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Avocado Leaf Surface Morphology

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Introduction

Our laboratories are interested in understanding the relationship between the environment and leaf gas-exchange in avocado. Ultimately, we are interested in prolonging the daily period of maximum gas-exchange between the avocado leaf and the environment in an effort to increase total carbon fixation. This, in turn, will hopefully lead to decreased flower and fruit drop, and larger, more consistent yields. Part of deciphering the relationship between leaf gas-exchange and the environment is to examine the basic structural properties of the avocado leaf to determine how they might affect the physiology of the leaf. Relatively little work has been done on anatomy and surface characteristics of avocado leaves. We used scanning electron microscopy to examine the leaf surface characteristics of leaves of various ages from several released and unreleased avocado varieties. Our goal was to examine the basic surface characteristics of avocado leaves and to determine any differences in these characteristics between leaf ages and among varieties.

Materials and Methods

Avocado trees in southern California typically exhibit two shoot growth flushes per growing season: one in spring and one in late summer (Mickelbart et al., 2001). Leaves from the current season's spring flush, and from the previous year's summer flush were collected from several varieties growing at the University of California, Riverside in May 2001. Leaf disks were punched and prepared for examination by surface electron microscopy (SEM) as previously described (Bytnerowicz et al., 1998).

Two- to three-month-old leaves were collected from five mature 'Hass' trees to determine stomatal density. Stomatal density was estimated from SEM micrographs of leaf surfaces (leaf area of 0.98mm^2). Micrographs were printed and covered with a transparency on which stomata were marked and counted.

The conductance and assimilation were measured as previously described (Heath et al., 1985) except that the system has less pressure build-up due to specific vents of the air flow. Leaves used to measure gas exchange were collected from lathhouse-grown 'Hass' plants on Duke 7 clonal rootstock.

Results and Discussion

Leaf surface characteristics

An early description of avocado leaf anatomy reported that stomata on the abaxial (lower) surfaces of the leaf were "relatively few in number" (Heismann, 1939). Since then several reports have confirmed that many stomata are located on the abaxial surface of the leaf (Blanke and Lovatt, 1993; Whiley et al., 1988). In SEM micrographs of leaves of 10 varieties of avocado ('Hass', 'Lamb Hass', 'Fuerte', 'Reed', 'Bacon', 'Zutano', 'Pinkerton', 'Harvest', 'OA184',and 'GEM'), stomata were observed only on the abaxial surface. Figure 1 shows representative micrographs of the adaxial (upper) and abaxial surfaces of 'Hass' leaves. The lack of stomata on the adaxial surface correlates with very low gas-exchange rates on that surface (Table 1).

There are clear differences in the surface topology of the various avocado varieties examined. Figure 2 shows representative micrographs of the abaxial surface of recently matured spring flush leaves of several avocado varieties. The trees are all approximately the same age (>10 years) and leaves (which developed in full sunlight) were, collected from the northeast quadrant of each tree. 'Hass' leaves appear to have a very smooth abaxial surface compared to the other varieties, which often have noticeable depressions around individual epidermal and guard cells. 'Pinkerton' and 'Lamb Hass' leaves have trichomes (hairs) on the abaxial leaf surface, and while trichomes were fewer on 'Hass' and 'Gwen' leaves, they were not observed on leaves of 'Fuerte'. Blanke and Lovatt (1993) also reported observing very few trichomes on fully expanded 'Hass' and 'Fuerte' leaves. Previous studies have demonstrated that very young avocado leaves may have a thick covering of trichomes, but these structures are lost as the leaf matures (Blanke and Lovatt, 1993; Heismann, 1939).

We have not characterized any differences in wax load or chemistry among the observed varieties, but visual observations indicate that they may in fact be different (Figure 3 and Barthlott et al., 1998). Schroeder (1950) noted varietal differences in wax structure in avocado fruit. Differences in wax load or structure may partially explain differences in gas exchange observed in different varieties (Liu et al., 2000), due to varying limitations of gas movement around and into the leaf (see below).

Stomatal density of 'Hass' avocado

We estimated Stomatal density of 'Hass' avocado leaves growing in both sun and shade (Table 2). Our estimates of between 40,000 and 45,000cm^{-2} fall in the range previously reported for both 'Hass' and 'Fuerte' leaves. Estimates of stomatal density of fully-matured 'Fuerte' leaves range from 40,000cm^{-2} (Scholefield and Kriedmann, 1979) to 73,000cm $^{-2}$ (Whiley et al, 1988), and estimates of 35,000 to 51,000cm $^{-2}$ were given for leaves of 'Hass' and 'Fuerte' (Blanke and Lovatt, 1993). Interestingly, there was no difference in stomatal density of leaves that developed in sun versus those that developed in shade. To our knowledge, this has not been examined in avocado. Hirano (1931) found an approximately two-fold difference in stomata on sun and shade leaves of 'Eureka' lemon. Sun leaves of that species had an average stomatal density of 63,600cm, $^{-2}$ while shade leaves had 32,200-crrr^{2}. Since avocado is an understory tree in its native habitat, it may be that leaf anatomy does not adapt to increased sunlight under orchard situations. Certainly, avocado leaves reach a light-saturated level of photosynthesis at low light levels compared to sun-adapted species (Liu et al., 2000; Heath lab, unpublished data).

Leaf age and surface wax

Adaxial surface wax accumulates with age in avocado leaves (data not shown), as has been shown in other plant species (letter and Schaffer, 2001). We also observed differences in the amount of wax accumulating around and within stomata between leaves of consecutive flushes. Figure 4 shows typical micrographs of stomata from 'Hass' leaves of the current spring flush and of the previous summer season's flush. Wax accumulation tends to be greater in stomata of older leaves than in recently matured leaves. The increase in wax per leaf area during leaf development has been observed previously, but the timing seems to be species dependent (Riederer and Markstadter, 1996). This may partially explain the observed decrease in avocado leaf stomatal conductance with time (Shaffer et al., 1991). We have observed differences in gas-exchange between leaves from consecutive flushes measured concurrently (Heath lab, unpublished data). One potential reason for gas exchange differences might be differences in wax covering of the abaxial surface and wax accumulation on the guard cells and in the stomatal cavity. This may also explain why stomatal opening in response to light appears to be slower in older avocado leaves than in younger leaves (Heath lab, unpublished results). This may not be the case for all varieties, however. Figure 5 shows micrographs similar to Figure 4 for an unreleased variety, 'OA184'. Neither young nor older leaves show heavy stomatal cavity wax accumulation. This variety has not been tested yet for gas exchange properties.

Conclusions

We have conducted a preliminary examination of avocado leaf surface topography and wax accumulation, and discussed how this may relate to gas-exchange of avocado leaves. It seems clear that there are differences in both surface and stomatal characteristics among varieties and there are indications that wax characteristics may be different as well. These critical factors may substantially influence avocado leaf gas-exchange by their interaction with the boundary layer outside the leaf, especially under low wind speed. Furthermore, any changes in the surface near the stomata during leaf development will alter gas-exchange of the leaf with the outside environment and thus alter the leaf's productivity (Aphalo and Jarvis, 1993). Future work will focus on the physiological development of avocado leaves and the interactions between leaf development, environmental conditions, and gas exchange.

Literature cited

Aphalo, P. J. and P. G. Jarvis. 1993. The boundary layer and the apparent responses of stomatal conductance to wind speed and to the mole fractions of CO_2 and water vapour in the air. Plant, Cell Environ. 16:771-783.

Barthlott, W., C. Neinhuis, D. Cutler, F. Ditsch, I. Meusel, I. Theisen, and H. Wilhelmi. 1998. Classification and terminology of plant epicuticular waxes. Bot. J. of Linnean Soc., 126:237-260.

Blanke, M.M. and C.J. Lovatt. 1993. Anatomy and transpiration of the avocado inflorescence. Ann. Bot. 71:543-547.

Bytnerowicz, A., K. Percy, G. Riechers, P. Padgett, and M. Krywult. 1998. Nitric acid vapor effects on forest trees—deposition and cuticular changes. Chemosphere 36: 697-702.

Heath, R.L., R.T. Furbank, and D.A. Walker. 1985. Effects of polyethylene-glycol-induced osmotic stress on transpiration and photosynthesis in pinto bean leaf discs. Plant Physiol. 78:627-629.

Heismann, P. 1939. Notes on avocado anatomy. California Avocado Society Yearbook 24:87-91.

Hirano, E. 1931. Relative abundance of stomata in citrus and some related genera. Bot. Gaz. 92:296-310.

Jetter, R. and S. Schaffer. 2001. Chemical composition of the *Prunus laurocerasus* leaf surface. Dynamic changes of the epicuticu-lar wax film during leaf development. Plant Physiol. 126:1725-1737.

Liu, X., M.V. Mickelbart, P.W. Robinson, R. Hofshi, and M.L. Arpaia. 2000. Photo synthetic characteristics of avocado leaves. Acta Horticulturae (in press).

Mickelbart, M.V., P.W. Robinson, C. Adams, G.W. Witney, and M.L. Arpaia. 2001. Avocado tree phenology in California. HortScience 36(3):527.

Riederer, M. and C. Markstadter. 1996. *Cuticular waves: a critical assessment of current knowledge,* in *Plant Cuticles,* G. Kerstiens, (ed.), BIOS Scientific Publishers Ltd, Oxford, pp. 189-200.

Schaffer, B., A.W. Whiley, and R.R. Kholi. 1991. Effects of leaf age on gas exchange characteristics of avocado *(Persea americana* Mill.). Scientia Horticulturae 48:21-28.

Scholefield, P.B., andP.E. Kriedmann. 1979. Stomatal development in avocado leaves. CSIRO Australian Division of Horticultural Research, Report 1977-1979, pp. 50-51.

Schroeder, C.A. 1950. The structure of the skin or rind of the avocado. California Avocado Society Yearbook 35:169-176.

Whiley, A.W., K.R. Chapman and J.B. Saranah. 1988. Water loss by floral structures of avocado (*Persea americana* cv. Fuerte) during flowering. Aust. J. Agric. Res. 39:457-467.

Table 1. Stomatal and trichome density on the abaxial (lower) side of 'Hass' leaves growing in sun or shade. Micrographs (surface area of 0.978 mm^2) of five sun leaves and four shade leaves were examined for calculations.

Exposure	Stomatal density (stomata·cm^{-2})	Trichome density (trichomes·cm^{-2})
Sun	43,900 ± 4,130	818 ± 323
Shade	42,525 ± 6,040	757 ± 271

Table 2. Stomatal conductance of the adaxial (upper) and abaxial (lower) sides of 'Hass' leaves under laboratory conditions.[1]

Leaf Surface	Stomatal Conductance (mmol m^{-2} sec^{-1})	Assimilation (μmol m^{-2} sec^{-1})
Adaxial	0.5 ± 1.1	0.02 ± 0.03
Abaxial	76.1 ± 1.5	4.42 ± 0.04
Blank[2]	0.0 ± 1.5	0.0 ± 0.04

[1] The air flow for the back surface and the front surface of the leaf chamber were nearly the same and stable. Only the adaxial surface is illuminated during all measurements.

[2] "Blank" refers to measurements of gas exchange with no leaf in the gas stream and represents a background level.

Figure 1. Adaxial (A) and abaxial (B) surfaces (500x magnification) of recently matured 'Hass' leaves from mature trees growing in Riverside, California.

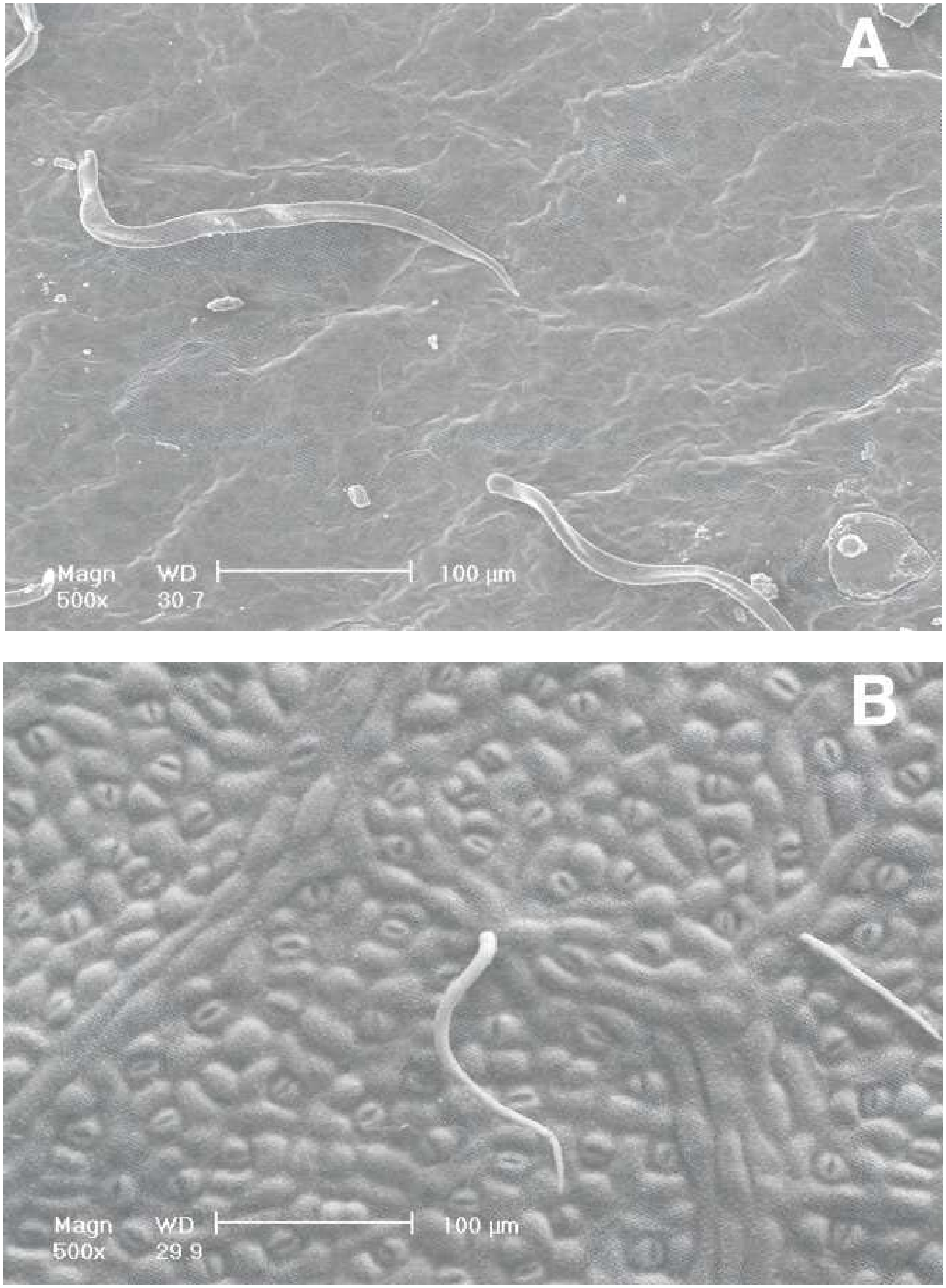

Figure 2. Abaxial surface (200x magnification) of recently matured 'Hass' (A), 'Lamb Hall' (B), 'Fuerte' (C), 'Gwen' (D), and 'Pinkerton' (E) leaves from mature trees growing in Riverside, California.

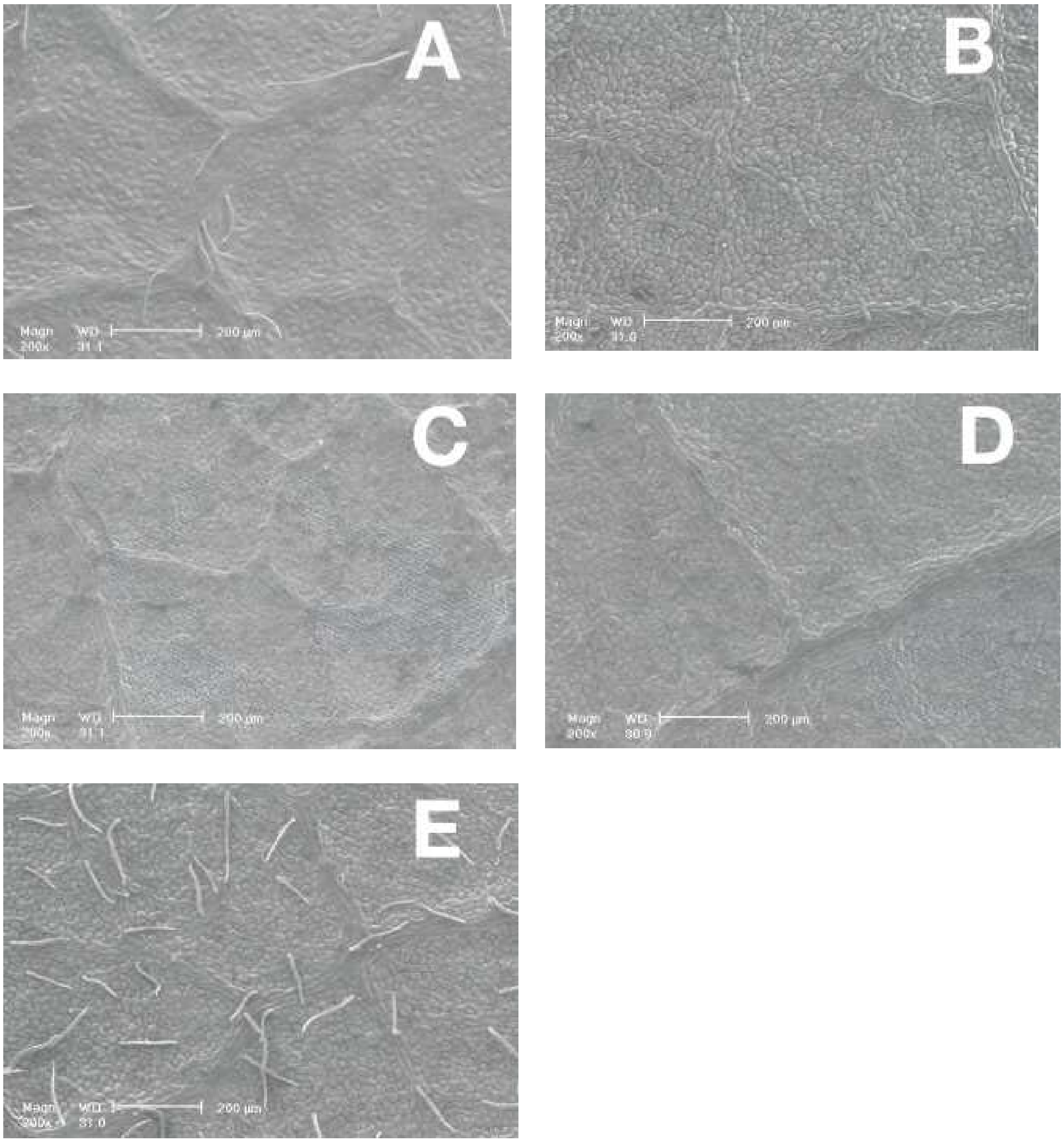

Figure 3. Representative micrographs of stomata (10,000x magnification) from 'Hass' (A), 'Lamb Hass' (B), 'Fuerte' (C), 'Gwen' (D), and 'Pinkerton' (E) recently matured leaves harvested in spring 2001 from mature trees growing in Riverside, California.

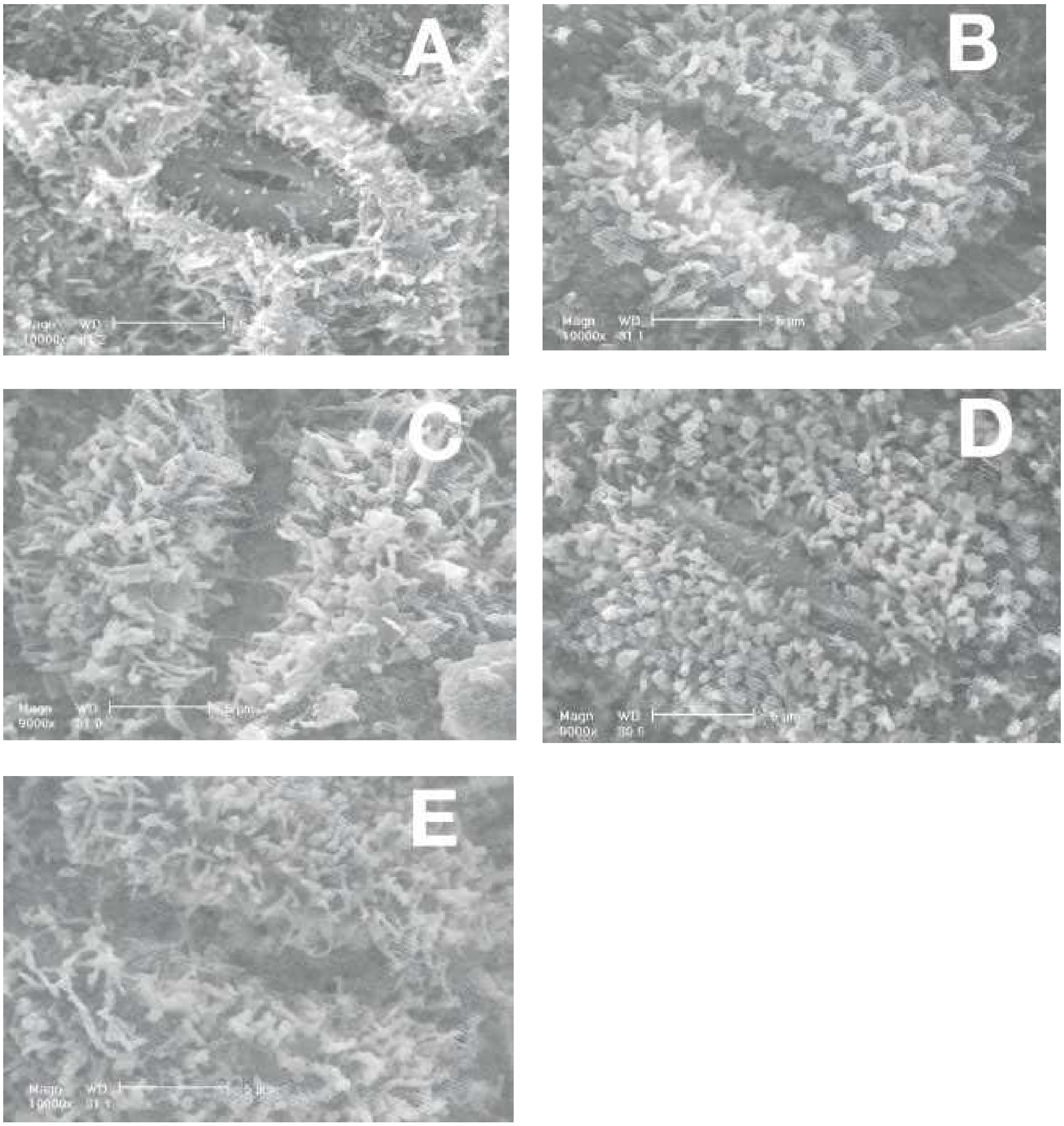

Figure 4. Representative micrographs of stomata (10,000x magnification) from recently matured (A) and previous season's (B) leaves harvested in Spring 2001 from mature 'Hass' trees growing in Riverside, California.

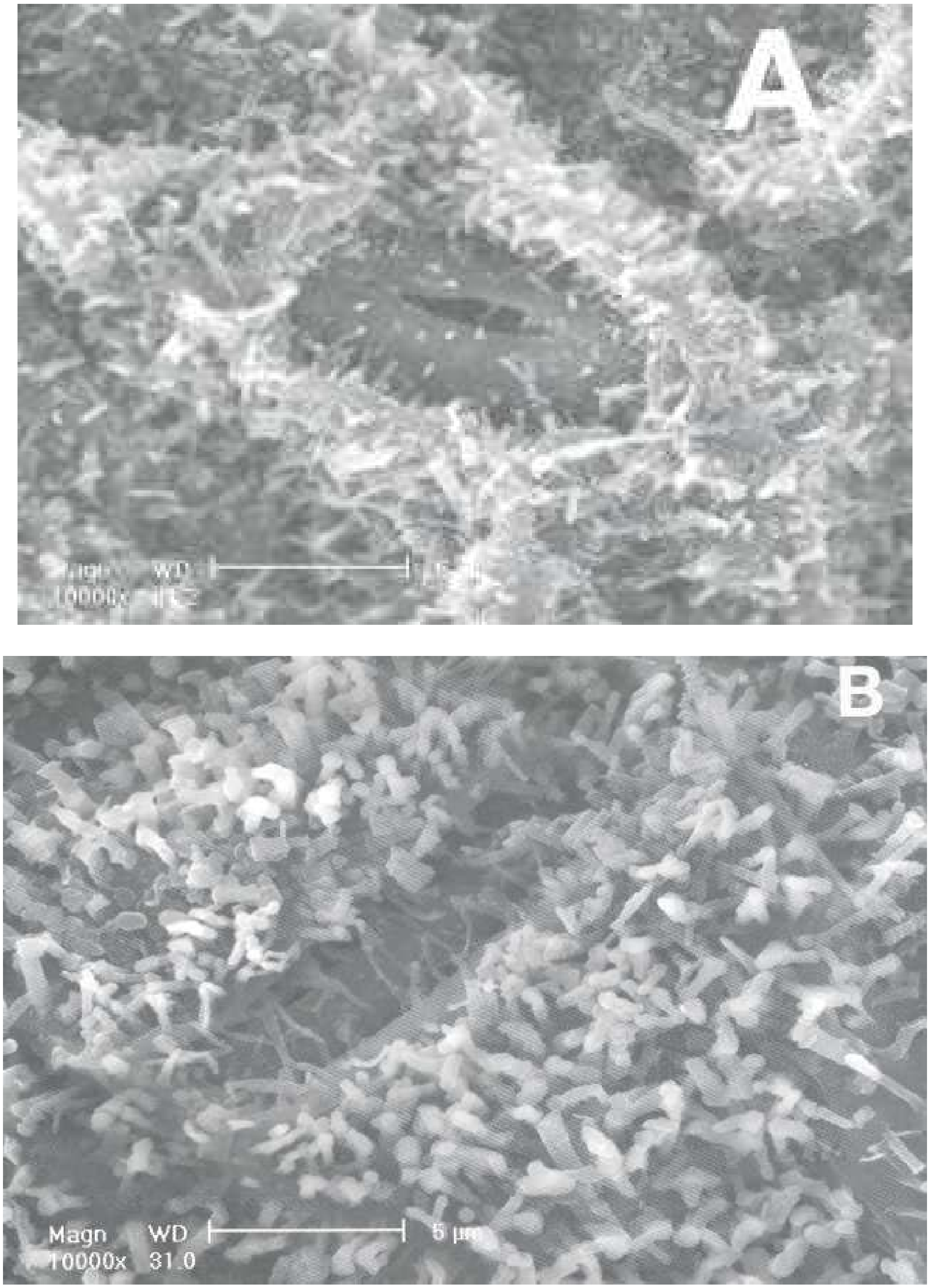

Figure 5. Representative micrographs of stomata (10,000x magnification) from recently matured (A) and previous season's (B) leaves harvested in Spring 2001 from mature 'OA184' trees growing in Riverside, California.