ray pits had elongated apertures (Fig. 1B and E). In narrow latewood vessels, perforation plates were simple, and helical thickenings were densely present throughout the body of these vessels. Vessel-ray pits had large circular to elongated apertures (Fig. 1C and F). Wood porosity and vessel anatomy traits (ring-porous wood, simple perforations, alternate intervessel pitting, pits elongate to circular in outline) found in both stock types fully agreed with the diagnostic microscopic characteristics described for the majority of temperate species of the genus *Ulmus* (Sweitzer, 1971; Wheeler et al., 1989).

Quantitative data for the examined vascular anatomy traits are presented in Table 5. Both stock types performed similarly for traits such as *N*, *F*, and *RC*. Significant differences were found for traits *A* and *S*, when the micropropagated plants

Table 3. Macromolecular traits of underivatized cellulose isolated from the wood of the dutch elm hybrid 'Dodoens'.

Trait ^z	Stock type		<i>t</i> test (<i>P</i>)
	Micropropagated	Grafted	
M_n (mean \pm SD)	19,538 \pm 269	19,357 \pm 308	0.89 (0.4091)
M_w (mean \pm SD)	131,134 \pm 424	126,496 \pm 375	16.39 (0.0001)
PDI (mean \pm SD)	6.71 \pm 0.08	6.54 \pm 0.09	2.85 (0.0291)
DP_w (mean \pm SD)	810 \pm 3	781 \pm 3	15.80 (0.0001)
LOI (A_{1422}/A_{897}) (mean \pm SD)	1.19 \pm 0.09	1.28 \pm 0.19	0.84 (0.4321)
TCI (A_{1371}/A_{2884}) (mean \pm SD)	2.40 \pm 0.29	1.63 \pm 0.83	1.76 (0.1296)

^z M_n = number-average molecular weight; M_w = weight-average molecular weight; PDI = polydispersity index; DP_w = degree of polymerization; LOI = lateral order index; TCI = total crystallinity index.

Table 4. Absolute yields of saccharides (% of oven-dry weight per unextracted wood) and their relative proportions (%) in the wood of the dutch elm hybrid 'Dodoens'.

Saccharide ^z	Stock type		<i>t</i> test (<i>P</i>)
	Micropropagated	Grafted	
Absolute yields			
Glc [mean \pm SD (%)]	52.99 \pm 0.27	51.96 \pm 0.08	7.10 (0.0004)
Xyl [mean \pm SD (%)]	16.21 \pm 0.18	16.43 \pm 0.18	1.75 (0.1304)
Ara [mean \pm SD (%)]	2.25 \pm 0.24	2.08 \pm 0.02	1.34 (0.2708)
Man [mean \pm SD (%)]	1.76 \pm 0.03	1.93 \pm 0.02	8.72 (0.0001)
Gal [mean \pm SD (%)]	1.62 \pm 0.02	1.67 \pm 0.01	4.24 (0.0054)
Total yield [mean \pm SD (%)]	74.82 \pm 0.18	74.06 \pm 0.18	5.96 (0.0010)
Relative proportions			
Glc [mean \pm SD (%)]	70.82 \pm 0.21	70.16 \pm 0.21	4.47 (0.0042)
Xyl [mean \pm SD (%)]	21.66 \pm 0.20	22.18 \pm 0.19	3.81 (0.0089)
Ara [mean \pm SD (%)]	3.01 \pm 0.32	2.81 \pm 0.02	1.20 (0.3174)
Man [mean \pm SD (%)]	2.34 \pm 0.05	2.60 \pm 0.02	9.44 (0.0001)
Gal [mean \pm SD (%)]	2.17 \pm 0.03	2.25 \pm 0.02	5.42 (0.0016)

^zGlc = D-glucose, Xyl = D-xylose, Ara = L-arabinose, Man = D-mannose, Gal = D-galactose.

reached significantly higher values than the grafts. The trait *S* measures variation in the vessel composition within the water transport space (Zanne et al., 2010). Mean water-conducting area in the micropropagated plants was comprised of a smaller number of larger vessels. A direct impact of higher *A* and *S* in the micropropagated plants could be seen in an increased value of RC for this stock type. In addition, the differences in vascular

anatomy and hydraulic conductivity among the rootstocks used for grafting can often play a role in the subsequent scion (i.e., stem) vascular growth. Changes in vessel lumen areas over time in the graft union of various *P. avium* heterograft and homograft combinations were reported by Olmstead et al. (2006). The authors found that wound-related callus formation response after grafting led to reduced vessel lumen areas in scion and graft union tissues.

Smaller vessels in the graft union of *Malus × domestica* ‘McIntosh’ scions grafted back onto the same rootstocks were also reported by Poniedziałek et al. (1979). In the grafts, these anatomical constraints may be responsible for lower stem hydraulic conductivity, which in turn affects stomatal conductance and net CO₂ assimilation rate in leaves (Bayramzadeh et al., 2008; Santiago et al., 2004).

FIBER TRAITS. Libriform fibers showed a similar anatomy in both stock types. The occurrence of simple to slightly bordered slit-like pits clearly seen on radial cell walls (Fig. 2A and B) agreed with the anatomical description of fibers for temperate species of the genus *Ulmus* provided by Sweitzer (1971). The quantitative data pertinent to fiber length, fiber width, shape factor, and the proportion of fiber length classes are presented in Figure 3. Fiber length was slightly higher in the micropropagated plants, whereas the grafts had slightly wider fibers. It is known that the properties of cellulose chains also determine the properties of wood fibers. In the micropropagated plants, biosynthesis of longer cellulose chains (quantified by a higher value of DP_w) resulted in the formation of longer fibers for this stock type. Fiber length and strength have been shown to be particularly important for tearing resistance of wood pulps (Seth and Page, 1988). Fiber width, quantified also by the cell wall thickness, is of particular interest for describing

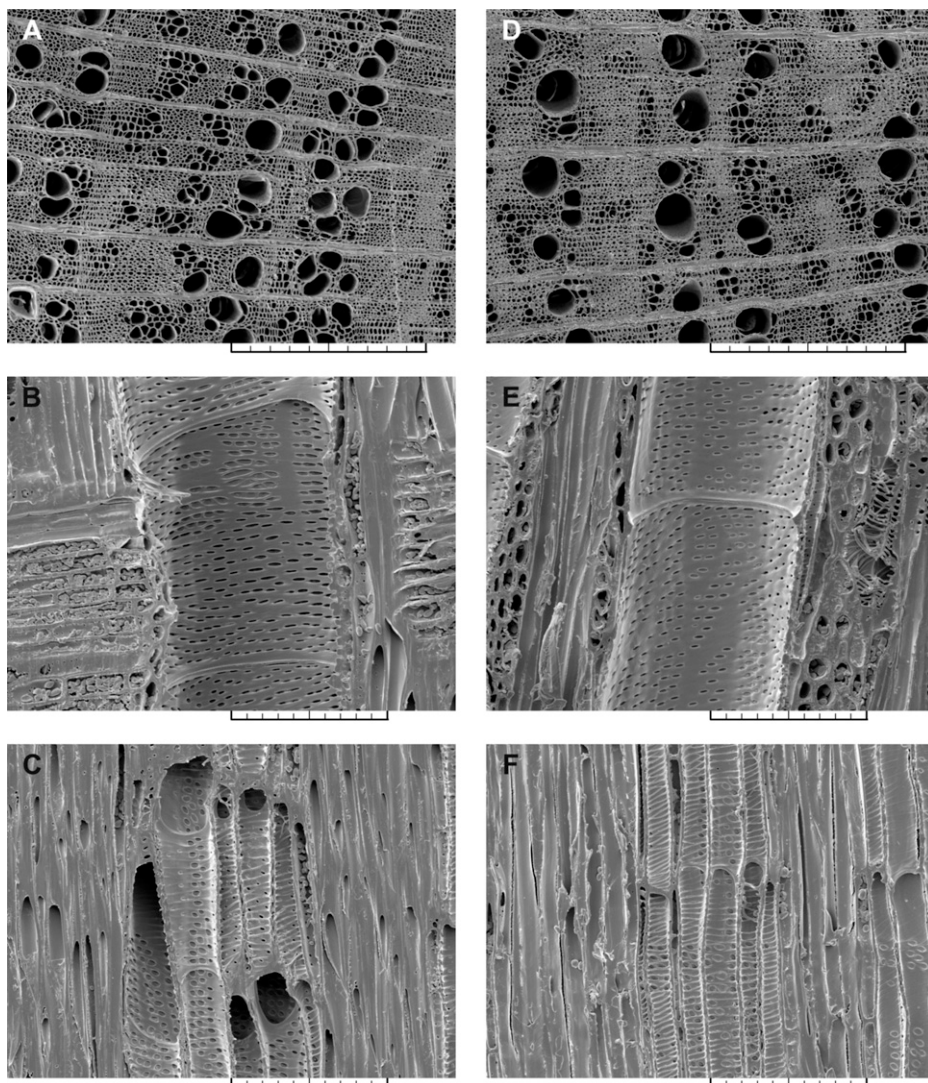


Fig. 1. Scanning electron microscopy images of vascular anatomy in micropropagated (A–C) and grafted (D–F) dutch elm hybrid ‘Dodoens’. (A, D) Ring-porous wood in the examined stock types; cross-sections, scale bars = 500 µm. (B, E) Wide earlywood vessel showing a simple perforation and an alternate pitting arrangement; radial section (B), tangential section (E), scale bars = 100 µm. (C, F) Narrow latewood vessels showing simple perforations and distinct helical thickenings; radial sections, scale bars = 100 µm.

Table 5. Vascular anatomy traits (per square millimeter of wood) in the dutch elm hybrid ‘Dodoens’.

Trait ^z	Stock type		<i>t</i> test (<i>P</i>)
	Micropropagated	Grafted	
Vessel lumen area [mean ± SD (10 ⁻⁴ mm ²)]	7.4 ± 1.1	5.8 ± 0.5	2.58 (0.0418)
Vessels [mean ± SD (no./mm ²)]	248 ± 27	266 ± 9	1.22 (0.2696)
<i>F</i> [mean ± SD (mm ²)]	0.18 ± 0.04	0.15 ± 0.01	1.53 (0.2150)
<i>S</i> [mean ± SD (10 ⁻⁶ mm ⁴)]	3.0 ± 0.5	2.2 ± 0.2	2.71 (0.0353)
RC [mean ± SD (10 ⁻⁴ mm ⁴)]	15.6 ± 9.0	9.4 ± 3.6	1.27 (0.2505)

^z*F* = vessel lumen fraction; *S* = vessel size: number ratio; RC = relative conductivity.

fiber quality differences (Paavilainen, 1993) with thick-walled fibers forming bulky sheets of low tensile but high tearing strength (Wardrop, 1969). With regard to shape factor, there were non-significant differences between the stock types. In both stock types, from the four examined fiber length classes, the class of 0.50 to 0.99 mm was represented by the highest proportion. However, a significantly higher percentage for this class was found in the micropropagated plants. The prevailing proportion for the length class of 0.50 to 0.99 mm was also reported for libriform fibers of *Sorbus aucuparia* (Đurković et al., 2011). The grafts had a higher proportion of the classes less than 0.20 mm and 0.20 to 0.49 mm. There was no difference found between the stock types for the length class of 1 to 3 mm.

ASSOCIATIONS AMONG WOOD TRAITS. PCA was done to evaluate how wood traits were associated (Fig. 4). The first axis explained 49% of the variation and showed strong positive loadings for M_w , DP_w , and syringic acid. The negative side of the axis indicated strong loadings for the relative proportions of

Man, Xyl, Gal, and lignin content. The second axis explained 18% of the variation and showed strong positive loadings for vanillic acid, F , and N . The negative side of the axis indicated strong loadings for syringaldehyde, the S/G ratio in lignin, and M_n . The relative proportion of Glc together with the macromolecular traits of cellulose such as M_w and DP_w were closely associated with both the fiber length and fiber shape factor to ensure the tensile strength for the wood. In addition, both stock types formed compact homogeneous clusters clearly separated from each other in the multivariate wood trait analysis.

Summary

We found no significant differences in lignin monomer composition and the relative proportion of G units between micropropagated plants and the grafts. Seen from a phytopathological point of view, no advantage could be attributed to either stock type toward an enhanced tolerance to DED. However, the grafts did reach a higher lignin content, which could provide minor advantages in terms of mechanical and physical properties to the cell walls of this stock type. Taken together, the micropropagated plants reached significantly higher values for 13 traits (32.5%), primarily associated with the relative proportion of Glc and the macromolecular traits of cellulose to compensate for a lower content of holocellulose. The grafts reached higher values for 10 traits (25%), including the contents of both lignin and holocellulose as well as the relative proportions of Xyl, Man, and Gal. The effect of the

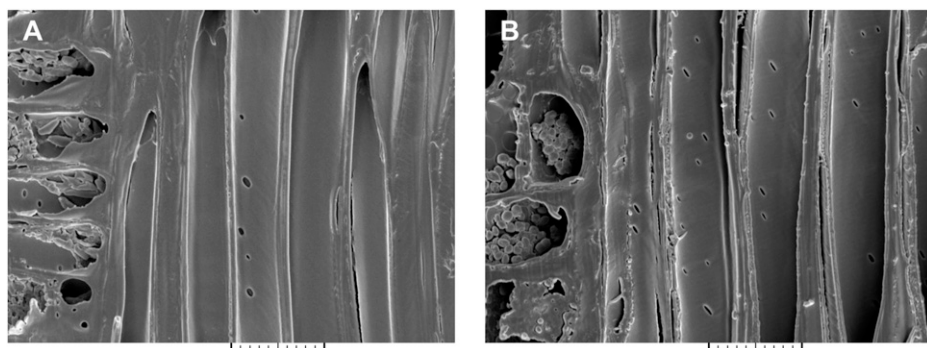


Fig. 2. Scanning electron microscopy images of libriform fibers in micropropagated (A) and grafted (B) dutch elm hybrid 'Dodoens'; radial sections, scale bars = 20 μ m.

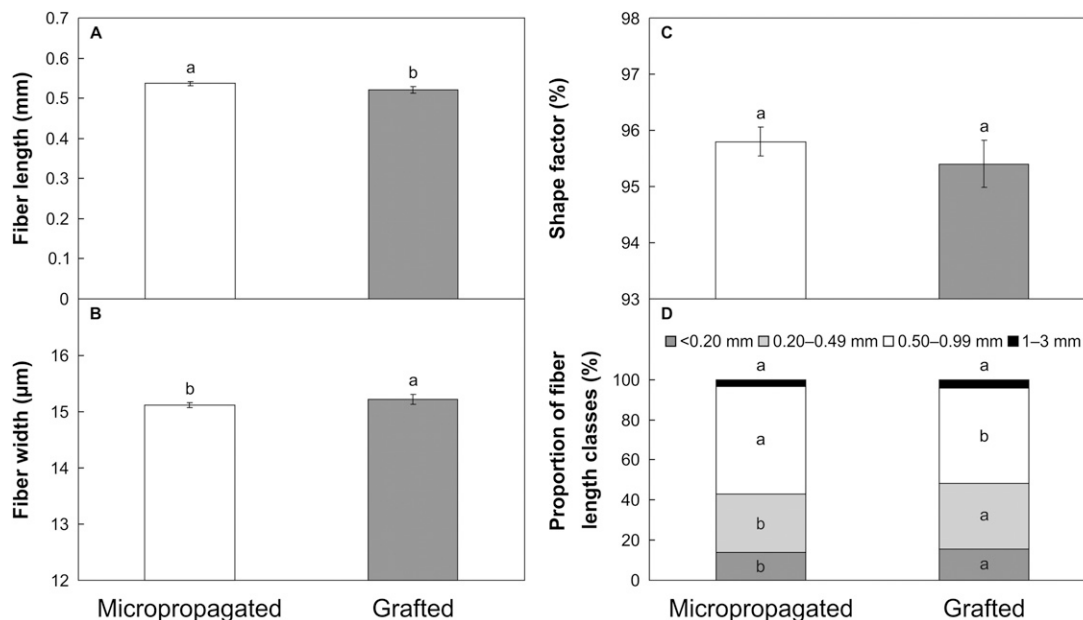


Fig. 3. Fiber morphology characteristics in micropropagated and grafted dutch elm hybrid 'Dodoens': (A) fiber length, (B) fiber width, (C) shape factor, and (D) proportion of fiber length classes. Histograms represent means \pm SD. Means followed by the same letter within a particular fiber length class and across stock types are not significantly different at $P < 0.05$ (t test).

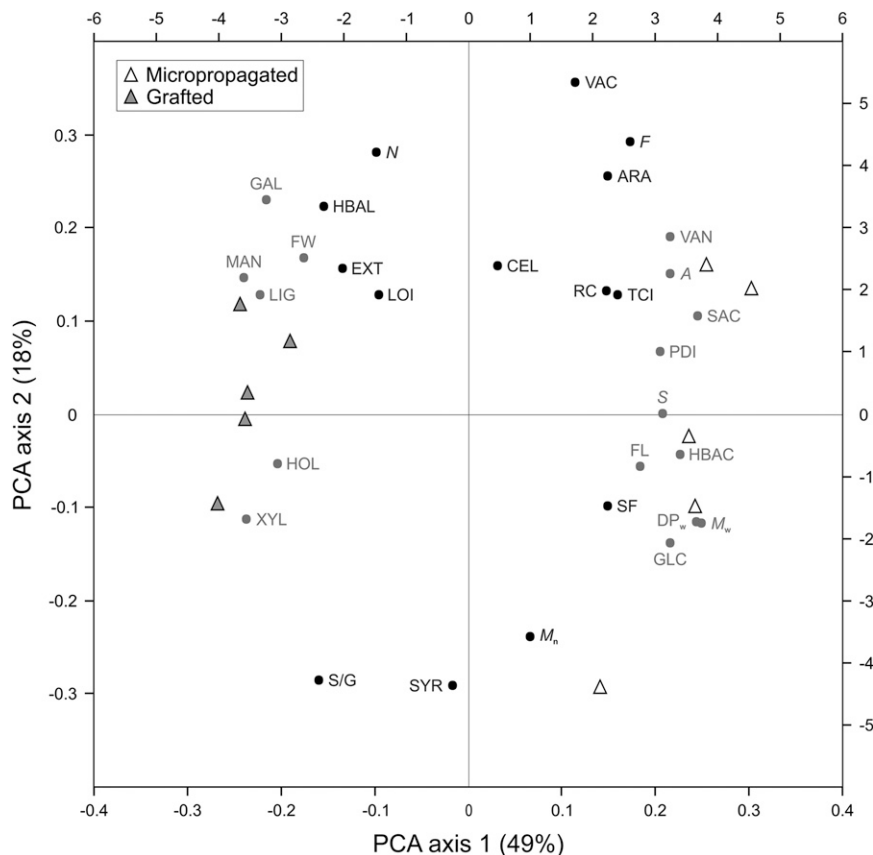


Fig. 4. Positions of 30 wood traits on the first and second axes of the principal component analysis (PCA). Traits associated with significant differences between micropropagated plants and the grafts are indicated in gray: *A* = vessel lumen area; *ARA* = l-arabinose ; *CEL* = cellulose content; DP_w = degree of polymerization of cellulose; *EXT* = extractives content; *F* = vessel lumen fraction; *FL* = fiber length; *FW* = fiber width; *GAL* = D-galactose ; *GLC* = D-glucose ; *HBAC* = *p*-hydroxybenzoic acid; *HBAL* = *p*-hydroxybenzaldehyde; *HOL* = holocellulose content; *LIG* = lignin content; *LOI* = lateral order index; *MAN* = D-mannose ; M_n = number-average molecular weight; M_w = weight-average molecular weight; *N* = number of vessels per square millimeter; *PDI* = polydispersity index; *RC* = relative conductivity; *S* = vessel size: number ratio; *S/G* = syringyl-to-guaiacyl ratio in lignin; *SAC* = syringic acid; *SF* = shape factor; *SYR* = syringaldehyde; *TCl* = total crystallinity index; *VAC* = vanillic acid; *VAN* = vanillin; *XYL* = D-xylose . Micropropagated and grafted plants of dutch elm hybrid ‘Dodoens’ are as indicated in the key.

rootstock might contribute to different amounts of these cell wall substances in the grafts. Similarities between the stock types were found for 17 traits (42.5%). Our observations showed that both stock types formed compact homogeneous clusters clearly separated from each other in the multivariate wood trait analysis.

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