Some circumstantial evidence is very strong, as when you find a trout in the milk.

Henry David Thoreau (1817-1862)

American author, poet and philosopher

Chapter 3

MORPHOLOGY AND CULTIVAR SPECIFICITY OF MANGO (*Mangifera indica* L.) LENTICELS

3.1 ABSTRACT

Lenticel discolouration of mango (*Mangifera indica* L.) fruit annually causes financial loss to growers. Cultivars 'Tommy Atkins', 'Kent' and 'Keitt' were investigated as part of a study into the inducement of this condition. Lenticels occur abundantly on the surface of mango fruit, and are important in regulating temperature and transpiration. They originate from stomata and differentiate as the fruit develop and mature. It was found that the morphology of the lenticels from different cultivars differs in stomate width, lumen depth and abundance of epicuticular wax. Mango lenticels are lined with cutin, and do not have any underlying meristematic tissue. An intra-lenticular layer of wax accompanies the cuticular membrane, with its abundance and complexity distinctive for each cultivar investigated. Sufficiently different morphologies were found between the studied cultivars for lenticels to be considered as a variable in cultivar susceptibility to the development of lenticel discolouration. Although discolouration of lenticels are quite visible with the naked eye and light microscopy, no discernable differences between affected and non-discoloured lenticels could be identified by scanning electron microscopy.

3.2 INTRODUCTION

Lenticels are superficial structures facilitating gaseous exchange between internal plant tissue and the environment, and are found on aerial parts of members from many plant families (Stern, 1994). They usually appear as raised or blistered, corky pores due to suberised tissue and are associated with the woody bark of perennial plants, located in the periderm of trunks, twigs and stems. Descriptions of lenticel morphology are limited to a classic model (Fig. 1) on which textbooks base their discussion of this structure. According to this classic model, a typical lenticel is stratified and consists of epidermal

cells, phellogen (cork cambium) that lines the substomatal chamber, and specialised complementary tissue (phellem and intercellular spaces) (Esau, 1977; Stern, 1994). The growth and development of stem lenticels are marked by meristem activity that initially increases the layers of non-suberised tissue closest to the phellogen. These new layers pushes outward, breaking the barrier tissue from previous growth seasons apart, and becoming suberised later during the same season. During this secondary process of suberisation, the tissue that was pushed into the newly exposed fissure, seals off the nutrient rich, living tissue inside from the atmosphere outside, while the size of the lenticel opening to the atmosphere is expanded (Guirguis *et al*., 1995; Batzli & Dawson, 1999; Rosner & Kartusch, 2003). Lenticels are therefore regarded as cork cambium derivatives. Phellem cells in the cortex are normally tightly packed, tangentially elongated and radially flattened. However, the eruptuous lenticels are differentiated from the rest of the cortex by rounded phellem cells and the resulting intercellular spaces.

Although the occurrence of lenticels on fruit such as pears, avocadoes, apples and mangoes are well documented, it is mostly in conjunction with physiological and pathogen related problems (O'Hare *et al*., 1999; Pesis *et al*., 2000; Amarante *et al*., 2001; Everett *et al*., 2001; Veraverbeke *et al*., 2003a & 2003b; Anonymous, 2004). The ontogeny and anatomy of the mango (*Mangifera indica* L.) fruit lenticels (cultivars 'Keitt' and 'Tommy Atkins') was described in a study by Bezuidenhout *et al*. (2005), confirming their stomatal origin. Other discussions on lenticels from various species are set around its value in identifying horticultural relationships (Guirguis *et al*., 1995; Rosner & Kartusch, 2003), as an indicator of flood stress (Larson, 1991; Kozlowski, 1997; Batzli & Dawson, 1999), and as a functional parameter in reforestation models (Vanclay *et al*., 1997). In each of these instances, the investigation focused on the hypertrophied growth and expression of physiological reactions, and anatomical appearance and frequency of stem and bark lenticels. As a structure functional in gaseous exchange, growth and expansion are important mechanisms in transpiration and temperature control (Kozlowski, 1997; Rosner & Kartusch, 2003). Temperature control is of utmost importance to mango fruit, of which surface temperatures can exceed 56 °C during fruit set and growth (Grové, 2004, pers. com.).

Although fruit up to 25 mm in length (point of peduncle attachment to style end) are dotted with stomata, lenticels of varying shapes and sizes rapidly develop from them (Bezuidenhout *et al*., 2005). Lenticels are a distinctive feature of mango fruit, but are also the cause of economic problems when a condition known as lenticel damage (Bezuidenhout *et al*., 2003; Du Plooy *et al*., 2003) or lenticel spotting (Bally *et al*., 1996)

develops. The condition manifests as red or blackened halos surrounding the lenticels. Discolouration is chronological in terms of colour development, with normal (non-discoloured) lenticels developing a red halo that will eventually turn black. Several investigations into the signalling for the development of the discolouration have been done, but no satisfactory explanation can be offered (Bally *et al*., 1996; O'Hare *et al*., 1999; Pesis *et al*., 2000). Prediction and management of the problem is therefore difficult and uncertain (Table 1). To complicate matters, cultivar susceptibility is highly significant in the development of the condition (Donkin & Oosthuyse, 1996). Based on differences in expression and severity of lenticel discolouration between cultivars, the purpose of this study was therefore to investigate the lenticel morphology of physiologically mature fruit from different cultivars, and to determine the possible connection between discolouration and fruit lenticel morphology.

3.3 MATERIALS AND METHODS

3.3.1 Plant material

Samples of physiologically mature mango fruit exhibiting lenticel discolouration (cultivars 'Tommy Atkins', 'Kent' and 'Keitt') was collected fortnightly from the packhouse of Bavaria Fruit Estates (Hoedspruit, Limpopo Province, South Africa) throughout the 2002/2003 and 2003/2004 seasons. Five fruit per cultivar and three sections per fruit was sampled, prepared and viewed during each collection. Dissections were done immediately upon and at the point of collection.

3.3.2 Methods

3.3.2.1 Microscopy

3.3.2.1.1 Electron Microscopy

Samples for both normal scanning electron microscopy (SEM) (JSM 840, JEOL, Tokyo, Japan) and field emission scanning electron microscopy (FE-SEM) (JSM 6000FE, JEOL, Tokyo, Japan) were dissected from mature mango fruit. For normal SEM, sections were viewed at 5 kV and a working distance of 12 mm, while for FE-SEM 5 -10 kV was used.

Samples were prepared using two parallel methods in order to exclude the interpretation of artefacts from preparation. According to the first method, sections were cut and fixed in a 1:1 mixture of 2.5 % glutaraldehyde and 2.5 % formaldehyde in 0.1 M $NaPO_4$ buffer (pH = 7.3 ± 0.05), postfixed with 1 % aqueous OsO_4, and dehydrated in an ethanol dilution series (30, 50, 70, 90 and 3 x 100 %). Sections were subjected to critical point drying

(Biorad E3000, Polaron, West Sussex, UK), before mounting on double-sided carbon tape on stubs, after which it was rendered conductive in the vapour of a 0.5 % RuO_4 solution (Van der Merwe & Peacock, 1999).

For the second method, small sections of fresh material were plunge-frozen in liquid propane at -180 °C, vacuum dried (Custom built, Tshwane University of Technology, Pretoria, South Africa) at -80 °C and 10^{-7} mBar for 72 hours. Finally, sample material was mounted on double-sided carbon tape on stubs and rendered conductive in the vapour of a 0.5 % RuO_4 solution.

3.3.2.1.2 Light Microscopy

Samples for light microscopy were dissected from physiologically mature fruit from all three cultivars and fixed in a mixture of 2.5 % glutaraldehyde and 2.5 % formaldehyde in a 0.1 M $NaPO_4$ buffer (pH = 7.3 ± 0.05). After standard rinsing and dehydration, the samples were embedded in L.R. White resin.

Thin sections (0.5 - 1.0 µm) of the embedded material were cut on a Reichert Ultracut E ultra microtome (Reichert AG. Vienna, Austria) and heat fixed (60 °C) to glass microscope slides. The material was stained with a 0.5 % aqueous solution of Toluidine Blue (O'Brien & McCully, 1981) while the microscope slides were still warm. Sections were viewed with a Zeiss Axiovert 200 microscope (Zeiss, Göttingen, Germany) fitted with a Nikon digital camera DXM1200 (Nikon Instech Co., Kanagawa, Japan) and the digital images captured with Nikon ACT-1 version 2.

3.3.2.2 Chemical retrieval of cuticular membranes

Cuticular membranes were obtained by enzymatically pretreating small sections of physiologically mature 'Tommy Atkins' and 'Keitt' fruit rind. To remove the subcuticular tissue, sections were submerged for 48 hours, using a 1:1 pectinase:cellulase solution (1mg/ml pectinase, 10mg/ml cellulase, 0.075 M $NaPO_4$ buffer at pH = 7.5 ± 0.05) (Peacock, 2000). After three rinses in distilled water, the sections were soaked in 78 % H_2SO_4 for a further 48 hours, followed by three rinses in distilled water. It was then air dried, mounted on double-sided carbon tape on stubs, made conductive with RuO_4 and viewed with SEM and FE-SEM.

Epicuticular wax was removed from air-dried cuticular membranes retrieved from 'Keitt' material by soaking small sections of previously prepared membranes in chloroform

($CHCl_3$) for 72 hours. It was then air dried, mounted on double-sided carbon tape on stubs, made conductive with RuO_4 and viewed with SEM.

3.4 RESULTS AND DISCUSSION

Although two different sample preparation protocols were followed, data comparison indicated negligible differences in results obtained with material from mature fruit. All micrographs used to discuss the results were obtained by plunge freezing.

General morphology of mango fruit lenticels does not follow the classic building plan of those found on stems and twigs (Fig. 1). Firstly, there is a total absence of the phellogen layer that ensures continuous growth and replacement of cells (Fig. 2A). The stomate chamber or lumen is cavernous and lined living cells that are exposed directly to the outer atmosphere. This is countered by a unique feature in the surface of the exposed cells covered with cutin and epicuticular wax (Fig. 2B). This cutin lining is extensive and completely envelops the central air space (Fig. 3). It also becomes part of the sub-epidermal tissue as a result of the enlargement of the fruit during which epidermal cells are pushed out of the embryonic layer order, and becomes deformed. In all cultivars, expansion during growth leads to tearing and cracking of the cuticular layers surrounding the fruit, because cell division in these layers has ceased long before the volumetric increase of the fruit has reached a maximum. Ridges and convolutions result as the cutin layer fills out the enlarging perimeter of epidermal tissue. Deposition of cutin also extends into the air spaces branching off from the lumen; evidence of hollow tubular structures forming a network beneath the cuticle proper was found (Fig. 4). This proves that the lenticels are linked to one another and share the fate of both environmentally and metabolically produced volatiles.

The wax fractions present in the lenticel lumen is another anomaly, since such an intimate relationship between cutin, cuticular wax and epicuticular wax crystalloids is not normally found in lenticels. The cuticular association and continuation of the epicuticular wax in the lumen is evident from Figure 5. Physical removal of the intra-lenticular wax is not a feasible option at this time, but the observable characteristics of morphology and quantity gave important indications of cultivar specific differences. In the early maturing cultivar ‘Tommy Atkins’, a rapidly diminishing layer of intra-lenticular wax crystalloids are seen on the inside of a chemically retrieved lenticel structure. This layer is also present in the other two cultivars. In ‘Keitt’ (a late maturing cultivar) it was found to have greater abundance

and density. It was the mid-season cultivar 'Kent', however, that had the highest abundance and greatest complexity of the intracuticular wax (Fig. 6). Although carried over to the inside surface from the epicuticular wax on the outside surface, the intra-lenticular wax is not necessarily chemically identical to the outer epicuticular wax, as indicated by Prinsloo *et al*. (2004).

Internally, the size and shape of the lumen varies greatly, while externally the size and shape of the lenticel stoma is also variable. However, these morphological characters have a distinctive depiction for each the three cultivars studied (Fig. 6 & 7). 'Kent' has a large, disorganised stomate with a shallow to intermediate lumen which is often congested by epicuticular wax and cutinised cells. These lenticels have pronounced air passage ways linking the lumens. In all cultivars, expansion during growth leads to some tearing or cracking of the surrounding cuticular layers, because cell division has ceased long before the volumetric increase of the fruit has reached a maximum.

In surface view, the lenticel area is flush with, or slightly depressed into the surface of fresh fruit. The absence of raised structures corresponds to the fact that there is no phellogen, and therefore no continuous growth from beneath. In some material prepared by plunge-freezing a small measure (< 15 %) of shrinkage occurred (Boyde & Maconnachie, 1979), creating a 'hillock'-effect around discoloured lenticels (Fig. 8). This concurs with the hardening effect of the phenolics present in the vacuoles and cell walls (Dai *et al*., 1996) of these discoloured lenticels and is not restricted to a specific cultivar. In the material studied using SEM, no other indications of phenolics and the accumulation thereof could be found. Within all three cultivars, non-discoloured, reddened and blackened lenticels appear structurally similar, both internally and externally.

Comparison of external structures revealed that 'Tommy Atkins' has predominantly small stomata, with limited suberisation taking place. The lenticel stoma has a rounded to irregular perimeter. Internally, the lenticel lumen is lined with intra-lenticular wax fractions that rapidly diminish vertically down the lenticel (Fig. 6 & 9), leaving bare cutin lining the lumen wall of the lenticel. Superficial subsidiary tissue, such as mesophyll cells filling the lenticel lumen, is mostly absent. The combined effect of the observations is that little light is reflected from the stoma, which will inhibit cutin and cuticular wax deposition (Jeffree, 1996), and that the lenticel lumen maintains a larger air volume than those with disorganised lumen contents. It can be deduced from the small stomate, interconnecting air passages and large air volume in the lumen, that the lumen will contain an accumulation of volatile metabolic by-products.

In a study by Bezuidenhout *et al*. (2005), a transverse section through “Tommy Atkins” material stained with Sudan Black B, showed little cutin in the stomate chamber. Their observations are consistent with the finding of this study that the wax layer diminishes. Although Sudan Black B is used to identify cutin, it is a lysochrome, specifically targeting neutral to slightly acidic triglycerides in lipids of the cutin layer (BioGenex, 2004). Embedding in paraffin wax dissolved and dispersed the small fraction of plant waxes present. Consequently, the small wax quantities in deeper lumen areas were no longer discernable.

‘Keitt’ is a cultivar with a medium to high incidence of lenticel discolouration. Lenticel appearance on this cultivar is variable, but present with a large, irregularly torn stomate. Due to the prolonged fruit development of ‘Keitt’ (Table 1), suberisation of its intra-lenticular cells is more prevalent than in any of the other two cultivars. Together with the wax layer partially covering the intra-lenticular, this would contribute to protection of the exposed mesophyll cells beyond the lenticel lumen (Fig. 10). Tracing the differential between the wax fractions in the various parts of the lenticel, regression from dense and structurally intricate epicuticular wax to sparse, simple wax crystalloids, and eventually, nude cutin can be seen. The change is not linear, with a sudden change in the amount of coverage and complexity at about halfway down the lumen. Mesophyll cells at the lower end of the lumen and in air passages leading away from the lumen are covered with a layer of cutin that is almost without crystalline wax. In a cross-section of a lenticel from an immature fruit, the wax fraction extended further into the lenticel cavity, yet still diminished in quantity as the depth increased (Fig. 2B). The lenticel cavity thus expands with the increasing volume and fruit size during growth, but it is uncertain what governs the gradient of wax deposition for cultivars with sparse intra-lenticular wax cover.

Transversely fractured sections of ‘Kent’ material exposed a lumen wall covered with large amounts of wax (Fig. 11). Although the wax layer consists of crystalloids with a more complex architecture than that of the other two cultivars in this study, it still becomes structurally simpler in deeper areas of the central cavity and the air passages leading away from it. Randomly organised, suberised mesophyll cells obstruct the open, more exposed lumen, thereby providing a barrier against harm inflicted from outside. It has a higher abundance of lenticels on the fruit surface than either ‘Tommy Atkins’ or ‘Keitt’ fruit. ‘Kent’ lenticels are also the largest of the three cultivars investigated. Despite these facts and the overall impression of structural disorganisation, this cultivar exhibits the lowest incidence of lenticel discolouration (Donkin & Oosthuyse, 1996).

3.5 CONCLUSION

During fruit set and growth, the surface area of mangoes exceeds a 2400-fold increase in 3 - 4 four months, depending on the cultivar. All structures associated with the fruit and rind must be able to deal with this massive growth rate, with meristematic tissue best adapted to meet the demand. However, the absence of meristematic tissue is one of the distinctive features of mango fruit lenticels. To compensate for this, the dynamic maintenance of cutin and its accompanying waxes ensure that the expansion of the epidermal and mesophyll tissue will not leave the developing pulp exposed to degradation by the environment and pathogens. This intra-lenticular layer of cutin and wax is distinctively different between the three cultivars studied, with 'Tommy Atkins' lenticels least developed, 'Keitt' lenticels variable and intermediary, and 'Kent' lenticels most developed. This finding correlates with cultivar susceptibility to lenticel discolouration. The mechanism for the development of the condition is still poorly understood (O'Hare *et al*., 1999), and other aspects of lenticel morphology may also prove to be influential.

The network of air space and tubular structures between the lenticels correlates well with the lenticel function of gas exchange. Current knowledge indicates that cutin is a product of the epidermal cells formed by an external, environmental signal or combination of signals (Martin & Juniper, 1970; Jeffree, 1996). However, the results of this study indicates a non-epidermal cell type bordering the lumen and air passages. According to Jeffree (1996) though, the presence of air, moisture and light in the lumen are signalling factors for the formation of cutin by the epidermal cells. The description of cutin and epicuticular wax lining the air channels of *Gloriosa rothschildiana* (Ponsamuel *et al*., 1998) support this statement. These signals are present in the cavernous lenticels of mango fruit, and could trigger a cascade of reactions for the formation of cutin by affected mesophyll cells. The amount of light and air that enter the lenticel will be limited by the size of the stomate. External dimensions of lenticel stomates vary considerably between the three cultivars, but can be generalised. Such a generalisation assigns the smallest stomate size to 'Tommy Atkins' fruit, while both 'Keitt' and 'Kent' lenticels are structured with large stomate sizes. The distinguishing features in the latter case are the lumen sizes and the significant differences in wax richness and deposition inside the lumen.

From the results of the microscopy, the amount of cutin present in the lenticel lumen is very similar for all three cultivars. With the epicuticular wax being the prevalent distinguishing factor, the amount of wax crystalloids present in the lenticel may be of far greater consequence than can be determined by visual methods. Barnes & Cardoso-

Vilhena (1996) summarised the importance of epicuticular wax in temperature canopy and surface control of leaves and fruit. A reflective wax coating facilitates lower temperatures, with subsequent lower transpiration rates. This may result in the overall metabolic rate of the leaf being lower, decreasing the production of secondary metabolites such as terpenoids. Such a wax coating also traps some volatile metabolic by-products passing through the cuticular membrane (Schmutz *et al*., 1994). Trapped metabolic derivatives can contribute to the protective nature of the epicuticular wax by enhancing UV protective properties.

A number of terpenoids, representing an array of chemical structures, are emitted as volatile compounds (Lalel *et al*., 2003; Narain & de Sousa Galvaõ, 2004). Most of these, however, contain one or more aromatic functional groups within the molecular structure. Although the presence of some aromatic components in mango wax was indicated by Prinsloo *et al*. (2004), the exact origin of these aromatics is unknown. Furthermore, several aroma volatiles previously described from mango may act as irritants on exposed cellular tissue (John *et al*., 1999). This fact creates another possibility, namely, that the intracuticular wax may trap some potentially harmful terpenoids emitted during normal metabolic action, contributing to lower lenticel discolouration incidence in cultivars with lenticels rich with wax.

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3.7 TABLES

Table 1 Comparison of the susceptibility to lenticel discolouration of mango cultivars 'Tommy Atkins', 'Kent' and 'Keitt' and some of their horticultural characteristics (Knight, 1997; Anonymous, 2000)

Characteristics	Tommy Atkins	Keitt	Kent
Susceptibility to lenticel discolouration	High	Medium to high	Low
External appearance of fruit	Regular ovoid to oblong, orange-yellow covered with red and heavy purple bloom	Ovate with slightly oblique apex and rounded base, pinkish green	Regular ovate, rounded base, greenish yellow with red shoulder
Harvest period (Average time from fruit set)	Early December to February (3 - 4 months)	March to April. (5 - 6 months)	Mid-February to March (4 - 5 months)
Tree characteristics	Medium to large tree; erect; early flush; usually bears well; early maturing fruit	Small to medium tree; erect; open to scraggly; very productive; late maturing fruit	Erect, slender tree; moderate size; early flush; bears well; fruit matures mid-season
Average marketable fruit size	Fruit medium to large 350 - 650 g	Fruit medium to large 350 - 650 g	Fruit large 500 - 750 g
Time of leaf flush	January to February	April to May	March to April
Susceptibility to latex burn	High	High	Low
Susceptibility to gall fly infestation	High	Medium	Low

3.8 FIGURE CAPTIONS

Figure 1 A lenticel depicting the stratified nature of the constituent tissue contributing to the enlargement of the structure (based on Stern (1994)).

Figure 2 Light microscopy of the general morphology of a mango (cultivar Tommy Atkins) lenticel, with no indication of cell differentiation or cambium tissue. The cuticular layers are situated flush over the lumen (1), which is lined with cutin (2) and epicuticular wax (3).

Figure 3 Abaxial view of a enzyme/acid treated cuticle section, with intact lenticel lining, stomate lumen (white arrow) and the network of radially expanding cutin ridges and air passageways traversing between lenticels (A). In (B), where the cutin lining (red arrow) has been torn away, epicuticular wax crystalloids can be seen (white arrow). The red arrow also indicates the convoluted and disorganised development of the cutin between deformed and irregularly spaced epidermal cells.

Figure 4 Close-up view of a tube extending from, and linking some lenticels (A). Damaged areas, as indicated by the arrows in (B), revealed the hollow extensions, giving evidence of tubular passageways.

Figure 5 Wax crystalloids lining the lenticel cavity (white arrow in A) confluent with the adaxial epicuticular wax (white arrows in B), and part of a cutinous membrane (black arrow in A). The cutin in B (indicated by a black arrow) is exposed due to physical handling of the sample material.

Figure 6 The external appearances of the lenticels of the three cultivars compared. ‘Tommy Atkins’ (A) has predominantly small lenticel stomas, with limited suberisation taking place. ‘Keitt’ (B) has stomas of varying sizes, but mostly develops a very large, torn structure with suberisation taking place towards the end of the period of pulp expansion. ‘Kent’ (C) lenticels are the most abundant of the three cultivars investigated, appearing predominantly large, with internal wax visible from outside (arrow).

Figure 7 Diagrammatic representation of the lenticel lumen of each of the three mango cultivars studied. Wax abundance in each type is depicted by the

red areas. ‘Tommy Atkins’ typically has a deep, more organised lumen with a small stomate that is often irregularly fissured. ‘Keitt’ has a deep to intermediately deep, disorganised lumen, and develops a larger stomate. ‘Kent’ has a large, disorganised stomate with a shallow to intermediate lumen.

Figure 8 Shrinkage (< 15 %) caused by plunge-freezing of material with discoloured lenticels, created a ‘hillock’-effect due to presence of mesophyll cells hardened by vacuoles and cell walls containing phenolic compounds (A). The material prepared for light microscopy confirms the presence of the phenolics (darkened cells in unstained section) (B).

Figure 9 A transversally fractured lenticel from ‘Tommy Atkins’. White arrows indicate the presence of wax, while the red arrows indicate bare cutin lining the lumen of the lenticel. The extracuticular wax rapidly diminishes and disappears from the cutin layers forming the lumen wall.

Figure 10 A transversely fractured lenticel from ‘Keitt’, with the wax gradient down the lumen wall sampled at points a - d. At point (a), the wax is similar to the epicuticular wax layer, although it appears less densely-packed and architecturally less intricate. Point (b) is on the surface of a mesophyll cell in the lumen, clearly lacking all the complexity of the adaxial wax. At this point, the wax suddenly diminishes in quantity and becomes even sparser. Point (c) is an area deep inside the lenticel, close to an air passageway, with almost no wax present, whilst the cell surface in the air passageway at point (d) clearly still has a cutin layer, but no more wax.
Scale bar (a - d) = 1µm.

Figure 11 A transversally fractured lenticel from ‘Kent’, showing the wax fractions extending deep into the lumen (two upper arrows), as well as air passageways (two lower arrows). The demarcated area indicates the epidermal cells of a resin duct in close proximity to the lenticel.

3.9 FIGURES

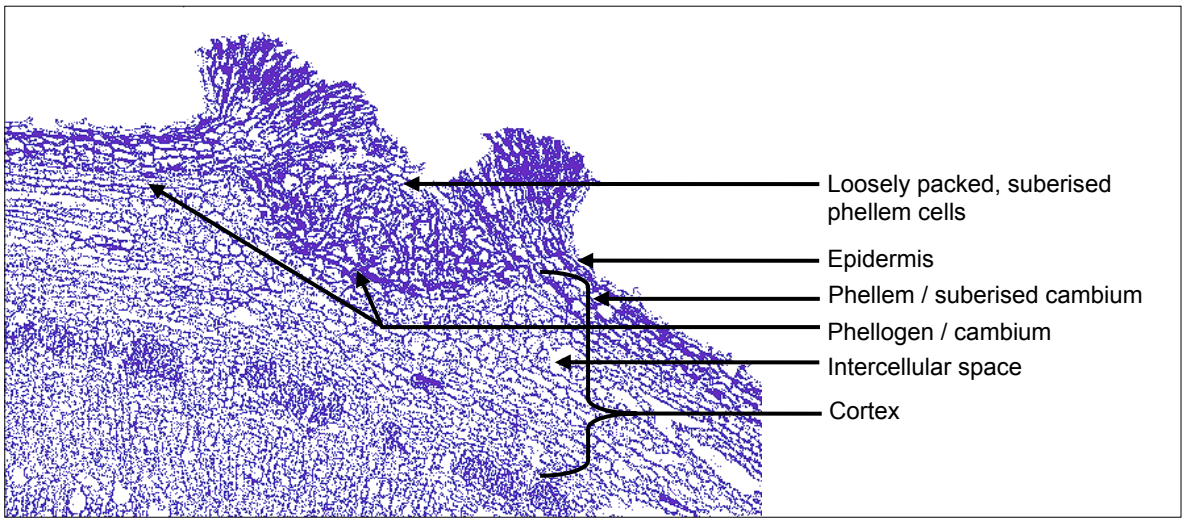


Figure 1

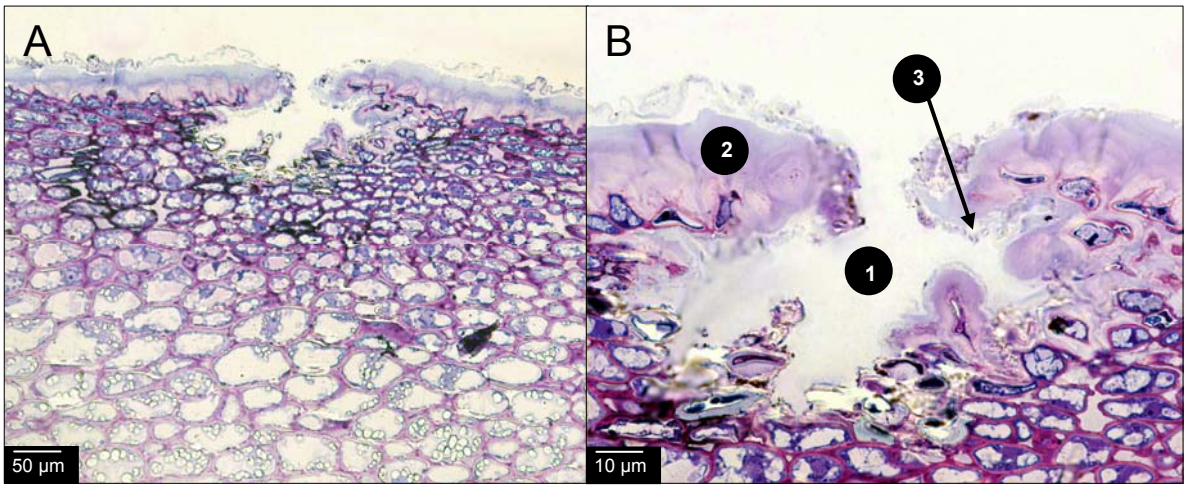
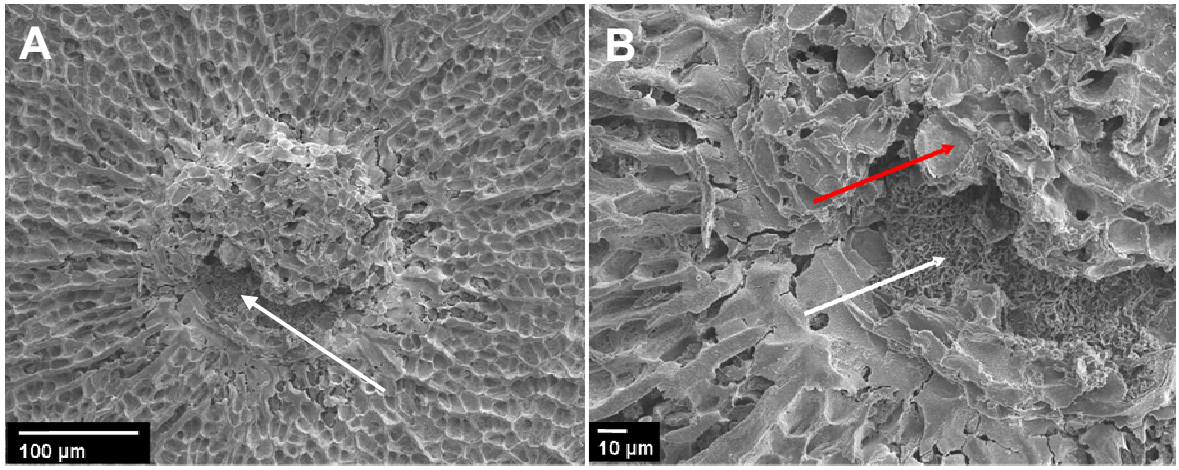


Figure 2



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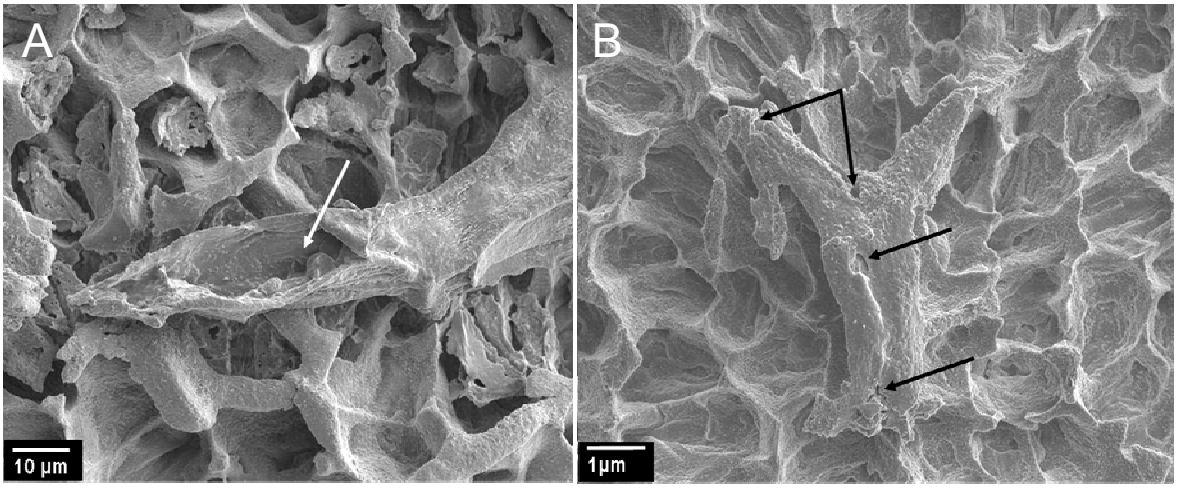

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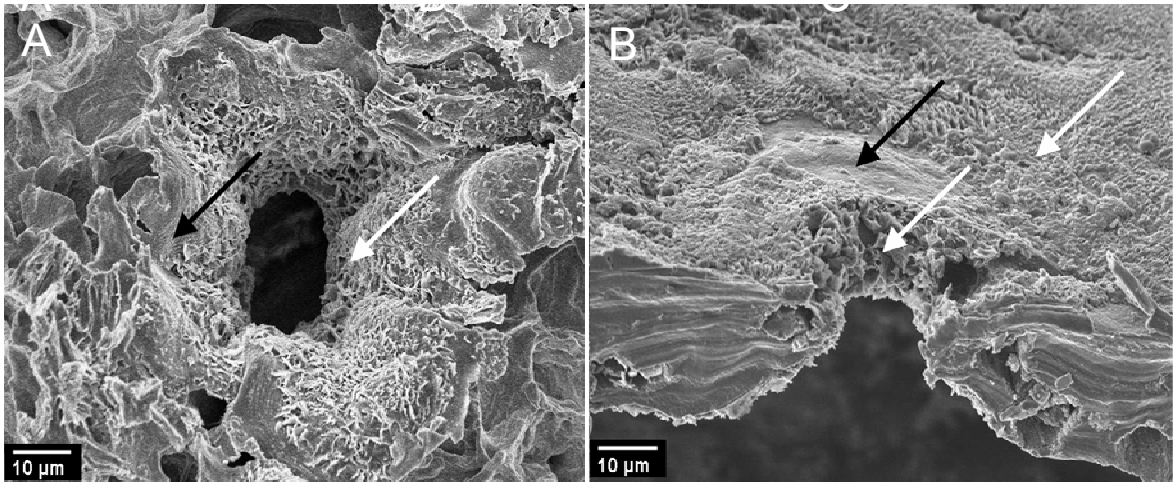

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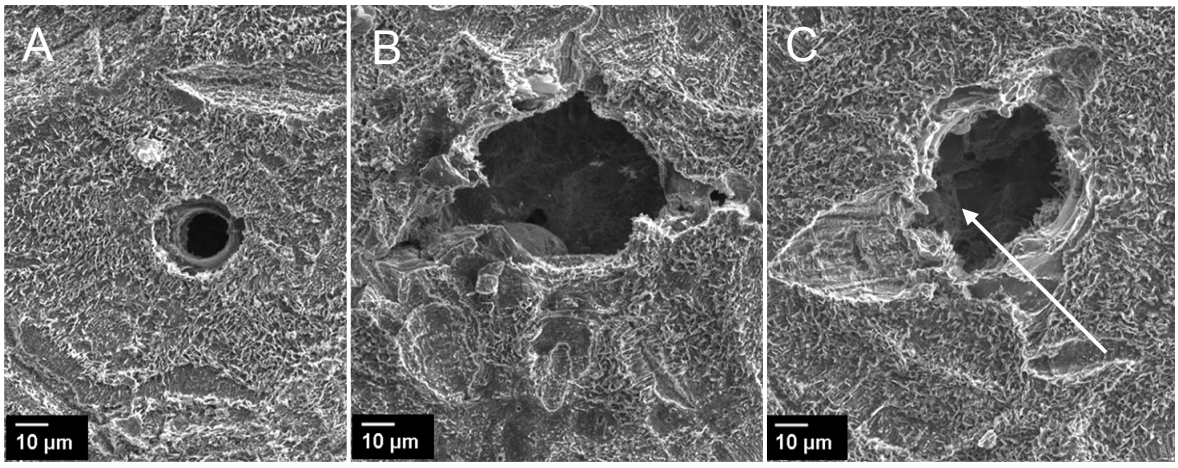

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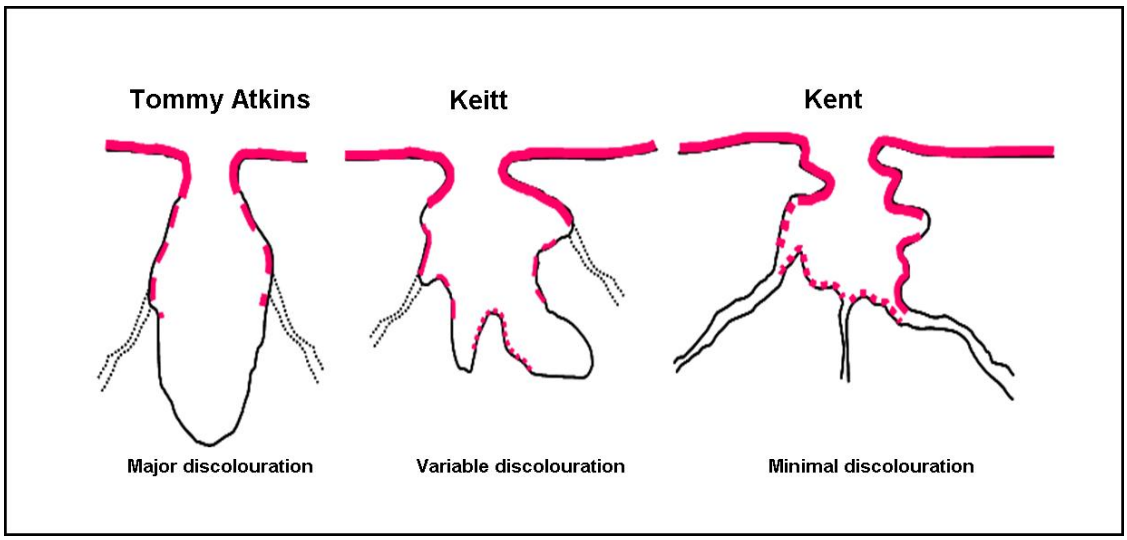
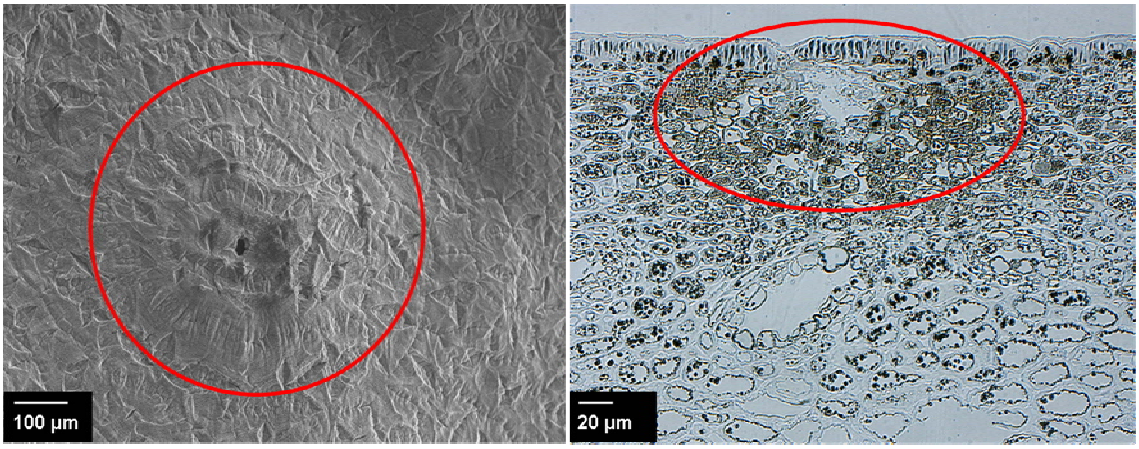


Figure 7



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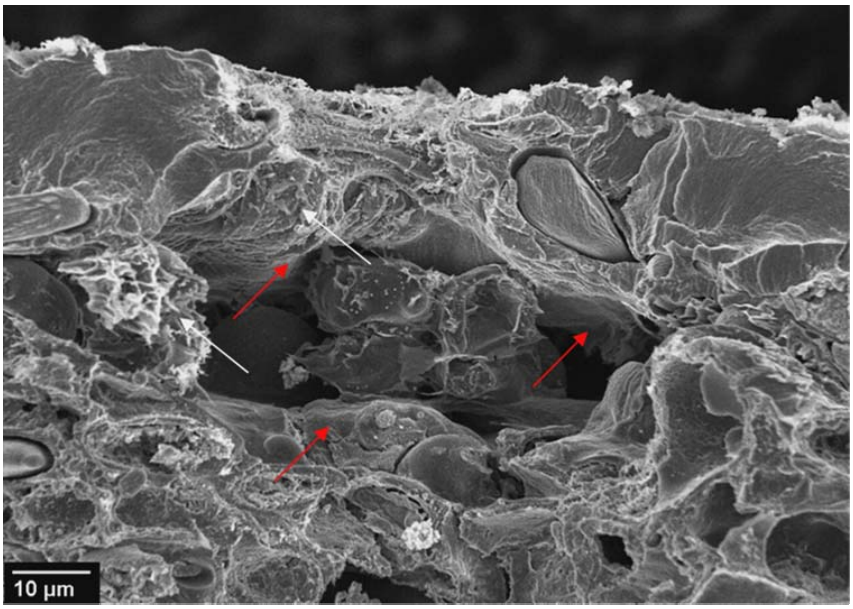

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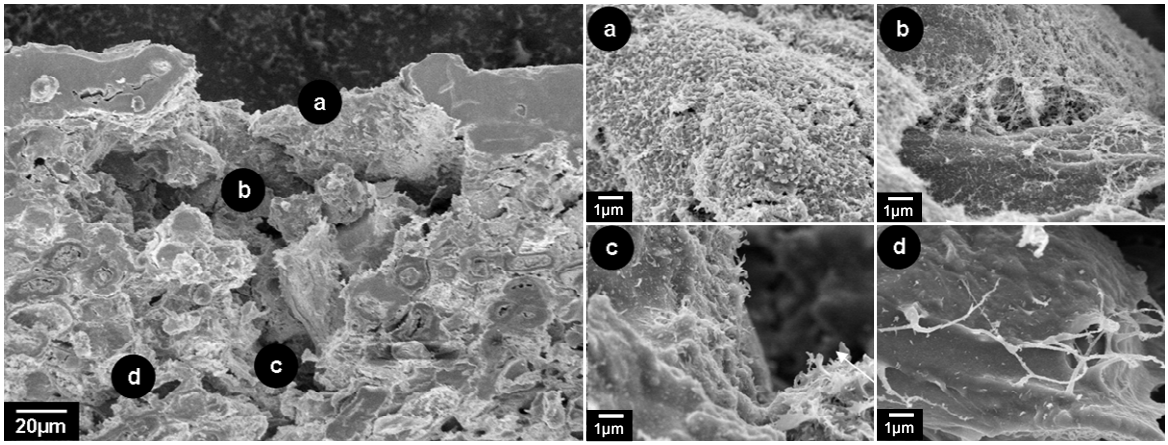

Figure 10

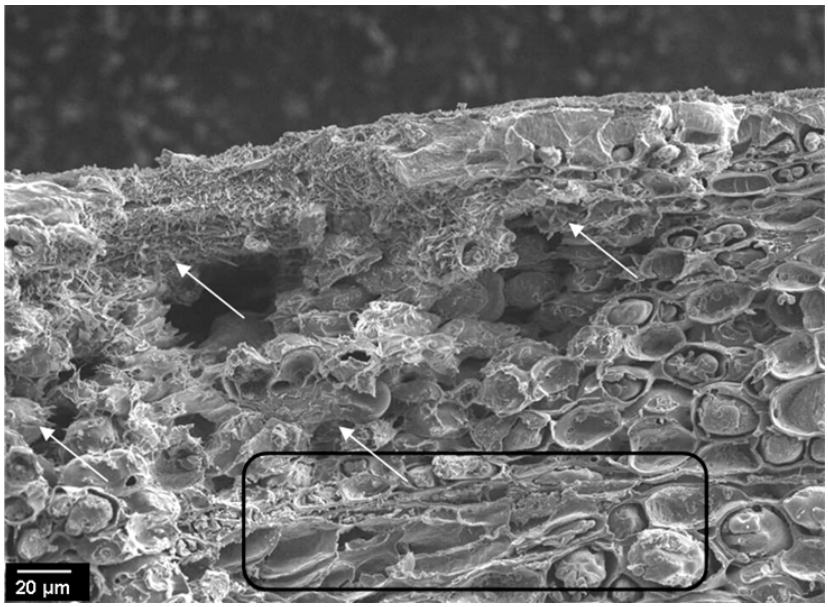

Figure 11