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# Interactions between rootstock, inter-stem and scion xylem vessel characteristics of peach trees growing on rootstocks with contrasting size-controlling characteristics

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### Abstract

Background and aims	The primary physiological mechanism influencing tree vigour in size-controlling rootstocks of peach has been related to the hydraulic conductance of the rootstock. Differences in rootstock hydraulic conductance are a function of rootstock xylem vessel characteristics. The present research examined whether the vigour and xylem vessel characteristics of the rootstock influence the xylem characteristics of the scion. We tested whether using a size-controlling rootstock genotype as an inter-stem influences the xylem vessel characteristics of either the rootstock below the inter-stem or the scion above it and vice versa.
Methodology	Anatomical measurements (diameter and frequency) of xylem vessels were determined above and below the graft unions of the trunks of peach trees with differing scion/rootstock combinations. The three peach rootstocks were 'Nemaguard' (vigorous), 'P30-135' (intermediate vigour) and 'K146-43' (dwarfing). The vigorous scion cultivar was 'O'Henry'. The inter-stem experiment involved trees with 'Nemaguard' (vigorous) as the rootstock, 'K146-43' (dwarfing) as the inter-stem and 'O'Henry' as the scion. Based on anatomical measurements, we calculated the theoretical axial xylem conductance of each stem piece and rootstock genotype with the Hagen–Poiseuille law.
Principal results	Xylem vessel dimensions of rootstocks varied in conjunction with tree vigour. Scion xylem vessel dimensions of different scion/rootstock combinations were only marginally affected by rootstock genotype. The inter-stem sections from the dwarfing genotype ('K146-43') had narrower vessels and a lower calculated hydraulic conductance than the xylem from either the vigorous rootstock below ('Nemaguard') or the scion above ('O'Henry').
Conclusions	Rootstock genotype only marginally affected scion xylem vessel characteristics. Thus the xylem vessel characteristics of the dwarfing rootstock genotypes appear to influence tree growth directly rather than through an effect on the xylem characteristics of the scion. A dwarfing rootstock genotype used as an inter-stem appeared to work as a physical restriction to water movement, reducing potential xylem flow and conductance of the whole tree.

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#### Introduction

Grafting is a common practice for propagating fruit trees. The use of vigour-controlling rootstocks is one method used to promote early fruit bearing, reduce vigour and increase yield (Webster, 2004). The use of inter-stems (grafting a third genotype in between a rootstock and a scion) is another method used to induce scion dwarfing, and also to induce tolerance to winter cold and resistance to disease (Rogers and Beakbane, 1957). 'Malling 9' ('M.9') inter-stems have been found to be effective for reducing the vigour of apple trees (Rogers and Beakbane, 1957) but the influence of 'M.9' as an inter-stem is generally less than that of 'M.9' used as a rootstock (Tukey and Brase, 1943). The effect of some vigour-controlling rootstocks used as inter-stems was reported to be related to the length of the inter-stem piece in apple (Parry and Rogers, 1972; Di Vaio et al., 2009) and peach (Rufato et al., 2006).

Dwarfing apple rootstocks reduced the formation of nodes during shoot growth (Seleznyova *et al.*, 2003) and the cumulative effect of such reduction over time can have a dramatic effect on branch development. Furthermore, dwarfing rootstocks and inter-stems reduced the number of extension shoots and promoted the formation of floral shoots (Seleznyova *et al.*, 2008).

The physiological mechanisms of vigour reduction by rootstocks or inter-stems are not clearly understood. During the last century, many mechanisms for dwarfing have been proposed (Webster, 2004). It has been proposed that dwarfing might be caused by water supply restrictions to the scion induced by anatomical characteristics of the rootstock (Beakbane, 1956; Atkinson et al., 2003). On the other hand, others suggested that dwarfing was caused by a reduction of solutes transported to the scion through the rootstocks (Bukovac et al., 1958; Jones, 1976). This theory contradicted a previous study that reported an increase of solutes in scions grafted on dwarfing rootstocks (Rogers and Vyvyan, 1934). A long series of studies reported possible mechanisms related to the production and translocation of hormones in the plant (Jackson, 1993). However, exactly how hormones act in the dwarfing process is not well understood (Webster, 2004). It has also been reported that in some cases dwarfing might be caused by partial incompatibility between the scion and the rootstock, which may alter the transport of minerals and hormones (Webster, 2004). It is well known that root restriction or pruning can influence scion vigour and the root:shoot ratio (Richards and Rowe, 1977; Ferree, 1992; Williamson et al., 1992). While the root:shoot ratio is sometimes less in trees on dwarfing rootstocks (Solari et al., 2006b), this is not always the case (Rogers and Vyvyan, 1934; Poni et al., 1992).

Atkinson et al. (2003) and Cohen and Naor (2002) hypothesized that dwarfing apple rootstocks may impose scion dwarfing by changing the hydraulic conductance of the whole tree. However, Cohen et al. (2007) later concluded that apple rootstock vigour is not related to hydraulic architecture or hydraulic conductance of the rootstock. They suggested that the graft union may play a central role in limiting water transport and thus induce scion dwarfing (Cohen et al., 2007). Experiments with selected peach and olive rootstocks have indicated that there was no influence of the graft union on stem hydraulic conductance but that conductance of the rootstock was apparently directly related to peach rootstock vigour (Solari et al., 2006b; Gascò et al., 2007). On the other hand, Clearwater et al. (2004) reported that differences in rootstock-induced vigour in kiwifruit vines were related to differences in the timing of phenological development of the rootstocks. Nardini et al. (2006) also reported that dwarfing in specific olive rootstocks was unrelated to hydraulic conductance, while Trifilò et al. (2007) suggested that the dwarfing characteristics of a dwarfing olive rootstock obtained through mutagenesis may also be related to delays in development. From these varied accounts it is clear that there is probably more than one mechanism involved in causing rootstockmediated dwarfing effects in different fruit crop species.

Recent studies with peach have demonstrated that daily patterns of stem water potential are directly related to patterns of shoot growth. The pattern of stem water potential occurring during the afternoon hours strongly affects shoot growth rates in the field (Berman and DeJong, 1997). Subsequently, a strong correlation was found between stem water potential and shoot growth over a day among trees on rootstocks that imparted differing amounts of vigour (Basile et al., 2003a). Vegetative growth was also found to be correlated with cumulative water potential differences during the first half of a growing season (Basile et al., 2003a). Subsequently, it was demonstrated that differences in stem water potential are causally related to differences in relative shoot growth rates among peach trees on different rootstocks (Solari et al., 2006a). Stem water potential is strongly influenced by stem hydraulic conductance (Tyree and Sperry, 1988), and differences in measured stem hydraulic conductance among rootstocks (Basile et al., 2003b; Solari et al., 2006b) correspond to differences in xylem vessel characteristics and theoretically calculated hydraulic conductance of the same rootstocks (Tombesi et al., 2010). Furthermore, it appears that xylem vessel diameter and number are the main rootstock characteristics influencing scion

vigour in peach trees growing on graft-compatible rootstocks (Tombesi *et al.*, 2010).

Xylogenesis has been hypothesized to be regulated by hormones produced both in leaves and in roots (Fukuda, 1996). Indoleacetic acid produced in leaves and transported basipetally appears to control xylem regeneration around wounds (Jacobs, 1952). In the basipetal direction, high concentrations of auxin in close proximity to leaves have been proposed to cause the formation of many vessels of small diameter while low auxin concentrations farther from leaves appear to promote the arowth of fewer large vessels (Aloni and Zimmermann, 1983). These results were confirmed by experiments on transgenic Petunia plants (Klee et al., 1987). Cytokinin appears to promote vessel regeneration in the acropetal direction in the presence of indoleacetic acid by increasing the sensitivity to auxin and stimulating xylem formation (Baum et al., 1991; Aloni, 1995). These results suggest that in compound trees the scion could influence xylogenesis in the rootstock or vice versa.

The comparison of xylem anatomy among peach rootstocks reported by Tombesi et al. (2010) focused on anatomical differences among rootstocks, but that research did not determine whether the xylem characteristics of the rootstocks influenced the xylem characteristics of a scion grafted onto those rootstocks, or whether the xylem characteristics of a vigorous rootstock could influence the xylem characteristics of a size-controlling rootstock genotype grafted onto it. The primary aim of this work was to study the influence of the rootstock on the xylem anatomical characteristics of the scion to determine whether the vigour and xylem vessel diameters of the rootstock induce changes in the xylem structural characteristics of the scion. Secondarily, we were interested in determining whether a vigorous rootstock can influence the xylem vessel characteristics of a dwarfing genotype used as an inter-stem. As a corollary, we wanted to know whether using a size-controlling rootstock genotype as an inter-stem influences the xylem vessel characteristics of either the rootstock below the inter-stem or the scion above it. Thus we attempted to test whether the mechanism proposed to explain vigour control by dwarfing rootstocks (Tombesi et al., 2010) is also applicable to rootstock genotypes used as inter-stems.

#### **Materials and methods**

Plant material was sampled from an experimental orchard of the University of California located at the Kearney Agricultural Centre, Parlier, CA, USA. The tested scion/rootstock combinations involved different rootstocks representative of three different vigour classes: high (*Prunus persica* L. Batsch × *Prunus* 

davidiana hybrid, cv. 'Nemaguard', seed propagated), intermediate (Prunus salicina Lindl.  $\times$  P. persica L. Batsch hybrid, cv. 'P30-135', vegetatively propagated) and low (P. salicina Lindl.  $\times$  P. persica L. Batsch hybrid, cv. 'K146-43', vegetatively propagated). Commercially, 'P30-135' and 'K146-43' are sold as 'Controller 9<sup>™</sup>' and 'Controller 5<sup>TM</sup>', respectively. The scion cultivar grafted on these three rootstocks was 'O'Henry'. Trees were 7 years old, trained to a perpendicular V (DeJong et al., 1995), and received normal horticultural care. In June 2009, a wood chisel was used to remove patches of bark and extract samples of xylem tissue ( $\sim$ 1.5 cm  $long \times 0.5$  cm wide  $\times 0.5$  cm deep) from the trunks of five trees per rootstock. The samples were taken  $\sim$ 5 cm above and  $\sim$ 3 cm below the graft union for the scion and rootstock samples, respectively.

To test inter-stem influence, we used five trees from a different block of 6-year-old trees. Trees had 'Nemaguard' as the rootstock, 'K146-43' as the inter-stem and 'O'Henry' as the scion. The sampling protocol and the training system of these trees were the same as described previously. Three samples of xylem tissue per tree were collected, one from each genotype.

Xylem tissue samples were sectioned with a manual microtome at a thickness of  ${\sim}150\,\mu\text{m}$  to obtain four cross-sections from each field sample. The sections were stained with toluidine blue O to increase the visual contrast. Photographs of the tissue cross-sections were taken with a camera (Leica, Model Lei 750, Solms, Germany) mounted on a light microscope (Nikon Eclipse E 600, Tokyo, Japan). Images were then acquired with Optronics DEI-750D software (Goleta, CA, USA). Two digital photographs were taken of each tissue crosssection to calculate the vessel density and dimensions. Two randomly selected view fields  $(0.276 \text{ mm}^2)$  of xylem tissue were analysed in the most recent annual ring of xylem because this zone of xylem tissue has been reported to account for as much as 90 % of the stem hydraulic conductance (Ellmore and Ewers, 1985).

Vessels were measured and counted in frequency classes, as described by Solla and Gil (2002), using a computer graphics program (The Gimp, freeware) to paste a ruled grid at the same magnification onto photographs of vessels. The frequency classes for xylem vessels were established in intervals of 30  $\mu$ m.

Theoretical hydraulic conductance  $(k_h)$  (kg m MPa<sup>-1</sup> s<sup>-1</sup>) was calculated with the modified Hagen-Poiseuille law described by Tyree and Ewers (1991):

$$k_{\rm h} = \left(\frac{\pi\rho}{128\eta}\right)\sum_{i=1}^{n} \left(d_i^4\right)$$

where *d* is the radius of the vessel in m,  $\rho$  the fluid density (assumed to be 1000 kg m<sup>-3</sup> or equal to that of water at 20 °C) and  $\eta$  the viscosity (assumed to be  $1 \times 10^{-9}$  MPa s or equal to that of water at 20 °C).

A weighted mean (Wm) diameter was calculated as

$$Wm = \frac{\sum_{i=1}^{n} Vn_{class i} \times \bar{d}_{class i}}{TN}$$

where Vn is the number of vessels in each class,  $\overline{d}$  the mean diameter in each class and TN the total number of vessels per visual field (Tombesi *et al.*, 2010).

Statistical analyses of the data were performed with SAS 9.1.3 statistical software (SAS Institute, Cary, NC, USA). Treatments were analysed with a one-way ANOVA model with significance level set at 0.05. Means were separated by Tukey's *w*-procedure at P = 0.05 (Sokal and Rohlf, 1969). The grand mean values (n = 5) were calculated using data from two randomly chosen visual fields in each of the four sections from each of the five stems per genotype analysed.

#### Results

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'Nemaguard' was the most vigorous rootstock followed by 'P30-135' and 'K146-43', which had 85 and 44 % of the trunk cross-sectional area of 'Nemaguard', respectively (Table 1). The trees that had 'K146-43' used as an inter-stem and 'Nemaguard' as a rootstock had vigour intermediate between trees on 'Nemaguard' and 'K146-43' directly.

Table 1 Trunk cross-sectional area (cm<sup>2</sup>) of the three rootstocks used in the experiment and of the inter-stem trees. Vigour percentage is related to the most vigorous one ('Nemaguard') used as a standard for the two experiments. Means of five trees (n = 5). Means with different lower case letters are significantly different at P = 0.05 (Tukey test)

Rootstock	Trunk cross-sectional area (cm²)	Vigour percentage
'Nemaguard'	155.0 ± 5.58a	100
'P30-135'	$131.3\pm2.61b$	85
'K146-43'	$67.7 \pm \mathbf{2.00c}$	44
Inter-stem		
Control	$145.2\pm13.75a$	100
('Nemaguard')		
'O'Henry'/	$\textbf{103.9} \pm \textbf{5.96b}$	72
'K146-43'/		
'Nemaguard'		

The xylem of 'Nemaguard' was characterized by vessels of a range of dimensions, from narrow to wide (Fig. 1). The medium vessel size class (30–60  $\mu$ m) had the greatest number of vessels. 'Nemaguard' also had a substantial number of vessels in the largest diameter classes (90–120 and 120–150  $\mu$ m). In contrast to the

'Nemaguard' 30 'O'Henry Number of vessels 20 a 10 a a a 0 P30-135 30 O'Henry Number of vessels 20 10 a a đ b a 0 K146-43 a 30 'O'Henry' a Number of vessels 20 10 a a b 0 30-60 60-90 90-120 120 - 1500 - 30Vessel diameter (µm)



other two rootstocks, 'Nemaguard' had the fewest vessels per visual field among the three rootstocks tested. 'P30-135' had 58 % of vessels in the second size class ( $30-60 \mu$ m), and 17 and 24 % in the smallest class ( $0-30 \mu$ m) and the third class ( $60-90 \mu$ m), respectively. 'P30-135' had only 1 % of total vessels in the 90–120  $\mu$ m class and none in the largest class. The number of vessels per visual field for 'P30-135' was intermediate between 'Nemaguard' and 'K146-43'. 'K146-43' was characterized by having 52 and 43 % of total vessels in the lower size classes (0–30 and 30–60  $\mu$ m, respectively). Only 5 % of vessels were in the medium class and there were no vessels in the largest classes. 'K146-43' had the most vessels per visual field among the three tested rootstocks.

Differences in vessel diameter among 'O'Henry' scions grafted on the three different rootstocks were non-significant (Table 1). The second vessel size class (30–60  $\mu$ m) was the most represented class in all the 'O'Henry' scions, and there were very few vessels in the largest diameter class (120–150  $\mu$ m). The total numbers of vessels per visual field in 'O'Henry' scions grafted on 'Nemaguard' and 'P30-135' were similar, but the scion grafted on 'K146-43' had more vessels per visual field.

The weighted mean xylem vessel diameter (Wm) of the rootstocks was greatest in 'Nemaguard' followed by 'P30-135' and 'K146-43' (Fig. 2). The scion Wm had a slight but non-significant tendency to decrease with the vigour of the rootstock that they were on. The absolute values were similar to those of 'Nemaguard'.

'Nemaguard' had the highest calculated hydraulic conductance of the three rootstocks (Fig. 3). The values of 'P30-135' and 'K146-43' were lower. The scion on the three rootstocks had calculated conductance values between 'Nemaguard' and 'P30-135'. No significant differences were found among the calculated hydraulic conductances of the scion on the three rootstocks.

In the trees with inter-stems, xylem vessels in the 'Nemaguard' rootstock and the 'O'Henry' scion were distributed among all five diameter classes (Fig. 4). The most representative class for both was the 60- $90 \,\mu\text{m}$  class. Few vessels were in the largest diameter class. Xylem tissue of the 'K146-43' inter-stem had many vessels in the middle diameter class (60-90  $\mu$ m) and in the second class (30–60  $\mu$ m). There were no vessels in the largest diameter class (120-150  $\mu$ m) while a few were in the first (0-30  $\mu$ m) and the fourth (90-120  $\mu$ m) classes. The distribution of xylem vessel diameters in the 'K146-43' inter-stem (Fig. 4) tended to be more prevalent in the intermediate size classes than in the 'K146-43' used as rootstock (Fig. 1). The calculated hydraulic conductance of the 'Nemaguard' rootstock and the 'O'Henry' scion was higher than that of the 'K146-43' inter-stem (Fig. 5).



Fig. 2 Weighted mean xylem vessel diameters ( $\mu$ m) per visual field in trunks of 'Nemaguard', 'P30-135' and 'K146-43' rootstock genotypes and their respective grafted scions ('O'Henry'). Each value is the mean of two view fields (0.276 mm<sup>2</sup>) of four sections from five trees (n = 5). Means with different lower case letters are significantly different among the same rootstock genotype and scion at P < 0.05 (Tukey test).



Fig. 3. Calculated axial hydraulic conductance (g m MPa<sup>-1</sup> s<sup>-1</sup>) per visual field in trunks of 'Nemaguard', 'P30-135' and 'K146-43' rootstock genotypes and their respective scions ('O'Henry'). Each value is the mean of two view fields (0.276 mm<sup>2</sup>) of four sections from five trees (n = 5). Means with different lower case letters are significantly different among the same rootstock genotype and scion at P < 0.05 (Tukey test).



Fig. 4. Frequency distributions of xylem vessel sizes per visual field in stem pieces of inter-stem trees with a 'Nemaguard' rootstock, a 'K146-43' inter-stem and an 'O'Henry' scion. Each value is the mean of two view fields  $(0.276 \text{ mm}^2)$  of four sections from five trees (n = 5). Means with different lower case letters are significantly different at P < 0.05 (Tukey test).



Fig. 5. Calculated axial hydraulic conductance (g m MPa<sup>-1</sup> s<sup>-1</sup>) per visual field in stem pieces of inter-stem trees with a 'Nemaguard' rootstock, a 'K146-43' inter-stem and an 'O'Henry' scion. Each value is the mean of two view fields ( $0.276 \text{ mm}^2$ ) of four sections from five trees (n = 5). Means with different lower case letters are significantly different at P < 0.05 (Tukey test).

#### Discussion

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As reported previously (Tombesi *et al.*, 2010), the xylem characteristics (i.e. the size class distributions of the xylem vessels (Fig. 1), Wm (Fig. 2) and the calculated xylem hydraulic conductance (Fig. 3)) differed among

peach rootstocks that confer differing amounts of vigour to the scion. 'K146-43', the more dwarfing rootstock (Table 1), had more vessels per visual field than 'P30-135' and 'Nemaguard' (Fig. 1), but Wm was smaller (Fig. 2). This had a direct consequence on calculated hydraulic conductance (Fig. 3), being higher in 'Nemaguard' and 'P30-135' than in 'K146-43'.

In spite of the large differences in the xylem characteristics of the rootstock, there were only slight but non-significant differences in the xylem characteristics among the vigorous scions of the same cultivar grafted on the three rootstocks. These data are in general agreement with data from field measurements of the hydraulic conductance of young peach trees. Solari *et al.* (2006b) reported that the roots of trees on dwarfing rootstocks had substantially lower leaf-specific hydraulic conductance than the roots on trees with higher vigour rootstocks, but non-significant differences in the leafspecific hydraulic conductance of scion branches.

In trees composed of a vigour-inducing rootstock, a size-controlling inter-stem and a normal vigour scion, the xylem vessel characteristics of the three parts corresponded to their relative characteristics as rootstocks or scions in the 'two-part' trees (Solari et al., 2006b). However, the absolute values of some measured parameters were higher in the inter-stem trees than in the two-part trees. Thus, it is possible that the vigorous rootstock under the size-controlling inter-stem genotype did have some influence on the xylem characteristics of the inter-stem. Alternatively, these absolute differences may have been due to the age of the trees and some differences in cultural practices (pruning, irrigation and fertilization) that went on in the two plots prior to collecting the current data. Thus, it is probably only valid to compare differences in the relative values between genotypes within plots rather than between trees in different plots.

Nevertheless, as expected based on previous research (Tombesi et al., 2010), the vigorous rootstock, 'Nemaguard', had the largest Wm and the highest calculated hydraulic conductance among the three rootstocks. The 'O'Henry' scion had xylem vessel characteristics similar to 'Nemaguard'. The 'K146-43' inter-stem grafted in between the two more vigorous genotypes had xylem yessel characteristics that were smaller than either the rootstock below or the scion above (Fig. 4), and consequently had a mean calculated hydraulic conductance that was less than either the rootstock below or the scion above (Fig. 5). Thus, it appeared as if the dwarfing rootstock genotype used as an inter-stem functioned as a kind of physical restriction to water movement and probably reduced the hydraulic conductance of the whole tree. If this is

correct, according to the Hagen-Poiseuille law, the interstem effect should depend on its length: doubling the length should halve the hydraulic conductance. This may partly explain why Parry and Rogers (1972), working with dwarfing apple rootstock genotypes, reported that a 35-cm inter-stem piece had a greater dwarfing effect than a 5-cm inter-stem and similar results were reported by Di Vaio et al. (2009). It may also explain why the vigour-reducing effect of dwarfing rootstock genotypes used as inter-stems is generally intermediate between the vigour obtained from using vigorous versus dwarfing genotypes as rootstocks (Webster, 2004). These results are in accordance with those of Rufato et al. (2006), who demonstrated a relationship between the vigour-controlling potential of a peach inter-stem and the length of the inter-stem.

### **Conclusions and forward look**

From these studies with peach, rootstocks did not modify scion xylem vessel diameter and number significantly. Therefore, the decreased growth of scions on the size-controlling rootstock genotype, and associated reductions in xylem vessel size and number, were a function of the rootstock genotype and were not related to rootstock-induced changes in the xylem anatomy of the scion genotype. Similarly, the use of the sizecontrolling rootstock genotype as an inter-stem had no significant effect on the xylem characteristics or calculated hydraulic conductance of either the rootstock below or the scion above the inter-stem. Previous research documented the association between xylem vessel characteristics and the size-controlling behaviour of specific rootstocks (Tombesi et al., 2010). This work focused on the influence of size-controlling rootstocks or inter-stems on the xylem characteristics of the attendant scion (or rootstock in the case of the interstem). In both studies there was a clear association between the size-controlling behaviour of the rootstock and the xylem characteristics of that same genotype. We realize that the xylem vessel numbers and diameters, and associated Hagen-Poiseuille-based estimates of potential hydraulic conductance, do not represent actual hydraulic conductance behaviour of the tissues studied. Vessel length and resistances associated with xylem pits and wall sculptures can reduce conductance by as much as 50 % compared with theoretical values obtained when considering xylem vessels as perfect capillaries (Tyree and Zimmermann, 2002). However, the Hagen-Poiseuille calculations do represent the physical limit of hydraulic conductance of xylem with specific vessel numbers and diameters, and these calculated

theoretical limits correspond with the size-controlling behaviour of the rootstock genotypes.

Now that the relationship between xylem anatomy and dwarfing by peach rootstocks used in these and previous experiments is better understood, the use of interstems may be an efficient method to obtain trees with a range of vigour by varying the length of the inter-stem while still using traditional rootstocks known to be adapted to specific edaphic conditions.

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# **Contributions by the authors**

S.T. and T.M.D. conceived and planned the study. S.T. collected and prepared tissue samples, made the microscopic measurements, analysed the data and wrote the first draft of the manuscript. R.S.J. and K.R.D. planted and managed the trees that were used in the study, and reviewed the manuscript. T.M.D. helped in the analysis of the data, revised and edited the manuscript, and obtained funds to support the project.

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# **Conflict of interest statement**

None declared.

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