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A SUPPLEMENTARY STUDY ON MELALEUCA PHELLEM BY ELECTRON MICROSCOPE[1]

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Abstract: The supplemental observation by EM is made for the phellem of *Melaleuca leucadendra* L. The EM survey clearly provides the site of "casparian strip" on the anticlinal wall of short cell. The "casparian strip" in the young cell is more conspicuous than that in the older cell. The wrinkled wall exhibits only on the anticlinal wall of young short cell. Basing on the absence of plasmodesmata, cytoplasmic contents and the presence of "casparian strip", the short cell is suggested as more specialized than the long cell. The cell expansion occurs after the appearance of suberized wall.

INTRODUCTION

Since the cork cells are easily slouphing off dead cells, they have not received as much attention as other tissues such as: wood. However the bark of *Melaleuca* is characterized by their thick multilayered structure (Chiang, 1980; Chiang & Wang, 1984; Metcalfe & Chalk, 1950). It is recently becoming as a center of interest for the material in the form of whole-tree chips (Smith & Dowd, 1981).

The origin, structure and development of the heterogeneous phellem in the stem of *Melaleuca leucadendra* have been described in the previous papers (Chiang, 1980; Chiang & Wang, 1984). It has a very peculiar bark both in structure and its large volume. The bark of this plant appears to be spongy in texture. Its phellem consists of alternating layers of cells: one cell layer of short suberized cells, and several layers of long cells with partly destroyed non-suberized cells (Fig. 1). The short cells are compactly arranged and bear "casparian strips" along the radial- and transverse-walls. One to several cells in a bundle run across the short cell layer and long cell layer radially. They are designated as "cork ray cells". The purpose of this report was to reveal the more clear morphology of cell wall in cork layer as well as some related structure as examined under EM.

MATERIALS AND METHODS

Bark from several different ages of the twigs of *Melaleuca leucadendra* L. were collected from the campus of National Taiwan University. The materials for SEM were fixed in FAA prior to embedding in paraffin. Sections were made at 20 to 50 μm thickness with a rotary microtome. After removing the paraffin they were coated with gold on the stubs. Photographs were taken with Hitachi S-550 SEM.

For TEM, materials were fixed in 2.5% glutaraldehyde followed by 1% $KMnO_4$, dehydrated with ethanol-acetone series and embedded with Spurr's resin. Sections were stained with

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methanolic uranyl acetate and lead citrate (Spurr, 1969; Reynold, 1963), viewed with Hitachi H-600 TEM at 75 KV.

RESULTS

I. General description

As described in the previous reports (Chiang, 1980; Chiang & Wang, 1984), the first periderm in the most twigs originates variously from 5th to 10th cortical cells (Fig. 2). Three to four subsequent periderms are formed in the stem of *Melaleuca leucadendra* in a growing season. Most of them are parallel to each other. Almost all of the cells in the periderm are cork cells.

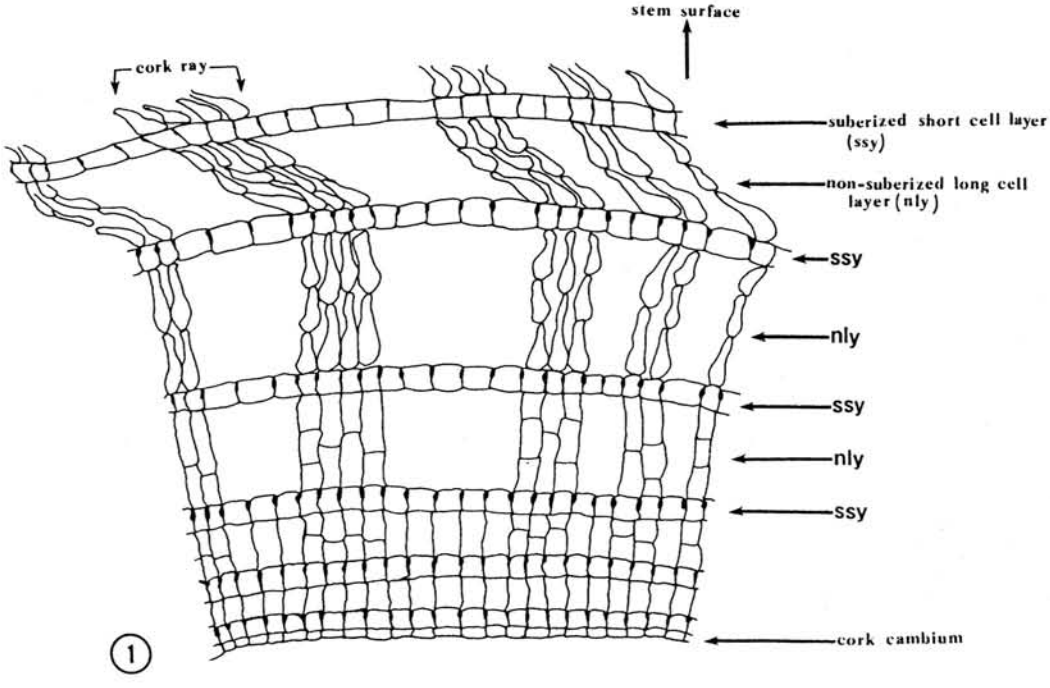


Fig. 1. Shematic drawing showing the formation of phellem.

Key to labeling (For Fig, 2~24)

C – cork layer
Cc – cork cambium
cr – cork ray
cs – casparian strip
cy – cytoplasm
e – epidermis
F – fiber cell
in – intercellular space
ls – long cell
m – middle lamella
nly – non-suberized long cell
nsu – non-suberized layer (long cell layer)
p – parenchyma
pe – periderm
r – ray
se – sieve tube element
sh – secondary phloem
ss – short cell
su – suberized layer (short cell layer)
T – trichome
w – cell wall

No phelloderm cells are found in the young twigs (Fig. 2). They are heterogeneous. Each periderm in its later stage is composed of layers of radially elongated thin-walled non-suberized "cork ray cells", alternating radially with a flattened casparian strip bearing suberized short cells. The long cells located between the cork ray cells become destroyed, and remain as intercellular spaces (Fig. 1). Consequently the aerenchymatous cork appears to be a latticework structure in its both transection and longisection (Figs. 3, 4, 5). The fibers in bundles are occasionally found located among the cork layers (Figs. 8, 9). The first derivative layer of the initial cork cambium give rise to suberized short cell layer in the most branches, whereas they become non-suberized long cell layer (or layers) in the others. The cells in short cell layer are compactly arranged and strictly one cell in thickness. But the long cell layer has one to several cells and is composed of cork ray cells alternating periclinally with intercellular spaces with the debris of destroyed cell walls (Figs. 4, 8).

II. Short cells in cork layer

The short cells in cork layer are isodiametric or slightly flattened tetrahedrals as shown in transection (Figs. 2, 3, 4, 8). They are somewhat elongated longitudinally (Figs. 5, 6). In addition to suberized characteristic, the cell wall of short cells bear "casparian strips" on their anticlinal walls (both transverse and radial). The casparian strip is very conspicuous even in the cell layer immediately next to the cork cambium (Figs. 2, 10, 11). The topographical position of casparian strip on the anticlinal wall varies from cell to cell. It occurs at the center of anticlinal wall in some cells, but in some others, it is centered 1/4, 1/3 of anticlinal wall length from its outer tangential wall (Figs. 11, 12). And still in some members, the thickened strip occurs at the junction of anticlinal and outer tangential-walls (Fig. 10). They appears as Y-shape in sectional view (Fig. 10). In general, the casparian strip is formed at the middle to the outer tangential wall rather than to the inner. In TEM the casparian strip appears as a thickened, homogeneous electron opaque wall region, and is uniformly shared by the adjacent short cells (Fig. 17).

The anticlinal wall including the casparian strip of the short cell is wrinkled (Figs. 10, 11). The wrinkled walls are wavy in tangential view (Figs. 14-16). Both the wrinkled pattern and the casparian strip are more conspicuous in the younger stage, and become more and more obscure as the tissue grows. In the later stage wrinkled pattern disappears and the cells become fully expanded with remain of a slightly thickened casparian strip (Fig. 13). The wrinkled or wavy pattern is restricted on the anticlinal wall. Both anticlinal and outer tangential walls are thicker than the inner tangential wall (Figs. 10, 11, 13). The anticlinal wall of the short cell is more firmly attached to each other than all the other walls on both short and long cells in cork layer. No plasmodesmata can be observed on all the faces of the cell wall of short cell. It appears to be homogeneous and the boundary between two adjacent short cells can not easily be recognized even under TEM (Figs. 13, 17). Though the plasmodesmata are not present on the wall contiguous with the long cells, the middle lamella, boundary between short and long cells, are clearly noted (Fig. 19). Although no direct measurement has been made on the thickening process of the cell wall, the thickness of the wall of short cell seems not to has changed. On the contrary, the young cell wall appears to be thicker than that of older one in the most cases.

III. Long cells in cork layer

The long cells are radially elongated. One to several long cells are radially linked between two short cell layers (Fig. 1). In a fully developed bark, majority of the long cells become

destroyed except the "cork ray cells" (Fig. 8). Most of the long cells are club-shaped with swollen ends facing internally (Fig. 7). They are jointed one to the other by their end walls. But their lateral walls are in contact with the intercellular spaces, occasionally remain partly in contact with other long cells (Fig. 20). Their walls are evenly thickened. Nor pits neither plasmodesmata have been seen on their lateral walls which are in contact with intercellular space, other long cells and short cells (Figs. 18, 19, 20). But the plasmodesmata are definitely present on the end wall connecting two long cells (Fig. 20). In other words, the plasmodesmata are present only on the wall being contiguous with the other long cell. The middle lamella located either between two long cells, or long cell and short cell can be identified. The plasmodesmata in the wall of the long cell become obscure as the tissue grows.

IV. Secondary phloem elements

Almost all layers of the periderms other than the initial periderm originate in secondary phloem. The secondary phloem consists of four tissues, i.e., fiber cells, ray cells, parenchyma and sieve tube members. These four kinds of cells are more or less orderly arranged. One partly two celled layer of fibers are to alternate with bands of parenchyma, sieve tube members and parenchyma again (Figs. 21, 22). The sieve tube members are very rarely to be found in contact with fiber cells. The ray cells are parenchymatous, uniseriate rarely biseriate, usually contain denser protoplasm (Fig. 23). The fiber cells are thick walled, but not as thick as that of cork fiber (Figs. 9, 22). Parenchyma are in full of cytoplasmic inclusion in younger phloem and tern to more "empty" in the later stage (Figs. 21-24). The crystal containing phloem parenchyma are often seen in all the ages of phloem. The crystals in parenchyma are more or less cubical in shape and one single crystal almost occupies the entire cell lumen in transection (Figs. 22, 23). The sieve tube members contain crystals only in the later stage of development. The crystals in the sieve tube members occur in groups. They are much smaller than that in the phloem parenchyma (Fig. 24). The sieve plates are transversely oriented. Numerous sieve pores of various diameters are uniformly distributed on the end wall, i.e., sieve plate (Figs. 21-24). The largest sieve pore is measured as 1.5 μm in diameter.

DISCUSSION

The cork cambium is composed of one type of cell which gives rise mainly to two different types of cells, i.e., suberized short cells and non-suberized long cells. These two kinds of cells are quite different in morphology but difinitely arranged in alternating order. The cork layer in this plant probably exhibits relatively high specialization in morphology, and may be concerned with some unrevealed physiological functions.

This report provides unequivocal evidence to support the previous finding on the presence of casparian strip on the anticlinal wall of short cell (Chiang, 1980; Chiang & Wang, 1984). Though cell is considered to be "dead" after the complete deposition of suberin, the short cell wall has apparently undergone expansion after the deposition of suberin. This phenomenon has also been described in some other plants (Sifton, 1945). The function of casparian strip based on its fine structure in the living cell, endodermis, has been widely discussed (Bonnett, 1968; Clarkson, Robard & Sanderson, 1971; Haas & Carothers, 1975). Very few studies have been concerned with the function of casparian strip in dead cell. The morphological evidence for cell expansion comes from the characteristics such as: reduction in bulgy area of casparian strip and disappearance of wrinkled wall in older cork cell. Since the casparian strip in young cell is more conspicuous than that in the older cell. The suberin appears on the short cell wall

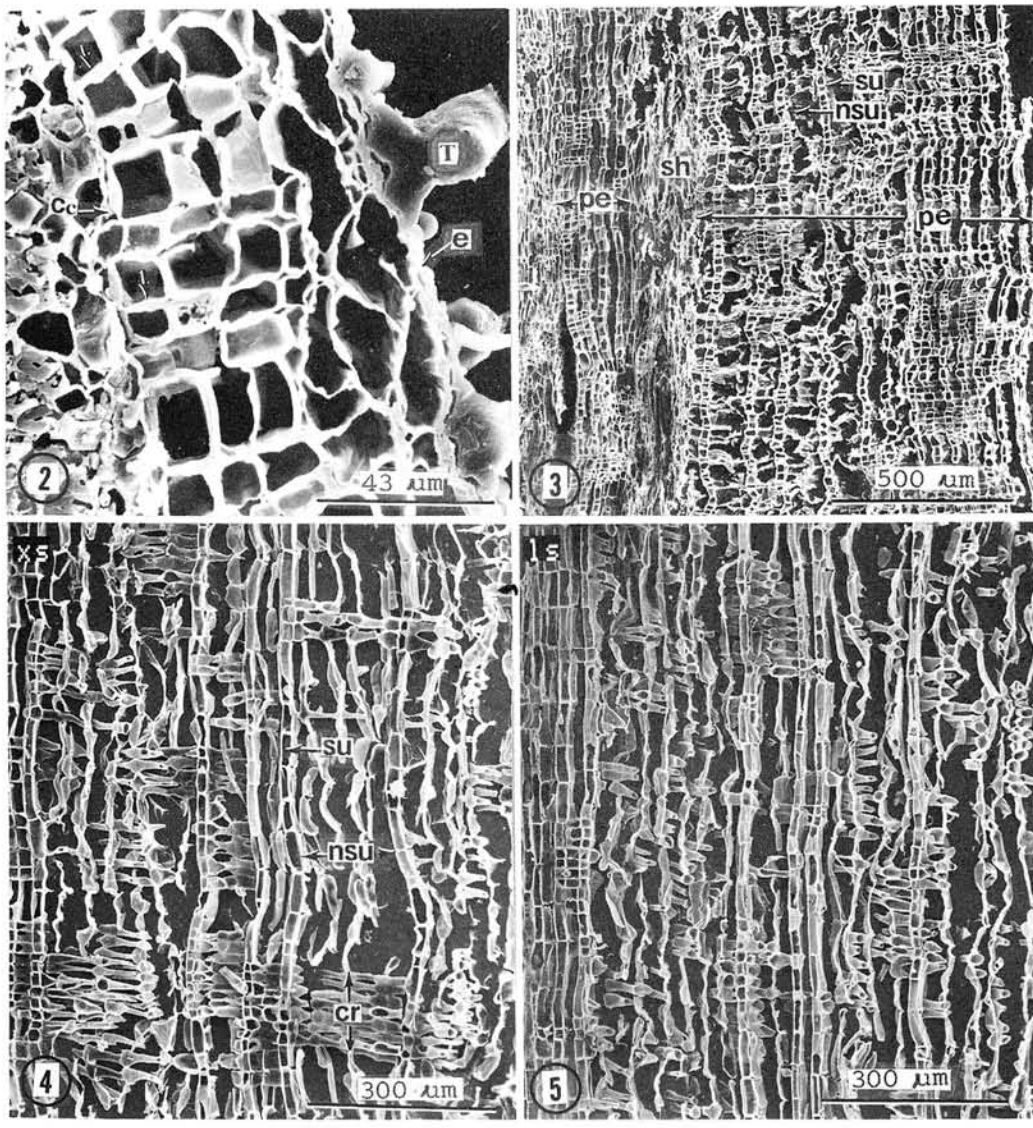
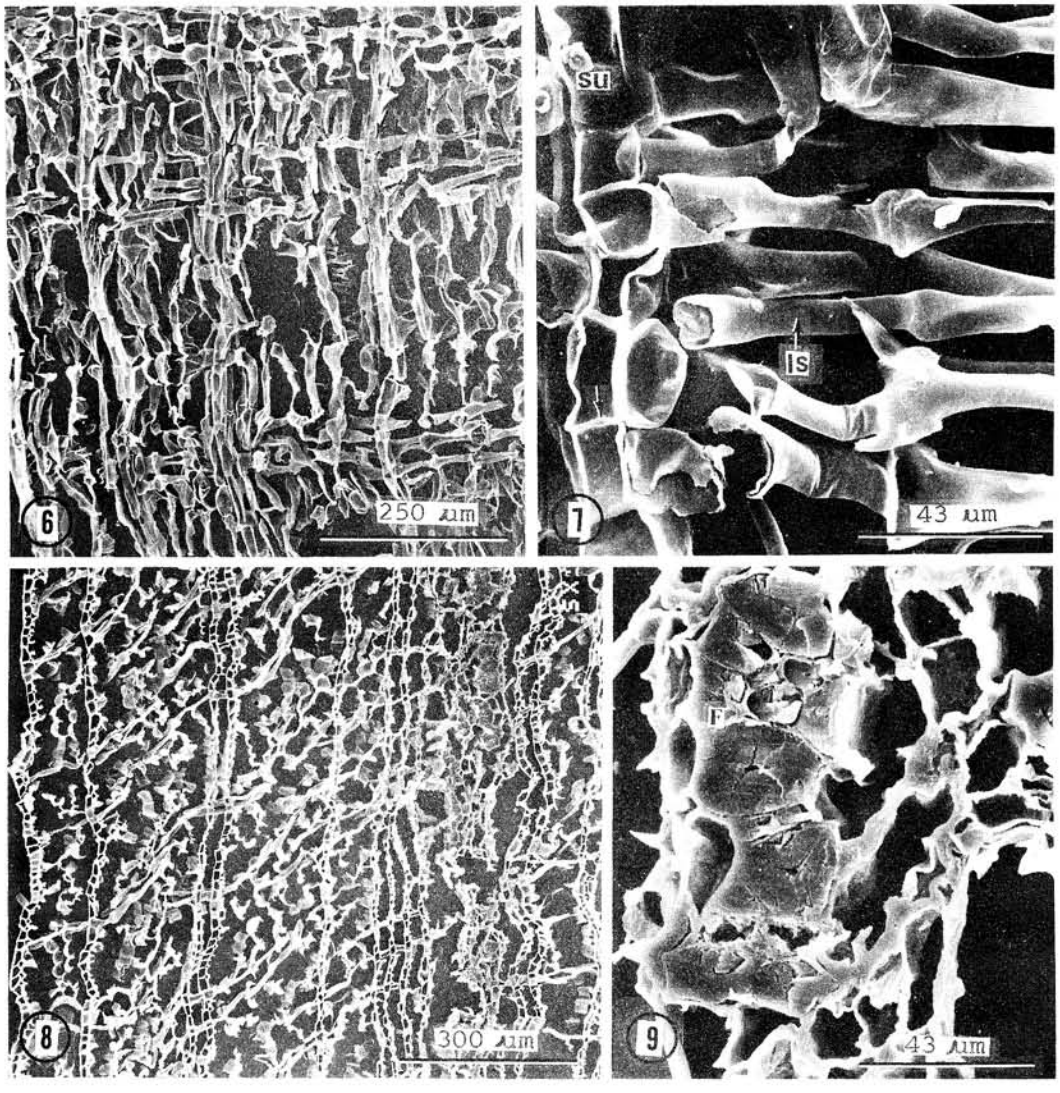


Fig. 2. Transection of a young branch bearing initial periderm (arrows=casparian strip).

Fig. 3. Longisection of a young stem bearing two subsequent periderm.

Figs. 4. 5. Transection and longisection through phellem at the stage later than that in Fig. 2.



Figs. 6, 7. Longisections through phellem at mid-stage of development (arrows= casparian strip).
Fig. 8. Transection of phellem at late stage of development.
Fig. 9. Same stage as Fig. 8, showing the fiber cells in sectional view.

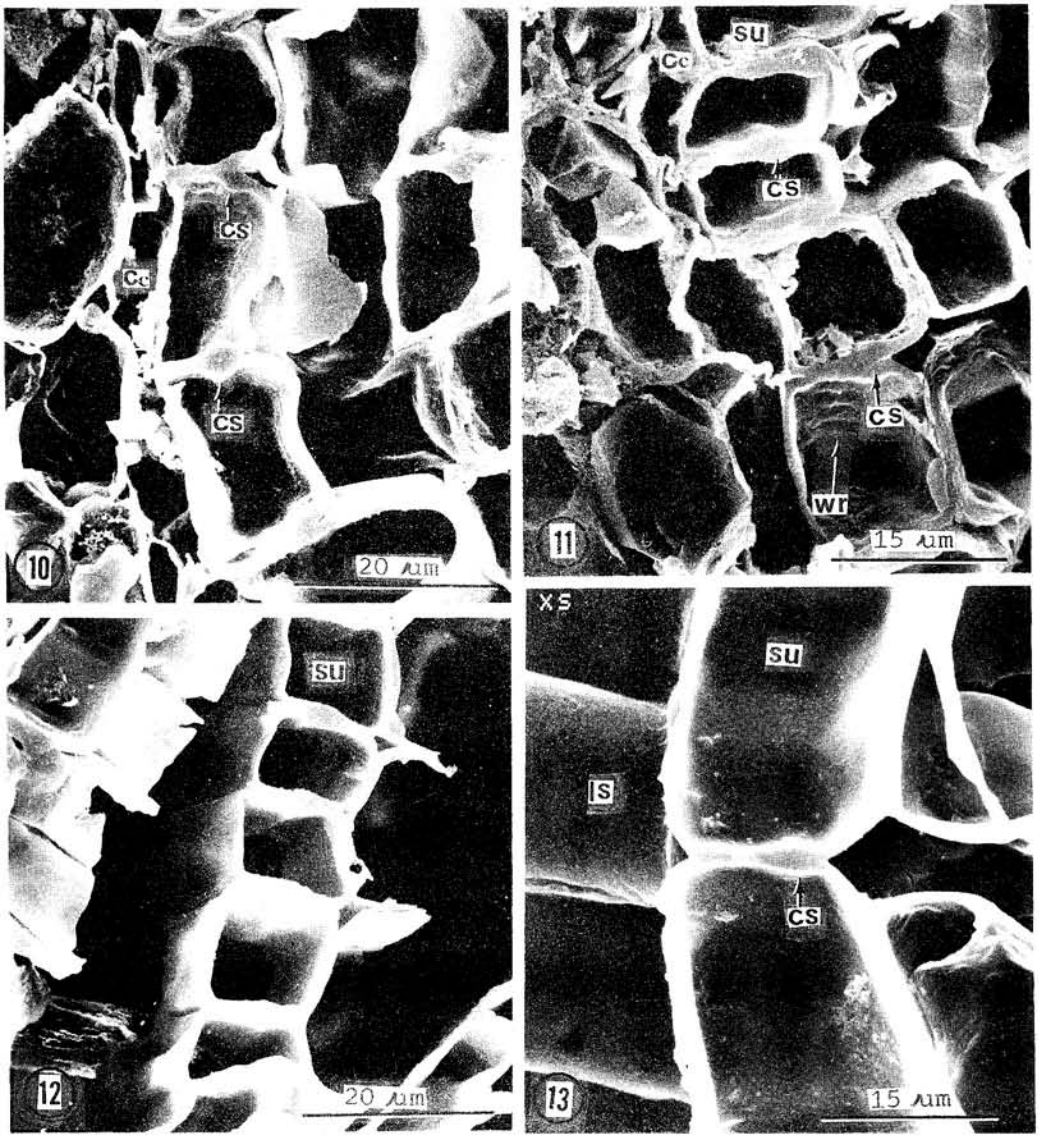
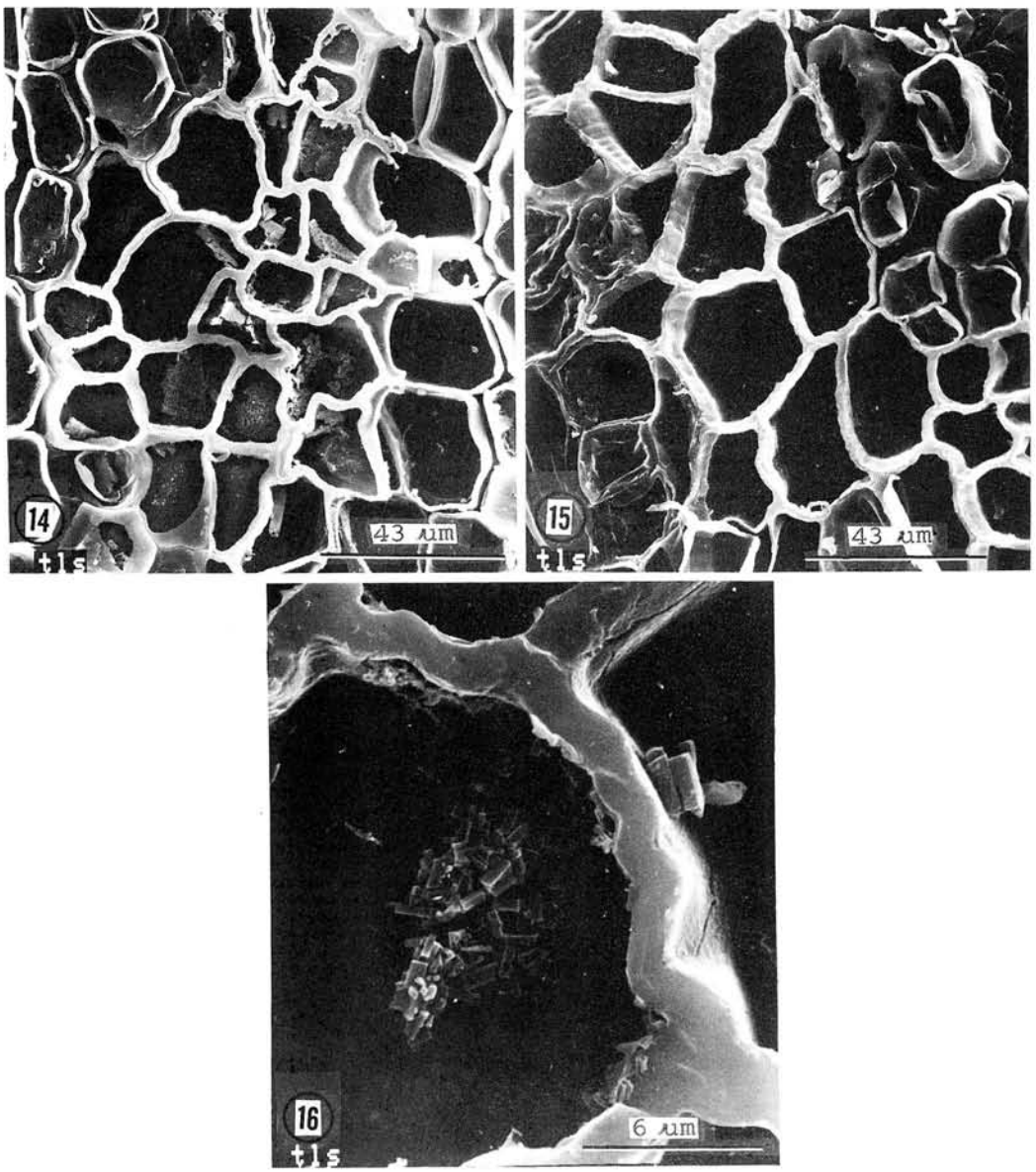
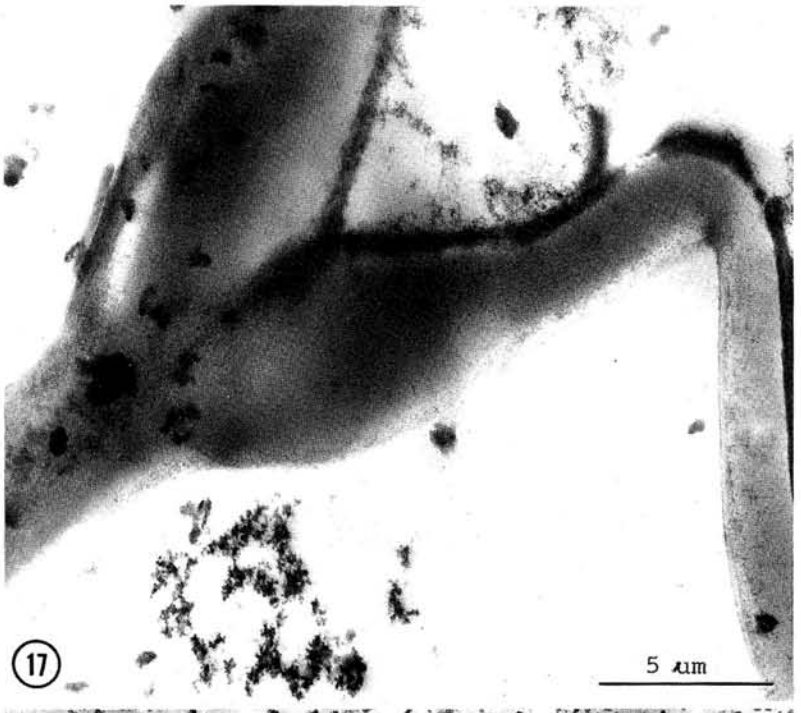


Fig. 10. Transection showing the phellogen and its adjacent tissues.
Fig. 11. Same as Fig. 10.
Fig. 12. Transection through a short cell layer at the mid-stage.
Fig. 13. Transection through a short cell layer at the later stage.



Figs. 14, 15. Tangential longisection through the short cell layer; note the wrinkled anticlinal wall.
Fig. 16. Enlarged view of a wrinkled short cell.



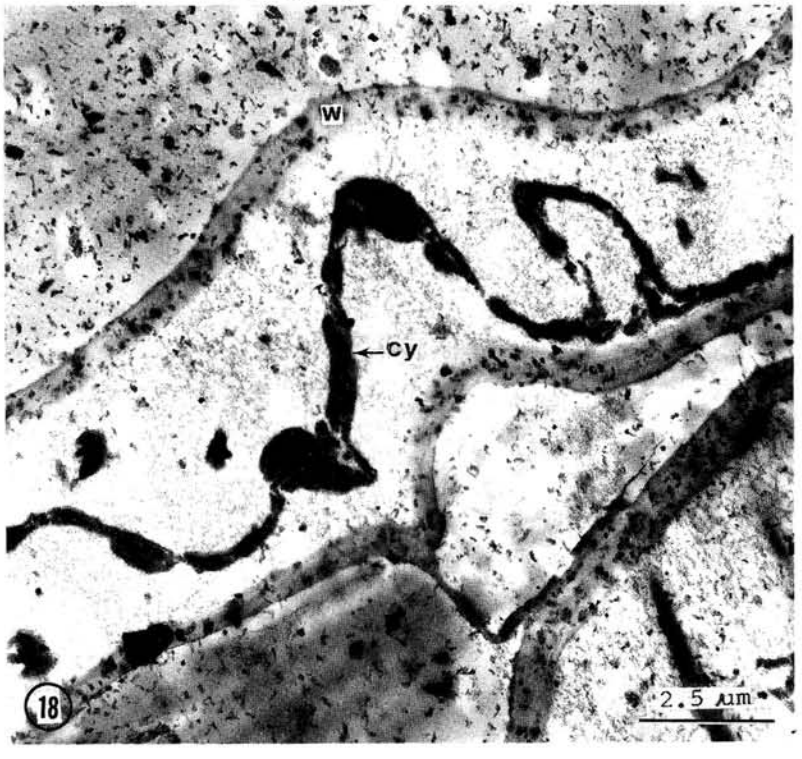


Fig. 17. TEM, transection of a "casparian strip" in young cell.
Fig. 18. TEM, transection, a part of long cell.

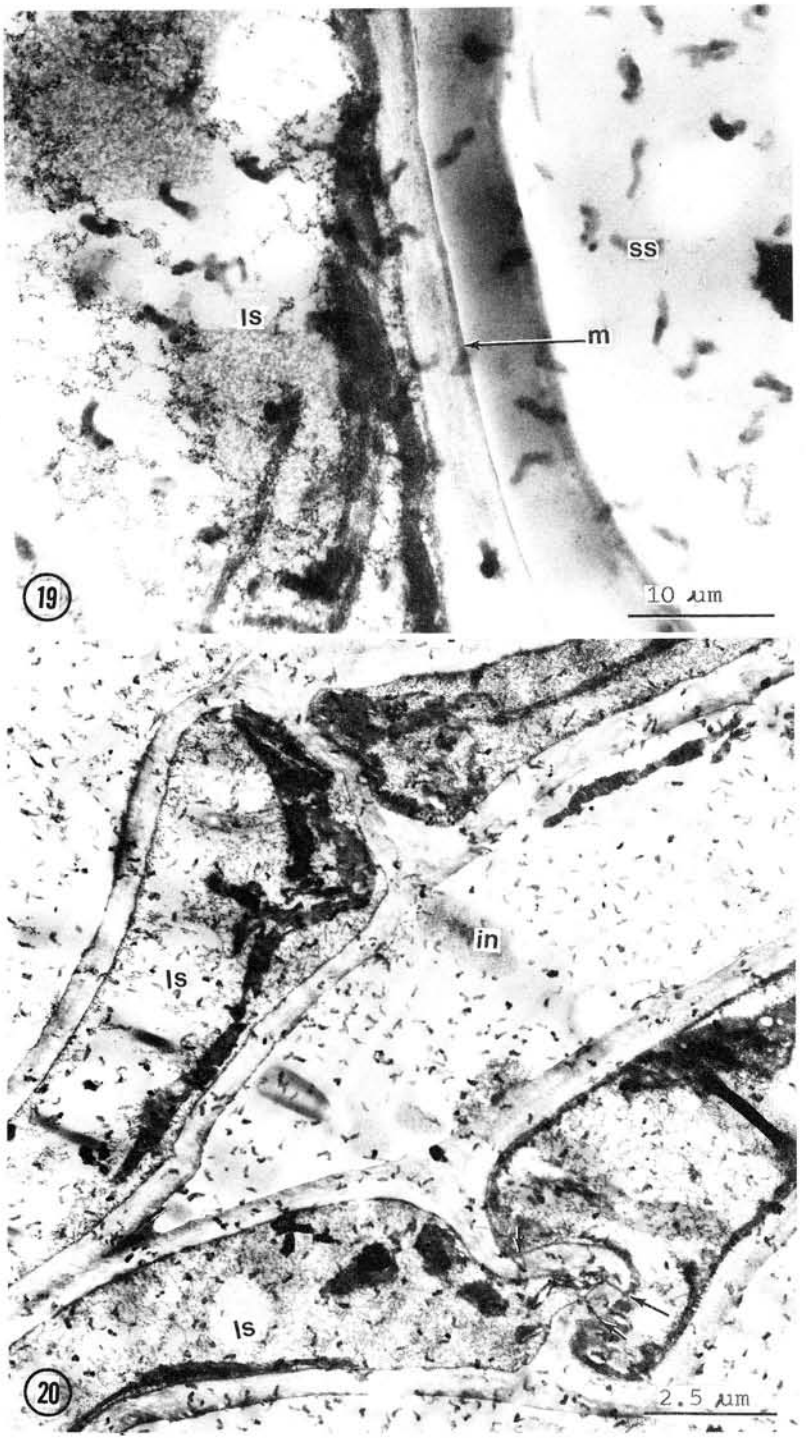


Fig. 19. TEM, transection through the wall between short and long cells.
Fig. 20. TEM, transection through the long cell layer. (arrows=plasmodesmata).

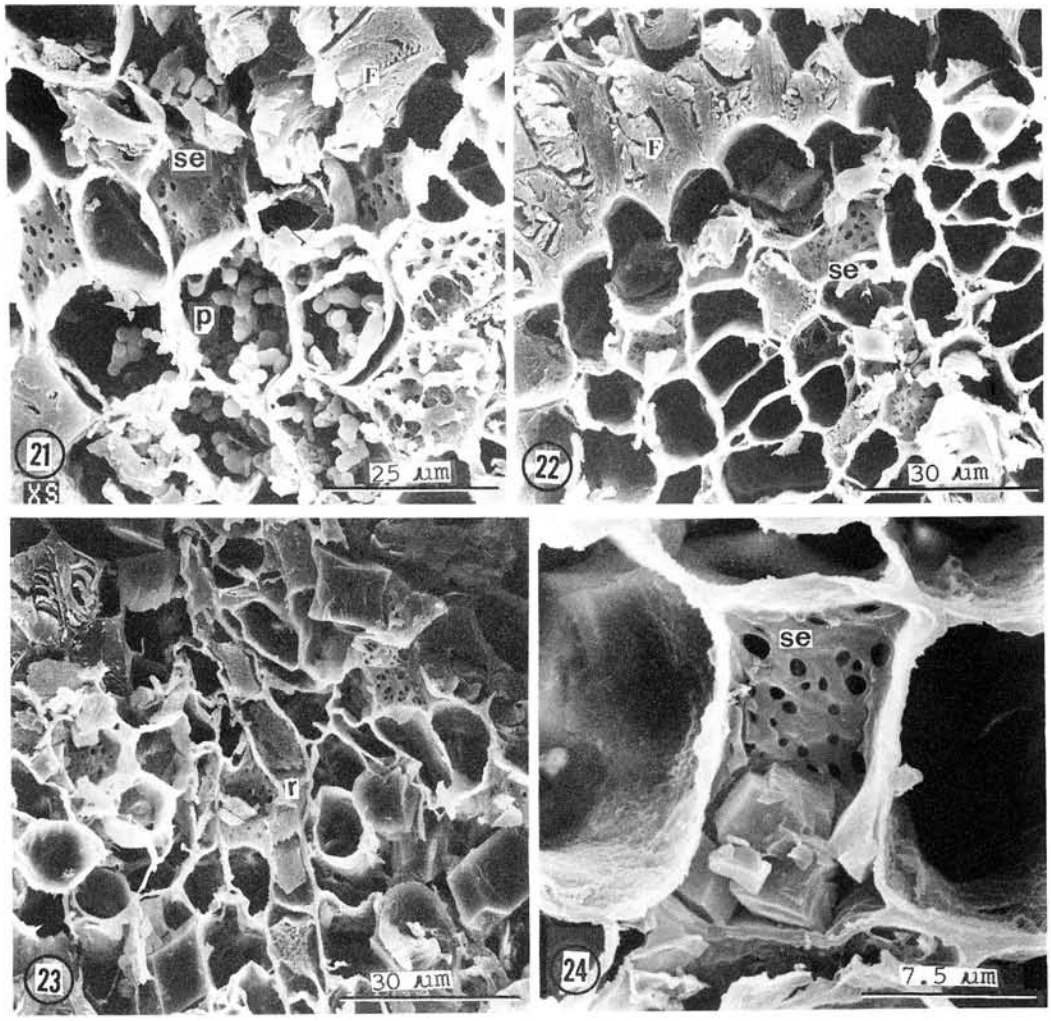


Fig. 21. Transection through the functional secondary phloem.
Figs. 22-24. Transections of older secondary pholem.

very early. The suberin has been detected on the wall of immediate derivatives of cork cambium (Chiang, 1980). The short cells could be suggested as more specialized members than the long cells. The short cells lack pits and plasmodesmata on all the faces of cell wall. Its cytoplasmic contents diasppear before that of the long cell. The presence of cytoplasmic inclusions of long cell after the suberization of short cell indicates that the suberized wall in the developing stage of this tissue is apparently permeable to some materials. The permeable suberized wall has also been described by earlier workers (Kramer, 1946; Schonherr & Ziegler, 1980). The peculiar area, casparian strip, seems not be the barrier of transportation either, since the cytoplasmic contents in long cell become disintegrated long after the formation of casparian strip and disappearance of cytoplasmic inclusion in the short cell located internally. It is suggested that the materials may move from the places other than the casparian strip. As pointed in earlier report, the short cells do not separate from each other in macerating reagent (Chiang, 1980). In addition to the presence of casparian strip, the homogeneous appearance of the wall structure between two adjacent short cells could also play an important role in the fact that they are firmly attached to each other anticlinally.

In summary, the main findings provided in the present report are: 1) casparian strip is more conspicuous in young stage; 2) wrinkled wall occurs only on the anticlinal wall of young short cell; 3) short cell is more specialized than the long cell.

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白千層木栓組織之電顯補充觀察

江蔡淑華　陳淑宇

摘　要

以電顯觀察白千層（*Melaleuca leucadendra* L.）木栓組織作爲光顯之補充研究。顯示「卡氏帶」固定存在於短型細胞之垂周細胞壁上。且在幼細胞上之「卡氏帶」較老細胞上者更爲明顯。波浪式皺紋細胞壁也只於短型細胞之垂周壁上才可找到。基於原生質連絡絲、細胞內含物和「卡氏帶」之存否等事實，短型細胞應認爲是較長型細胞更爲特化之細胞。木栓質出現後，細胞仍行擴大生長。