Sap flow in ‘Hass’ avocado trees on two clonal rootstocks in relation to xylem anatomy
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1. Introduction
Avocado (Persea americana Mill.) is a very genetically diverse species comprised of three botanical races and their hybrids, viz. Mexican, Guatemalan and West Indian ecotypes (Newett et al., 2002). Avocado rootstocks have been selected from those races based fundamentally on the tolerance to Phytophthora root rot caused by the oomycete, Phytophthora cinnamomi, to salinity, and on adaptability to calcareous soils (Ben Ya'acov and Michelson, 1995). To the authors' knowledge, there are no published reports that relate the physiological and anatomical differences among avocado rootstocks to their ability to absorb and transport water.

Rootstocks should exert significant physiological effects on scions, including affecting the rate of water and nutrient absorption and translocation. If water availability is a problem in the leaves, the stomata will close and assimilation will be reduced. Therefore, the rootstock may significantly influence the productivity of the scion by affecting the tree water balance (Giulivo et al., 1985; Olien and Lakso, 1986; Klamkowski and Treder, 2002). Solari et al. (2006) suggested that the effect of rootstock on vegetative growth of peach trees was associated with balanced water relations, specifically to differences in hydraulic conductance in the vascular system of the rootstock. Studies that have examined hydraulic conductance in whole trees as well as individually in roots, stem and leaves showed that hydraulic resistance to water flow is higher in the roots than in the above ground shoots of the tree (Olien and Lakso, 1986; Tsuda and Tyree, 1997; Basile et al., 2003).

The anatomical characteristics of the water conduction system in plants can have a profound impact on the hydraulic conductivity of the tree (Tyree and Zimmerman, 2002). From an engineering point of view, the xylem is the water distribution network that transmits water from the root collection system to the main consumers, the leaves, in the upper parts of the plant (Karam, 2005). Recent anatomical studies of avocado (P. americana Mill.) trees have elucidated differences in vessel anatomy among races and cultivars. If the scion of a grafted tree is a different cultivar than the rootstock, which is generally the case in commercial groves, the difference in anatomical features between the scion and the rootstock seems to cause a discontinuity in the water conduction system that negatively impacts water transport (Reyes Santa Maria et al., 2002).

The objective of this research was to determine the effect of the xylem vessel network of two clonal avocado rootstocks on water consumption of ‘Hass’, the most widely planted avocado cultivar throughout the world.
2. Material and methods

2.1. Water use

This part of the research was conducted in a shaded glasshouse at the University of California, Riverside from June through August, 2005 and 2006. Climatic variables within the glasshouse during the experiment were recorded with Hobo H8 data loggers (Onset, Pocasset, Massachusetts, USA). Average day/night temperatures were 18 °C/36 °C and relative humidity ranged from 40 to 100%. The photosynthetic photon flux (PPF) was recorded above the canopy with two quantum sensors (Model Li 190, Li Cor, Inc., Lincoln, NE, USA) which were connected to an Li 1000 data logger (Li Cor, Inc.). The average PPF at midday was between 250 and 300 μmol m⁻² s⁻¹. The vapor pressure deficit ranged from 0.48 mol m⁻³ in the morning to 1.57 mol m⁻³ in the afternoon.

Two-year-old avocado trees were used in this experiment. Treatments consisted of four rootstock/scion combinations were used, nongrafted clonal Duke 7 (D7) and Toro Canyon (TC) trees, and ‘Hass’ grafted onto clonal D7 (H/D7) or TC rootstock (H/TC). Duke 7 is a Mexican race rootstock and Toro Canyon is a Mexican × Guatemalan hybrid rootstock. One group of plants (used during 2005) was provided by ACW Farm (Fallbrook, CA) and propagated by the Hofshi method (Hofshi, 1996) and the second group (used in 2006) was provided by Brokaw Nursery, Inc. (Ventura, CA) and propagated by the Brokaw method (Brokaw, 1987). Trees were grown in 15-l plastic pots filled with 33% sand, 33% clay and 33% silt. During the experiment the plants were watered and fertilized daily at 9:00 a.m. with Hoagland’s solution (Hoagland and Arnon, 1950). Tensiometers placed at a 20-cm depth in the soil were used for monitoring soil matric potential.

Trees with similar trunk diameters and leaf areas were used in this study because preliminary tests showed that trunk diameter and leaf area affect sap flow, which was measured in this study. At the end of the experiment, root dry weight was measured to determine the relationship between root biomass and sap flow. Prior to dry weight determination the root systems were washed and dried in a forced draft oven at 60 °C for 48 h.

The area of every leaf of each tree and the calculation of the total leaf area per tree were determined non-destructively by multiplying the squared value of the length of the leaf midrib by a pre-determined conversion factor. Separate conversion factors were determined for each cultivar because these factors were related to the shape of the leaf which was different for each cultivar. It was determined that it was not necessary to use both the length and width to get a better estimation of leaf area because the width was related to the length for each cultivar and so an area proportional to the length x width was similar to the area proportional to the square of the length. To determine the conversion factors, 10 mature leaves from each of 3 trees per cultivar were collected from potted trees in a glasshouse. The detached leaves were immediately transported in a plastic bag to a copy machine. A photocopy was made of each leaf. The image of each leaf was cut from the photocopied page and the leaf image was weighed. The midrib length was determined from this copy. A piece of graph paper of a known area (30 cm²) was also photocopied and weighed to determine the surface area: weight ratio. The surface area/weight ratio of the graph paper photocopy was then used to determine the unknown surface area of the leaf photocopy. This scaling allowed each conversion factor to be calculated (dividing the leaf area by the squared value of the length of the midrib). The conversion factors of all 30 sampled leaves per cultivar were averaged to obtain the mean conversion factor for converting midrib length to leaf area for each cultivar.

The leaf area conversion factors (measured area/midrib length²) were 0.36, 0.30 and 0.31 for ‘Hass’, D7 and TC, respectively.

Three experiments were conducted with trees in containers, each with four single-tree replications of each tree or scion/rootstock combination (D7, TC, H/D7, H/TC). Two experiments were conducted during 2005, from July 26 to 28 and from August 4 to 6. The third experiment was conducted from August 19 to 21, 2006. Trees were arranged in the glasshouse in a completely randomized design.

Sap flow rate was monitored with a Dynagage sap flow system (Dynamax, Inc., Houston, TX, USA) based upon the heat balance technique (Steinberg et al., 1989) with SGA13 sensors linked to a CR10 data logger (Campbell Scientific, Inc., Logan, UT, USA). A four-channel stem gauge was attached to the base of the stem, approximately 5 cm above the graft union and was covered with insulation to prevent extraneous heat flow over which was placed aluminum foil to reduce the effect of external radiation. The data logger was programmed to record the output every 15 min and the data were downloaded and analyzed every day using Flow32 WIN analysis software (Dynamax, Inc.). All trees were monitored for 3 days and the sap flow rate per day was calculated between 8:00 and 20:00 h, when a majority of the total sap flow occurred.

To test the operation and accuracy of the equipment, daily water loss was determined from gravimetric measurements of soil water content for 8 days. For soil water content determinations during the 8-day period, pots with the soil and trees were placed on an electronic balance and weighed continuously during the day. The pots were wrapped in aluminum foil, which covered the soil surface throughout the experiment so that the only water loss was from transpiration.

The experiment was arranged in a complete randomized block design with three replications and periods of tree days as a blocking factor. All data were statistically analyzed with SPSS software 11.5 for Windows TM (SPSS Inc., Chicago, IL, USA) and significance differences among treatment means were determined with Duncan’s multiple range test at P ≤ 0.05. The relationship between sap flow and weight loss, measured gravimetrically, was determined by linear regression analysis.

2.2. Xylem vessel anatomy

This part of the research was conducted in the Propagation and Histological Laboratories at the Catholic University of Valparaiso, Chile.

Histological sections of the xylem were prepared from three of the trees used in the water use experiment plus three additional trees (at approximately the same state of development as those used in the water use experiment) of each of H/D7 and H/TC to determine the diameter and number of vessels in the stem of the scion and the stem and roots of the rootstock on the grafted trees. Root and stem samples were fixed in a formalin, acetic acid and alcohol solution (10 formalin:5 acetic acid:50 ethanol, by volume) (Ruzin, 1999). The tissue was embedded in a water-soluble wax. Sections that were 16–18 μm thick were cut from the embedded stem and 5 μm thick sections were cut from the embedded root using a rotary microtome (Spencer 820 Microtome, American Optical Co., Buffalo, NY, USA). Sections were stained with safranin and fast green. For ‘Hass’ (scion) stem analysis, six cross sections were obtained from 5 cm above the graft union. For analysis of the stem of the rootstocks of grafted trees, six cross sections were obtained from 5 cm below the graft union. For root sections, five root samples were collected 2.5 cm above root tips from second-order roots (diameters between 1 and 2 mm) from each tree (30 samples per rootstock) (Fig. 1).

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Histological sections were observed at 10× using an Olympus vanox BX40 compound microscope attached to a computer and camera (Sony, CCD model DXC 107A). A total of 6 stem sections, one from each single-tree replication from each scion and rootstock were prepared and 20 microscope fields were examined and photographed for each stem section resulting in 120 stem section photographs of the scion and rootstock of each H/D7 and H/TC. From each of the six single-tree replications of H/D7 and H/TC, a total of five root sections were prepared and five microscope fields were examined and photographed per section for a total of 150 root section photographs for each H/D7 and H/TC. The size of each field was 250 μm × 250 μm.

Images were analyzed for average vessel diameter using Sigma Scan Pro 5.0 software (Systat Software, Richmond, CA, USA). Scion Image for Windows Beta 4.02 (Scion Corporation, Frederick, MA, USA) was used to determine the average number of vessel per square millimeter.

All data were statistically analyzed with SPSS software (SPSS Inc., Chicago, IL, USA). Significant of differences among treatment means were determined with Duncan’s multiple range tests at $P \leq 0.05$.

### 3. Results

#### 3.1. Tree morphology

All the trees in each treatment were approximately the same size, leaf area, stem diameter and root dry weight (Table 1).

#### 3.2. Sap flow and cumulative weight loss

There was a strong positive linear correlation between sap flow measured by the steady state heat balance technique and transpiration determined by cumulative weight loss of plants and soil in the pots ($r^2 = 0.98$; Fig. 2). There was no difference among cultivars for the correlation between the sap flow and water loss.

##### 3.2.1. Daily sap flow

There were significant differences in daily sap flow (from sunrise to sunset) among the different avocado varieties tested (Table 2). The nongrafted trees had 40% higher sap flow rates than the grafted trees in both cases (D7 and TC). Nongrafted D7 had a 29% higher sap flow rate than nongrafted TC and the same pattern was observed for grafted D7 (H/TD7), that had a 29% higher sap flow rate than grafted TC (H/TC).

The diurnal pattern of sap flow, measured with the sap flow gauge, showed a typical bell shaped curve for each nongrafted or scion/rootstock combination tested; a steep rise during the morning with the maximum rate at about midday (when vapor pressure deficit was its maximum), followed by a sustained gradual decrease until late in the afternoon. Duke 7 exhibited the highest rate of sap flow (91.6 kg h$^{-1}$ at midday), TC and H/D7 had intermediate rates (60–75 kg h$^{-1}$), and H/TC the lowest rate (50 g h$^{-1}$) (Fig. 3).

![Fig. 1. Diagram showing the locations of histological sample collection.](image)

![Fig. 2. Relationship between sap flow and weight loss due to transpiration of potted 'Hass' trees on Duke 7 and Toro Canyon clonal rootstocks. Symbols represent individual data points for the two rootstocks combined and lines represent the linear regression line and 95% confidence intervals.](image)

<table>
<thead>
<tr>
<th>Tree or rootstock/scion combination</th>
<th>Root dry weight (g)</th>
<th>Stem diameter (mm)</th>
<th>Leaf area (m$^2$)</th>
<th>Tree height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H/D7</td>
<td>72.3 a*</td>
<td>14.5 a</td>
<td>35.0a</td>
<td>1.1 a</td>
</tr>
<tr>
<td>D7</td>
<td>73.9 a</td>
<td>13.9 a</td>
<td>34.0 a</td>
<td>1.2 a</td>
</tr>
<tr>
<td>H/TC</td>
<td>69.2 a</td>
<td>14.1 a</td>
<td>38.0 a</td>
<td>1.2 a</td>
</tr>
<tr>
<td>TC</td>
<td>76.7 a</td>
<td>14.3 a</td>
<td>36.0 a</td>
<td>1.2 a</td>
</tr>
</tbody>
</table>

* Data are means of three replications per combination. Means in each column with the same letters indicate no significant difference according to Duncan’s multiple range test ($P \leq 0.05$).
3.3. Xylem vessel features

The anatomical differences in the xylem vessel features in root, and stem of scion and rootstocks are shown in Fig. 4. Table 4 shows the quantitative measurements of the xylem from each of the varieties. No significant differences were found between H/TC and H/D7 in xylem vessel area, number of vessel per square

<table>
<thead>
<tr>
<th>Combination</th>
<th>Sap flow (kg day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7</td>
<td>2.8 a</td>
</tr>
<tr>
<td>TC</td>
<td>2.0 b</td>
</tr>
<tr>
<td>H/D7</td>
<td>1.7 c</td>
</tr>
<tr>
<td>H/TC</td>
<td>1.2 d</td>
</tr>
</tbody>
</table>

* Different letters indicate significant differences among means according to Duncan’s multiple range test (P ≤ 0.05).

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Table 2
Daily sap flow (kg H₂O) measured with a stem gauge.

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Fig. 3. Diurnal course of sap flow on ‘Hass’ trees grafted on Duke 7 rootstock (H/D7), ungrafted Duke7 (D7), ‘Hass’ grafted on Toro Canyon rootstock (H/TC) and ungrafted Toro Canyon (TC) trees on 6 August 2006.

Fig. 4. Light micrographs showing the xylem vessel network in transversal sections; (A) stem of ‘Hass’ grafted onto Duke 7; (B) Stem of ‘Hass’ grafted onto Toro Canyon; (C) Duke 7 (stem); (D) Toro Canyon (stem); (E) Duke 7 (root); (F) Toro Canyon (root) (10×).
millimeter and total conduit area of the ‘Hass’ scion. There were significant differences in xylem vessel area, number of vessel per square millimeter and total conduit area between the ‘Hass’ scion and the stems of the D7 and TC rootstocks. ‘Hass’ had narrower vessel diameters and a higher frequency of vessels, and total conduit area than D7 and TC. Also, D7 exhibited narrower vessel diameter than TC but there was no difference between D7 and TC in the frequency of vessels and the total conduit area (Table 3). In the roots, D7 had widest vessels and a lower frequency of vessels than TC (Table 4). Also total conduit area was 19% greater in D7 than in TC.

4. Discussion

The heat balance method for measuring sap flow in intact plants yielded reliable estimates of the water consumption in avocado trees, which is similar to results of studies with other tree species (Valancogne and Nass, 1993; Hussein and McFarland, 1994; Massai and Gucci, 1997; Bethenod et al., 2000). Therefore, this may be a useful nondestructive, relatively inexpensive monitoring technique for the automated management of irrigation in an orchard.

In the present study, avocado rootstocks affected water use by ‘Hass’. Between nongrafted trees, D7 had higher water consumption than TC and when they were grafted the same pattern was showed. However, in both cases the graft had the same effect in terms of the restriction of the water flow through the whole tree. This response could be due to the fact that no differences were found between D7 and TC in stem xylem vessel features (xylem vessel area, number of vessel per square millimeter and total conduit area), but significant differences were found between those rootstocks and the scion (‘Hass’).

Atkinson et al. (2001) showed that the graft union affects water relations in apple trees due to the development of morphological and anatomical changes in this zone that creates a discontinuity of the vascular tissues. Olmstead et al. (2006) provided evidence that a combination of smaller and fewer vessels in the scion and graft union as well as irregular vessel orientation in the vascular tissue could contribute to hydraulic resistance in the graft union. This discontinuity caused a significant resistance to water flow from the root system to the canopy. Citrus rootstocks that promote vigor in the scion, have high root conductivity, higher rates of leaf gas exchange, increased leaf N and P concentration and higher shoot to root ratios when grown as nongrafted plants (Syvertsen, 1981; Syvertsen and Graham, 1985). According to Massai and Gucci (1997), the hydraulic restrictions to the xylem caused by the graft union might have induced a more rapid water deficit in the scion tissue which caused a partial stomatal closure, thereby slowing the water movement.

The different sap flow in D7 and TC was probably due to the vigor of the rootstocks and the anatomy of the roots. Toro Canyon rootstocks have a moderate size under normal edaphic conditions. On a rating scale of 0–5 relative to tree size, with 5 being the largest, D7 was rated as a 5, whereas TC was rated a 4 (Rose, 2003). Solarì et al. (2006) found that peach trees on rootstocks with a size controlling effect had lower hydraulic conductance than trees on vigorous rootstocks. Clearwater et al. (2004) showed in Kiwifruit that whole plant hydraulic conductance and xylem sap flow were slower in the low vigor rootstocks than in rootstocks with more vigor. Olien and Lakso (1986) suggested that the low hydraulic conductivity in dwarfing apple rootstocks might be related to low hydraulic conductivity in the root system of these rootstocks, and/or reduced hydraulic conductivity caused by the graft union.

Studies by Hussein and McFarland (1994) of apple trees indicated that the cause of the differences in sap flow rates between different rootstocks might be related to differences in root characteristics, xylem anatomy, or other features related to the hydraulic architecture from the roots to the bud union. Variation in citrus water relations that have been attributed to rootstocks are probably due to differences in root quantity, distribution and/or apparent efficiencies in water uptake and transport (Syvertsen, 1981). Water uptake by the root is complex, depending on root structure, root anatomy and the pattern by which different parts of the root contribute to overall water transport (Steudle et al., 1987). There has been some research on root morphology and anatomy in relation to root hydraulic conductance in fruit trees. According to anatomical studies by Beakbane and Thompson (1939), dwarfing rootstocks in apple tend to have roots with fewer and smaller xylem vessel than vigorous rootstocks. Wang et al. (1994) reported that flower promoting rootstocks, tended to have a higher total cross sectional area of xylem vessels in the stele than non-flower promoting rootstocks, implying higher root axial conductivity.

In the present study, the greater conduit areas in the roots of D7 than TC can result in more rapid transport of water through the root system of D7 compared to TC. Since xylem vessels are the major water conducting element in avocado roots, vessel size, vessel number and total vessel area might well affect the efficiency of water conductance. The larger cross sectional area of vessels in D7 may facilitate transport of greater volumes of water per unit time to the scion. It is important to consider that, in terms of the anatomy of the roots, TC tended to have a greater proportion of cortex than stele in comparison to D7 (data not shown). Studies of different species by Rieger and Litvin (1999) showed the strong role of the cortex in root hydraulic con-

### Table 3

<table>
<thead>
<tr>
<th>Variety</th>
<th>Average xylem vessel area (µm²)</th>
<th>Frequency (number of vessel mm⁻²)</th>
<th>Total conduit area (µm²)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>H/D7 (stem)</td>
<td>733.1 c</td>
<td>312.6 a</td>
<td>200,734.7 a</td>
</tr>
<tr>
<td>H/TC (stem)</td>
<td>575.3 c</td>
<td>349.8 a</td>
<td>187,896.2 a</td>
</tr>
<tr>
<td>D7 (stem)</td>
<td>1248.7 b</td>
<td>124.1 b</td>
<td>154,469.0 b</td>
</tr>
<tr>
<td>TC (stem)</td>
<td>1536.1 a</td>
<td>107.1 b</td>
<td>161,243.0 b</td>
</tr>
</tbody>
</table>

ᵃ Different letters indicate significant differences among means according to Duncan's multiple range test (P < 0.05).

### Table 4

<table>
<thead>
<tr>
<th>Variety</th>
<th>Average xylem vessel area (µm²)</th>
<th>Frequency (number of vessels mm⁻²)</th>
<th>Total conduit area (µm²)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duke 7</td>
<td>598.4 a</td>
<td>185.1 b</td>
<td>110,386.3 a</td>
</tr>
<tr>
<td>Toro Canyon</td>
<td>265.8 b</td>
<td>336.9 a</td>
<td>89,630 b</td>
</tr>
</tbody>
</table>

ᵃ Different letters indicate significant differences among means according to Duncan's multiple range test (P < 0.05).
ductivity. The cortex can exert an appreciable resistance to water flow in the roots and species with a wider cortex had a lower root hydraulic conductivity. The root hydraulic system can be separated into a radial and axial conductance with the radial conductance generated by radial water movement from the root surface to the conductive tissue (through xylem vessels). Therefore, studies of water movement and the anatomy of the avocado roots need to be continued to determine the role of the cortex in avocado root hydraulic conductivity. Another anatomical aspect that has been associated with root hydraulic conductivity is the thickness of the exodermis (Eissenstat and Achor, 1999). As the exodermis becomes more impermeable, there should be greater capacity for mineral nutrient uptake and water absorption. However, in the present study with avocado, no anatomical differences were observed between roots of the rootstocks.

Studies done by Mickelbart and Arpaia (2002) showed that TC had a greater ability to exclude Na and Cl from the aerial part of the tree then D7 under saline condition. Also, significant differences were found between TC and D7 in the ability to absorb mineral nutrients (Mickelbart et al., 2007). The difference between those rootstocks may be due to differences in the rate of water movement and differences in root anatomy as observed in the present study and the fact that the D7 is a Mexican race rootstock and TC is a Mexican x Guatemalan hybrid rootstock. Thus, the different anatomical and physiological features of these varieties may be related to the adaptation of these different ecotypes to different geographical locations in their native habitats. Thus, more research is needed to identify phenotypic and physiological parameters that may be responsible for genotypic differences related to the different ecotypes.

5. Conclusion

The choice of an appropriate rootstock is one of the most crucial considerations in the production of fruit trees. The effect of the rootstocks on tree water movement is a critical concern for reducing water consumption but optimizing tree growth and productivity in orchards where the availability of water is limited during part of the growing season. The results of this study indicate that the differences in water consumption of ‘Hass’ avocado scions on different rootstocks may be associated with differences in the efficiency of the root to uptake water across conductive tissue, which may be linked to differences in the amount of xylem vessels in the root.

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References