CHAPTER 3

LENTICEL ONTOGENY OF ‘TOMMY ATKINS’, ‘KEITT’ AND ‘KENT’ FRUIT

ABSTRACT

Lenticels differentiate from existing stomata that lose their function and protrude above the fruit surface as a result of rapid anticlinal cell divisions in the epidermis of the exocarp. Based on the comparative study between different mango cultivars and mature marula fruit, it seems as if the absence of a cork cambium and cork cells in the mango lenticel could be one of the most important reasons for lenticel discolouration. An interaction between naturally occurring pigments and sap from the resin ducts in the exocarp appears to be another contributing factor for lenticel discolouration.

3.1 INTRODUCTION

Lenticels can be found on the surface of stems, old roots and on several fruit types, including apples, pears, avocados and mangos (Dietz *et al.,* 1988). In the absence of stomata will the lenticels take over the vitally important process of gaseous exchange needed for photosynthesis, respiration and transpiration (Mauseth, 1988). Postharvest discolouration of mango lenticels is a serious problem, since the resultant black markings on the fruit skin are unacceptable to consumers, consequently depreciating the economic value of the fruit (O’Hare and Prasad, 1992). The degree of lenticel discolouration may vary in different mango cultivars. In South Africa, ‘TA’ and ‘Keitt’ are two of the most important cultivars susceptible to lenticel discolouration, whereas ‘Kent’ is not known to problematic in that aspect.

According to Dietz *et al.* (1988), mango fruit lenticels may develop from either pre-existing stomata, or from rupturing of the epidermis. The sequence of events during the formation of lenticels from pre-existing stomata in fruits are: death of guard cells, loss of cuticular membrane in substomatal chambers, suberization of the cells lining the substomatal camber and the empty cavity of the lenticel chamber due to the absence of cork cambium (Dietz *et al*., 1988).

According to Tamjinda *et al.* (1992), the cuticle in mango fruit showed a discontinuity around the lenticels. The sublenticellular cells were also smaller in diameter than surrounding parenchymatous cells. A periderm was also absent in all but one cultivar where lenticels were not susceptible to lenticel discolouration.

Clements (1935) recognized two lenticel types, namely *open* and *closed* lenticels. Open lenticels lack a phellogen and therefore also the protecting cork cells, with or without an interrupted cuticle. By contrast, closed lenticels may a) have a cuticle sealing the sublenticellular cells or b) a phellogen may develop that results in formation of suberized cell layers or c) both the cuticle and phellogen may be present.

The limited and insufficient literature on the formation, development and detailed anatomy of mango lenticels (Tamjinda *et al.,* 1992) emphasized the need for a more detailed study on the ontogeny and structure of mango lenticels that could form a base for interpreting lenticel discolouration.

3.2 MATERIALS AND METHODS

Fully-bearing 9-year-old ‘Tommy Atkins’ (‘TA’), ‘Kent’ and ‘Keitt’ mango trees, grafted onto ‘Sabre’ seedling rootstocks, from commercial blocks at Bavaria Estate, Hoedspruit (24°22’32”S, 30°53’26”E), were used for this study. Representative fruit samples over two seasons were collected regularly, from anthesis to fruit maturity and during harvesting.

During the early stages of fruit growth and development, fruit was collected randomly at intervals of three to four days while, during the later stages of fruit development, two weekly intervals were employed. Young fruit was also sampled from young trees grown under controlled environmental conditions at the research farm of the University of Pretoria (25°45’8”S, 28°15’32”E).

For comparative purposes, fruit was sampled from mature marula (*Sclerocarya birrea* (Richard) Hochst. subsp*. caffra* Kokwaro), also belonging to the mango family (Anacardiaceae). Sections of *Phytolacca dioica* L. (Phytolacaceae) petioles where obtained from the slide collection of the Botany Department, University of Pretoria. These were used for comparing mango fruit lenticels with “typical” lenticels.

Several sections of the exocarp (side of fruit exposed to direct sunlight) tissue were cut in 2 to 3 mm sections to be embedded in “LR White” and 5 to 12 mm sections were cut to be embedded in paraffin wax. The material was fixed in paraformaldehyde (4% formaldehyde in 0.15 M phosphate buffer) or FAA (5% Formalin, 5% Acetic acid and 50% Ethanol, 1:1:18). Thereafter, samples were dehydrated in a graded ethanol and xylene series and embedded in paraffin wax (Sass, 1966). A microtome (Reichert-Jung 2040, Germany) was used to make sections of 7 µm thick. Other samples were embedded in LR White resin, following fixation in paraformaldehyde and dehydration in a graded ethanol series (Sass, 1966). Sections of 0.5 µm were cut using an ultramicrotome (Ultracut E, Reichert, Vienna, Austria). Wax preparations were stained with Toluidine Blue, Sudan IV, Sudan Black B or a combination of Safranin O and Fast Green (O’Brien and McCully, 1981) and viewed under a Leitz Biomed microscope. Photographs were taken with an Olympus Camedia C-4000 Zoom digital camera.

For scanning electron microscopy (SEM), material was fixed in 2.5 % glutaraldehyde 0.1 M $NaPO_4$ buffer (pH 7.4), followed by three rinses (10 minutes each) with the same buffer. Postfixation was done with 1 % OsO_4 for two hours and was removed with three rinses (10 minutes each) of distilled water. Material was dehydrated in a graded ethanol series, followed by critical point drying in a Polaroid critical point dryer. Dried samples were coated with gold, using a Polaron E5200C sputter coater for conductivity. Specimens were viewed with a JOEL 840 scanning electron microscope, operated at 5 kV. Images were recorded digitally.

3.3 RESULTS

3.3.1 Fruit development from anthesis to 3 mm in length

During anthesis, stomatal guard cells of ‘TA’, ‘Keitt’ and ‘Kent’ were already differentiating on the ovary surface (Fig. 3.1A and D) (Chapter 2). At this stage, stomata were still covered with cutin and obviously not yet functional. The epidermis consisted of a single layer of approximately isodiametric cells undergoing active anticlinal cell division. Branched resin ducts (canals) had already formed (Fig. 3.1A) and could be seen throughout the ovary wall. Complete guard cells and associated cells of the substomatal cavity were completely differentiated in fruit of 3 mm in length and guard cells were still flush with the surrounding epidermal cells (Fig. 3.1B and C). No significant differences between the three cultivars could be seen at this stage.

3.3.2 Fruit length, 4 - 20 mm

Epidermal cells of ‘TA’, ‘Keitt’ and ‘Kent’ fruit appeared tangentially flattened, radially elongated and covered by a waxy cuticle, staining black with Sudan Black B. Stomatal guard cells were probably still functional at this stage, with well developed guard cells and a substomatal cavity (Fig. 3.1C). Continued anticlinal cell division of epidermal cells resulted in the fruit surface of ‘TA’ and ‘Keitt’ taking on an undulating appearance (Fig. 3.2A - C) and stomata became elevated above the fruit surface, resulting in volcanic-like protuberances on the fruit surface in fruit of 12 to 15 mm in length. ‘Kent’ fruit surface of this size also took on an undulating appearance (Fig. 3.2D), but not to the same extent as in ‘TA’ and ‘Keitt’.

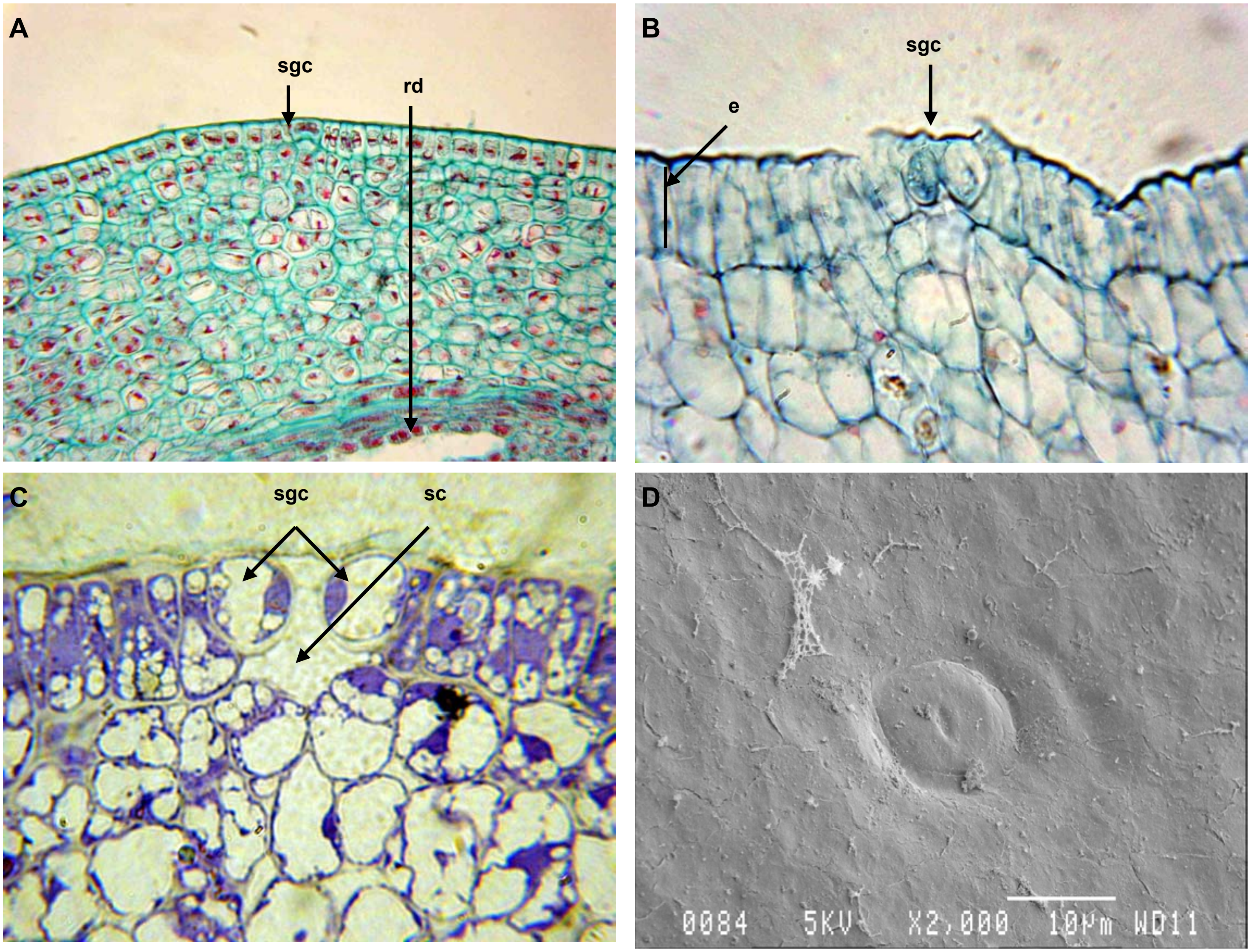

Figure 3.1 Sections of: (A) 1 mm ‘TA’ ovary, showing differentiating stomatal guard cells (sgc) and resin ducts (rd) already formed (at anthesis); (B) 2 mm ‘Kent’ fruitlet with differentiated guard cells; (C) 3 mm ‘TA’ fruitlet showing differentiated guard cells and substomatal cavity. Active cell divisions of epidermis cells are clearly visible in (A, B and C). (D) SEM micrograph of a 1 mm ‘TA’ ovary showing differentiated stomata. Schizogenic opening between the guard cells is just starting to form underneath the wax/cuticle layer. e - epidermis.

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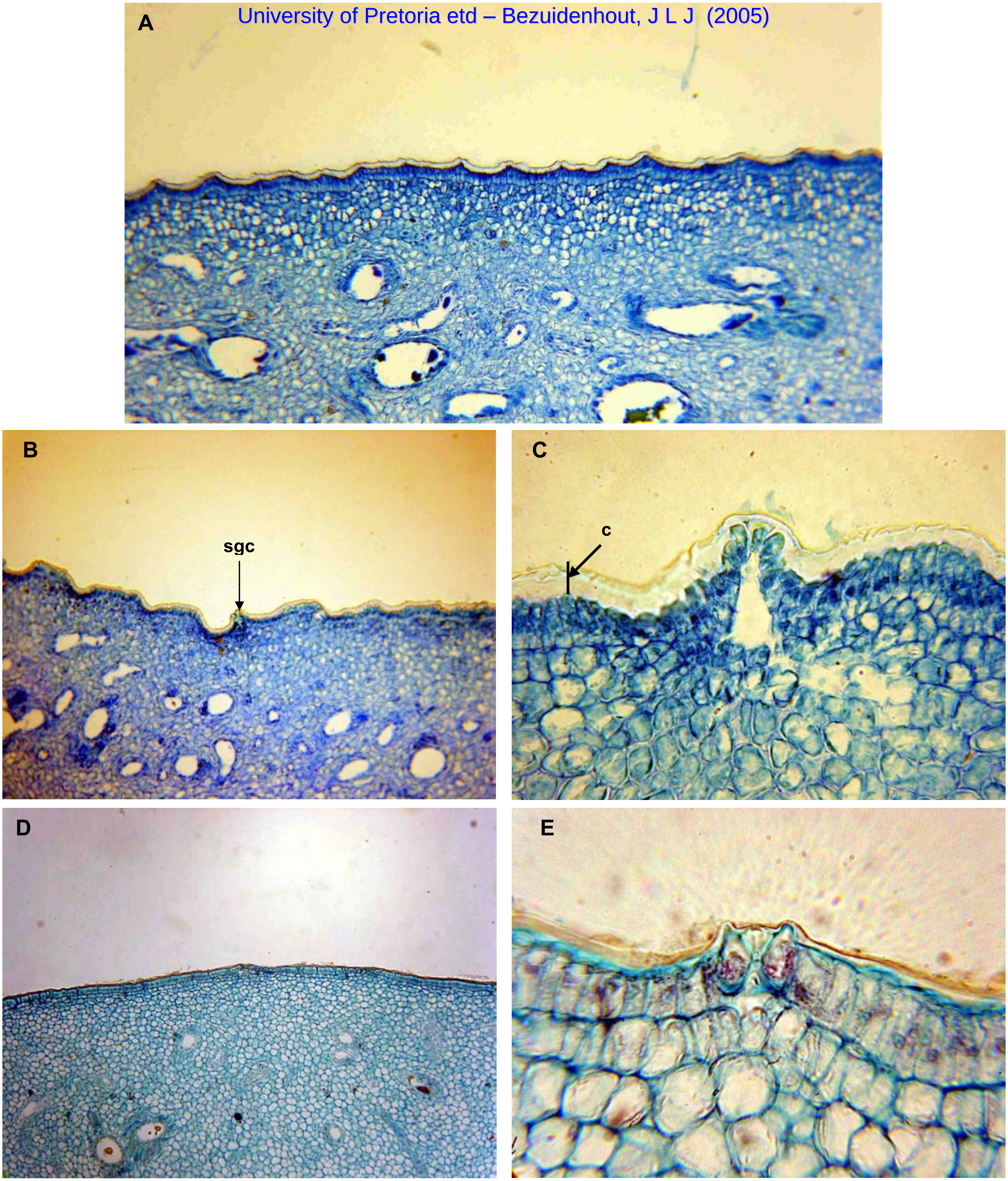

Figure 3.2 (A) 13 mm ‘TA’ fruit showing undulating epidermis; (B and C) Stomatal guard cells being forced upward. (D) 12 mm ‘Kent’ fruit with smooth surface. (E) Stomatal guard cells of a 14 mm ‘Kent’ fruit seem to be still functional. Note the abundance of resin ducts in (A, B and D). c = cuticle

Stomata of 'Kent' fruit were therefore not pushed upwards, which means that their stomata were not subjected to the same pressure as 'TA' and 'Keitt' and remained functional at this stage of development (Fig. 3.2E). The reason for this could be that cell division of the subepidermal cells of 'Kent' fruit keeps up with the cell division of epidermal cells.

In 'TA' and 'Keitt' fruit up to 20 mm in length, there was a marked decline in anticlinal cell division of epidermal cells, concurrent with the enlargement of the subepidermal cells, resulting in loss of undulation of the fruit surface (Fig. 3.3A) and rupturing of the stomata (Fig. 3.3B). Stomatal guard cells did not return to their original position, but remained raised above the now almost smooth epidermis, isolated on top of some epidermal cells. The stomata possibly lost their function due to the rupturing of the stomatal opening. This led to a permanent opening in the epidermis, apparently a vulnerable area that needs to be closed from the environment. Under normal circumstances, a phellogen would originate under such damaged stomata (Fahn, 1974).

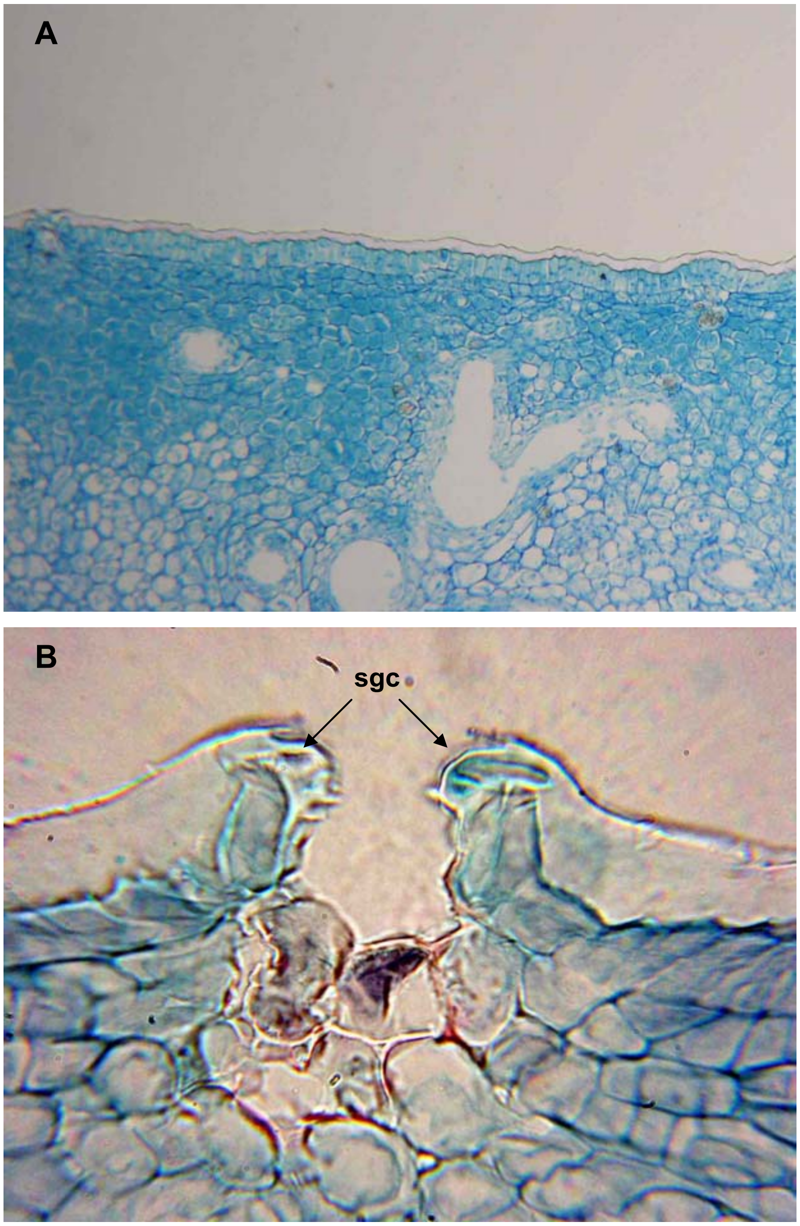

Figure 3.3 (A) Epidermis of 20 mm 'TA' fruit lost its undulating appearance. Resin ducts close to the fruit surface are also visible in this figure. (B) Stomatal guard cells (sgc) elevated above the now smooth epidermis.

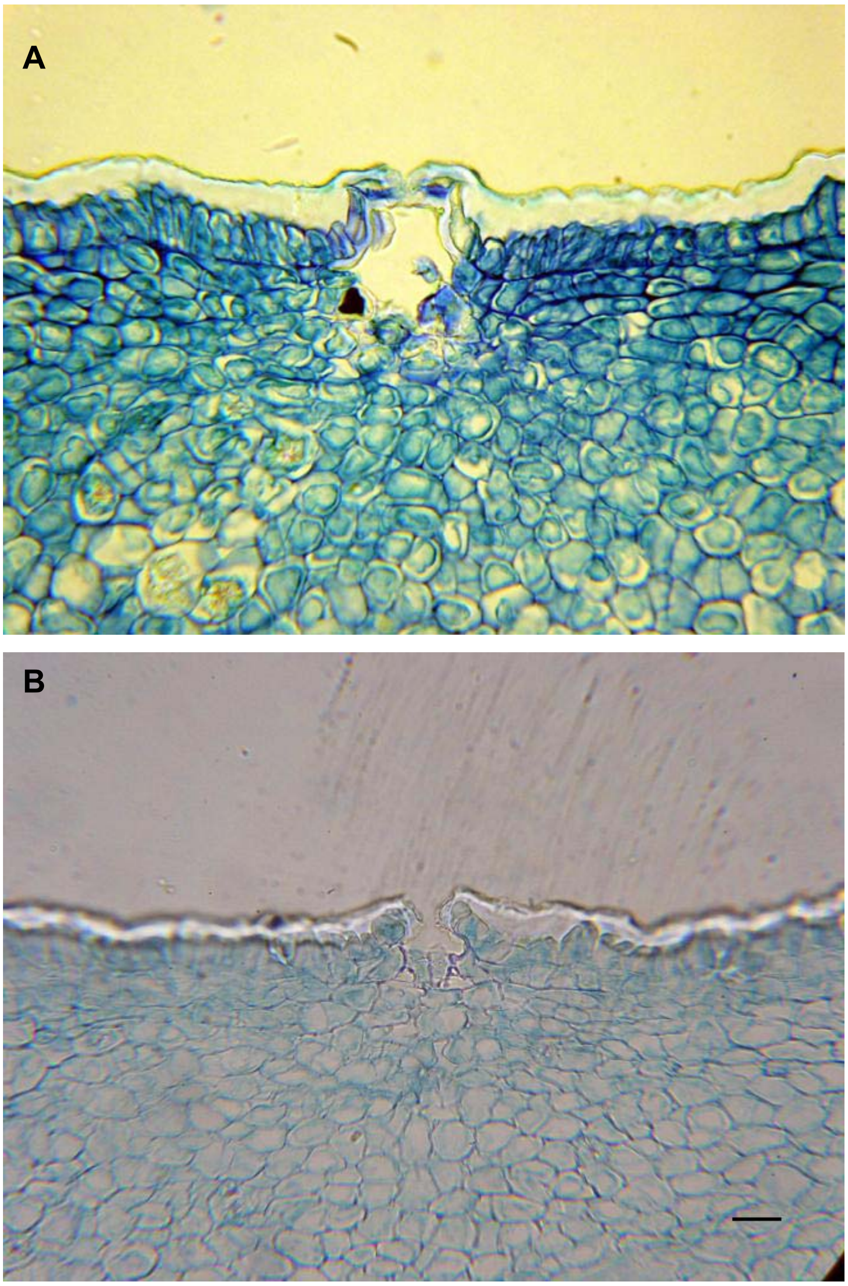

Figure 3.4 35 mm ‘TA’ fruit with stomatal guard cells still raised above epidermis (A) and 40 mm ‘Kent’ fruit (B). Enlarging lenticel cavity only protected by a thin cuticle. Scale - 0.02 mm.

3.3.3 Fruit length, 20 to 50 mm:

As the growing of the fruit progresses, the substomatal cavity, (now lenticel cavity) was exposed due to the absence of phellem. The inability to close the substomatal cavity with phellem caused the lenticel cavity to enlarge as the fruit grew and resulted in the forming of an atypical lenticel in all cultivars examined (Fig. 3.4A and B). Epidermal cell division nearly stopped, but cell enlargement continued both in the epidermis and in the subepidermal cells. The cuticle continued to thicken, entering the exposed lenticellular cavity and sealing it off. In addition, cells below the lenticel had thinner cell walls and larger intercellular spaces, an observation in keeping with Dietz *et al.* (1988).

At this stage of 'Kent' fruit development, stomata also ruptured due to the rapid increase of fruit size. The consequent cavity in the epidermis was very small by comparison to those of 'TA' and 'Keitt' at the same stage (Fig. 3.4A and B).

3.3.4 Fruit length 50 to 100 mm:

Signs of limited cell division were still detected and the lenticel cavity still increased in size due to increased cell enlargement (Fig. 3.5A and B). The entire epidermis, including the lenticels, was covered with a cuticle. Pigmentation appeared in the sublenticellular cell vacuoles of larger fruit (Fig. 3.7A). The latter phenomenon was also observed by Loveys *et al.* (1992).

'Kent' lenticels, however, did not enlarge as much as 'TA' and 'Keitt' lenticels. Lenticels of 'Kent' were better insulated than both 'TA' and 'Keitt' lenticels. The surface of 'Kent' fruit lenticels were covered with a rather thick cuticle (Fig. 3.7B) while 'TA' and 'Keitt' lenticels were covered with a thin and, sometimes, interrupted cuticle (Fig. 3.6). 'Kent' lenticels also contained suberized cells whereas ‘TA’ and ‘Keitt’ only had loose, dead cells in their lenticel cavities (Fig. 3.5A and B).

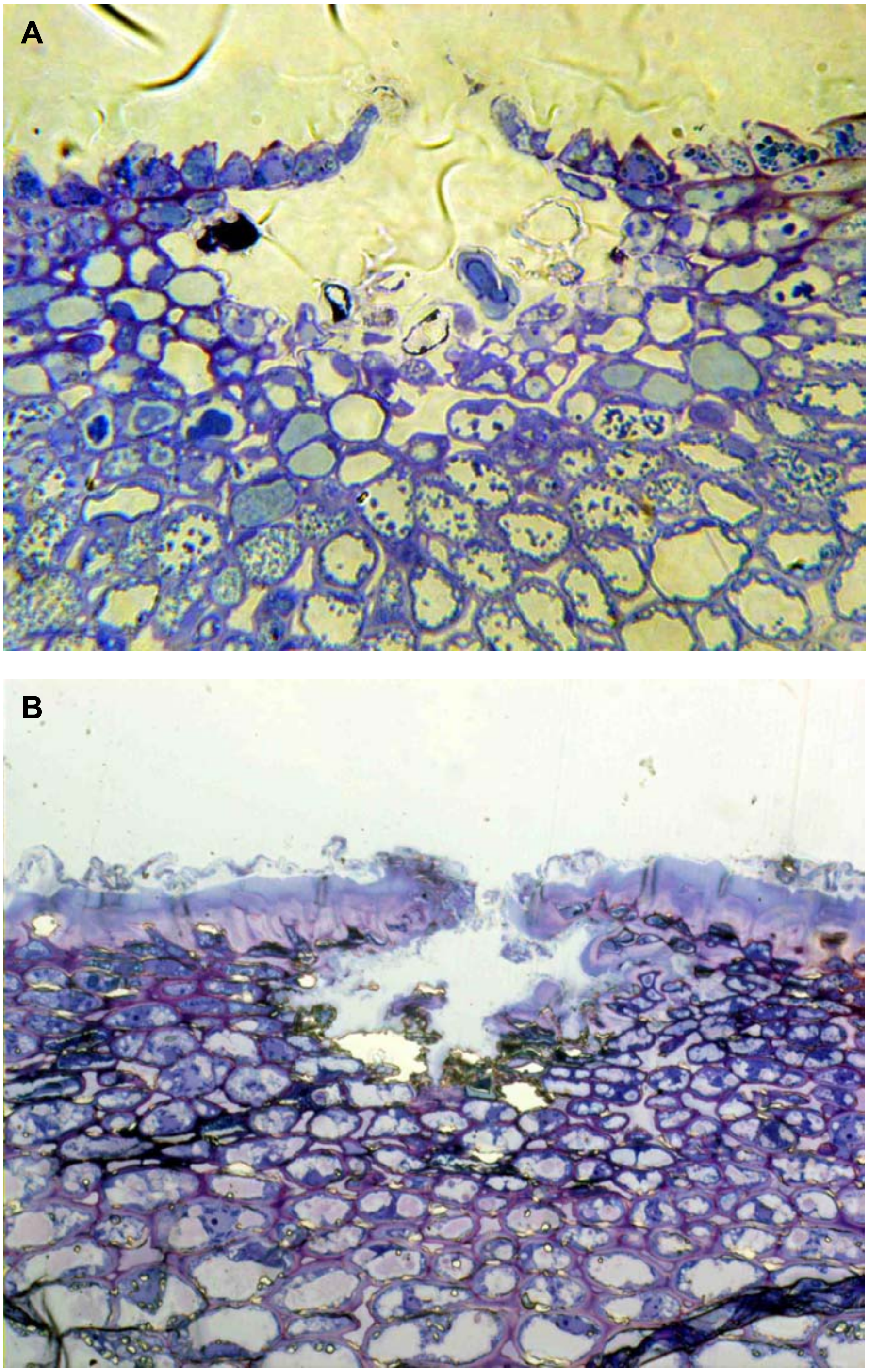

Figure 3.5 Lenticels of (A) 90 ‘TA’ and (B) 70 mm ‘Keitt’ fruit. Lenticel cavity contains dead, loose cells and it is clear that a periderm is absent.

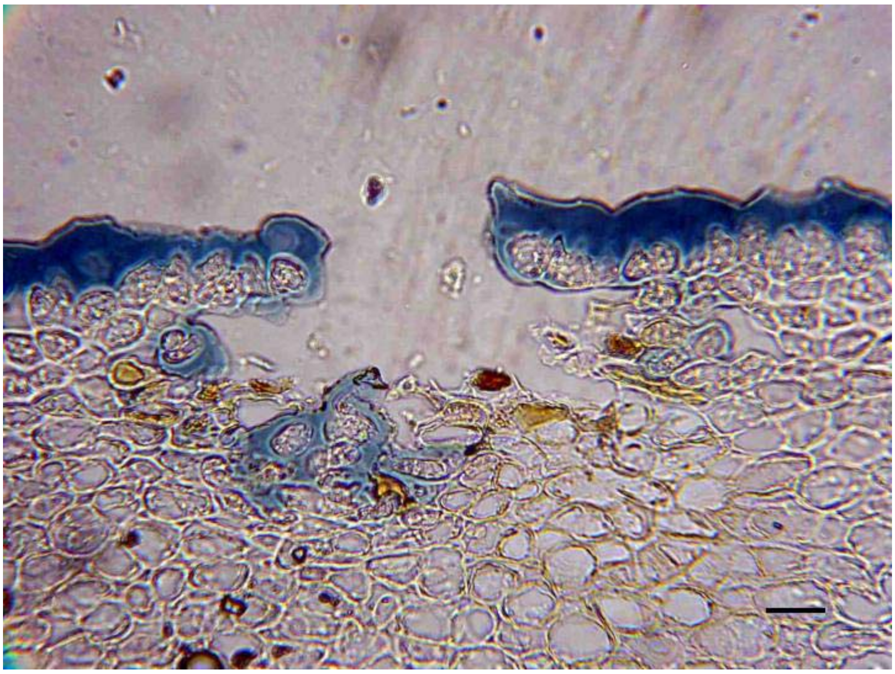

Figure 3.6 Section of a 100 mm 'TA' fruit. Lenticel cavity is only partially covered with cutin (staining black with Sudan Black B), making it more susceptible for penetration of foreign objects. (Scale = 0.02 mm)

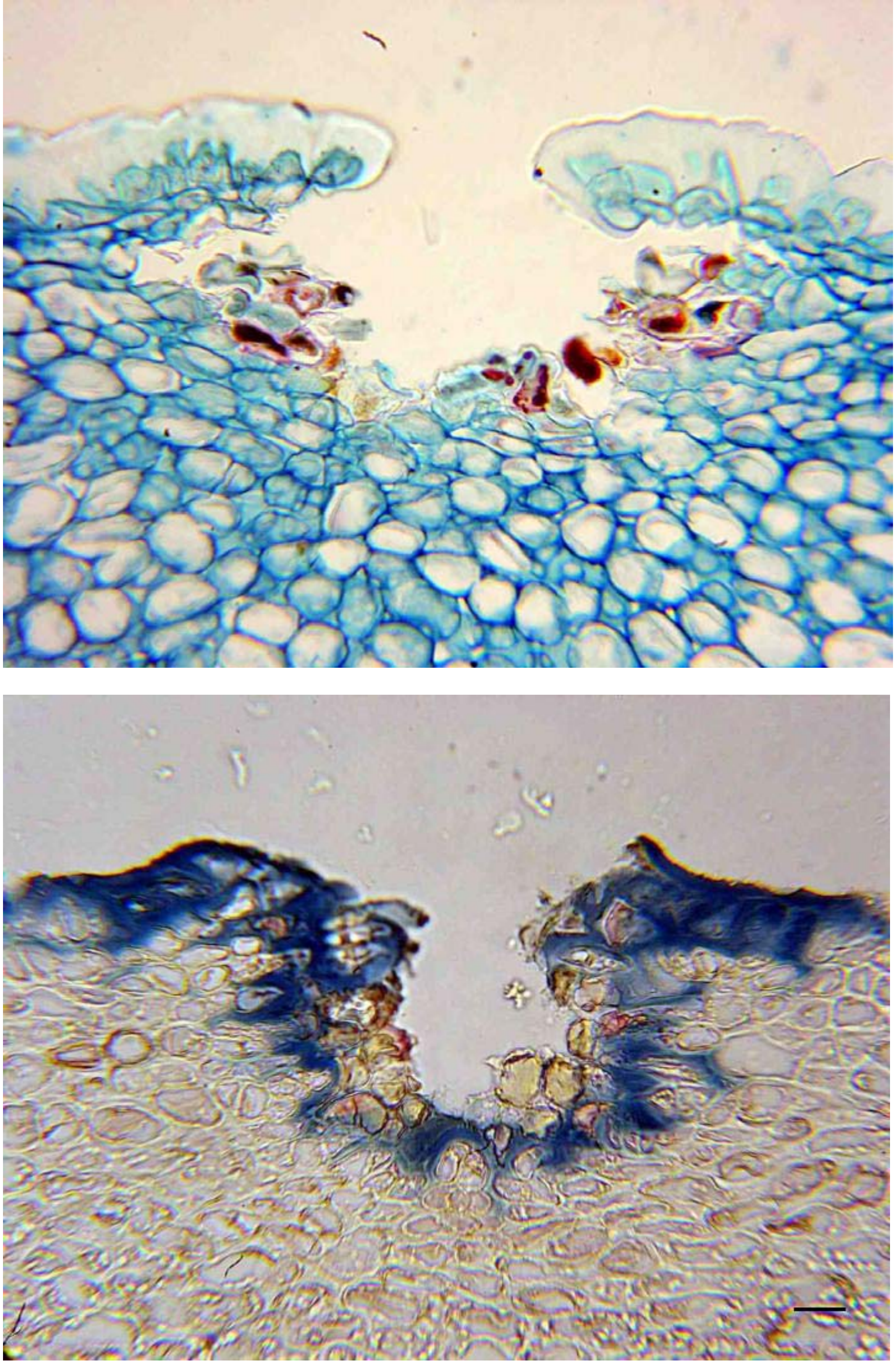

Figure 3.7 (A) 100 mm ‘TA’ fruit lenticel with pigments in vacuoles in sublenticellular cells. (B) 100 mm ‘Kent fruit. Continuous cuticle, stained black with Sudan Black B are not interrupted at the lenticel. Scale - 0.02 mm.

3.3.5 Lenticels on mature fruit

Lenticels of mature ‘TA’ and ‘Keitt’ fruit contained pigments in vacuoles of sublenticellular cells (Fig. 3.8A.), probably phenolics that are anti-microbial and therefore protect the fruit against pathogens (Robinson *et al.*, 1993). With ‘Kent’, these pigments were absent, which might be due to the fact that ‘Kent’ lenticels are physically better protected than those of ‘TA’ and ‘Keitt’. It is clear that a thick cuticle (stained black with Sudan Black B) completely covers the lenticel cavity and is continuous with the epidermal cuticle. These lenticels are therefore *closed* lenticels as termed by Clements (1935) (Fig. 3.8B). The cavity of ‘Kent’ lenticels was also smaller in size than those of ‘TA’ and ‘Keitt’.

3.3.6 Second type of lenticels on ‘Kent’ fruit

2 mm - 15 mm

Lenticels from another origin have been observed in ‘Kent’ fruit. The origin of these lenticels was not from existing stomas, but from resin ducts developing too close to the surface of the fruit. These resin ducts developed three or four cells from the fruit surface. Enlargement of the fruit led to increased tension on the cells above these resin ducts and therefore caused the epidermis and accompanying cells above the resin duct to rupture, which left an opening in the fruit surface. Content in the ruptured resin duct was still visible in figure 3.9F. In figure 3.9B – E, it is clear that the resin duct, which can be distinguished on the base of the accompanying vascular bundle (Fig. 3.9A), is situated close to the surface of the fruit, eventually breaking through the surface in figure 3.9F.

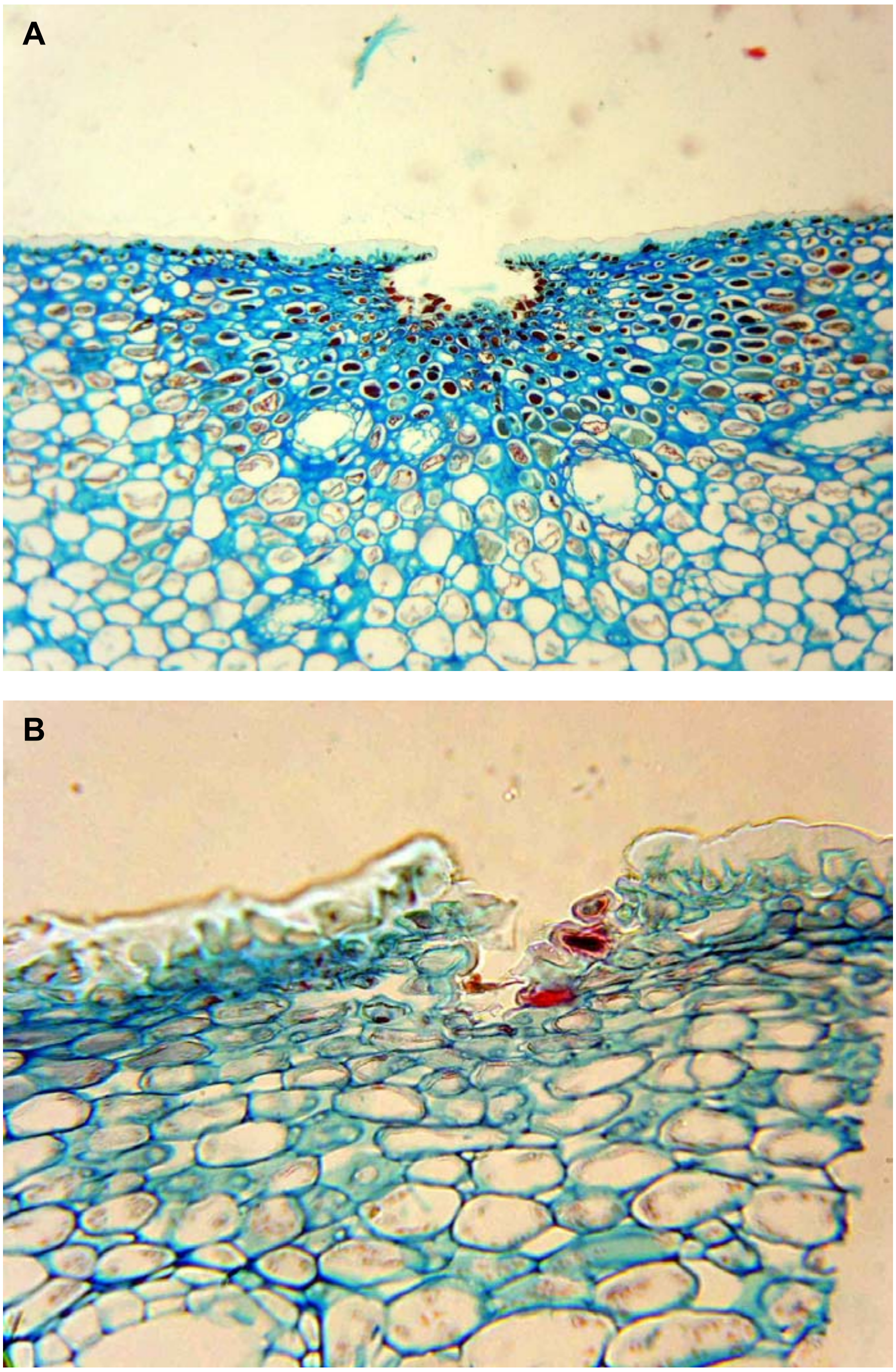

Figure 3.8 Lenticels of mature (A) 'TA' and (B) 'Kent' fruit. Note the abundance of pigments around 'TA' lenticel in contrast to the absence thereof in 'Kent'. It is also marked how closely situated resin ducts are to the lenticel.

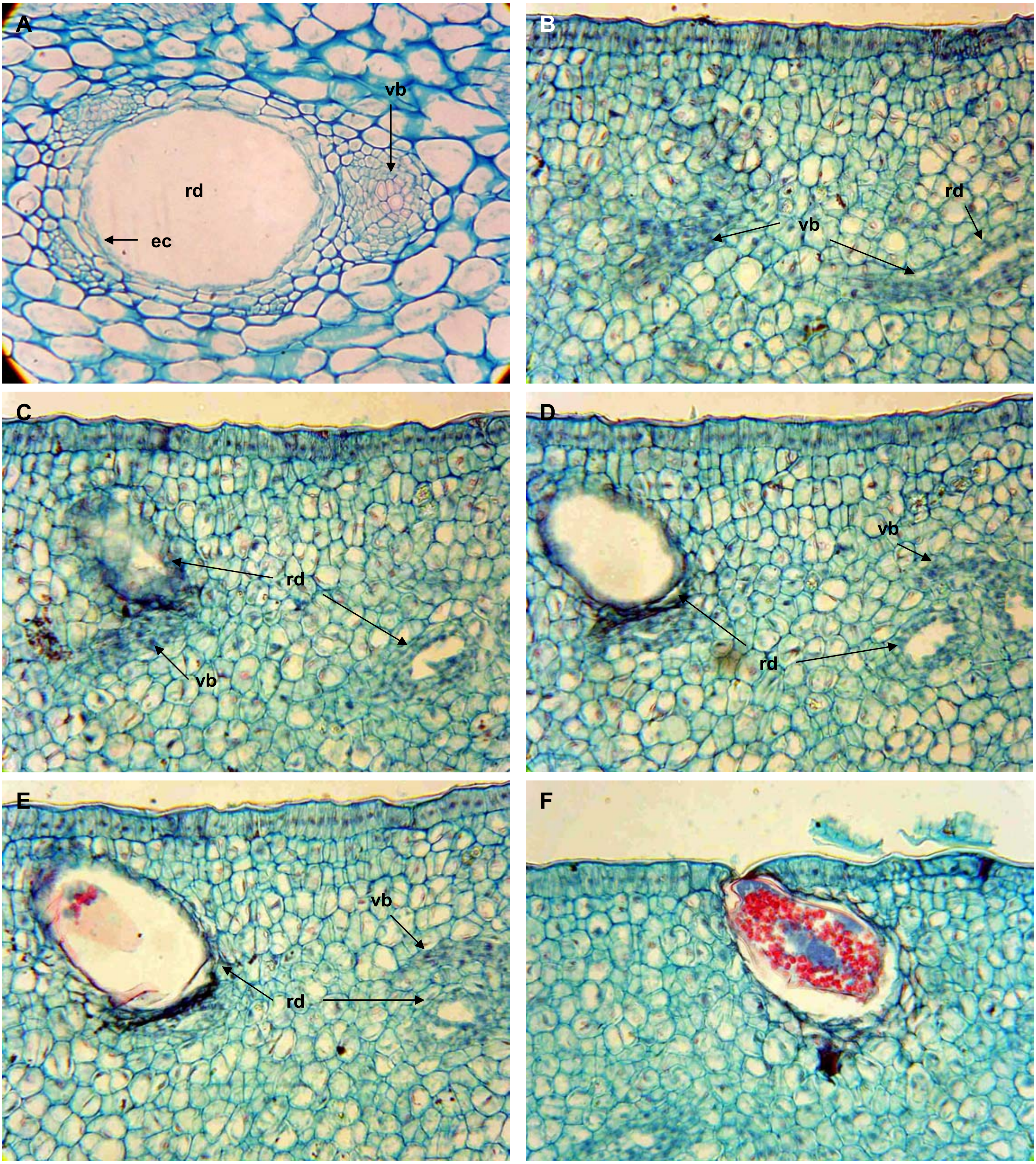

Figure 3.9 (A) Part of mature fruit skin of 'TA' showing a resin duct (rd) subtended with epithelial cells (ec) and bordering vascular bundle (vb), always associated with a resin duct. (B – F) Sequential sections of a 6 mm 'Kent' epidermis showing a resin duct breaking through the epidermis.

15 mm - 35 mm

The lenticels opened up and formed a neat, cup-like lenticel (Fig. 3.10A) with a cuticle already covering the lenticel cavity. First signs of cells arranged in rows, anticlinal to adjacent surface, was becoming visible. This is the first stage of the development of a phellogen (in this instance, a wound cambium due to the rupture in the epidermis). In contrast to lenticels originating underneath existing stoma, these lenticels develop a periderm.

35 mm - 70 mm

The phelloderm, consisting of rays of cells, is now clearly visible around the lenticel cavity (Fig. 3.10B). At this stage, no phellem has been formed, but the surface of the cavity has been sealed with cutin.

70 mm - Mature fruit

In most instances the lenticels are partly filled with cells, densely packed and originating from the phellogen (Fig. 3.11A and B). The structure of these lenticels resembles the structure of typical lenticels as described by Mauseth (1988). Loose cells, characteristic of ‘TA’ and ‘Keitt’ fruit, are absent in these lenticels. Again, a thick and uninterrupted cuticle is evident in these lenticels.

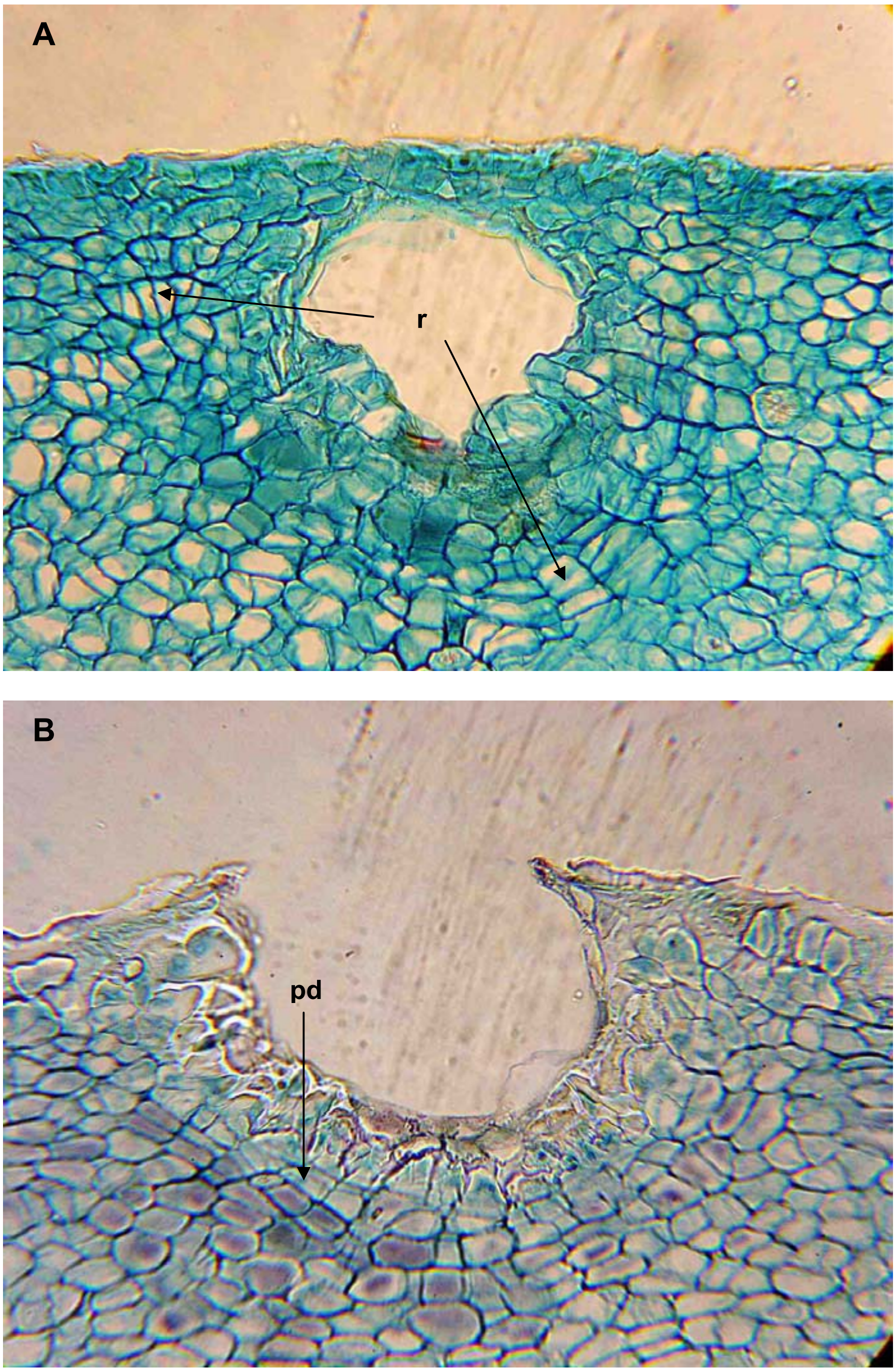

Figure 3.10 Sections of A) 30 mm ‘Kent’ fruit showing rays (r) of cells where a phelloderm (pd) are starting to develop; (B) 40mm fruit lenticel showing a well-developed phelloderm around the lenticel cavity as well as a cuticle present in the lenticel cavity, continuous with the epidermis.

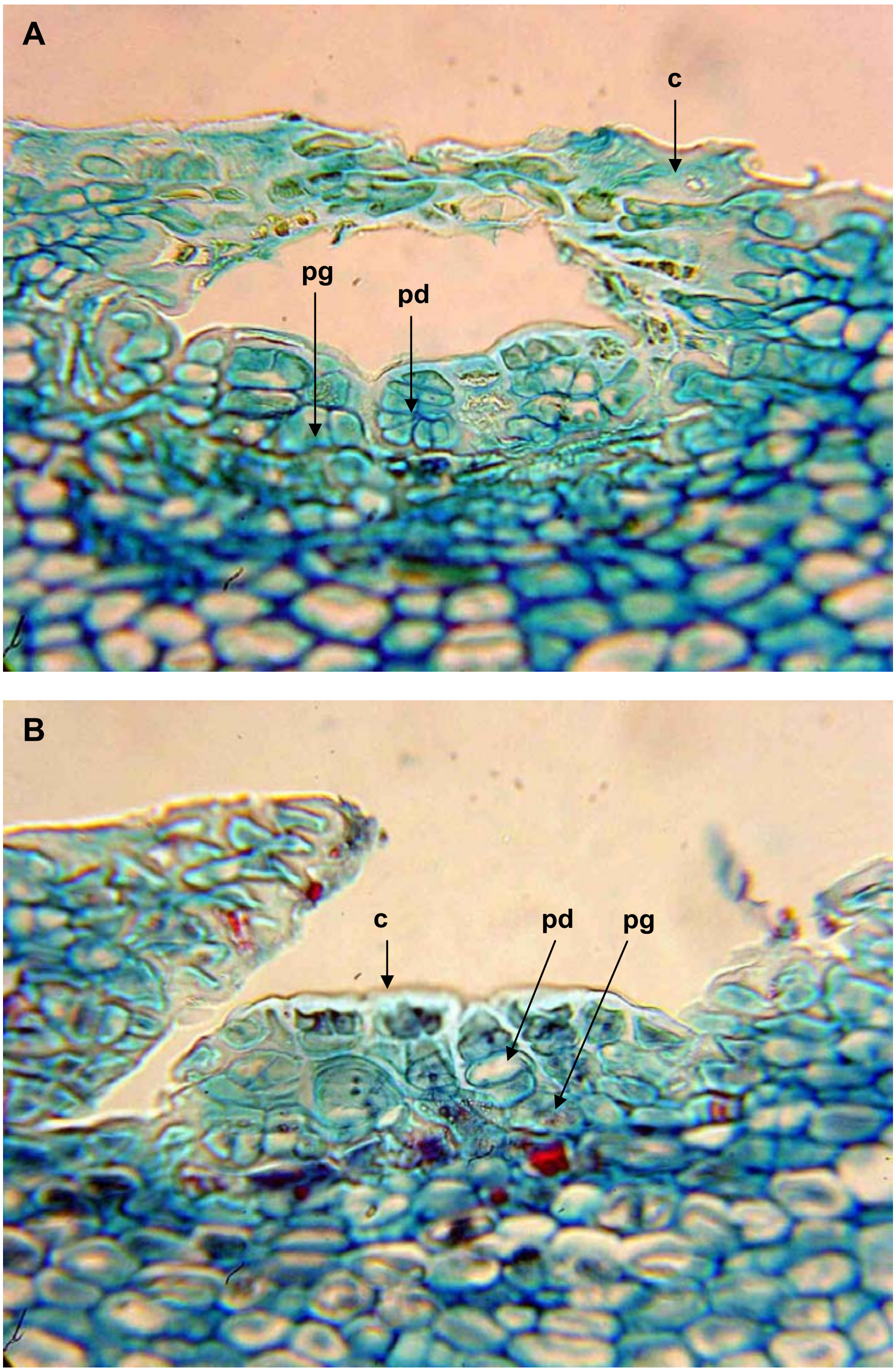

Figure 3.11 (A and B) Lenticels originated from resin ducts of mature 'Kent' fruit. In both lenticels phelloderm (pd) are clearly visible, filling the lenticel cavity with living cells. Lenticel cavities are also covered with the characteristic thick cuticle (c), extending into intercellular spaces. pg – phellogen.

3.3.7 Lenticels of different plant species

Fully developed lenticels of mature marula fruit (Fig. 3.12A) and young petioles of *Phytolacca dioica* L. (Fig. 3.12B) were compared with those from 'TA' (Fig. 3.5A) and 'Keitt' (Fig. 3.5B) mango fruit. A noticeable difference between mango lenticels and the other two species was that lenticels of both *P. dioica* and marula fruit were subtended by phellogen. However, mango lenticels were subtended by several degenerate cells, clearly lacking a phellogen, except those developing from resin ducts.

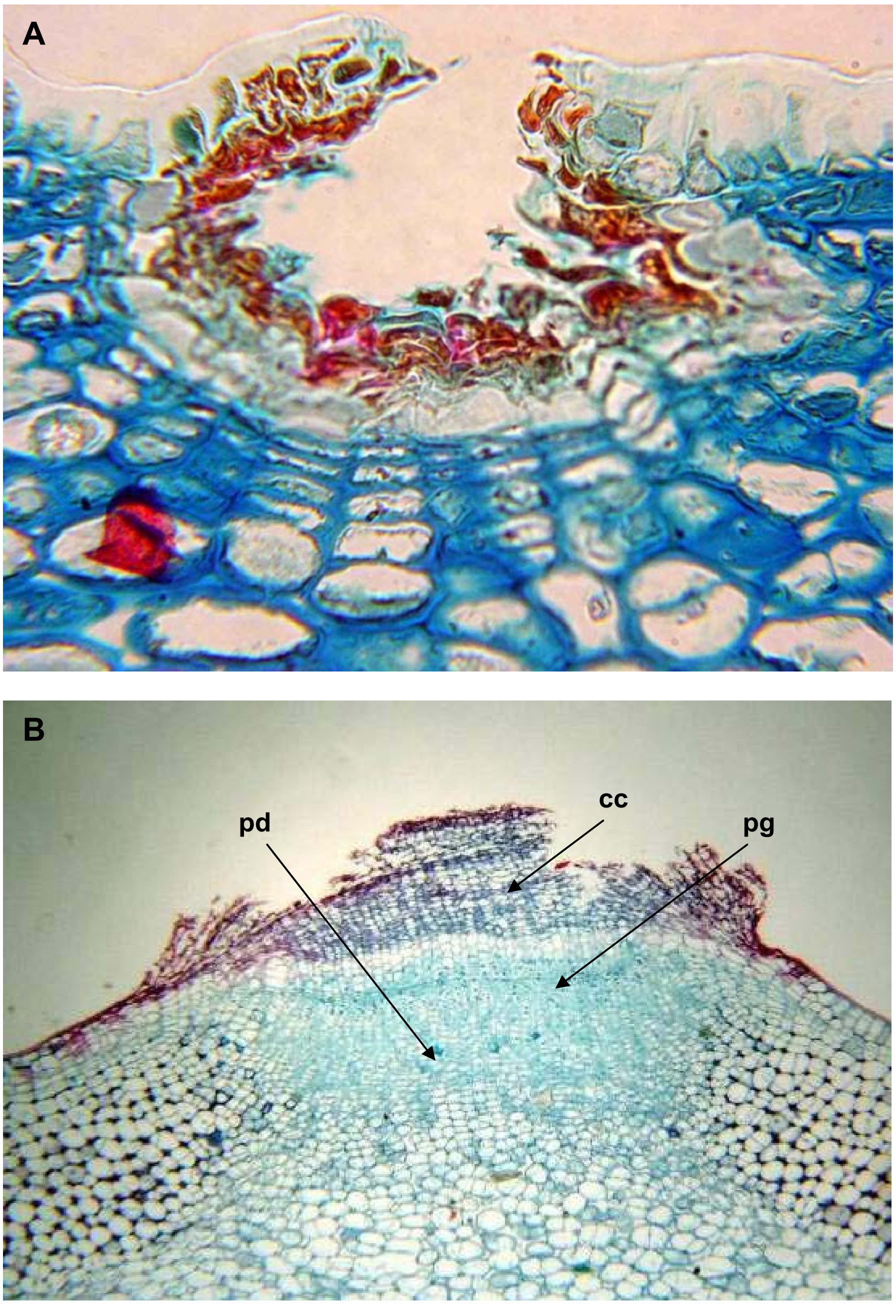

Figure 3.12 (A) Lenticel of mature Marula fruit. Radial cells of phelloderm (pd) are neatly arranged to the outside. The lenticel cavity is covered by a phellem (cork cells). (B) Petiole lenticel of *Phytolacca dioica* with a very active phellogen (pg). cc - complementary cells, pg – phellogen.

3.4 DISCUSSION AND CONCLUSION

The structure and function of a typical lenticel have previously been described by Mauseth (1988), concurring with those of *Phytolacca dioica* L. (Belhombra), (Fig. 3.12B) and marula in the current study (Fig. 3.12A). Here, an active cork cambium gives rise to loosely packed cork cells, enabling gaseous exchange and preventing microbial infection of the plant organ. Radial cell division of the cork cambium also enables expansion and elongation of the tissue surface. When these typical lenticels are compared to those of the mango fruit (Fig. 3.5A and B), it is clear that mango fruit lenticels are atypical, lacking a cork cambium. Mango lenticels are thus not able to elongate and expand to cope with tissue growth. This results in cell wall shearing and cell collapse of sublenticellular cells. With 'TA' and 'Keitt', this in turn results in cell rupturing, allowing contact between cytoplasmic contents and, presumably, resin from resin ducts. The fact that marula lenticels do not discolour, despite the presence of resin ducts, is supporting evidence for this hypothesis.

During the initial stages of rapid fruit growth (up until 20 mm in length), mango fruit has several stomata, which, except for 'Kent', become forced onto the surface of the fruit due to logarithmic radial growth of the exocarp. Because of the physical shape of these protuberances and, presumably, the resultant pressure on them, stomatal guard cells cannot retain their integrity, collapse and are torn apart, leaving the substomatal cavity exposed to the environment. The mango fruit has adapted to this phenomenon by producing cuticular cutin that enters the stomatal cavity, permitting gas exchange and forming an atypical lenticel by the time fruit has reached 20 to 30 mm in length. These lenticels lack cork cambia, but, due to this adaptation, have the ability to limit fungal penetration and prevent excess moisture loss from fruit during fruit growth and development. Furthermore, cells directly under the lenticels had thinner cell walls and larger intercellular spaces than surrounding tissues, enabling gaseous exchange and transpiration. One of the reasons for

‘Kent’ fruit being less subjected to lenticel discolouration may possibly be the comparatively thick cuticle as well as the lenticels which originated from resin ducts containing a phellogen.

Subsequent vacuolar pigment accumulation (possibly phenolics) takes place in the cells, subtending the lenticels. The subsequent rapid growth of the mango fruit of up to 100 mm in length results in shearing of sublenticellular cells and staining of lenticel cell walls. Interestingly, Tamjinda *et al.* (1992) examined ‘Falan’ a mango cultivar, which did not exhibit lenticel discolouration. They found that it did indeed have a cork cambium which prevented shearing of cells and subsequent discolouration.

Clearly, mango fruit lenticels perform important functions, viz. enabling gaseous exchange while preventing fungal attack. However, it is a paradox that, where mango fruit lenticels lack a cork cambium, a structural “fault” has arisen, leading to shearing of pigment containing vacuoles and subsequent discolouration of the lenticels.

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