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Anatomy and Transpiration of the Avocado Inflorescence

MICHAEL M. BLANKE* and CAROL J. LOVATT

Department of Botany and Plant Sciences, University of California, Riverside, California 92521, USA

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Structure and function of the inflorescence of cv. 'Hass' and 'Fuerte' avocado (Persea americana Mill.) were examined by scanning electron microscopy (SEM) and by porometry. Sepals and petals could not be distinguished by their position in the flower, by visual gross morphology or by microscopic surface structure and were hence designated as tepals. These tepals were arranged in two whorls of three, followed by two whorls of three outer and three inner stamens, each opposite a tepal. The most conspicuous feature of tepals, developing leaves and peduncles was the dense cover of hairs which were most frequent on the adaxial tepal surface (925–1200 trichomes mm^{-2}), followed by their abaxial surface (625–1000 mm^{-2}) and peduncles (375–655 mm^{-2}). Stomata were absent from the adaxial surfaces of both tepals and leaves, as well as peduncles. On the tepals, abaxial stomata appeared functional, small (8–9 × 11–13 μm) and scarce with 2·8–3·4 stomata mm^{-2}, i.e. very low relative to avocado leaves (350–510 stomata mm^{-2}) or young fruit (50–75 stomata mm^{-2}). However, flowers including tepals transpired 1·2–1·3 mmol under field conditions in Southern California (1·6–2 kPa), i.e. in excess of leaves (0·7–1·1 mmol) and peduncles (0·6–0·8 mmolH_2O m^{-2} s^{-1}). This situation was attributed to the few small but functional abaxial stomata on the tepal, in contrast to 80% closed stomata and dense epicuticular wax cover in form of rodlets on young and dendritic crystals on old leaves including the guard cells, and absence of stomata from the peduncle.

Key words: *Persea americana* Mill., avocado, bioenergetics, flower, fruit, leaf, peduncle, scanning electron microscopy, stomata, transpiration, petals, sepals, tepals.

INTRODUCTION

Flowering is particularly water and energy expensive in many fruit crops. In avocado (*Persea americana* Mill.), flowering is associated with increased transpiration (Whiley, Chapman and Saranah, 1988) and may impose alternate bearing (Scholefield, Sedgley and Alexander, 1985). Avocado trees bear excessive numbers of flowers in relation to final number of fruit harvested per tree, e.g. 1–2 million flowers, but only 200–300 fruit (Whiley *et al.*, 1988). Thus, fruit yield in avocado represents only 0·002–0·02% of the original flowers formed.

Avocado growers in Southern California are advised to increase their orchard irrigation during flowering from bud break to fruit set (Whitney and Bender, 1992). This recommendation, however, is based more on empirical rather than scientific evidence, since data on anatomy and transpiration of the avocado inflorescence and its leaves are scarce and contradictory. The perianth of the avocado flower is composed of an outer whorl of three sepals and an inner whorl of three similar petals (Scholefield, 1982). 'The only way that they can be differentiated with certainty is the placement of each of the three sepals opposite an inner stamen, while each petal is opposite a staminode' (Bergh, 1975). These criteria became inadequate, when Sedgley (1985) reported as many as nine stamens in three whorls rather than six stamens in two whorls. On the sepals or petals, 'the occasional stoma could be located on the abaxial surface of these organs' (Whiley *et al.*, 1988). Whiley *et al.* (1988) measured transpiration gravimetrically as reduction in fresh weight of detached avocado inflorescences under laboratory conditions.

In the light of the alleged costs in terms of energy and water loss, the present work examines the surface of avocado inflorescences and its leaves by scanning electron microscopy. This is to differentiate sepals from petals, to determine their stomatal densities and to examine the surfaces for the presence of hairs and wax which may also affect transpiration. The results are used to aid interpretation of transpiration rates obtained in parallel porometry measurements under typical conditions of Southern California.

MATERIALS AND METHODS

Plant material

Avocado cv. 'Hass' and 'Fuerte' grafted to Duke 7 rootstock flowered in the last two weeks of Apr. 1992 on the campus of the University of California, Riverside (UCR). Apical inflorescences were collected in the morning when flowers opened and examined for gross morphology by SEM in the Department of Biological Sciences of UCR within 2 h of sampling to produce Figs 1–6. For examination of the epicuticular wax fine structure of foliage leaves, they were sent to Long Ashton Research Station, Bristol, UK (LARS) by air courier.

* Present address for correspondence: Institut für Obstbau und Gemüsebau der Universität Bonn, Auf dem Hügel 6, D-5300 Bonn, Germany.

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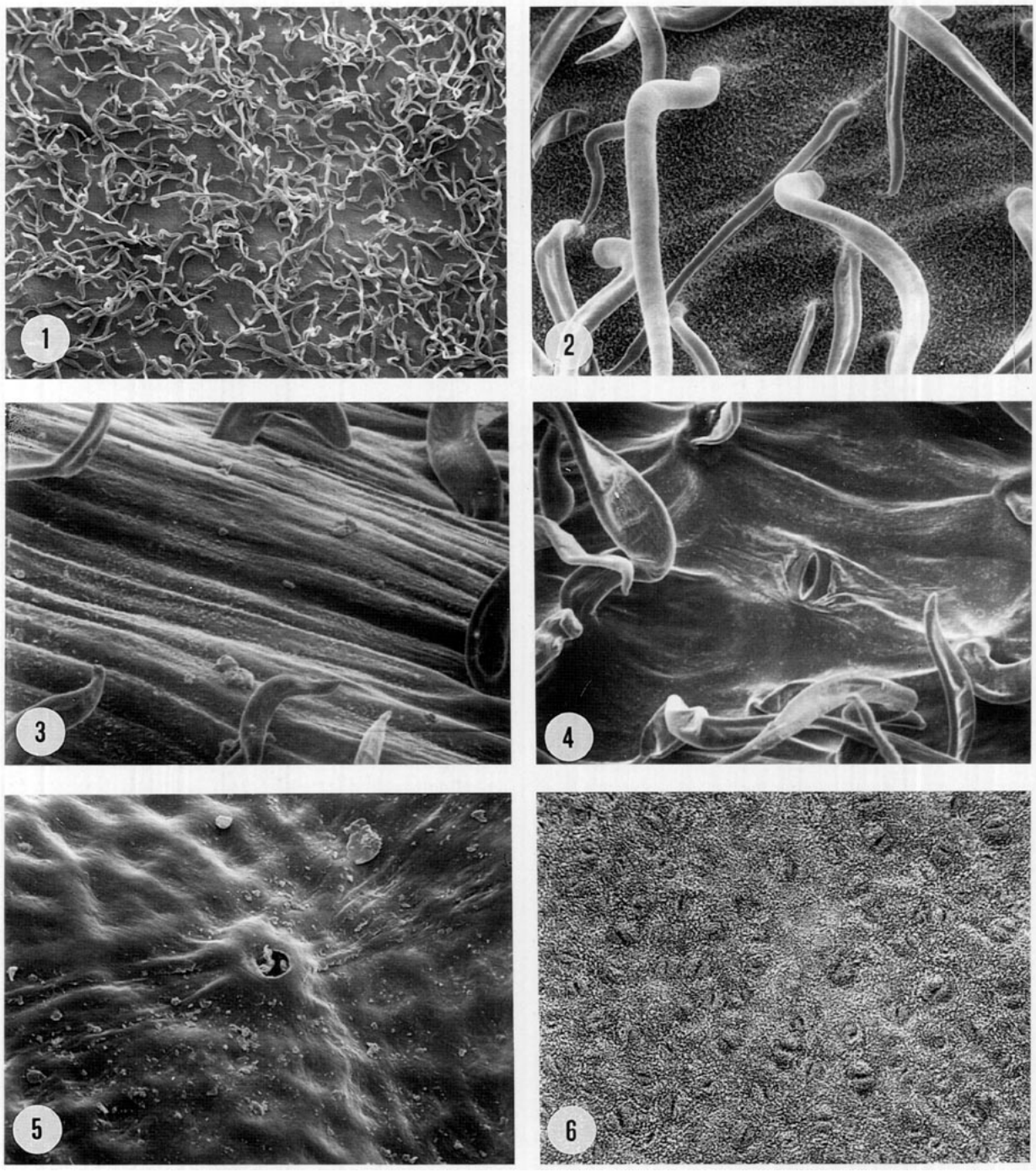

FIGS 1–6. SEM micrographs of a 'Hass' inflorescence and its leaves at anthesis showing non-stomatal (Figs 1–3 and 5) and stomatal (Figs 4 and 6) surfaces.

FIG. 1. The *adaxial* surface of the **tepals** is densely covered by trichomes and devoid of stomata. × 62.

FIG. 2. The *abaxial* surface of a developing avocado **leaf** (10% of final size) was similarly covered by trichomes as the tepals, but stomata had not yet started to develop. × 500. (The *adaxial* surface of developing avocado leaves appeared very similar and is hence not shown.)

FIG. 3. Surface of the **peduncles** exhibited fewer trichomes and was devoid of stomata. The undulated pattern was parallel to the growing axis. × 620.

FIG. 4. The gross morphology, but with apparently functioning stomata, of the *abaxial* surface of the **tepals** resembled the adaxial one shown in Fig. 1 with many trichomes. × 745.

FIG. 5. The *adaxial* surface of fully expanded avocado **leaves** exhibits hair base cells with hairs broken off. × 500.

FIG. 6. *Abaxial* surface of fully expanded avocado **leaf** showing the distinct and dense epicuticular wax which also covers the stomata. × 185.

Scanning electron microscopy

Avocado inflorescences and their leaves were examined by scanning electron microscopy (SEM) as described by Blanke and Leyhe (1987). For each flower under examination, all petals/sepals of a flower were mounted on a microscope stub using colloidal silver (Biorad). Mounted specimens were gold-coated in an EMSCOPE SC 500 sputter coater for 3 min at 14 mA. Coated specimens were studied in a Philips SEM 515 at an accelerating voltage of 5 kV and a spot size of 200 nm. Micrographs were taken with a Polaroid camera using 18 DIN 4 × 5 Polaroid 55 film. Abaxial leaf or tepal stomata were covered by crystalline epicuticular wax or hairs, respectively, and could not be accurately counted. Leaves were hence dewaxed by two 30-s dips in chloroform (E. A. Baker, pers. comm.) or trichomes removed from pubescent tissue. Stomata were counted on each petal/sepal (4·5 mm^2) of each avocado flower examined and on 0·25 × 0·25 mm squares for inflorescence leaves. Trichomes were counted on 0·25 × 0·25 mm squares of pubescent tissue. Counts were repeated for 20 flowers or 20 leaves picked randomly from different avocado trees.

Transpiration measurement

Transpiration and stomatal conductance of attached tepals, peduncles or leaves were measured in the field with a portable porometer type LCA 3 (ADC, Hoddesdon, Herts., UK) and a specially designed cuvette, both shipped from Bonn, Germany. Cuvette air was vigorously stirred to give a boundary layer resistance (r_b) of 0·19 m^2 s mol^{-1}. Flow rates into the cuvette and into the LCA 3 were controlled by two mass flowmeters of the LCA 3. Transpiration and stomatal conductance were determined by the differences in humidity of air entering and leaving the cuvette. One apical flower, peduncle or leaf section was enclosed in the cuvette. Measurements were repeated three times on typical mornings between 0800 and 1000 h in Apr. 1992 with temperatures of 24–28 °C, vapour pressure deficits (VPD) of 1·6–2·0 kPa and a PAR of 1200–1400 μmol m^{-2} s^{-1}.

RESULTS

Gross morphology

In flowers of both the 'Hass' and 'Fuerte' avocado, sepals and petals were indistinguishable by either visual or by microscopic observations. Both floral parts were 3·2–3·3 mm long and 1·6–1·7 mm at their widest point. In addition to size, their shape and colour were the same. Petals and sepals also could not be distinguished by their position in the flower. They were arranged in two whorls of three, followed by two whorls of three outer and three inner stamens, each opposite a petal/sepal. Six staminodes were located opposite the overlap between two petals/sepals in the whorl intermediate to the two whorls of stamens.

Surface structures

The most conspicuous feature of the adaxial and abaxial surfaces of the petals/sepals (Fig. 1) or developing leaves in the inflorescence (Fig. 2) was the dense cover of hairs. This pubescence was due to long, thin and unicellular trichomes, 5–8 μm in diameter and 80–220 μm long. On the adaxial petal/sepal surface, 925–1200 trichomes mm^{-2} were observed without differences in frequency between petals or sepals (Table 1). Trichomes were less frequent on the abaxial surface of the petals/sepals (625–1000 mm^{-2}) (Fig. 1) and on the peduncles (375–655 mm^{-2}) (Fig. 3), but scarce on fully expanded leaves (1–8 trichomes mm^{-2}) (Table 1). The surface of the peduncles appeared undulating (Fig. 3) with the structures parallel to the growing axis, while the abaxial surface of young avocado leaves (Fig. 2) resembled that of the petals/sepals (Fig. 1). Epicuticular crystalline wax structures in form of 0·4–0·6 μm rodlets densely covered the abaxial surface of young leaves and formed dendritic irregular crystals (not shown) on fully expanded leaves including the stomata, thereby preventing accurate assessment of stomatal densities and sizes (Fig. 6). Two 30-s dips in chloroform removed crystalline epicuticular wax and parts of the underlying amorphous wax layer making the guard cells clearly visible. The adaxial surface of the mature leaf exhibited an amorphous appearance with little crystalline wax. Hair base cells were protuberant, sometimes partially wax-plugged (Fig. 5), and 6–10 μm in diameter.

Stomata

For both 'Hass' and 'Fuerte', stomata in the avocado flower were found exclusively on the abaxial surface, i.e. the distal or outer side of the petals/sepals (Fig. 4). Stomata were clearly identified and easily distinguished from the smaller (6–10 μm diameter) and raised hair base cells lacking elliptical guard cells. All stomata observed on the petals/sepals, i.e. approx. 300, were not raised and appeared regulatory. The guard cells were of the common elliptical type with vestibules mostly open and not plugged. Stomatal size was uniformly 8–9 × 11–13 μm (vestibules 1–2 × 6–8 μm). Stomata were widely, but evenly, distributed on the abaxial surface, i.e. not patchy. Their density was 2·8–3·4 stomata mm^{-2} (Table 1), equivalent to 13–16 stomata per petal/sepal and 78–96 stomata for the petals/sepals of an avocado flower. Stomata, however, were absent from the adaxial surface of petals/sepals (Fig. 1) and leaves (Figs 2 and 5), and from the peduncle (Fig. 3). Leaves in the avocado inflorescence had 350–510 stomata mm^{-2} on their abaxial surface; most of them were not open (Fig. 6).

Transpiration

Petals/sepals and leaves were hypostomatic (Table 1), whereas stomata were evenly distributed over the avocado fruit surface (Blanke and Bower, 1990). Hence, transpiration is given for one side of the leaf, but on per total fruit surface in Table 1. The avocado flower (mostly petals/sepals) transpired 1·2–1·3 mmolH_2O m^{-2} s^{-1} compared to 0·7 –1·1 mmolH_2O m^{-2} s^{-1} for the leaves.

DISCUSSION

Sepals and petals of the avocado flower closely resembled foliage avocado leaves by having a protecting cuticle and structures such as stomata and trichomes (Fig. 4). Sepals

TABLE 1. *Surface features of avocado inflorescence*

Feature (Unit)	Tepal		Peduncle	Leaf		Fruit
	Adaxial	Abaxial		Adaxial	Abaxial	
Trichomes (mm^{-2})	925–1200	625–1000	375–655	⩽ 1	1–8	50–100*
Stomata (mm^{-2})	0	2·8–3·4	0	0	350–510	50–75*
Transpiration ($mmolH_2O\ m^{-2}\ s^{-1}$)	1·2–1·3		0·6–0·8	0·7–1·1		4·5–4·7

Field conditions of transpiration measurements (0800–1000 h in Apr. 1992) were temperatures of 24–28 °C, vapour pressure deficits (VPD) of 1·6–2·0 kPa and PAR of 1200–1400 $\mu mol\ m^{-2}\ s^{-1}$.
* Data from Blanke and Bower, 1990.

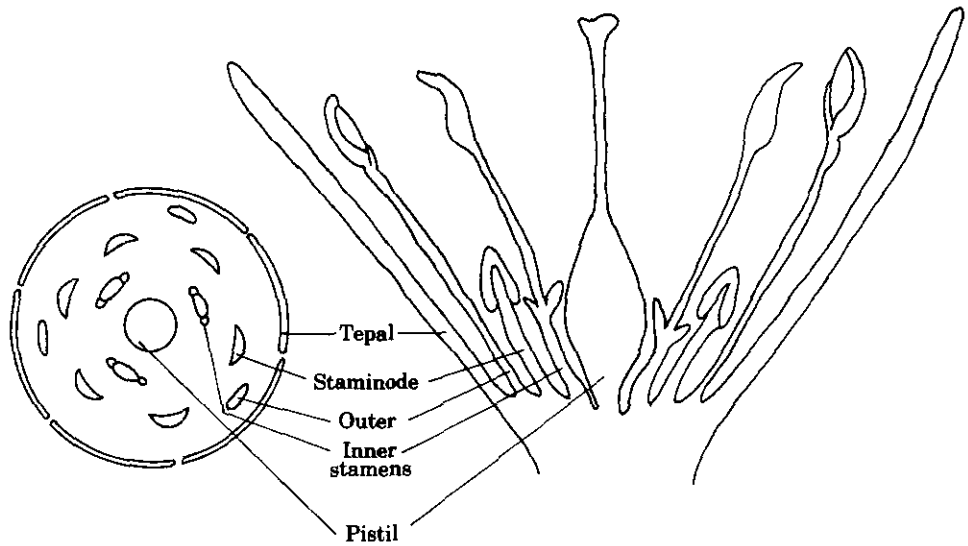

FIG. 7. Cross section (left) and vertical projection (right) of floral organs in avocado.

and petals were indistinguishable on the basis of surface morphology after visual and microscopic observation. The differentiation into sepals and petals by their position relative to inner stamens (sepals) or staminodes (petals) also proved unreliable. In observations on a large number of representative flowers from cvs Hass and Fuerte, we found each staminode aligning with the junction of the tepals (Fig. 7). We also found six staminodes instead of the reported three (Bergh, 1975), and six stamens instead of nine as reported by Sedgley (1985).

Attempts to separate sepals and petals at the differentiation stage (Inoue and Takahashi, 1989) of the cv. Fuerte flower resulted in the use of the term perianth. If a perianth cannot be differentiated into sepals and petals, as in the flower of onion (*Allium cepa*) (Esau, 1965) or grape (*Vitis vinifera* L.) (Blanke and Leyhe, 1989), the individual members of the perianth are called tepals (Esau, 1965), a term also employed for convenience by Reece (1939) and Schroeder (1952) in parts of their work on the avocado flower. We, hence, propose the term tepals be substituted for sepals and petals in describing the morphology of the avocado flower. This proposed change in terminology aids understanding the physiology, *viz.* photosynthesis and transpiration of the avocado flower as sepals often have stomatal densities similar to those of foliage leaves, whereas tepals and petals have fewer or no stomata. In flowers of apple, pear, strawberry or raspberry, sepals and petals can be easily distinguished by colour, thickness and surface structures. In strawberry, trichomes and stomata were exclusively distributed over the abaxial epidermis of the sepals and stomatal frequencies resembled those of the leaves, while the strawberry petals had no stomata, trichomes or cuticle (Blanke, 1991).

The observed abaxial stomata of the tepals (Fig. 4)

TABLE 2. *Stomatal frequency (mm^{-2}) and size (μm) in avocado*

Organ, stage Cultivar	Stomatal frequency	Stomatal size
Leaves, adaxial*†‡§‖ Fuerte, Hass	0	n.a.
Leaves, abaxial* Fuerte, Hass	350–510	6–12 × 11–13
Leaves, abaxial† Fuerte	400	n.d
Leaves, abaxial‡ Fuerte	700–760	n.d.
Fruit, post-anthesis§ Fuerte	50–75	9–12 × 16–19
Fruit, pre-mature‖ Fuerte	1·1	n.d.
Fruit, mature‖ div.	0·25–2·7	14 × 21
Fruit, mature‖ Hass	0·7	n.d.
Tepal, adaxial* Fuerte, Hass	0	n.a.
Tepal, abaxial* Fuerte, Hass	2·8–3·4	8·9 × 11–13
Tepal, abaxial‡ Fuerte	'occasional'	n.d.

n.a., not applicable; n.d., not determined; * this study; † Scholefield and Kriedemann, 1979; ‡ Whiley *et al.*, 1988; § Blanke and Bower, 1990; ‖ Schroeder, 1950.

confirms the report by Whiley *et al.* (1988) who found abaxial stomata on both 'Fuerte' 'sepals' and leaves. We examined stomata from flowers of avocado cv. 'Hass' and 'Fuerte' to clearly identify the independency of our results of cultivar employed and enable comparison of our observations with those of Scholefield and Kriedemann (1979), Sedgley (1985) and Whiley *et al.* (1988). The stomata of 8–9 × 11–13 μm in size observed on fully expanded tepals (Fig. 4) were smaller than on the respective young fruit post-anthesis with 9–12 × 16–19 μm reported by Blanke and Bower (1990). Tepal stomatal frequencies of 2·8–3·4 stomata mm^{-2} were low relative to the 350–510 (this study), 400 stomata mm^{-2} (Scholefield and Kriedemann, 1979) and 700–760 stomata mm^{-2} for the abaxial leaf surface of 'Fuerte' (Whiley *et al.*, 1988) or 'Fuerte' fruit (50–75 mm^{-2}) (Blanke and Bower, 1990) (Table 2). Abaxial leaf hair base cells, if connected to the intercellular space and at least partially open (Fig. 5), may contribute unregulated to CO_2 exchange and transpiration.

The trichomes of avocado tepals (5–8 μm diameter) were thinner (Fig. 1) than those of young fruit (8–10 μm; Blanke and Bower, 1990), but with a length commensurate with those of young avocado fruit (60–140 μm; Blanke and Bower, 1990). However, the trichome densities on the tepals (625–1200 mm^{-2}) was much greater than the 50–100 mm^{-2} observed on young fruit by Blanke and Bower (1990). The dense trichome cover on the tepals and young leaves may increase the boundary layer resistance and diminish transpiration. Transpiration of tepals exceeded that of the foliage leaves, which in turn exceeded that of the peduncle. The peduncle was devoid of stomata and showed the least transpiration per surface area. On the tepals, stomata were open. The low transpiration rate of the avocado leaves may be a combination of (*a*) more than 80% closed stomata and (*b*) stomata being covered by the conspicuous epicuticular crystalline wax structures in form of 0·4–0·6 μm rodlets or irregular dendritic crystals and may reflect the tropical origin of this species which is now grown in a subtropical environment (Whiley *et al.*, 1988). Transpiration of young fruit at the time when they exhibited the same surface area as the flower bud (0·3 cm^2; 5–7 d before and after anthesis) was 4·5–4·7 $mmol H_2O\ m^{-2}\ s^{-1}$ and exceeded transpiration of both tepals and leaves.

Stomata on the abaxial surfaces of both tepals and leaves were difficult to observe and count due, respectively, to the trichomes and wax structures present. This may have been the cause of a past overestimate of 730 abaxial stomata mm^{-2} (Whiley *et al.*, 1988) suspected under the wax cover.

CONCLUSION

The transpiration measurements and anatomical examinations presented in this study contribute to our understanding of water partitioning to flowers, fruits and leaves. This work provides scientific evidence for the recommendations in Southern California (Whitney and Bender, 1992) to increase orchard irrigation during flowering, a time when an avocado tree produces up to 2 million transpiring flowers with a surface area of 54 m^2 in the periphery of the tree. This study also shows the peculiar structure of the avocado flower, relative to the flowers of other fruit crops, with respect to the identity of petals and sepals and their large stomatal transpiration.

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