CASPARIAN STRIPS IN THE LATTICE-WORK PHELLEM OF *MELALEUCA LEUCADENDRA* (L.) L.

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Abstract: The presence of casparian strips on cork cells, and the structure and the development of the lattice-work phellem are described. Three to four subsequent periderms are formed during a growing season in the young branch. Most of these are parallel to each other. Each periderm is composed of layers of radially elongated thin-wall non-suberized 'cork ray cells', alternating radially with a flattened casparian strip bearing suberized cells. The significance of the casparian strip in the cork of the stem is also described.

INTRODUCTION

The periderm plays the important role in the protection of the plant axis. The occurrence of the periderm in both the stem and root is very commonly observed in woody plants. The tissue constituents in the phellem in the majority of species are relatively simple. They have been described to be homogeneous, usually compactly arranged, prismatic in shape, tetrakaidecahedral in form, and with the presence of suberin in the walls (Esau, 1965, 1977). In these the cell type is the same throughout the whole width from the innermost layer to outermost layer in the phellem; but some special types of pellem have been observed. In the roots of the Hypericaceae, Myrtaceae, Onagraceae and Rosaceae (Luhan, 1955; Neslon and Wilhelm, 1957) this zone is known as the polyderm, and in some other plants, the phellem is heterogeneous in cell constitution (Esau, 1977; Metcalfe and Chalk, 1957). But the most spectacular case thus far described is in some of the Myrtaceae, which includes *Melaleuca leucadeudra*, where the phellem is usually stratified, being composed of layers of radially elongated thin-walled cells alternating with radially flattened cells (Metcalfe and Chalk, 1957). The origin of the first phellogen in *Melaleuca leucadendra* was mentioned in a previous report (Chiang, 1978). It shows a very peculiar pattern in its topographical location within the primary tissue. The first phellogen is deep-seated in the inner cortex, and arises nearly parallel to the stem surface in some twigs; but in other branches it appears to be curved or wavy within the cortical zone. The occurrence of casparian strips is well known in roots, underground stems, leaves of ferns and in the root polyderm of some seed plants (Esau, 1965, 1977; Guttenberg, 1943; Luhan, 1955; Nelson and Wilhelm, 1957; Ogura, 1972; Van Fleet, 1961), however no detailed description of the occurrence of the casparian strip in the cork cells of a stem has been previously reported. The structure and development of the periderm in both young branches and the main trunk is presented in this investigation.

MATERIALS AND METHODS

The materials for this study were collected from the campus of the National Taiwan University in Taipei, from September, 1977 to May, 1979. The buds started to form in December, and branches ceased to grow by the next September. The new twigs are always

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lighter in color than those which grew in the preceding year. In order to study the successive development of the periderm within the same growing season, the base of the new twig was harvested at monthly intervals at the beginning of every month. The materials were fixed in FAA, dehydrated and run through the TBA series in paraffin. Sections were cut at 10 μ and stained with safranin and fast-green.

Some materials of the bark were obtained from a bigger trunk. Some of these were sectioned by the same method as that for the young twigs, and others were macerated in superoxal then stained with safranin. The detection of suberin and fatty substances was by staining with sudan IV in 1:1 glycerol alcohol, then destained by 1:1 glycerol alcohol.

RESULTS

The Origin of Subsequent Periderm

As described in the previous report (Chiang, 1978), the first periderm of some twigs originates as deep as in 10th cortical layer, almost immediatly external to the pericyclic fiber groups (Figs. 1, 2), and almost parallel to the plant surface, but curved and wavy phellogens were also frequently observed. The observation of the development of subsequent periderms in the present report has mainly been made on the twigs where the first periderm is parallel to the stem surface. The initial phellogen appears in a region about 5–6 cm from the tip of the twig of the first growing year. As the cell layers of the first periderm became as much as about 12 cells thick, the second phellogen started to divide in the secondary phloem (Fig. 3). Three to four subsequent periderms are formed during the first growing year. Most of these are parallel to each other (Fig. 4), but restricted overlapping strata of the subsequent periderms can also be found (Fig. 5). All of the subsequent periderm originates from the secondary phloem which is constantly being formed from the vascular cambium (Fig. 2). So the subsequent periderms alternating with the disintegrating phloem layers come to nearly surround the axis (Fig. 4). The secondary phloem consists of three main tissues, ie, thick-walled fiber groups, darkly stained ray cells, and thin-walled phloem elements. The darkly stained material in the ray cells was detected to be fatty substances. The fiber groups occur in tangential strands more or less alternating with the phloem elements (Figs. 2, 4, 5). Both the rays and phloem parenchyma become dilated at later stages.

The Structure and Development of the Components in a Young Branch

Though a relatively wide phellem is produced from the phelloderm may be only one cell deep in some periderm (Fig. 3) while in others there is no indication of any phelloderm (Figs. 7, 8). The dividing activity initiating the periderm begins around almost the entire circumference. The phellogen gives rise to a layer of suberized cells alternating with a layer of non-suberized cells. The non-suberized cells become radially elongated except for the early formed cork cells in the first periderm (compare Fig. 1 with Figs. 7, 8, 9). They become elongated soon after they are cut from the phellogen (Figs. 7, 8). The suberized cells remain as a shorter layer, radially flattened, and characterized by the presence of casparian strips on their anticlinal (both transverse and radial) walls (Figs. 9, 10, 11) The casparian strip differentiates during an early stage (Fig. 1). In most cases, the first derivatives of the initial phellogen appear to be the suberized cells bearing conspicucous casparian strips (Fig. 1), but occasionally they produce a non-suberized layer as the first derivative. Both the radial and outer tangential walls appear to be thicker than their inner tangential walls as seen in transections (Fig. 8). The non-suberized cells possess thinner walls than the suberized ones. In addition to bearing casparian strips, the suberized cells appear to be wrinkled. They look like they had scalariform pitting at first glance (Figs. 10, 11); but the wrinkled pattern can be clearly identified in the radial section (Figs. 13, 14), and becomes more and more obscure in the periderms located

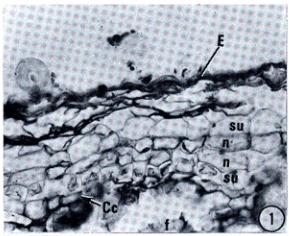
nearer to the surface of the stem (Fig. 12). The cork cells in the outer periderm appear to be fully expanded. The wavy wrinkles on the cell wall disappear eventually completely.

Based on the length of the cork cells in the direction parallel to the stem surface (tangential direction), the mature phellem cells can be classified into two kinds, ie, the tangentially expanded cells and the narrow cells (Figs. 4, 5, 7, 8, 9). Though the narrow cells are not definitely linked with the vascular ray, they are comparable to rays in the secondary vascular tissue. In the longitudinal view the suberized layer has a pattern very similar to storied cambium (Fig. 12). It consists of rather vertically elongated cells (comparable with fusiform initials in the cambium), and has both uniseriate and multiseriate ray-like cells. The former are expanded cells, and the latter are narrow cells as seen in transection (Figs. 4, 7, 8, 9). For convenience the narrow cells are termed as 'cork ray' in the present description. The 'cork ray' exhibits stronger stainability than the expanded cork cell. Both uniseriate and multiseriate 'cork rays' are seen. The expanded cork cells in the non-suberized layer disintergrates first as the axis continues to increase in circumference (Figs. 4, 5, 7, 9). The non-suberized 'cork ray cells' are the next to disintergrate. The cell walls of the suberized layer are more firmly attached to each other along their anticlinal walls where the casparian strips are seated. They do not readily separate from each other when they are put in a macerating reagent which usually causes the separation of cells (Fig. 12). In addition to the cell types mentioned above, fibers are very often seen within the phellem (Fig. 6). These always appear in groups and are vertically elongated. The cell wall of the fiber is very thick and lignified.

The Structure and Development of the Components in the Trunk

As the tree grows bigger, the branches and the main trunk always bear numerous layers of subsequent periderms. The outer periderms appear to be spongy in texture. They consist of alternating layers of cells: one cell layer of suberized cells (with casparian strips), and several layers of cells with partly destroyed non-suberized cells (Figs. 17, 18). Similar to that seen in the young branch, the cells in the suberized layer are compactly arranged, with

Fig. 1. Transection through the first periderm of a young branch, ×315.
Fig. 2. Transection through the secondary phloem and its adjacent tissues, the second layer of the periderm is visible on the upper side of the photo, ×150.
Fig. 3. Early development of the second layer of the periderm, ×150.
Fig. 4. The stem bearing three layers of periderm, ×80.
Fig. 5. Overlapping periderms, ×80.
Fig. 6. Fiber containing periderm, ×150.
Key to labeling: cc-cork cambium; cr-cork ray; Cs-casparian strip; E-epidermis; f-fiber; n-non-suberized layer; nsr-non-suberized cork ray; pe-periderm; ph-phloem element; r-ray; sh-secondary phloem; su-suberized layer; v-vascular cambium; W-wood (secondary xylem); wr-wrinkled wall.
Figs. 7, 8. Transections of stem, showing the growing phellems and the arrangement of the young phellem cells, Fig. 7 ×170; Fig. 8 ×350.
Fig. 9. Young phellem stained with sudan IV, showing the casparian strips and wrinkled suberized cell layers, ×170.
Figs. 10, 11. Enlarged views from Fig. 9, ×900.
Fig. 12. Tangential longisectional view of the well-expanded suberized cell layer from the macerated material, ×88.
Fig. 13. Tangential longisection of the wrinkled cells in the suberized cell layer from the young portion of stem, ×170.
Fig. 14. Enlarged view of Fig. 13, ×900.
Fig. 15. The innermost layer of the periderm from the trunk, ×170.
Fig. 16. The immediate preceding periderm of that in Fig. 15, ×170.
Fig. 17. Transection through the outermost periderm in a region some distance from the crack in the stem surface, ×88.
Fig. 18. The same periderm of Fig. 17, in the region near the crack in the stem surface, ×88.
Fig. 19. Enlarged view of Fig. 18, ×350.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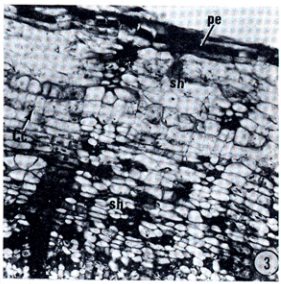
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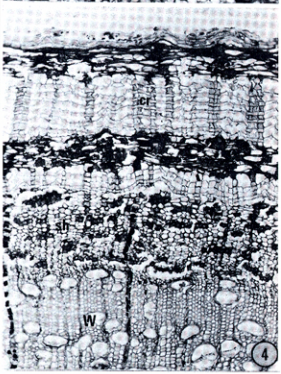
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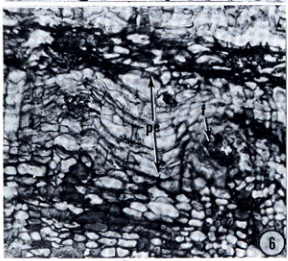
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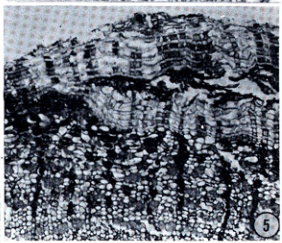
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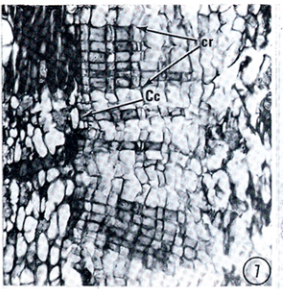
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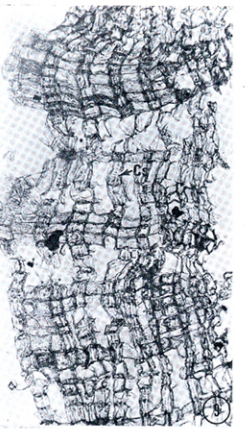
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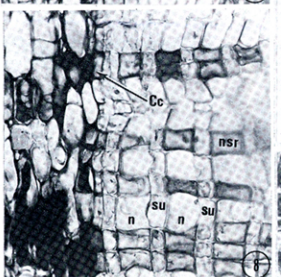
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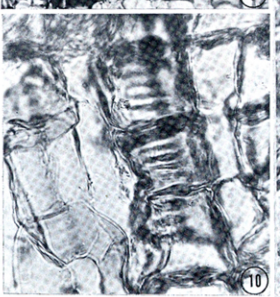
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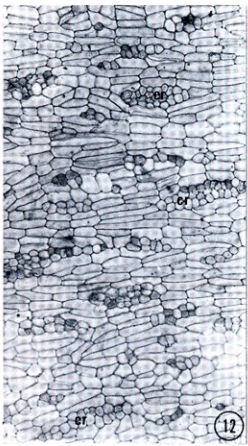
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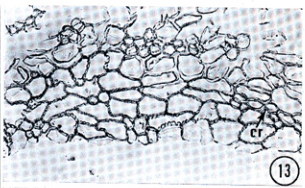
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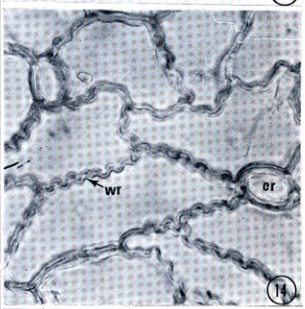
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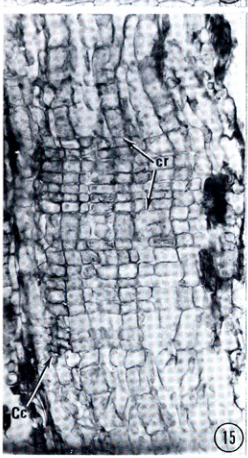
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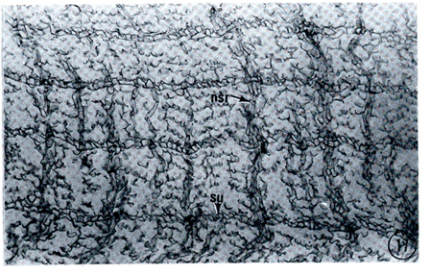
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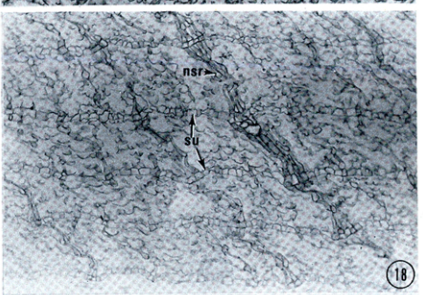
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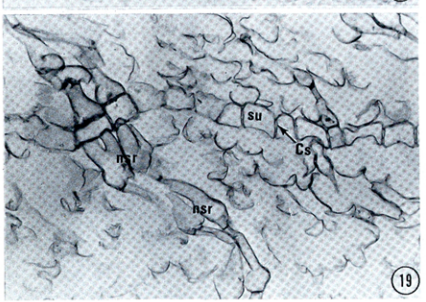
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casparian strips, and thicker radial and outer tangential walls (Figs. 17, 18, 19). The non-suberized cells become destroyed except for the 'cork ray cells'. The majority of the 'cork ray cells' are radially elongated, club-shaped and with the swollen end facing the center of the stem axis. Some of them appear to forked (Fig. 19). In most cases, four to five radially attached non-suberized 'cork ray cells' are located between two successive suberized cell layers. As the tree grows older, the increase in the circumference causes the crack formation on the stem surface where the successive periderms accumulate to a great depth. The angles formed between the non-suberized 'cork ray cells' and the suberized layer become oblique in the region near the crack (Fig. 18). But they are almost perpendicular with each other in the periderm farther from the crack (Fig. 17).

The phellogen gives rise to one layer of non-suberized cells after it forms one layer of the suberized casparian bearing cells. This "one after one" pattern becomes obscure in the bigger branches as well on the main trunk. At later stages the phellogen produces one to two, rarely three, layers of the non-suberized cells between the successive single layered suberized cells (Fig. 15). This pattern is occasionally seen in the first periderm of the young twig as well (Fig. 1). The newly produced cells of the subrized layer from the deeper layers of the phellogen do not show a flattened appearance in the bigger branches or on the main trunk (Figs. 15, 16). The length of the tangential and the radial walls are almost the same at first but the cells become radially flattened as they grow older. The multicellular pattern of the radially linked non-suberized cork ray' in the fully developed periderm of the bigger branches are mainly caused by the cell division together with the radial elongation of these cells. The cell elongation plays a more important role than cell division. The expanding process of the suberized layer and the elongating process of the 'cork ray cells' do not stop until long after the subsequent periderm has born a considerable number of cell layers (compare Fig. 15 and Fig. 16). During the process of the formation of the aerenchymatous periderm the non-suberized expanding cells do not divide. Finally they become destroyed, and remain as scattered debrises with intercellular spaces (Figs. 17, 18, 19). The aerenchymatous cork has a lattice-work appearance in transverse section, with the uniseriate flattened suberized layers located tangentially and 'cork ray cells' radially.

DISCUSSION

Heterogeneous cork has been found in both the stem and the root of many plants. The place of the origin of the initial phellogen as well as the structure of the periderm have been briefly described for some Myrtaceae including *Melaleuca leucadendra* (Metcalfe and Chalk, 1957). The formation of one after another periderm in the successively deeper secondary phloem causes the rhytidome to be a prominent part of the axis in this plant. Though some restricted overlapping strata of the sequent periderms can be seen, most of them occur parallel to each other. Nemerous layers of the subsequent periderms alternating with parallelly seated dead secondary phloems remain on the stem surface for a rather long time. The formation of a large amount of intercellular spaces by the destruction of the non-suberized expanding cell layers makes the mature cork highly compressible and spogy in texture. The stem of *Melaleuca leucadendra* produces three to four subsequent periderms in an year. The phellogen in this plant is actively dividing. Once the preceeding phellogen ceases to divide, a subsequent phellogen initiates actively in the deeper secondary phloem. The phellogen has been observed to divide more than ten times. Consequently the cell divisions occurring in these phellogens which are formed in a single growing season are estimated to be more than 40.

Cork cells are considered to be dead cells, but at least in *Melaleuca leucadendra*, the elongation precess and the cell division in the non-suberized 'cork ray cells' are visible even

at a time when the subsequent additional phellogen is forming a considerable amount of the cork in its deeper tissues. Though the expansion of the suberized cell layer may be caused simply by smoothing out the creases on their cell walls, the cell divisions occurring in the non-suberized 'cork ray cells' are physiological rather than mechanical. As a matter of fact, the wrinkles or folded anticlinal walls of the suberized layer has a great reserve of the expanding ability. This makes it possible for cell layer to increase in surface area and keep pace with the increase of the circumference of the axis. So the cells in periderm, or at least some of them, remain alive after their subsequent deeper phellogen has preduced an amount equal to its preceding periderm. The wrinkled process of the growing cells is widely distributed among the roots of some herbaceous angiosperms (Esau, 1977; Davey, 1946; Rimbach, 1899). The wrinkled walls of these roots mainly occurs in the cortical and phloem parenchyma at a certain stage of development. Wrinkle formation in *Melaleuca leucadendra* also makes the cork cells remain on the surface of the stem for a longer period during the growth of the stem in circumference.

Little is known of the occurrence of the casparian strips in the stem cork. A very typical casparian strip is constantly visible in this plant. The possible physiology of the casparian strip has been studied in the root (Bonnett, 1968). Morphologically it is merely a thicker area of the cell wall. The function of the casparian strip in the stem cork of this plant is questionable. The presence of a casparian strip may connect a cell more tightly with the walls of the neighboring cells. It prevents the suberized cell layers from separating from each other too easily. The formation of the wrinkled cell wall during the development of this tissue together with the tightly connecting casparian strip on the suberized cell layer are the key reasons why the periderm remains on the growing axis for a long time.

Though cork with heterogeneous cell types has been found yet cork exhibiting a structure comparable to that of the vascular cambium has not been previously seen. All the subsequent periderms, except the initial periderm, constantly originate from the secondary phloem in this plant. The secondary phloem is known as one of two derivatives of the vascular cambium. It seems that the cork developing from the derivatives of the vascular cambium appears to be more similar to the vascular cambium in cell arrangement than the cork which initiates in other tissues. The confirmation of this idea needs to be based on the further exploration of the structure of the various plants which possess the periderms originating in the secondary phloem.

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