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VASCULAR AND ASSOCIATED TISSUE OF 'HASS' AVOCADO (*PERSEA AMERICANA* MILL.) FRUIT

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ABSTRACT

In the avocado fruit mesocarp (flesh), the lipid or oil levels are found in greatest concentrations below the pedicel and near the micropyle, with a distinct gradient between these two regions. These lipid levels closely parallel vascular distribution, with the highest lipid levels associated with the highest densities of vascular tissue.

Semi-mature Hass avocado fruit were passively infused with 1% Eosin red, which permeated the vascular tracts of the fruit. Hand-cut sections of these fruit revealed numerous vascular traces arranged in two concentric rings. TEM and SEM techniques were applied to semi-mature and mature fruit and adapted until good fixation was achieved. It was found that annular and helicular ringed xylem vessels were concentrated in the mesocarp, while pitted and latticed vessels were found only in the micropylar and pedicel regions of all the fruit examined. This is probably because the nature of the ringed vessels allows some elasticity. Consequently these vessels are not likely to rupture with the massive expansion of the mesocarp during fruit growth. The micropylar and pedicel regions of the fruit do not expand to the same extent as the mesocarp and elasticity is thus not essential. In ultramature fruit and those with degenerate testas, large concentrations of phenolics were found concentrated at the micropylar region indicating possible degeneration of the vascular traces at the micropyle.

INTRODUCTION

Lipid or oil levels in the avocado fruit mesocarp (flesh) are found in greatest concentrations distal to the pedicel and near the micropyle, with a distinct gradient between these two regions (Schroeder, 1987). This gradient closely parallels vascular distribution, where the highest lipid levels are associated with the highest densities of vascular tissue. It has also been observed that "browning" in mature avocado fruit flesh begins and remains pronounced in the micropylar region and below the pedicel, and then spreads throughout the mesocarp (Cutting *et al.,* 1988). Again, both these regions are associated with a high density of vascular tissue. Consequently, the vascular tissue was investigated at both the morphological and ultrastructural levels to clarify the situation.

MATERIALS AND METHODS

Morphology

Sixteen 3 month old fruit were harvested with long pedicels and then transported to the laboratory where the pedicels were trimmed to a length of 15 mm. Strips of silicone tubing, approximately 40 mm long, were forced over each pedicel and connected to separate 10 ml syringes. Four fruit each were then passively infused with 1 % eosin red for periods of 15, 30 and 60 min and 24 hr. For each treatment period, two fruit were sectioned serially in both the longitudinal and transverse planes and then photographed. Noteworthy results are presented as Figs. 1 to 6.

Ultrastructure

Samples were taken from three distinct regions of the mesocarp (viz. below the pedicel; in the midsection of the fruit, and in the micropylar region) from young fruit (±3 months old), fruit of intermediate maturity (±6 months old) and very mature fruit, which had been left to hang on a tree for at least 15 months.

Transmission electron microscopy

(TEM). Specimens were fixed for 8 hr in 3% glutaraldehyde in a 0.05 M sodium cacodylate buffer (pH 7.1), postfixed for 4 hr in 2% osmium tetroxide and dehydrated through a graded ethanol series (from 10% to 100%). Some TEM specimens were dehydrated further in propylene oxide and then resin infiltrated using a graded series of propylene oxide and Spurr's resin (Spurr, 1969). Other TEM specimens were block stained in 2% aqueous uranyl acetate (Reynolds, 1963) after postfixation, and then resin infiltrated using a graded series of only ethanol and Spurr's resin. All specimens were viewed in a Jeol 100CX® TEM at an accelerating voltage of 80kV and noteworthy results are presented as Figs. 7 to 12.

Scanning electron microscopy

(SEM). Preparation for critical point drying, using liquid CO_2, was identical to TEM preparation for fixation, post-fixation and dehydration. Coating with gold/palladium was followed by viewing in an Hitachi S570® SEM at an accelerating voltage between 8 and 12 kV. Where cryofreezing was undertaken, some specimens were hand-cut from the fruit, mounted and then frozen using liquid nitrogen. Other specimens were first mounted, frozen and then fractured using a cooled razor blade (Bruton *et al.,* 1991). Coating with gold/ palladium was done under vacuum and specimens were viewed in an Hitachi S570 SEM® cooled to -175 °C by a dewar of liquid nitrogen, via a stranded copper rope. Noteworthy results are presented as Figs. 13 to 20.

RESULTS AND DISCUSSION

Morphology.

Transverse sections through the pedicel revealed a central core of stained vascular tissue (Fig.1), supporting Valmayor's (1967) results. Serial transverse sections through the fruit showed that the vascular tissue enters the fruit and immediately ramifies and permeates the entire mesocarp. The vascular strands at the proximal end of the fruit were asymmetrical (Fig.2) but differentiated into two concentric rings in the mesocarp, around the seed (Fig.3). The latter findings disagree with those of Valmayor (1967), as none of the fruit sectioned in the present study had only six major strands. In addition, all the vascular traces in any one fruit coalesced and entered the testa through the funiculus (Fig.5) and no strands ended blindly towards the distal end of the fruit. Ramification of the vascular traces occurred in functional testas, which were thick, spongy and creamy in colour (Fig.5). In contrast no activity could be seen in degenerate, brown testas (Fig.6), in which the vascular strands had degenerated to become nonfunctional.

Infusion of eosin red for 15 min. led to faint staining only of the mesocarp vascular strands of fruit with functional testas (Fig.4). Where fruit with functional testas were infused for periods longer than 15 min., staining of vascular traces in the fruit mesocarp was more pronounced, while the vascular traces in the functional testas also became stained (Fig.5). This indicates that water uptake occurred as usual during infusion. In fruit with brown, degenerate testas there was very little infusion of eosin red, even after 24 hr (Fig.6). This was probably because the seed had become functionally isolated with a minimal demand for water and assimilates. Drying out of the testa causes the vascular strands to lose their translocation ability. In this sense then, the seed/mesocarp plus endocarp connection ceased to exist in physiological terms. Where this occurs prematurely or relatively early in fruit development, it may contribute to the small fruit problem of 'Hass'. A future study on seed maturity is implicated.

Ultrastructure

Transmission Electron Microscopy

In assessment of the techniques employed, when samples were dehydrated with propylene oxide, leaching of the lipids resulted and the lipid bodies were transparent and distorted with many indentations (Fig.7). Subsequent resin infiltration of fresh samples without a propylene oxide step gave better results, as all organelles were intact (Figs. 8,9 and 10).

In immature, 3 month old fruit, lipid accumulation in the parenchyma cells adjacent to the phloem was undetectable (Fig.8). Subsequent lipid accumulation in lipid bodies occurred in the parenchyma cells of 6 month old fruit of intermediate maturity (Fig.9). Following this, lipid accumulation in phloem parenchyma cells of ultramature 15 month old fruit had increased dramatically and reached copious amounts (Fig.10). Phloem tissue in fruit with functional testas was extremely rich in mitochondria (Fig.8) signifying considerable physiological activity. This was especially true for 6 month old fruit of intermediate maturity (Fig.9). In some 6 month old fruit however the testas were brown

and degenerate and phloem cells in the micropylar region were practically inactive. There were some mitochondria at the peripheries of these inactive phloem cells, but the pronounced decrease in the number of mitochondria signifies greatly reduced physiological activity (Fig. 11).

In addition, polyphenol oxidase (PRO) activity was seen in significant amounts in the mesocarp tissue of the micropylar region of those with degenerate testas (Fig. 12), and in ultramature fruit. The black, stained PPO deposits formed as a result of the reaction between PPO and osmium tetroxide. Since the testas had degenerated, phenolics which would normally have entered the seed, probably accumulated in the plastids of parenchyma cells in the micropylar region. Consequently, degeneration of the testa resulted in a buildup of phenolics in the micropylar region. This may help to explain the apparent increase in PPO activity observed i n the micropylar region of the mesocarp of late-hung fruit by Cutting *et al.,* (1988). Supporting evidence for this was that seed development in late-hung fruit was complete and the testas had started degenerating or were dry, thin and membranous.

Scanning Electron Microscopy

Critical point drying produced excellent fixation of lipids /ns/fu(Figs. 13,14and 15) as did cryochamber fracturing (Fig.16). However, both techniques made identification of vascular tissue extremely difficult, while cryochamber fracturing also resulted in ice crystal damage. Hand-cut and cryofrozen samples resulted in good detail of the lignified xylem vessels (Figs. 17 to 20), although phloem cells could not be distinguished from parenchyma tissue. In addition the cell contents were dislodged probably because of the 'boiling' effect of liquid nitrogen.

Visible lipid accumulation in lipid bodies of parenchyma cells and idioblasts was not apparent until after 6 months of 'Hass' fruit growth (Fig.13). However in ultramature, 15 month old fruit, the majority of parenchyma cells were filled with lipid globules (Figs. 14 and 15). Pitted tracheids were found in both the micropylar region (Fig.19) and the pedicel. Sclerified, lattice tracheids were only found in the micropylar region (Fig.20). Helicular (Fig.17) and annular ringed (Fig.18) vessel members were also identified but both were found only in the mid-sections of the mesocarp. All the tracheary elements identified in this study are consistent with those identified by Cummings & Schroeder (1942). However, the importance of their distribution in the fruit was overlooked.

Esau (1977) maintained that the distribution of tracheary cell types is related to their function. The pitted and latticed tracheids of the avocado fruit are presumed to function primarily in the conduction of water and other soluble substances, but they are also thought to provide some support, due to their rigidity. In contrast, the helicular and annular ringed vessel members are likely to be involved only in conduction. Structural lignification of rings and helices allows for cell elongation, and stretching of the vessel members in the mesocarp is imperative as the mesocarp is the region which expands most during fruit growth. Consequently, rigid tracheids in the mesocarp have been selected against.

CONCLUSION

This investigation casts new light on the vasculature of the avocado fruit. The vascular traces enter the fruit through the pedicel as a cylindrical core but then ramify and permeate the entire mesocarp. Distal to the pedicel the strands are asymmetrical but then differentiate into two concentric rings around the seed. All the strands then coalesce at the distal end of the fruit and enter the testa as a solid core. The vascular strands ramify in the testa and form a complex network.

When fruit were infused with eosin red solution, staining of the vascular traces in fruit with functional testas was regular, as staining of all the traces became darker with time. However, in fruit with thin, dry, brown testas, staining of mesocarp vascular strands was incomplete even after 24 hr, indicating that uptake of water and assimilates is severely retarded when testas are degenerating. Indeed, the transmission electron microscopy study provided supporting evidence for retarded partitioning, as the phloem cells seen in fruit with degenerate testas were almost inactive. In addition, PPO activity increased in the micropylar regions of these fruit with degenerate testas, and this is most likely contributing to "physiological browning" of the avocado fruit.

Morphologically, the accumulation of lipid globules in parenchyma cells and idioblasts only became apparent in 6 month old 'Hass' fruit, where as structural xylem tracheids were only found in the micropylar and pedicel regions of the mesocarp. Clearly, the xylem vessels in the mid-region of the mesocarp need to stretch with fruit growth and elongation. Rigid tracheids in the mid-region would rupture with fruit growth, and cause a breakdown in assimilate flow.

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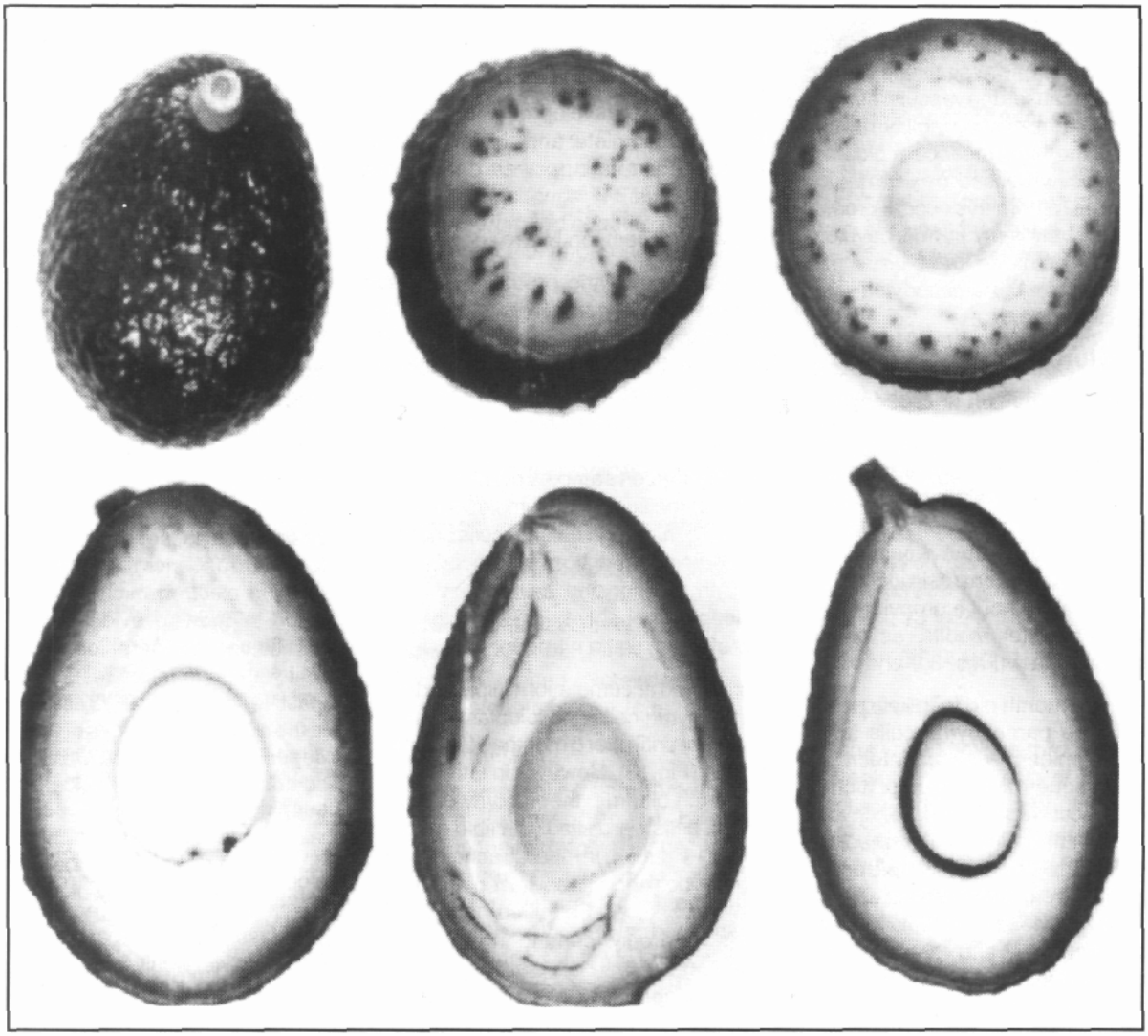

FIG. 1 Transverse section of intact pedicel of 4 month old 'Hass' avocado fruit, showing central core of vascular tissue stained with 1% eosin red solution (space bar = 1 cm).

FIG. 2 Transverse section of proximal end of 4 month old 'Hass' avocado fruit, showing asymmetry of vascular traces, stained with 1% eosin red solution, after ramification, distal to the pedicel (space bar = 1 cm).

FIG. 3 Transverse section of mid-region of 4 month old 'Hass' avocado fruit, showing two concentric rings of vascular traces, stained with 1% eosin red solution (space bar = 1 cm).

FIG. 4 Longitudinal section of 4 month old 'Hass' avocado fruit showing eosin red staining of the vascular traces in the mesocarp after 15 min. (space bar = 1 cm).

FIG. 5 Longitudinal section of 4 month old 'Hass' avocado fruit showing eosin red staining of the vascular traces in the mesocarp after 60 min. (space bar = 1 cm).

FIG. 6 Longitudinal section of 4 month old 'Hass' avocado fruit with a degenerating testa, showing faint eosin red staining of the vascular traces in the mesocarp after 24 hr (space bar = 1 cm).

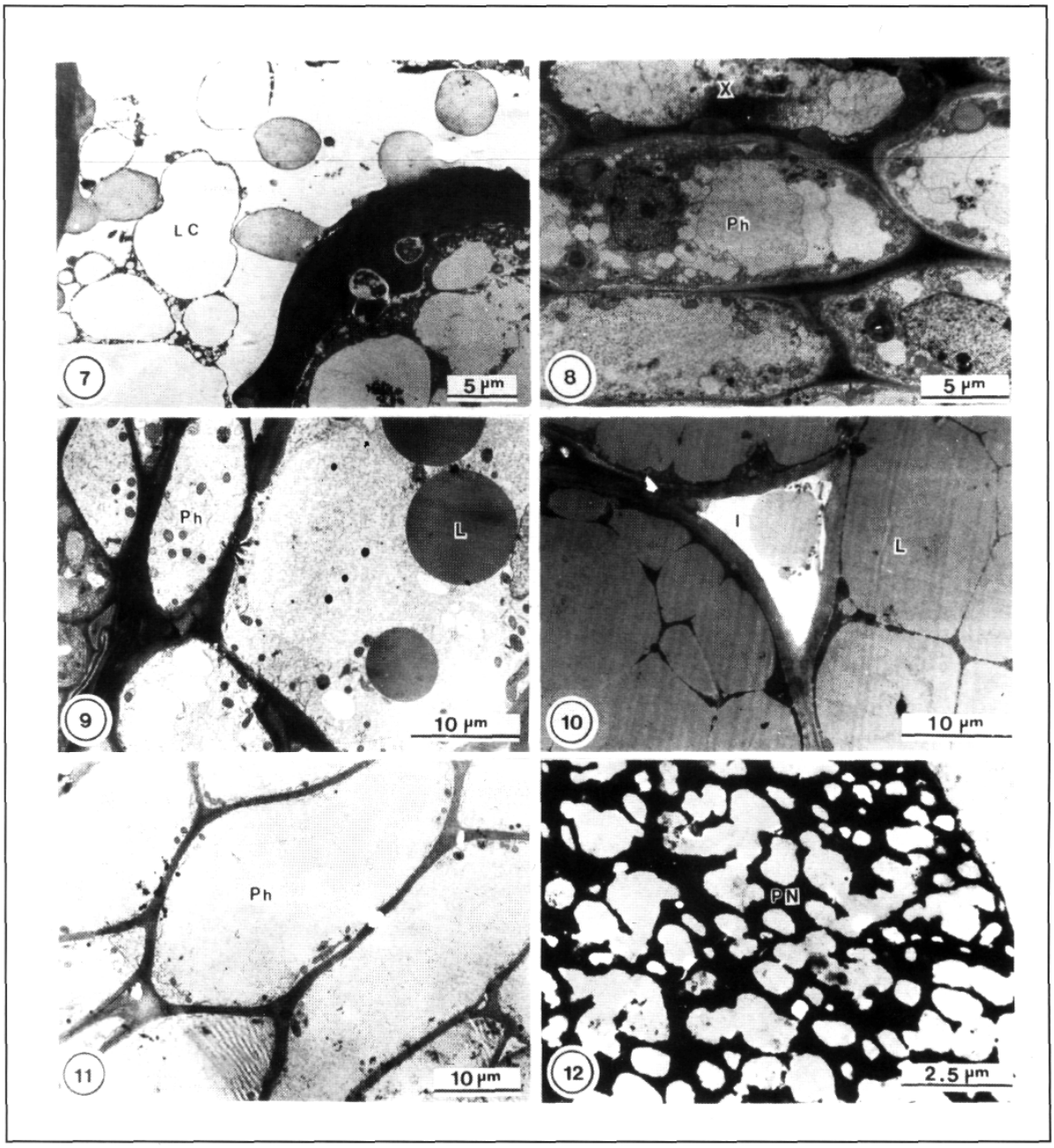

FIG. 7 Transmission electron micrograph of parenchyma cell of 'Hass' fruit mesocarp, showing leaching of lipids from lipid bodies (LC = leached lipid body).

FIG. 8 Transmission electron micrograph of very active phloem cells (Ph) and xylem vessel (X) of a 3 month old 'Hass' fruit mesocarp.

FIG. 9 Transmission electron micrograph of phloem and parenchyma cells of a 6 month old 'Hass' fruit mesocarp, showing lipid accumulation in lipid bodies (L) of a parenchyma cell.

FIG. 10 Transmission electron micrograph of phloem parenchyma cells of a 15 month old 'Hass' fruit mesocarp, showing lipid accumulation (I = intercellular space).

FIG. 11 Transmission electron micrograph of inactive phloem cells of a 4 month old 'Hass' fruit mesocarp, in a fruit with a brown, degenerating testa.

FIG. 12 Transmission electron micrograph of osmium tetroxide-stained PPO deposits in the plastid of a parenchyma cell in the micropylar region of the mesocarp, of a 4 month old 'Hass' fruit with a degenerating testa (PN = PPO deposit).

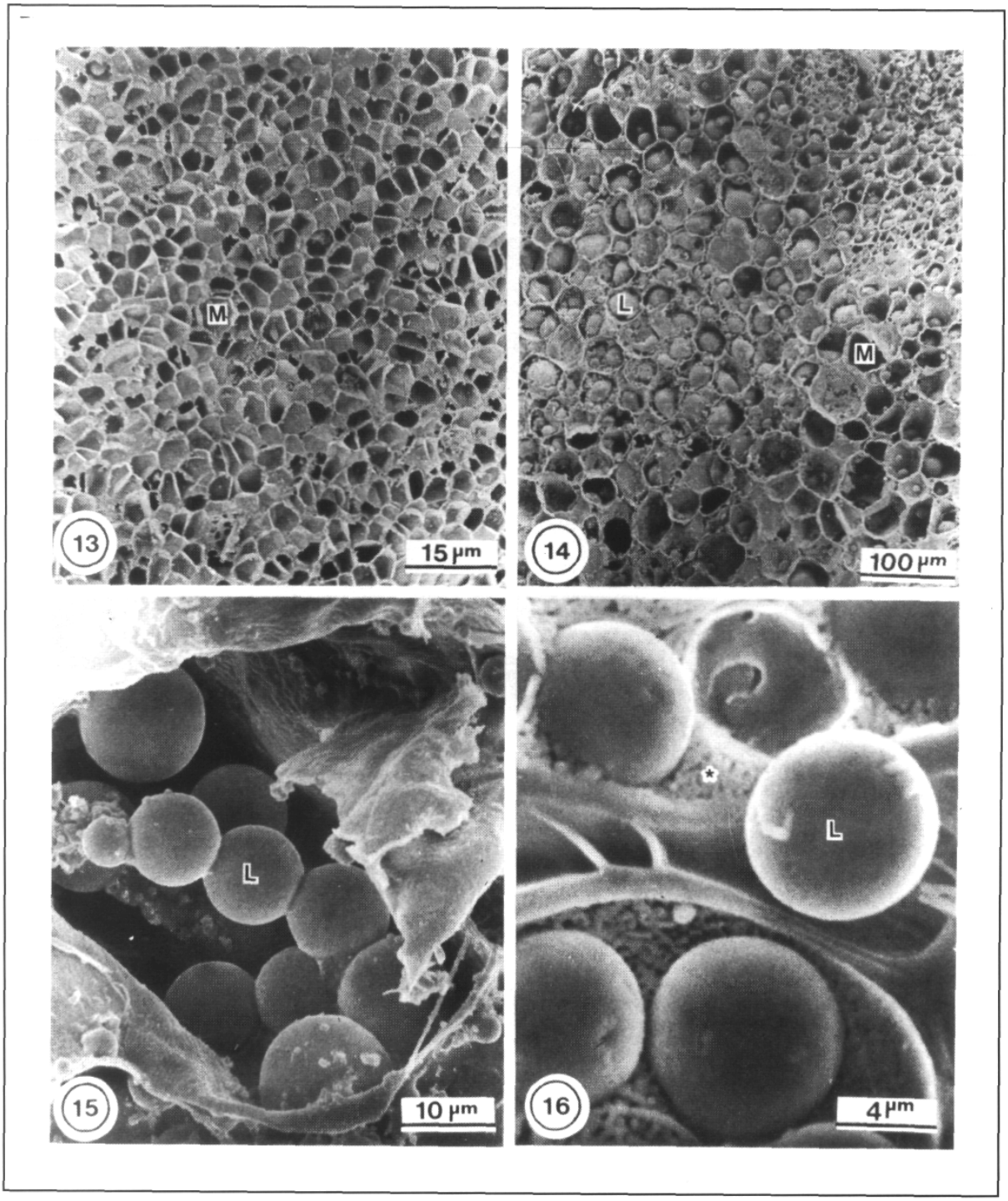

FIG. 13 Scanning electron micrograph (critical point drying) of mesocarp of a 6 month old 'Hass' fruit (M = mesocarp parenchyma cell).

FIG. 14 Scanning electron micrograph (critical point drying) of mesocarp of a 15 month old 'Hass' fruit (L = lipid body, M = mesocarp parenchyma cell).

FIG. 15 Scanning electron micrograph (critical point drying) of numerous lipid bodies within a typical parenchyma cell of a 15 month old 'Hass' fruit.

FIG. 16 Scanning electron micrograph (cryofreezing) of lipid bodies of two parenchyma cells of a 15 month old 'Hass' fruit (* = ice crystal damage).

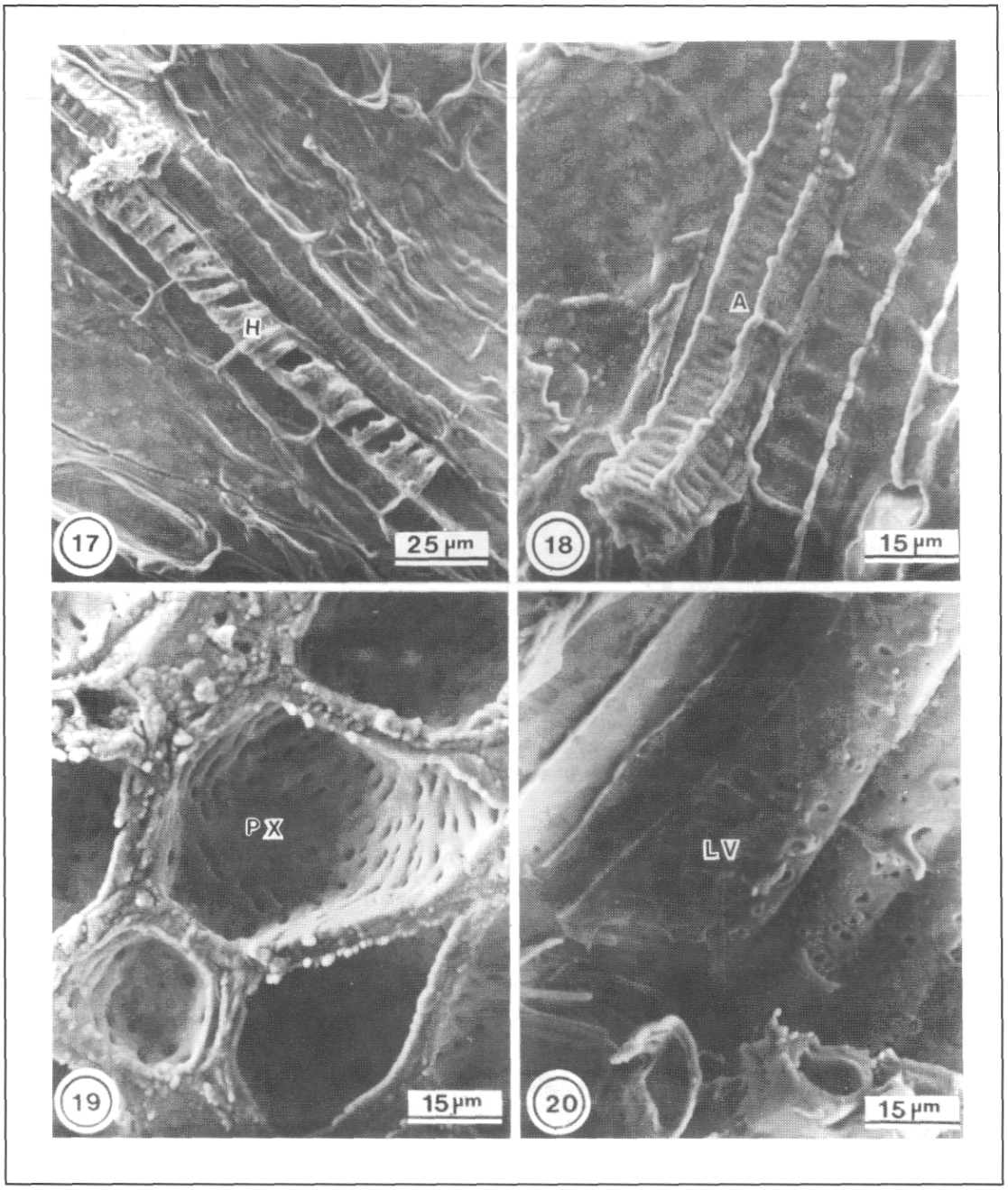

FIG. 17 Scanning electron micrograph (cryofreezing) of an helicular ringed xylem vessel member (H) in the mid-region of the mesocarp, of a 6 month old 'Hass' fruit.

FIG. 18 Scanning electron micrograph (cryofreezing) of an annular ringed xylem vessel member (A) in the mid-region of the mesocarp, of a 6 month old 'Hass' fruit.

FIG. 19 Scanning electron micrograph (cryofreezing) of a pitted xylem tracheid (PX) in the pedicel region of the mesocarp, of a 6 month old 'Hass' fruit.

FIG. 20 Scanning electron micrograph (cryofreezing) of a sclerified lattice xylem tracheid (LV) in the micropylar region of the mesocarp, of a 6 month old 'Hass' fruit.