RESEARCH PAPER

Does fertilization explain the extraordinary hydraulic behaviour of apple trees?

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Abstract

Fertilization of woody plants plays a central role in agriculture and forestry, but little is known about how plant water relations are thereby affected. Here we investigated the impact of fertilization on tree hydraulics, and xylem and pit anatomy in the high-yield apple cultivars Golden and Red Delicious. In fertilized trees of Golden Delicious, specific hydraulic conductivity of branch xylem, hydraulic conductance of the root system, and maximum stomatal conductance increased considerably. In Red Delicious, differences between fertilized and control trees were less pronounced. In both cultivars, xylem embolism resistance of fertilized trees was significantly lower and stomatal closure occurred at lower water potentials. Furthermore, water potential at turgor loss point and osmotic potential at full saturation were higher and cell wall elasticity was lower in fertilized plants, suggesting reduced drought tolerance of leaves. Anatomical differences were observed regarding conduit diameters, cell wall reinforcement, pit membrane thickness, pit chamber depth, and stomatal pore length, with more pronounced differences in Golden Delicious. The findings reveal altered hydraulic behaviour in both apple cultivars upon fertilization. The increased vulnerability to hydraulic failure might pose a considerable risk for apple productivity under a changing climate, which should be considered for future cultivation and management practices.

Keywords: Drought tolerance, embolism, hydraulic conductivity, pit anatomy, stomatal closure, stomatal conductance, turgor, water potential, xylem anatomy.

Introduction

In commercial orchards, high-yield cultivars are grown under optimized irrigation and fertilization regimes to achieve the best results in terms of quality and productivity (Jones, 1992; Naor and Girona, 2012; Beikircher et al., 2013). In a previous study, Beikircher et al. (2013) reported that under these conditions, trees can exhibit an extraordinary hydraulic behaviour. Measurements on three economically important apple cultivars revealed an adjustment of hydraulic traits in order to optimize gas exchange and thus productivity—even at the risk of hydraulic failure: 50% loss of hydraulic conductivity in leaves and branches, turgor loss point, and initial damage to mesophyll cells occurred around or even at less negative water potentials than full stomatal closure (Beikircher et al., 2013). Such a late stomatal closure poses a considerable risk for plant survival, and the authors suggested that this striking behaviour is based on the selection of high-yield cultivars and optimized water supply. High-yield apple cultivars are artificial plant systems, with scions, bred for high quantity and quality,
granted on selected rootstocks, which in turn strongly control tree vigour and fruit characteristics. As for water supply, daily irrigation of the orchards ensured optimal water supply, with constant soil water potentials around −0.2 MPa. In addition to the nature of the plant material and daily irrigation, it is possible that fertilization plays a role in the unusual hydraulic behaviour of apple cultivars.

For the most part, fertilization with plant-available nitrogen (N) increases growth rates and leaf area (Cooke et al., 2005; Pallardy, 2008), which in turn require significant anatomical and hydraulic adjustments to maintain sufficient water supply to the whole plant. Accordingly, in several species, an increase in specific hydraulic conductivity ($k$) of stems or branches was observed upon fertilization (e.g. Bucci et al., 2006; Hacke et al., 2010; Plavcová and Hacke, 2012; Plavcová et al., 2013; Villagran et al., 2013; Medeiros et al., 2016). However, positive effects of N on growth and $k$ are not universal (Clearwater and Meinzer, 2001; Villagran et al., 2013; Faustino et al., 2016; Medeiros et al., 2016; Wang et al., 2016). Also, as indicated by a similar or even reduced leaf-specific hydraulic conductivity ($k$), the increase in $k$ was often not sufficient to compensate for the higher leaf area in fertilized plants (Ewers et al., 2000; Clearwater and Meinzer, 2001; Bucci et al., 2006; Domec et al., 2009; Hacke et al., 2010; Faustino et al., 2013). This in turn bears consequences for other hydraulic parameters as, according to the Ohm’s law analogy (Tyree and Ewers, 1991; Hubbard et al., 2001), $k$ is tightly coupled with leaf water potential ($\Psi_l$) and, in consequence, stomatal conductance ($g$). In several studies, no effect (Clearwater and Meinzer, 2001) or a decrease in $\Psi_l$ and/or $g$ (Ewers et al., 2000; Bucci et al., 2006; Domec et al., 2009) was observed upon fertilization. Besides N, fertilization with phosphorus (P) and potassium (K) was also found to alter hydraulic conductivity, although effects were often less pronounced (Harvey and Driessche, 1997; Bucci et al., 2006; Faustino et al., 2013; Medeiros et al., 2016). In the case of K though, changes are related to an ionic effect (e.g. Nardini et al., 2011; Oddo et al., 2011) rather than to structural changes (see below).

From an anatomical point of view, the observed increase in hydraulic efficiency upon fertilization in some studies was related to an increase in conduit diameter (e.g. Hacke et al., 2010; Plavcová and Hacke, 2012; Plavcová et al., 2013). This is not surprising because conductivity is proportional to the fourth power of conduit diameter (Tyree and Zimmermann, 2002). Also, intervessel pit characteristics affect hydraulic efficiency (e.g. Choat et al., 2008; Lens et al., 2011), but potential changes of bordered pits due to fertilization are unclear. Conduit and pit anatomy may also influence a trees’ resistance to xylem embolism. BORDERED pit characteristics such as pit chamber depth, pit aperture area, pit border area, and pit membrane ultrastructure are known to affect air-seeding, although this process is only partly understood (Sperry and Tyree, 1988; Lens et al., 2011; Jansen and Schenk, 2015). In particular, pit membrane thickness was found to be highly variable and suggested to be an important determinant of resistance against drought-induced embolism (Jansen et al., 2009; Li et al., 2016). Furthermore, embolism resistance has also been reported to correlate with the mechanical strength of conduit walls; that is, the thickness to span ratio $[(t/b)^2]$; Hacke et al., 2001; Lens et al., 2011, 2013).

There are less than a dozen studies investigating changes in embolism resistance upon fertilization, and reported findings are contradictory (see Supplementary Table S1 at JXB online for a synthesis). In hybrid poplar, embolism resistance has been found to decrease upon N fertilization, slightly increase with P fertilization, and to be unaffected by K (Harvey and Driessche, 1997, 1999; Hacke et al., 2010; Plavcová and Hacke, 2012; Plavcová et al., 2013). Vice versa, in tropical trees, an increase in xylem embolism resistance has been observed in N-fertilized trees, but no effect was found in trees fertilized with P or K (Bucci et al., 2006; Villagran et al., 2013). Also, upon an elaborated fertilization regime with P, K, Ca, and Mg, Ewers et al. (2000) observed an increase in embolism resistance of roots, but no effect on branches of *Pinus taeda*.

It is clear that fertilization does impact tree hydraulics, but how, and to what extent, appears to be highly variable. The effect on plant productivity and hydraulics depends on various factors such as plant species (e.g. Villagran et al., 2013; Medeiros et al., 2016), fertilizer (type and amount), growing conditions (e.g. water supply, light), plant age, and experimental design (e.g. Faustino et al., 2015; Wang et al., 2016). Moreover, since different outcomes have been observed even under similar controlled conditions for a single species, the effect of fertilization on plant water relations might be highly dynamic (Hacke et al., 2010). Many studies were done on pine species or hybrid poplar, with the main focus on hydraulic efficiency and gas exchange. Little is known about embolism resistance of different organs, root and leaf hydraulics, stomatal control and hydraulic safety margins, and xylem anatomy including pit properties and stomata.

Our study was aimed at analysing the impact of fertilization on hydraulic and underlying anatomical traits of two high-yield apple cultivars, in order to scrutinize whether it might explain their extraordinary hydraulic behaviour. Analyses were carried out on Red Delicious, which in a previous study showed low embolism resistance and a remarkably late stomatal closure, and the more drought-tolerant Golden Delicious (Beikircher et al., 2013). We studied hydraulic parameters of different organs of fertilized and control trees, and correlated them with various anatomical traits. Based on well-known positive fertilization effects on growth and productivity of apple trees (Jackson, 2003), we hypothesized that fertilization leads to increased hydraulic efficiency, but also, due to associated anatomical changes, to reduced hydraulic safety of high-yield apple cultivars. As a result, we expected adjustments to occur at different levels (cell to whole organs) and to be more pronounced in Red Delicious.

**Materials and methods**

*Plant material, experimental design, and study site* Measurements were made on the *Malus domestica* Borkh. cultivars ‘Golden Delicious’ and ‘Red Delicious’ grafted on M9 rootstocks. The general experimental design was based on management practices in commercial orchards, but only half of the plants were fertilized and plants were not irrigated. For a list of acronyms with respective definitions and units used in the following, see Supplementary Table S2.
In April 2014, a total of 60 Golden and Red Delicious trees were planted in a south-exposed test field in Innsbruck (600 m a.s.l.; 47°16'22"N, 11°23'34"E); cultivars were randomly distributed in six rows. The distance between trees within a row was 1 m, while the distance between tree rows was 1.5 m. In the course of planting, in three randomly chosen rows, 15 g of organic nitrogen fertilizer (Ikosan, 13–14% N) per tree were worked into the soil (fertilized trees). Trees in the remaining rows were not fertilized (control trees). Plants were watered every second day throughout the 2014 growing season to ensure optimal plant establishment, but in the following years plants were not irrigated. In spring 2015 and 2016, on an area of ~50×50 cm around trees chosen for fertilization treatment, 15 g of an inorganic NPK fertilizer (Compo, Novatec, 14%N) per tree was applied. A wash-out of nutrients could be excluded because (i) the fertilizer was directly worked into the soil; (ii) there was a distance of at least 75 cm to the neighbouring row; and (iii) no height level difference existed between rows. Furthermore, in a previous study on the same cultivars on the same rootstock but older trees, the main rooting area was within 30 cm from the stem. Thus, the distance between the rows was assumed to be sufficient to avoid roots of control trees reaching fertilized soil. This assumption is confirmed by our observation that within control trees no effect of the position in the test field (i.e. neighboured by control or fertilized trees) on growth was observed. In all years, trees were pruned in spring and all but 10 flowers per tree were removed to avoid reduced shoot growth due to high allocation of resources in fruits. The main study year 2015 was characterized by an exceptionally warm and dry summer, with a precipitation and temperature deviation of –24% and +3.2 °C from the long-term mean in July (127 mm, 18.5 °C) and a deviation of ~20% and +2.1 °C from the long-term mean in August (122 mm, 17.8 °C), respectively (https://zamg.ac.at).

Sampling procedure
For measurements carried out in the laboratory, branches of randomly selected trees were harvested from mid August to mid September 2015. Harvesting and sampling were done according to the protocol by Beikircher and Mayr (2016) to maintain the native hydraulic state of individual tree branches. Branches per cultivar and treatment were cut, and turgid weight (TW) and fresh weight (FW) were measured before and after analysis, Sartorius AG, Göttingen, Germany). Leaves were then dehydrated on the bench and, at intervals, FW was measured before and after analysing the respective leaf water potential ($\Psi_l$; MPa) with a pressure chamber (Model 1000 Pressure Chamber, PMS Instrument Company, Corvallis, OR, USA). Leaves were then oven-dried at 80 °C for 24 h to obtain the DW, and the relative water saturation deficiency ($\Psi_{WSD}$) was calculated.

For each single leaf, $1/\Psi_l$ was plotted versus WSD. The turgescence section of the curve was fitted with a parabolic function and the osmotic section with a linear regression (see Tyree and Hammel, 1972) using Fig.P 2006 (Fig.P Software Inc., ON, Canada). The osmotic potential at full saturation ($\Psi_{sat}$; MPa) was determined as the intersection of the linear regression with the y-axis, the water potential at turgor loss point ($\Psi_{TPL}$; MPa) from the intersection of the parabolic and the linear regression function, and cell wall elasticity ($a_c$) was estimated by the opening width of the parabola (the lower the $a_c$, the more elastic the tissue; see Ganthaler and Mayr, 2015). Values of single leaves were then averaged per cultivar and treatment, respectively.

Hydraulic conductance of the branch xylem and the root system
The specific hydraulic conductivity ($k$; cm$^2$ s$^{-1}$ MPa$^{-1}$) of the branch xylem was measured in summer 2015 and 2016 on 8–10 shoots developed in the respective season per cultivar and treatment. Up to 8 cm long samples were cut from shoots under water, debarked, and sample ends accurately trimmed with a wood carving knife. Samples were then connected to a Xylem apparatus (Bronkhorst, Montigny les Cormeilles, France) and perfused with the same solution as used for vulnerability analyses. Hydraulic conductance was measured at 4.5 kPa and normalized by xylem area and sample length to obtain $k$. Samples were then flushed at 95 kPa for 20 min and $k$ re-measured to test for native or artefactual embolism due to sample preparation.

Root hydraulic conductance was measured in September 2016 on three trees per cultivar and treatment, using a high-pressure flow meter (HPFM Gen 3; Dynamax, Houston, TX, USA). The stem base was tightly sealed with plastic bags and the obtained receptacle filled with water. Stems were then cut under water ~10 cm above the graft (i.e. ~30 cm above the soil surface). The upper end of the tree stump was debarked over ~8 cm, connected to the HPFM flow meter, and perfused with the same solution used for branch conductivity and vulnerability analyses (see above). Immediately afterwards, 3–5 transient measurements (see Tyree et al., 1995) were made and the hydraulic conductance of the root system calculated ($K_{ro}$; kg s$^{-1}$ MPa$^{-1}$).

Vulnerability analyses
Embolism resistance was analysed on up to five shoots per cultivar and treatment developed in the 2014 and 2015 growing seasons. Analyses were done with the Cavirton method (Cochard, 2002; Cochard et al., 2005) using a 28 cm custom-built rotor. The use of 2-year-old shoots was necessary as younger shoots were too short for the 28 cm rotor, which was required to avoid open vessel artefacts (Choat et al., 2010; Cochard et al., 2010; Torres-Ruiz et al., 2014; for information on vessel length in the study cultivars, see Beikircher and Mayr, 2016). Samples were cut from branches under water by re-cutting branches repeatedly from both sides with a pruning knife. About 5 cm of both sample ends were debarked and sample ends finally trimmed with a sharp wood carving knife (see Beikircher and Mayr, 2016) before fixing them in the cuvettes of the rotor.

Cuvettes were then filled with distilled and filtered (0.22 µm) water containing 0.005% (v/v) ‘Micropur Forte MF 100F’ (Katadyn Products Inc., Wallisellen, Switzerland) to prevent microbial growth (Beikircher and Mayr, 2008), and rotational speed was set to a target xylem pressure (P) of –0.25 MPa. After an equilibration time of ~10 min, the water flow from the upstream to the downstream reservoir was measured and hydraulic conductance calculated (for details, see Beikircher et al., 2010). Hydraulic conductance was then repeatedly determined at gradually increased rotational speed (and thus decreased P) until flow was no longer measurable. Percentage loss of hydraulic conductivity (PLC) was calculated as the ratio of the actual and the first (i.e. maximum) measured hydraulic conductance value and plotted versus the respective xylem pressure. Cuvettes were fitted using an exponential sigmoidal equation (Equation 1) given in Pammenter and Vender Willigen (1998):

$$PLC = 100 / \left\{1 + \exp \left[ a \left(PP_{50}\right) \right]\right\}$$

where $P$ is the corresponding xylem pressure (MPa), $a$ is related to the slope of the curve, and $PP_{50}$ is the pressure value corresponding to 50% loss of conductivity. Additionally, the values of $P$ at the onset ($P_{12}$; $P_{50}$) and at full embolism ($P_{88}$; $P_{90}$) were calculated (Beikircher et al., 2010, 2013).
Stomatal behaviour

Stomatal behaviour was measured directly in the field on five branches per cultivar and per treatment on a sunny day in August 2015. To prevent incomplete stomatal opening due to possible drought stress, trees were irrigated the previous evening. First, stomatal conductance ($g_s$; mmol m$^{-2}$ s$^{-1}$) and $Ψ_l$ were repeatedly measured on randomly chosen leaves using a steady-state leaf porometer (SC-1, Decagon Devices, Pullman, WA, USA) and a pressure chamber (see above), respectively. When $g_s$ reached constant values (~12.00 h central European time (CET)), branches were cut and measurements repeated at intervals until complete stomatal closure, $g_s$ was measured first, and the respective leaf was then cut to determine $Ψ_l$. During dehydration, cut branches were exposed to similar conditions to branches attached to the tree, and measurements were made within a 2 h period to ensure that stomatal closure occurred in response to decreasing $Ψ_l$ (Hubbard et al., 2001; Beikircher and Mayr, 2009). Percentage stomatal conductance was then calculated as the ratio of actual and maximal $g_s$, and plotted against $Ψ_l$. Curve fitting was done according to xylem vulnerability analyses (Equation 1), whereby PLC was substituted by percentage stomatal conductance and $P_{50}$ corresponds to $Ψ_l$ at 50% $g_s$. The water potential at full stomatal closure ($Ψ_{s\text{max}}$) was defined as the value of $Ψ_l$ at 12% $g_s$ and that at onset of stomatal closure as the value of $Ψ_l$ at 88% $g_s$ (Beikircher et al., 2013).

Diurnal courses and maximum stomatal conductance

Diurnal courses of $Ψ_l$ and $g_s$ were measured on a sunny day at the end of August 2015. From 05:00 to 18:00 h CET, 5–8 trees per cultivar and treatment were chosen randomly for measurements of $Ψ_l$ and $g_s$ (see above) at ~2 h intervals. In addition to hydraulic measurements, air temperature ($°C$), air humidity (%), and global radiation (W m$^{-2}$) were measured with a meteorological station (uEMSet99) and soil water potential with a gypsum block sensor (MicroLog SP; all sensors and datalogger from EMS, Brno, Czech Republic). Climatic measurements were made at 1 min intervals, and 30 min means were calculated for analyses.

Maximum operating stomatal conductance ($g_{s\text{max}}$) for a given cultivar and treatment was calculated from the eight highest $g_s$ values measured in the daily course and the eight highest values of the in situ analyses of stomatal behaviour (see the respective paragraph above). To obtain $Ψ_{s\text{max}}$, the eight lowest values measured in the daily course (but independent of time) were averaged per cultivar and treatment.

Xylem anatomy

Anatomy of the branch xylem was analysed on eight shoots per cultivar and per treatment. The samples, which were previously used for hydraulic conductivity analyses, were soaked in an ethanol–glycerol–water solution (1:1:1, v/v/v/v) for several weeks before cutting transverse sections with a microtome (Sledge Microtome G.S.L. 1, Schenkung Dapples, Zurich, Switzerland). Transverse sections were stained with Etzold’s staining solution, and anatomical parameters were analysed with a light microscope (Olympus BX 41, System Microscope, Olympus Austria, Vienna, Austria) interfaced with a digital microscope camera (ProgRes CT3, Jenoptik, Jena, Germany) and image analysis software (ImageJ, 1.37, National Institutes of Health (NIH), Bethesda, MD, USA, public domain). In radial sections, individual lumen areas ($A$) were measured directly and the respective diameters ($d$) calculated assuming a circular conduit shape. The mean hydraulic diameter ($d_h$; µm) was calculated from individual diameters according to Hacke et al. (2004), Equation 2:

$$d_h = \frac{\sum d^4}{\sum d^3} \quad (2)$$

Conduit wall reinforcement ($t/b_h$) was analysed according to Hacke et al. (2001) by directly measuring the double wall thickness ($t$) between adjacent conduits and the respective conduit diameter ($b$).

To avoid possible unbalanced statistical weighting of samples with larger numbers of conduits, the mean diameter ($d_m$; µm) as well as $d_h$ and ($t/b_h$) were first averaged per sample and then means per cultivar and treatment were calculated from averages (Beikircher et al., 2013).

Pit analyses were made on shoots developed in spring 2016 using TEM. In July 2016, three shoots per cultivar and treatment were chosen randomly, harvested according to the protocol given above, and transported to the laboratory. Shoots were cut into 3 mm$^3$ blocks in phosphate-buffered saline (PBS) and fixed with 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.3. After washing with the latter, samples were post-fixed in 2% osmium tetroxide (OsO$_4$) which was then removed by washing with a graded ethanol series (30, 50, 70, and 90%). Finally, samples were block-stained with uranyl acetate before embedding in Epon™. Upon polymerization, ultrathin transverse sections were cut with an ultramicrotome (Ultracut, Reichert-Jung, Austria) and observed with a JEOLEMM-1400 transmission electron microscope (Jeol Germany GmbH, Freising, Germany). On intervessel pits, pit membrane thickness ($T_m$; nm) and depth of the pit chamber ($L_m$; nm; see Fig. 2) were measured using the image analysis software ImageJ (see above). $T_m$ was measured in the centre and at both sides near the annulus, and for $L_m$ the distances from the non-aspirated membrane surface to the inner edges of the pit apertures were measured. In the latter two cases, averaged values per pit were used to calculate means per cultivar and treatment.

Leaf anatomy

For leaf anatomical analyses, 10 leaves per cultivar and treatment were chosen randomly, harvested, and saturated in distilled water overnight. Trichomes on the adaxial surfaces were removed with adhesive tape and a coat of clear nail varnish was applied on intercostal areas. Upon drying, the nail varnish was peeled off with adhesive tape, placed on a microscope slide, and analysed with a light microscope interfaced with a digital microscope camera and image analysis software (see ‘Xylem anatomy’). To determine stomatal density (SD; number per mm$^2$), stomata were counted on defined areas. Stomatal pore length ($Ψ_l$) at 12% $g_s$ and that at onset of stomatal closure as the value of $Ψ_l$ at 88% $g_s$ (Beikircher et al., 2013). Similar to xylem anatomical parameters, mean values per cultivar and treatment were calculated from values averaged per leaf.

Statistics

Depending on normal distribution and variance (tested with the Kolmogorov–Smirnov and the Levene test), the following tests were applied to detect significant differences between fertilized and control trees within a cultivar: $P_{12}$, $P_{55}$, $P_{88}$, parameter $a$, $Ψ_{sc}$, $g_{s\text{max}}$, $K_R$, year ring width, $d_{max}$, $d_h$, $d_{min}$ and ($t/b_h$) were tested with the Welch test. All other parameters were analysed with the Student’s t-test. All tests were made pairwise at a probability level of 5% using SPSS version 21.

Results

Tree growth

Fertilized trees of Golden Delicious had ~5 cm longer current year tree tops and shoots than control trees (Table 1). In Red Delicious, differences were smaller and amounted to ~2 cm. In Golden Delicious, the year ring width was significantly higher in fertilized plants (~615±34 µm) than in the controls (502±36 µm). In Red Delicious, the overall increment was about half that of Golden Delicious, and similar between fertilized and control trees.

Hydraulic efficiency, xylem embolism resistance, and stomatal closure

Fertilization resulted in a significant increase in $k$, of the branch xylem (Table 2). In Golden Delicious, $K_R$ also strongly
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Table 1. Current year length increment of tree tops and side shoots and current year ring width of control and fertilized plants of Golden and Red Delicious

| Parameter                  | Golden control | Golden fertilized | Red control | Red fertilized |
|----------------------------|----------------|-------------------|-------------|----------------|
| Tree top (cm)              | 31.75±2.57     | 36.56±4.00        | 20.50±2.78  | 22.62±3.92     |
| Shoots (cm)                | 19.73±1.20     | 24.69±1.20*       | 18.50±1.13  | 20.27±1.73     |
| Year ring width (μm)       | 502±36         | 615±34*           | 316±19      | 327±44         |

Mean ±SE. Asterisks indicate significant differences within a cultivar.

Table 2. Hydraulic and anatomical parameters of control and fertilized plants of the apple cultivars Golden and Red Delicious

| Organ    | Parameter                  | Golden control | Golden fertilized | Red control | Red fertilized |
|----------|----------------------------|----------------|-------------------|-------------|----------------|
| Leaf     | Ψ_{lmp} (MPa)              | –2.97±0.09     | –2.65±0.08*       | –2.37±0.05  | –2.43±0.11     |
|          | Ψ_{low} (MPa)              | –2.56±0.09     | –2.39±0.06        | –2.14±0.05  | –2.21±0.08     |
|          | a_{l}                      | 1.38±0.27      | 1.63±0.24         | 0.96±0.19   | 1.44±0.24      |
|          | g_{max} (mmol m^{-2} s^{-1})| 497±44         | 617±30*           | 540±30      | 571±54         |
|          | Ψ_{s} (MPa)                | –3.75±0.17     | –4.41±0.21*       | –4.19±0.23  | –4.88±0.26*    |
|          | Ψ_{lmin} (MPa)             | –2.73±0.11     | –3.04±0.07*       | –2.52±0.08  | –2.55±0.05     |
|          | SD (no mm^{-2})            | 265±17         | 267±22            | 255±9       | 249±11         |
|          | l (μm)                     | 15.03±0.59     | 17.61±0.47*       | 17.27±0.51  | 15.33±0.36     |
| Branch   | k (cm^2 s^{-1} MPa^{-1})   | 6.36±0.45      | 10.48±1.36*       | 4.74±0.40   | 7.70±1.09*     |
|          | P_{12} (MPa)               | –2.00±0.22     | –1.16±0.20*       | –2.85±0.17  | –2.12±0.17     |
|          | P_{50} (MPa)               | –5.00±0.07     | –3.65±0.07*       | –4.77±0.06  | –4.29±0.06*    |
|          | P_{88} (MPa)               | –7.11±0.07     | –6.14±0.06*       | –6.60±0.06  | –6.46±0.05*    |
|          | Parameter a                | 0.95±0.06      | 0.80±0.04         | 1.04±0.06   | 0.92±0.05      |
|          | d_{wall} (μm)              | 22.00±0.74     | 25.15±1.83        | 19.78±0.61  | 20.71±2.37     |
|          | d_{s} (μm)                 | 24.52±0.95     | 26.86±1.91        | 22.33±0.57  | 23.18±2.56     |
|          | d_{wall} (μm)              | 30.80±1.00     | 32.89±2.62        | 27.93±0.58  | 28.45±3.24     |
|          | (l/b)_{max}×10^{-3}        | 5.44±0.40      | 4.01±0.31*        | 5.80±0.24   | 4.10±1.00      |
|          | T_{l} (nm)                 | 459±50         | 415±17            | 382±31      | 320±40         |
|          | L_{p} (mm)                 | 367±24         | 516±38*           | 318±38      | 342±23         |
| root     | K_{os} (kg s^{-1} MPa^{-1})×10^{-6} | 0.48±0.09    | 5.00±2.46         | 0.82±0.25   | 0.51±0.22      |

Water potential at turgor loss point (Ψ_{lmp}), osmotic potential at full saturation (Ψ_{osat}), cell wall elasticity (a_{l}), maximum operating stomatal conductance (g_{max}), water potential at stomatal closure (Ψ_{sc}), minimum leaf water potential (Ψ_{lmin}), stomatal density (SD), stomatal pore length (l), specific hydraulic conductivity (k), xylem pressure inducing 12, 50, and 88% loss of hydraulic conductivity (P_{12}, P_{50}, P_{88}), slope of the vulnerability curve (a), mean, hydraulic, and maximum conduit diameter (d_{wall}, d_{s}, d_{wall}), cell wall reinforcement [(l/b)_{max}], pit membrane thickness (T_{l}), pit chamber depth (L_{p}), and whole root conductance (k_{os}). Means ±SE. Asterisks indicate significant differences within a cultivar.

Increased, however, due to the high variation in K_{R} (values ranging from 9×10^{-6} kg s^{-1} MPa^{-1} to 5×10^{-7} kg s^{-1} MPa^{-1}) between trees, differences were not significant. In contrast, in Red Delicious, a slight, non-significant decrease in K_{R} upon fertilization was observed.

Embolism resistance of the branch xylem was significantly decreased upon fertilization in both cultivars. Water potential at 50% loss of hydraulic conductivity (P_{50}) decreased from –5.00 MPa and –4.77 MPa in control trees to –3.65 MPa and –4.29 MPa in fertilized trees of Golden and Red Delicious, respectively. Accordingly, vulnerability thresholds representing embolism onset and full embolism were also shifted –3.04±0.07 MPa and 0.7 (P_{12}) MPa and 0.73 (P_{50}) MPa and 0.23 (P_{88}) MPa towards less negative values in fertilized trees of Golden and Red Delicious, respectively (Table 2; Fig. 1). Moreover, upon fertilization, Ψ_{s} was shifted significantly –0.7 MPa towards more negative leaf water potentials (Table 2).

Stomatal conductance and cell osmotic parameters

Diurnal courses of g_{s} and Ψ_{l} of fertilized and control plants on an average summer day in 2015 followed similar patterns, but differed between cultivars (Supplementary Fig. S1). In both cultivars, g_{s} increased during the morning, and the highest values were reached around noon. In Golden Delicious, g_{s} then decreased steadily, and the reduction was faster in fertilized plants. In Red Delicious, g_{s} remained high until late afternoon and decreased at ~17.00 h CET. In both cultivars, we observed pre-dawn water potentials (Ψ_{PD}) close to 0 MPa, and, following stomatal opening, a decrease in Ψ_{l} towards noon. In Golden Delicious, the lowest values were reached at ~16.00 h CET, and in Red Delicious at around midday. In Golden Delicious, significantly lower Ψ_{lmin} and higher Ψ_{max} were measured in fertilized (~3.04±0.07 MPa and 617±30 mmol m^{-2} s^{-1}) compared with control trees (~2.73±0.11 MPa and 497±44 mmol m^{-2} s^{-1}; Table 2). In Red Delicious, Ψ_{lmin} and Ψ_{max} ranged around ~2.5 MPa and 555 mmol m^{-2} s^{-1}, respectively.

In fertilized trees of Golden Delicious, turgor loss occurred at significantly less negative leaf water potentials (~2.65 MPa) compared with control trees (~2.97 MPa). A similar though not significant trend was observed for Ψ_{osat} (~2.39 MPa and ~2.56 MPa; Table 2). In Red Delicious, Ψ_{lmp} and Ψ_{osat} were slightly higher than in Golden Delicious, but no significant differences between treatments were found (Table 2). In contrast,
differences in $a_{eli}$ between treatments were more pronounced (though not significant) in Red Delicious (1.5 times higher in fertilized plants). In both cultivars, the higher $a_{eli}$ points to a lower cell wall elasticity in fertilized trees.

**Xylem and leaf anatomy**

Larger $d_{mean}$, $d_h$, and $d_{max}$ were found in fertilized trees (Table 2; Fig. 2), corresponding to higher hydraulic efficiency. Differences were not significant due to high variation in fertilized trees. For instance, $d_{mean}$ ranged from 20 µm to 29 µm in fertilized plants of Golden Delicious, and from 16 µm to 27 µm in Red Delicious, while in control plants values ranged from 21 µm to 25 µm, and from 17 µm to 21 µm, respectively (data not shown). Higher values of fertilized trees were caused by higher fractions of wide conduits and, particularly in Golden Delicious, by a higher fraction of narrow conduits in control plants (Fig. 3). In both cultivars, $(t/b)_m^2$ was reduced from ~5.6 in control trees to 4.0 in fertilized trees (Table 2). Due to the high variation in fertilized trees of Red Delicious (values ranging from 1.7 to 6.3), differences were only significant in Golden Delicious.

Intervessel pit membranes appeared as electron-transparent structures with dark particles after OsO$_4$ treatment. The thickness was more or less homogeneous across the membrane, but typically highest in the centre near the pit aperture. In Golden and Red Delicious, $T_m$ tended to be lower in fertilized trees (415±17 nm and 320±40 nm) compared with control trees (459±50nm and 382±31 nm). $L_p$ increased upon fertilization, but differences were only significant in Golden Delicious.

Regarding leaf anatomy, I was significantly higher in fertilized plants of Golden Delicious (17.61±0.47 µm) than in controls (15.03±0.39 µm), while in Red Delicious the opposite was observed (15.33±0.36 µm versus 17.27±0.51 µm; Table 2). SD was ~260 stomata mm$^{-2}$ regardless of cultivar and treatment.

**Discussion**

A well-balanced fertilization management is of utmost importance in apple orcharding to obtain high fruit quality and quantity. It also positively influences tree growth, although to what extent strongly depends on plant age, cultivar, rootstock, soil, and other management practices (Jackson, 2003; Carranca...
Influence of fertilization on apple trees

In our study, effects on growth were relatively low which might be related to the cultivars’ growth patterns as well as to growing conditions. Both cultivars were grafted on the dwarfing rootstock M9, which supports weak to moderate vigour, and trees were carefully pruned. Furthermore, Red Delicious plants were spur-types, which are characterized by a particularly compact growth with closely spaced fruit spurs and not particularly dominant trunks (Jackson, 2003). This might explain the even weaker response to fertilization in Red Delicious than in Golden Delicious (Table 1). A weak or lack of response to fertilization might also be attributed to a sufficiently high N content in control trees. The focus of this study was on potential consequences of fertilization on growth and hydraulic traits; thus, we did not consider nutrient uptake or tissue nutrient concentrations per se. However, partially highly significant differences between treatments were observed (see below) and, based on the knowledge that young fruit trees in particular strongly depend on sufficient nutrient supply for growth (Carranca et al., 2018), we assume that the observed weak growth was attributed to limited water supply. The study year 2015 was relatively warm and dry. Although no prolonged and threatening drought periods occurred due to frequent thunderstorms, conditions might have been limiting, especially for Red Delicious, which strongly depends on high water supply for optimal performance. Despite its limited impact on growth, fertilization affected tree hydraulics considerably.

**Hydraulic efficiency of the branch xylem**

In both cultivars, specific hydraulic conductivity ($k$) of the branch xylem was significantly higher in fertilized than in control trees (Table 2). This was related to overall larger and most probably longer conduit diameters (Liu et al., 2017), higher fractions of large diameter conduits, and, in the case of Golden

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**Fig. 2.** Transverse wood anatomical sections of control (A, C) and fertilized (B, D) trees of Golden Delicious. The light microscope images (A, B) show variation in conduit diameter and cell wall reinforcement, and the TEM images (C, D) demonstrate pit membrane thickness ($T_m$) and pit chamber depth ($L_p$). (This figure is available in colour at JXB online.)
Delicious, lower fractions of small diameter conduits compared with control trees (Table 2; Figs 2, 3). The also observed high variation of \( k_i \) in fertilized trees is related to a high variation in conduit diameters. Both increases as well as decreases in \( k_i \) have been reported upon fertilization (e.g. Clearwater and Meinzer, 2001; Bucci et al., 2006; Hacke et al., 2010; Plavcová and Hacke, 2012, 2013; Villagra et al., 2013; Medeiros et al., 2016). However, a comparison between studies is difficult due to different experimental setups (from selective fertilization under controlled conditions to manipulation of nutrient availability of naturally nutrient deficient soils), plant age (from rooted cuttings or seedlings to mature trees), fertilizer (single nutrient to multinutrient fertilizers), fertilizing treatment (from moderate to excessive supply of fertilizer), and growing conditions (e.g. different light or water regimes).

Since \(~58\%\) of the total hydraulic resistivity of xylem is attributed to bordered pits between adjacent conduits (Choat et al., 2008), pit properties, such as pit membrane porosity and thickness or pit aperture/pit membrane area, play a key role for flow resistance (Wheeler et al., 2005; Choat et al., 2008; Lens et al., 2011). In the present study, the more conductive fertilized trees showed thinner pit membranes with deeper pit chambers, with the effects more pronounced in Golden Delicious (Table 2; Fig. 2). These observations are in line with Lens et al. (2011), who reported hydraulic conductivity to be negatively correlated with pit membrane thickness \( (T_{mb}) \) and positively with pit chamber depth \( (L_9) \). A functional explanation for these correlations is still unclear. It has been assumed that thinner pit membranes provide less hydraulic resistance than thicker ones, possibly due to higher pit membrane porosity (e.g. Jansen et al., 2009), and that deeper chambers increased embolism resistance (Lens et al., 2011). Alternatively, pore sizes could be determined by how much a pit membrane is shrunken, which is not necessarily related to pit membrane thickness but could be affected by dehydration and frost (Li et al., 2016; Schenk et al., 2017; Zhang et al., 2017).

**Hydraulic conductance of the root system**

Although it is known that N supply often leads to a shift in biomass allocation from root towards stem and leaf tissues (e.g. Ewers et al., 2000; Cooke et al., 2005; Wang et al., 2016), little is known about root hydraulics under different nutrient supply, and results are contradictory. In some studies, a reduction in the hydraulic conductance of roots \( (K_R) \) of fertilized trees has been observed (e.g. Ewers et al., 2000), although the decrease was often more pronounced under moderate levels and diminished with higher levels of N supply, or differences were only found under full sunlight but not under shaded conditions (Luis et al., 2010; Wang et al., 2016). In contrast, Trubat et al. (2006, 2012) reported a higher \( K_R \) for *Pistacia lentiscus* seedlings with sufficient N supply compared with plants under N deficiency conditions. Our study cultivars showed contrasting responses: while in Red Delicious, a slight reduction in \( K_R \) was observed upon fertilization, we found a strong increase in Golden Delicious (Table 2). At first glance, this seems interesting as both cultivars are grown on the same rootstock. However, recent studies have shown that scions of grafted plants have large influences on root biomass via different levels of endogenous abscisic acid (e.g. Chen et al., 2002; McAdam et al., 2016). Accordingly, the higher \( K_R \) of fertilized Golden Delicious plants might be related to a larger amount of fine roots and the consequently lower resistance in radial pathways (Tyree and Zimmermann, 2002).

**Embolism resistance**

The ability to prevent xylem embolism is one crucial factor for a plant’s drought resistance. In both study cultivars, water potentials inducing 12, 50, and 88% loss of hydraulic conductivity \( (P_{12}, P_{50}, \text{and } P_{88}) \) were significantly less negative in fertilized trees (Table 2; Fig. 1). Again, differences were more pronounced in Golden Delicious. This outcome is in line with most studies dealing with the effects of fertilization on embolism resistance in trees. Nevertheless, contradictory findings have been reported for different organs (Ewers et al., 2000) and different nutrients (see the Introduction and Supplementary Table S1).

The decrease in embolism resistance was related to lower cell wall reinforcement \( ([t/b]_h^2) \); Table 2). Positive correlations between embolism resistance and \( (t/b)_{h}^2 \) have been reported in several studies and on different taxonomical levels (Hacke et al., 2001; Lens et al., 2011). In some other fertilization studies, wood density has been positively correlated with embolism resistance (Hacke et al., 2010; Plavcová and Hacke, 2012; Plavcová et al., 2013). However, as wood density is influenced more by the thickness-to-span of fibres than conduits (Ziemnińska et al., 2013), this correlation is weaker and should not be used as a single determinant of embolism resistance (Cochard et al., 2008; Lens et al., 2011). Besides cell wall reinforcement,
embolism resistance is also known to be coupled to pit characteristics, especially to $T_m$ (Jansen et al., 2009; Lens et al., 2011; Scholz et al., 2013; Li et al., 2016), although the underlying mechanisms are largely unknown. It has been assumed that thicker pit membranes are less likely to have larger pores, thus increasing air-seeding pressure. However, this has not been proven yet and, as already discussed in context with hydraulic efficiency, porosity may largely be determined by the microfibril arrangement and be independent from $T_m$ (Li et al., 2016). Also, pit membranes are three-dimensional structures and thus air-seeding is rather determined by the most narrow pore throat within the largest pore pathway (Schenk et al., 2017). Similar to Lens et al. (2011), Scholz et al. (2013), and Li et al. (2016), in our study embolism resistance was positively correlated with $T_m$ and negatively with $L_p$ (Table 2). Thereby, with values ranging from 320 nm to 459 nm, $T_m$ was relatively high compared with other temperate tree species. Li et al. (2016), for instance, reported a mean $T_m$ of 211 nm for a total of 49 temperate woody species, and Lens et al. (2011) observed a mean of 263 nm for seven Acer species. The high $T_m$ was in accordance with the relatively negative $P_{so}$ values found in our study cultivars. However, vulnerability thresholds to drought-induced embolism are only one aspect and by themselves not sufficient to characterize a plant’s drought tolerance. Another key trait is stomatal regulation as it determines whether a plant tends to a rather isohydric (sensitive stomatal control) or aniso-hydric (tolerating negative $\Psi_l$) behaviour.

Stomatal behaviour and drought tolerance of leaves

Individual plants can adjust their hydraulic behaviour with respect to water supply (e.g. Franks et al., 2007; Domec and Johnson, 2012; Zhang et al., 2012). We assume that this also applies to nutrient supply because in fertilized plants both onset and full stomatal closure occurred at more negative $\Psi_l$ than in control trees (Table 2; Fig. 1). Unless low $\Psi_l$ is avoided by a sufficiently high $k_l$ and/or down-regulation of $g_o$, late stomatal closure ($\Psi_m$) poses considerable risk of runaway embolism (Tyree and Sperry, 1988). In Red Delicious, neither $\Psi_{lmin}$ nor $g_{max}$ differed between treatments (Table 2). In Golden Delicious, however, $g_{max}$ of fertilized trees was significantly higher, which might be related to significantly higher stomatal pore length ($l$) for similar stomatal density (SD) values (Table 2). A positive correlation between $l$ and $g_o$ has also been reported by Aasamaa et al. (2001) for various temperate deciduous trees, although higher $g_{max}$ is generally achieved with larger numbers of smaller but faster stomata (e.g. Franks et al., 2009; Drake et al., 2013; Elliott-Kingston et al., 2016; Sack and Buckley, 2016). The increase in $g_o$ resulted in significantly lower $\Psi_{lmin}$ (Table 2). $\Psi_{lmin}$ of fertilized trees was already in the range of embolism onset (Table 2), which might also explain the sharp drop in $g_l$ in the afternoon (Supplementary Fig. S1). Although $\Psi_l$ of transpiring leaves was probably more negative than actual water potential of the branch xylem, this cultivar follows a risky hydraulic strategy because of late stomatal closure (Supplementary Fig. S2).

Fertilization or water supply: what matters most?

In the present study, considerable adjustments in tree hydraulicities of two high-yield apple cultivars have been observed upon fertilization. Based on changes in xylem and pit anatomical traits, hydraulic efficiency was increased at the cost of embolism resistance in fertilized trees. Due to the parallel shift towards less negative $\Psi_{sc}$ and the reduced drought tolerance of leaves (Golden Delicious), fertilized trees thus operated at the edge of hydraulic failure. A similar and remarkable risky hydraulic behaviour has also been observed in trees growing in commercial apple orchards (Beikircher et al., 2013), where trees were irrigated daily. Thus, we suppose that optimal water supply might further increase the effect of fertilization. Nevertheless, given the pronounced cultivar-specific differences in various hydraulic and anatomical parameters reported by Beikircher et al. (2013), structure–functional changes in some cultivars might be limited by reduced water availability. Accordingly, we assume that in Red Delicious additional irrigation might have resulted in more pronounced differences in xylem and pit anatomy, maximum stomatal conductance, as well as leaf hydraulic parameters. In this context, the outcome of the present study is also of relevance for future orchard management. In view of altered precipitation patterns and reduced water availability due to climate change, careful selection of apple cultivars is required to ensure high productivity even under drier conditions.

Supplementary data

Supplementary data are available at JXB online.

Table S1. Synthesis of studies investigating the effect of fertilization on the xylem embolism resistance of woody plants.

Table S2. List of parameters measured with acronyms, definition, and units.

Fig. S1. (A, B) Diurnal course of stomatal conductance and leaf water potentials of control and fertilized Golden Delicious and Red Delicious. (C) Air temperature, relative air humidity, and global radiation on the measurement day. Means ± SE.

Fig. S2. Sequence of characteristic hydraulic parameters during dehydration of control and fertilized plants of Golden Delicious and Red Delicious.

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