Water Loss by Floral Structures of Avocado (*Persea americana* cv. Fuerte) during Flowering

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Abstract

Transpiration rate (E) and epidermal conductance (gₑ) were determined for avocado leaves and floral parts under controlled environmental conditions (28°C; PAR 60 μmole quanta m⁻² s⁻¹; VPD 1.14 kPa). E of hydrated flowers was shown to be approximately 60% that of similarly treated leaves. Around 13% of total transpirational water loss from tree canopies could be attributed to floral organs. The mean gₑ of leaves and flower panicles was similar at 0.028 and 0.023 mm s⁻¹ respectively. The available canopy surface area for water loss increased by c. 90% during flowering.

Xylem water potentials in panicle segments with open flowers were lower than those of mature leaves measured on trees in the field. Maximum transpiration rate of leaves measured in the field was 6.7 μg cm⁻² s⁻¹ at 0700 hours, declining to 3.0 μg cm⁻² s⁻¹ at 1100 hours, while stomatal conductance (gₛ) on the same leaves fell from 10.2 mm s⁻¹ at 0700 hours to 2.52 mm s⁻¹ at 1100 hours.

Scanning Electron Microscope studies of leaves and floral structures highlighted morphological and anatomical features for water conservation. Mature leaves have an epicuticular wax-like layer on the adaxial surface. Stomates were located only on the abaxial surface, which was also covered with wax-like deposits. Stomate density was estimated at 73,000 cm⁻² on sun leaves. Stomates were also located on abaxial surfaces of flower sepals and petals. All floral structures were densely pubescent, thereby increasing the effective boundary layer depth.

Introduction

The avocado is a perennial evergreen tree indigenous to the rainforests of the humid subtropics and highland tropics of Central America (Popenoe 1920), but is now widely grown in semi-arid regions of the world. A massive leaf canopy gives a large evaporative surface, yet root growth is shallow with a limited absorptive surface (Rowell 1979). While productivity is generally lower in semi-arid regions than the humid subtropics, the general success of commercial avocado cultivar over a wide environmental range suggests morphological, anatomical and/or physiological adjustment to water stress.

Woody species have inefficient water transport systems, with leaf water potential dropping markedly as transpiration rate increases (Camacho-B et al. 1974). Scholefield et al. 1980 and Whiley et al. 1986 describe diurnal changes in avocado leaf water potential. They show that significant water deficit occurs in leaves during the light period, even though soil water is non-limiting.

The role of the leaf as the major photosynthetic organ has been widely reported. More recently photosynthetic activity in growing fruits has been studied (Bazzaz et al. 1979). However, the contribution of flowers per se to photosynthesis and potential water loss from plants has largely been ignored (Vu et al. 1985).

Avocado flowering is mostly terminal to the last vegetative flush and occurs during late winter and spring. The inflorescence is classified as a cymose panicle (Scholefield 1982). The flowers show marked synchronous alternating dichogamy (Robinson and Savage 1926), which is strongly influenced by temperature (Sedgley 1977; Sedgley and Grant 1983). This outbreeding...
mechanism is aided by complementary flowering types to allow cross-pollination (Robinson and Savage 1926), and profuse flower production. Cameron et al. (1952) estimated that mature trees produced up to 1.6 million flowers. In south-east Queensland flower panicles of cv. Fuerte are well-developed by mid-July, with flowering occurring over an eight week period (Whiley, unpublished data).

Thus floral growth in the avocado is a major event of tree phenology, with the potential to substantially increase water demand during the critical period of fruit set if there is significant water loss from flowers. The intention of this study was to examine water loss from avocado floral structures and to define their relative contribution to water loss during flowering.

Materials and Methods

Cultural and Environmental Details

Both laboratory and field experiments were carried out on 6-year-old avocado trees, cv. Fuerte, growing in a commercial orchard in south-east Queensland, approximate latitude 27° S. The soil type is described as a deep, free-draining sandy loam and the climate humid/subtropical (1800 mm average annual rainfall; January mean maximum/minimum temperatures: 28-2/18.9°C; June mean maximum/minimum temperatures: 21.2/8.5°C).

Rainfall was supplemented with irrigation, using one microsprinkler per tree. Irrigation was scheduled with readings from tensiometers placed inside the dripline at 300 mm deep. Trees were irrigated from when soil matric potential reached −0.4 MPa until it fell to −0.1 MPa.

Water Loss and Epidermal Conductance

Water loss from leaves and panicles were measured using the detached leaf method described by Slavik (1974) and Sinclair and Ludlow (1986). The transpiration rate \( \varepsilon \) of fully hydrated tissues and epidermal conductance \( g_e \) as defined by Sinclair and Ludlow (1986), when stomates are hydroactively closed (asymptote point on the water loss curve) was quantified under standard conditions.

Flowering terminals were harvested from field grown trees, cv. Fuerte, at 0600 hours when water deficit in leaf tissues is minimal (Whiley et al. 1986). Terminals harvested included matured summer flush leaves, as well as panicles, and these were placed in clear polyethylene bags and taken directly to the laboratory. The youngest fully expanded leaf (approximately 7-8 months old at the time of this study) and panicles with opened flowers were selected, and hydrated by standing the re-cut surfaces in distilled water under clear polyethylene film for 2 h at 28°C.

During hydration, leaves and panicles were illuminated at near the light compensation point of net photosynthesis (63 µmole quanta m\(^{-2}\) s\(^{-1}\); Scholefield et al. 1980) to minimize weight loss due to respiration.

Individual organs used in this study were leaves, pedicel peduncle components (p.p.c.) and panicles consisting of p.p.c. and four to six open flowers plus flower buds. Panicles were used to study inflorescence water loss, as individual flowers were too small for this technique. Ten replicates of each organ were measured.

Immediately prior to the commencement of water loss measurement, leaf petioles and p.p.c. were re-cut to remove the surface wetted length which had been immersed in water, and the cut surfaces sealed with bees-wax. They were then placed on a Mettler AK160 electronic balance, with the weighing pan enclosed in an airtight glass chamber held at a constant temperature of 28°C and PAR at 60 µmole quanta m\(^{-2}\) s\(^{-1}\). Chamber wet and dry bulb temperatures measured for vapour pressure deficit (VPD) calculations, were remotely monitored with an Esterline Angus E1124E multipoint recorder, while PAR was measured with a Lambda LI-185 light meter. The VPD in the chamber, calculated from \( e (1 - RH/100) \), where \( e \) = saturated vapour pressure of H\(_2\)O at 28°C and RH = relative humidity, was stabilized at 1.14 ± 0.6 kPa by the inclusion of three 60 ml containers of coarse grade silica gel. Fresh silica gel was used for each determination, and there was only a minor colour change in silica gel on the surface of each container during measurement. Weight loss from leaves and panicle components was measured at 1-min intervals over a 30 min period and recorded on a Mettler GA40 printer. Surface areas of leaves, open flowers and flower buds were measured on a Li-Cor Area Meter (LI 3000). The surface area of p.p.c. was calculated from \( r d l \), where \( d \) was the diameter of the stalk and \( l \) the length.

Water loss calculated for each sample was expressed in µg cm\(^{-2}\) s\(^{-1}\) and plotted against time. Mitscherlich
curves ($y = b_1 + b_2 e^{b_3 x}$) were fitted to each replication via non-linear regression to obtain estimates of the asymptotes. $g_c$ (mm s$^{-1}$) was calculated from asymptotes (Sinclair and Ludlow 1986) using standard diffusion equations from weight losses of a detached leaflet measured under defined evaporative conditions (Willis and Jefferies 1963). Calculated $g_c$ values were subjected to ANOVA.

**Leaf and Floral Water Status in the Field**

Field measurements of xylem water potential ($\Psi_0$) of leaves and panicle segments using the pressure chamber method (Scholander et al. 1965) were taken on 23 September 1986 from 10 trees, when soil moisture was non-limiting ($32.5 \pm 2.5\%$ vol., means $\pm$ s.e., $n = 30$; as determined by a neutron moisture probe in the 20-40 cm zone). The youngest fully expanded leaf and panicle segments with 3-5 open flowers were selected and enclosed in clear polyethylene bags prior to cutting (Turner and Long 1980), and transferred to the chamber for $\Psi_0$ determinations. Three $\Psi_0$ measurements were made on each organ from each tree at 2-h intervals from shortly after sunrise (0600 hours) to late afternoon (1600 hours). Temperature and PAR were measured during the day and VPD's calculated (Fig. 2b). Stomatal conductance ($g_s$) and leaf transpiration ($E_l$) were also measured on abaxial surfaces at 2-h intervals from 0700 hours to 1500 hours (Fig. 2c) with a LI-COR Null-balance Porometer (LI-1600). Field measurements taken earlier in September gave similar results which are not presented. Field measurements of $E_l$ were also made between 0800 hours and 1200 hours over several days during the flowering period when air temperatures were predicted to exceed 28°C.

**Area and Nature of Water Loss Surfaces**

To determine comparative areas of leaf and floral surfaces, 20 terminals consisting of spring and autumn vegetative flushes with subtending panicles, were harvested from random positions around the tree during peak anthesis. Terminals were separated into leaves, flowers and p.p.c., and their respective surface areas defined. Leaf area was measured with the LI-COR Area Meter, while flowers and p.p.c. surface areas were calculated separately from an area: dry weight (cm$^{-2}$ : g) relationship previously established by measuring areas and then dry weight of specific organs, viz. flowers, 1 cm$^2$, $7.23 \times 10^{-3} \pm 2.4 \times 10^{-4}$ g, means $\pm$ s.e., $n = 10$; p.p.c., 1 cm$^2$, $1.16 \times 10^{-2} \pm 7.1 \times 10^{-4}$ g, means $\pm$ s.e., $n = 10$.

Cuticular development differed between the two surfaces of the leaf with the adaxial surface having a well-developed wax-like layer (Fig. 3f). To test the differences in water loss between the two surfaces, hydrated leaves had one surface coated with vaseline immediately before placement in the environment chamber, and water loss was measured as previously described. It was established that the abaxial surface was the only one contributing significantly to water loss, and all leaf calculations were based accordingly. In contrast, cuticular development on floral organs was less definitive, and the assumption was made that significant water loss could occur from all floral surfaces (Table 2).

**Electron Microscopy**

A Philips 505 Scanning Electron Microscope (S.E.M.) was used to examine anatomical water conservation features of leaves, flowers and p.p.c. of the avocado, cv. Fuerte. Freeze-dried sections were made of leaf, and floral parts, viz. petal, sepal and p.p.c., which were inserted into an aluminium S.E.M. stub and examined for cuticle development, pubescence and stomate distribution. Standard scanning magnifications ($\times$221) producing black and white prints from several leaf samples were used to estimate stomate density.

**Results and Discussion**

**Water Loss and Epidermal Conductance**

Water loss data from leaves and panicle segments in the constant environment chamber showed two distinct stages (Fig. 1). First, there was the stage of rapid but declining water loss rate interpreted as a period during which stomates closed. Stomates were located on the abaxial surfaces of leaves and sepal and petals of flowers (see electron microscopy section). Second, there was a stage of linear but constant water loss when it was assumed that stomates were closed but the saturated vapour pressure inside the organs was still maintained (Sinclair and Ludlow 1986). In contrast the p.p.c. only exhibited the stage two pattern where the rate of water loss was linear and constant (Fig. 1). The rate of water loss was higher ($P < 0.01$) from leaves than for floral structures over the period studied, while panicle segments had a higher ($P < 0.01$) rate of loss than p.p.c.
The highest rates of water loss (E) measured for leaves and panicle segments in the chamber (28°C; VPD 1.14 ± 0.01 kPa; PAR 60 μmole quanta m⁻² s⁻¹) were 1.94 μg cm⁻² s⁻¹ and 1.14 μg cm⁻² s⁻¹ respectively (Fig. 1). Epidermal conductance (gₑ) of the leaves (Table 1) was higher than in panicle segments and p.p.c. (P < 0.01), while gₑ of panicle segments was greater (P < 0.01) than that of p.p.c. (P < 0.01)

![Graph showing water loss from detached hydrated cv. Fuerte leaves, panicle segments, and pedicel peduncle components over 30 min at 28°C, PAR 60 μmole quanta m⁻² s⁻¹ and VPD 1.15 kPa. Vertical bars represent 1.s.d. (P < 0.01).](image)

Table 1. Epidermal conductance (gₑ) of detached leaf, panicle and pedicel peduncle components of avocado cv. Fuerte, at 28°C, 60 μmole quanta m⁻² s⁻¹ and VPD of 1.14 kPa.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>gₑ (mm s⁻¹)</th>
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<tbody>
<tr>
<td>Leaf</td>
<td>0.028ᵃ</td>
</tr>
<tr>
<td>Panicle (flowers + p.p.c.)</td>
<td>0.023ᵇ</td>
</tr>
<tr>
<td>Pedicel peduncle components</td>
<td>0.013ᶜ</td>
</tr>
<tr>
<td>s.e. of mean</td>
<td>0.0025</td>
</tr>
</tbody>
</table>

Reasons for significantly higher gₑ of stomatous organs, i.e. panicles and leaves which were 43 and 54% greater than the p.p.c., were not established. However, contributing factors to increased rates of water loss from dehydrating tissues may include differences in cuticle composition, anatomical structural differences between tissues and/or stomatal leakage.

The gₑ measured for mature avocado leaves in our study is in agreement with the higher values for citrus (0.025-0.0006 mm s⁻¹) reported by Schonherr and Schmidt (1979). However, considerably higher values were reported by Elfving et al. (1972) for citrus leaves where gₑ was estimated at 0.1 mm s⁻¹. Kriedemann and Barrs (1981) suggest that this latter value may be the higher limit, as stomates may not have been completely closed. However, it is difficult to accurately compare values where data have been measured by different techniques and with leaves from diverse environmental conditions.

Leaf and Floral Water Status in the Field

Air temperature and VPD reached a maximum of 26°C and 1.91 kPa respectively at 1300 hours on the day that field Wₖ of leaves and panicle segments were measured (Fig. 2a), while PAR rose to 1930 μmole quanta m⁻² s⁻¹ at 1100 hours and then declined as cloud cover increased.
Field estimates of $\Psi_x$ were highest (least negative) at 0600 hours ($-0.32$ MPa leaves and $-0.49$ MPa panicle segments), falling to their lowest measured value at 1200 h ($-1.02$ MPa leaves and $-1.15$ MPa panicle segments), and then recovering during the afternoon (Fig. 2b). Measurements of $\Psi_x$ were significantly ($P < 0.01$) lower in panicles than in leaves at all defined points between 0600 and 1600 hours.

A disproportionally higher loss of water from fruit and stems when compared to leaves, during periods of high transpirational activity, has been demonstrated for many species (Schroeder and Weiland 1956; Till 1965). There is no apparent explanation from our water loss studies on detached tissues for the significantly lower $\Psi_x$ of flower panicles when compared to leaves on orchard trees, i.e. floral parts lose water at lower rates than leaves, yet the $\Psi_x$ of panicles became more negative than leaves during the day. However, it is suggested that a greater transpirational loss from leaves preferentially withdraws water from floral structures or replacement water moves preferentially to leaves owing to a more negative osmotic pressure which may occur in these organs.

Environmental conditions were not conducive to high water loss on the day the $\Psi_x$ of leaves and panicles were measured (Fig. 2a). More stressful daily conditions do occur during the flowering period of cv. Fuerte in south-east Queensland when $\Psi_x$ deficits may induce permanent damage to floral organs (viz. 25% of days exceed 28°C and 2.2 kPa VPD). Sedgely (1977) in growth chamber studies reported flower abscission with the cv. Fuerte at day temperatures of 33°C.

Transpiration rate ($E_t$) ($6.71 \pm 0.53 \mu g \, cm^{-2} \, s^{-1}$) and stomatal conductance ($g_s$) ($10.20 \pm 2.54 \, mm \, s^{-1}$) were highest when first measured at 0700 hours, and thereafter declined to $2.99 \pm 0.35 \mu g \, cm^{-2} \, s^{-1}$ and $2.52 \pm 0.41 \, mm \, s^{-1}$ respectively at 1100 h (Fig. 2c). A significant increase in both of these rates had occurred by 1500 hours.
The fall in $E_1$ and $g_s$ from 0700 hours to 1100 hours indicates some stomatal closure which is supported by Klepper's (1968) results for woody plants. The $g_s$ measured at 1200 hours on leaves in our study was similar to that reported by Bower (1978), but approximately twice the value of Scholefield et al. (1980), where measurements were taken under much more stressful conditions.

From measurements on shade leaves taken over 3 days in the field an $E_1$ of 2.0 ± 0.49 μg cm$^{-2}$ s$^{-1}$ (means ± s.e., $n = 10$), was defined at 28°C, 1.05 ± 0.08 kPa VPD and PAR in full sun 1850 μmole quanta m$^{-2}$ s$^{-1}$. This compares favourably with the $E$ (1.94 μg cm$^{-2}$ s$^{-1}$) measured on hydrated leaves at similar temperature and VPD in the controlled environment chamber.

**Area and Nature of Water Loss Surfaces**

From dissection and measurement of flowering terminals, it was established that leaves contributed the greatest surface area for water loss (Table 2). However, reproductive structures contributed significantly to tree canopy surface area having potential for water loss. The p.p.c. and flowers contributed equally to the surface area of the panicle with the combined surface areas of floral organs increasing canopy surface area by 90% over existing leaf surface area during peak anthesis.

On the assumption that the relationship between the transpiration rate of leaf and panicle under our controlled environment (1.94 μg cm$^{-2}$ s$^{-1}$, leaf: 1.44 μg cm$^{-2}$ s$^{-1}$, panicle) holds for field conditions, we estimate that flowering in this cultivar contributes c. 80% of the trees transpirational water loss (viz. flowers' contribution to canopy surface area is 22%, flowers' transpiration rate is 59% of leaves, 22 x 0.59 = 13%). Also with the significant contribution of floral structures to canopy surface area, we anticipate some impact on tree water loss through $g_c$ from these surfaces. Indeed preliminary soil matric potential measurements in the avocado tree root zone have shown a rapid depletion in soil water as flowering begins (Trochoulias, personal communication).

### Table 2. Surface area$^A$ for potential water loss ($E$ and $g_s$) of leaves and panicles from flowering terminals of avocado, cv. Fuerte

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Surface area (cm$^2$)</th>
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<tbody>
<tr>
<td>Leaves</td>
<td>1229.3 ± 393.2</td>
</tr>
<tr>
<td>Panicles (flowers + p.p.c.)</td>
<td>1100.2 ± 364.4</td>
</tr>
<tr>
<td>Pedicel peduncle components</td>
<td>581.8 ± 174.6</td>
</tr>
<tr>
<td>Flowers</td>
<td>518.4 ± 163.7</td>
</tr>
</tbody>
</table>

$^A$Leaf surface area is calculated as an abaxial value. Flower surface area is calculated from both adaxial and abaxial surfaces.

Electron Microscopy

S.E.M. study of mature leaves and floral structures of avocado confirmed the presence of stomates on the abaxial surface of leaves (Fig. 3a). Stomates were also located on the abaxial surface of sepals (Fig. 3b) and petals (not shown). No stomates were found on the adaxial surfaces of leaves, petals and sepals or on the p.p.c. Stomate density on abaxial surface of leaves was 73 000 ± 3 000 cm$^{-2}$ (means ± s.e., $n = 6$). There was no epidermal wax-like deposit on the flower petals, sepals or p.p.c. All floral parts were densely pubescent (Fig. 3c), which prevented an accurate assessment of stomate density.

Confinement of stomates to the abaxial surface of the avocado leaf was also noted by Scholefield and Kriedmann (1979). A stomate density of c. 73 000 cm$^{-2}$ falls into the range reported for other subtropical and tropical evergreens, e.g. citrus species 32 000 to 87 000 cm$^{-2}$ (Hirano 1931), and mango c. 80 000 cm$^{-2}$ (Kriedemann 1986). However, our figure differs from...
Fig. 3. (a) Stomates on the abaxial surface of mature leaves (×221). (b) Stomate on abaxial surfaces of sepal (×1250).
Fig. 3.(c) Epidermal pubescence on abaxial surface of petal ($\times780$). (d) Epicuticular deposit (wax) on adaxial leaf surface ($\times655$).
Scholefield and Kriedemann (1979) who reported stomate density at c. 40 000 cm\(^{-2}\) on fully expanded avocado (cv. Fuerte) leaves. This anomaly may be explained by environmental differences under which the leaves for each study developed, i.e. moist subtropical versus arid Mediterranean, or variations in on-tree-sites from which leaves were chosen. Hirano (1931) found that stomate density of a sun leaf of ‘Eureka’ lemon (63 600 cm\(^{-2}\)) was nearly twice that of a shade leaf (32 200 cm\(^{-2}\)).

While the pubescence on the flower petals and sepals prevented estimates of stomate density, nevertheless the occasional stomate could be located on the abaxial surface of these organs. Chlorophyllous floral structures of citrus contribute to photosynthesis (Vu \textit{et al.} 1985), and it is likely that these chlorophyllous stomatal bearing organs of the avocado flower similarly contribute to CO\(_2\) assimilation.

Fig. 3 (e). Epicuticular deposit (wax) on abaxial leaf surface (\(\times 775\)).

A layer of epicuticular wax-like substance was found on both leaf surfaces (Fig. 3d, 3e). The wax-like substance on the adaxial surface appeared as a continuous layer, while on the abaxial surface it was globular, allowing stomate access for gas exchange. Epicuticular wax, an effective water barrier, is present on leaves of many plants. Its removal from citrus and pear leaves increases cuticular water permeability by a factor of 300-500 (Schonherr 1976). Likewise the dense cover of epidermal hair, as seen on the floral parts, induces a larger effective boundary layer which reduces water loss.

\textit{Horticultural Implications}

Flowering is a major physiological event in the growth cycle of the avocado tree with abscised flowers contributing approximately 8\% of annual dry matter production (Cameron \textit{et al.} 1952). During flowering, the levels of non-structural carbohydrate stored in the trunk and leaf concentrations of mobile nutrients decline rapidly (Scholefield \textit{et al.} 1985; Whiley unpublished data).

In addition, our study has shown that floral structures contribute significantly to water loss through epidermal surfaces of the panicle during flowering.
While these organs have some anatomical features which limit water loss, they are nevertheless likely to have higher water deficits than leaves during periods of moderate transpirational demand. Where excessive moisture stress is imposed, irreversible damage to floral organs may occur, limiting potential fruit set and retention.

Hence, with the significant increase in canopy surface area, water requirements of well-foliated flowering trees will rise, especially in environments experiencing periods of high evaporative demand. Attention should be given to irrigation scheduling and environmental modification (e.g. windbreaks) to reduce the potential adverse impact of this physiological event on orchard productivity.

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