

**LENTICEL DEVELOPMENT AND DISCOLOURATION IN THE
FRUIT OF SOME MANGO (*Mangifera indica* L.) CULTIVARS**

by

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ABSTRACT

Lenticels are macroscopic openings occurring on the surface of roots, shoots and some fruits like apples, pears, avocados and mangos and are responsible for gaseous exchange and transpiration. The discolouration of the lenticels of some mango cultivars is a serious problem, affecting the economic value of the fruit, especially in 'TA' and 'Keitt' while problems with lenticel discolouration are seldom found in 'Kent'. Mango fruit lenticels develop from ruptured stomata on fruit from about 20 mm in 'TA' and 'Keitt' and 30 to 40 mm in 'Kent'. Lenticels enlarge as the fruit grows due to stretching of the fruit surface, reaching their maximum size on adult fruit. Fully developed lenticels of 'TA' and 'Keitt' are larger in size than those of 'Kent'. 'Kent' lenticels are also better insulated than 'TA' and 'Keitt', having a thick cuticle in the lenticel cavity and in some instances a phellogen is also present, while both of above mentioned characteristics are absent in 'TA' and 'Keitt'. Resin present in the skin of the fruit plays an important role in the discolouration of 'TA' and 'Keitt' lenticels. The resin of both 'TA' and 'Keitt' fruit contain a considerable amount of an aggressive compound termed terpenes. These terpenes are volatile and are able to move out of the resin ducts via the sublenticellular cells to the outside of the fruit through the lenticels. The integrity of tonoplasts of the sublenticellular cells are lost due to the action of the terpenes, causing vacuolar bound phenols to come into contact with polyphenol oxidase present in the cell walls. The product of the resultant reaction is a quinone accumulating as a brownish deposit in the cell walls, visible from the outside as black markings around the lenticels. Lenticel discolouration may, however also occur due to maltreatment or rough handling of fruit, high temperatures in the warm water bath, extended brushing on packline or breaking of the cold chain and spilling of resin onto the surface of the fruit.

UITTREKSEL

Lentiselle is makroskopiese opening op die oppervlak van wortels, stingels en sommige vrugte soos appels, pere, avokado's en mango's en is verantwoordelik vir gaswisseling en transpirasie van die plant. Lentiselverkleuring by sommige mango kultivars is 'n ernstige probleem wat die ekonomiese waarde van die vrug beïnvloed, veral by 'TA' and 'Keitt' terwyl lentiselverkleuring selde by 'Kent' voorkom. Lentiselle ontwikkel vanuit beskadigde stomas op vruggies van ongeveer 20 mm by 'TA' en 'Keitt' en 30 tot 40 mm by 'Kent'. Lentiselle vergroot namate die vrug groei en die oppervlakkige selle onder spanning verkeer. Maksimum grootte word bereik wanneer die vrug volwassenheid bereik. Volwasse lentiselle van 'TA' en 'Keitt' vrugte is groter as dié van 'Kent'. 'Kent' lentiselle is ook beter geïsoleer as 'TA' en 'Keitt' lentiselle. 'n Dik kutikula is teenwoordig in die lentiselholte en soms is daar ook 'n fellogeen (kurk kambium) in 'Kent' lentiselle gevind, terwyl beide afwesig was in 'TA' en 'Keitt' lentiselle. Hars wat in die skil van die vrug voorkom speel 'n belangrike rol by lentisel verkleuring in 'TA' en 'Keitt'. Die hars van beide 'TA' en 'Keitt' vrugte bevat 'n aansienlike hoeveelheid aggresiewe verbindings wat terpene genoem word. Dié terpene is vlugtig wat dit moontlik maak om vanuit die harskanale, deur sublentisellêre selle via die lentisel na buite te beweeg. Die integriteit van die tonoplasts van die vakuole in sublentisellêre selle word versteur as gevolg van die teenwoordigheid van die terpene. Dit veroorsaak dat vakuoolgebonde fenole met die ensiem polifenooloksidase in die selmembraan in aanraking kom. Die produk van die reaksie is 'n bruinerige quinon wat in die selwande akkumuleer en die swart kleur aan die verkleurde lentiselle gee. Lentisel verkleuring kan ook veroorsaak word deur swak hantering van die vrugte soos byvoorbeeld: rowwe hantering, te hoë temperature in die warm water bad, verlengde periodes op die borsels in die paklyn, breek van die koue ketting en die mors van hars op die oppervlak van die skil van die vrug.

INTRODUCTION

In the 2001/02 season, the South African mango industry produced a total of 88 000 tons of fruit of which 16 851 tons were exported to foreign countries. An amount of about 5.25 million cartons were exported at an FOB price of R18.52 per carton, adding up to a total of R97.19 million worth of mangos, making up a substantial percentage of the income for a large number of mango farmers (Elphick, 2002).

Produce from and supply of a top quality product is therefore essential to ensure prime prices on the export market. To comply with this requirement, a series of rather strict criteria such as ripeness, taste, size, general appearance, minimum chemical residues present and a certain threshold of pest and disease incidence are needed to comply with. The outer appearance of the fruit plays in most instances a major roll in determining its economic value and therefore is an aesthetic attractive fruit of great importance.

In most mango producing countries around the world, producers have problems with the discolouration of lenticels on the fruit surface, especially after harvest and packaging (Tamjinda *et al.*, 1992). It is a serious problem because the dark coloured spots gives an undesirable impression and, secondly, it is incorrectly associated with pathogenic infections, consequently depreciating its economic value (O'Hare and Prasad, 1992).

Lenticels in general are macroscopic openings occurring on stems, old roots where the periderm (cork) has formed and on several fruit types (Dietz *et al.*, 1988). Lenticels are essential to the plant, since they control gaseous exchange for photosynthesis, respiration and transpiration in the absence of stomata (Mauseth, 1988).

On fruit like apples, pears and cherries, stretching and rupturing of the stomata due to fruit growth and enlargement can mostly be the beginning of lenticel

development, though, the lenticels can not always be regarded as “true” lenticels. This is due to the absence of a distinct phellogen below the lenticels (Clements, 1935 and Wilson, 1972).

Much research on different causal aspects on mango fruit discolouration has already been undertaken. Post-harvest treatments previously shown to increase lenticel discolouration include dipping fruit in hot water (45°C) for 30 min (Jacobi *et al.*, 2001); a combination of hot water and hot air (Jacobi *et al.*, 1996); washing fruit in one of several disinfectants or soaps including Agral[®], Cold Power[®] or Mango Wash[®] (Bally *et al.*, 1997); or washing fruit in ambient water (O’Hare *et al.*, 1999).

In order to understand the structure, function and discolouration of mango lenticels better, it is essential to know their origin and development. Dietz *et al.* (1988) maintained that lenticels may originate in one of two ways viz. from a preformed stoma, or from shearing of the fruit epidermis as a result of rapid fruit growth. Tamjinda *et al.* (1992) found that cells directly below the lenticel are smaller than surrounding cells and have larger intercellular spaces conforming to the situation in stomata.

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.

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CHAPTER 1 LITERATURE REVIEW

1.1 INTRODUCTION

In most vascular plants there are restricted areas of relatively loosely arranged cells, suberized or non-suberized, in the periderm which are called lenticels (Fahn, 1974).

Lenticels are lens-shaped (Kuo-Huang and Hung, 1995) macroscopic openings that occur on the surfaces of roots, shoots, some fruits like apples, pears, avocados and mangos (Dietz *et al.*, 1988; Esau, 1965; Fahn, 1974; Silvester and Harris, 1989) and even on vegetative leaves (Neish, 1995). Singh and Pant (1997) described the occurrence of lenticels on ovules of five *Cycas* species. The fruit of tomatoes, blueberries and persimmon were reported to be devoid of lenticels (Brown and Considine, 1982).

As seen from the surface, lenticels appear as masses of loose cells, usually protruding above the surface through a fissure in the periderm (Esau, 1965). Depending on the orientation of the fissure, transverse and longitudinal lenticels are recognized (Wetmore, 1926). In stems and roots, the fissures of the lenticels are usually closely related to the phloem rays (Kuo-Huang and Hung, 1995).

In perennial plants lenticels can survive for several years in which cases the phellogen is dormant during winter and regains activity every spring (Klebahn, 1884). The gross anatomy of mature lenticels in many plants has been described, but only a few published reports are referring to the development of lenticels (Jacob *et al.*, 1989).

As mentioned previously, lenticels also occur on mango fruit (Oosthuysen, 1998). Producers of mangos have a serious problem with postharvest discolouration of mango lenticels (Oosthuysen, 2002), because the dark coloured spots on the fruit give an undesirable appearance (Tamjinda *et al.*, 1992). Although superficial, it can substantially reduce consumer acceptance and the retail value of the fruit (Loveys *et al.*, 1992).

1.2 ORIGIN, FORMATION AND STRUCTURE OF LENTICELS

1.2.1 Lenticels on stems

The stage of plant organ development at which lenticel formation commences may differ from species to species and is dependent on the persistence of the epidermis on the organ (Esau, 1965). In the case of aboveground plant organs with secondary growth, the cuticle, stomata and epidermis are sloughed off as a result of periderm formation (Groh *et al.*, 2002). Lenticels may appear prior to periderm initiation or lenticels and periderm may arise simultaneously (Fahn, 1974). They usually arise below stomata (Adams, 1975) or under groups of stomata where the function of the stomata is gradually transferred to the lenticel (Fahn, 1974). In some species, with a low stomatal index, lenticels can form between stomata or, where there is a high stomatal index, lenticels may develop below some of them (Fahn, 1974).

During the ontogeny, parenchyma cells under the stomatal cavity divide in different planes, chlorophyll disappears and a mass of rounded thin-walled cells, with prominent intercellular spaces, is formed (Esau, 1965; Kuo-Huang and Hung, 1995). The division of the cells progresses inwards into the cortex and the orientation of the divisions become more and more periclinal until the phellogen of the lenticel is formed (Adams, 1975; Kuo-Huang and Hung, 1995). In

some species the phellogen is continuous with the outer cell layer of cortex just below the epidermis (Kuo-Huang and Hung, 1995).

The phellogen gives rise to the phelloderm in the interior and complementary cells towards the exterior (Langenfield-Heyser, 1997) (Fig. 3.12 A and B). Complementary cells may be suberized or non-suberized (Fahn, 1974). As the filling tissue and complementary cells increase in quantity, the epidermis is ruptured and filling tissue protrudes above the surface (Kuo-Huang and Hung, 1995). The exposed cells die and wither away but are replaced by others developing from the phellogen (Esau, 1965).

Intercellular spaces are present in the tissue of the lenticels and therefore they, like stomata, are prominent structures in the process of gaseous exchange (Esau, 1965; Fahn, 1974; Mauseth, 1988). Lenticels can therefore be regarded as passages for watervapour and gas exchange (Groh *et al.*, 2002).

The lenticellular phellogen of some species like *Phytolacca dioica* L. (Fig. 3.12B) also forms a seasonal closing layer apart from the phelloderm and complementary cells. These are seasonal layers of compact cells alternating with the complementary tissue (Fig. 3.12B) (Langenfield-Heyser, 1997). Despite their compact nature, the closing layers as well as the phellogen contain intercellular spaces for gaseous exchange (Fahn, 1974). The closing layer also ruptures as a result of the renewed production of new complementary cells (Fig. 3.12B) (Kuo-Huang and Hung, 1995).

Several structural features contribute to physiological functions of lenticels: (a) extent, structure and porosity of complementary cells; (b) diameter and extent of intercellular spaces in the lenticel phellogen; (c) extent of lenticel phelloderm and of chlorenchyma

radially adjacent, further their structure, porosity and metabolic activity; (d) continuity of intercellular spaces connecting metabolic active sites in the organ interior of lenticels to the outer environment (Langenfield-Heyser, 1997).

LENTICELS ON POTATO TUBERS

The apical tissues of a growing potato tuber bear stomata and as the tissue ages and lateral buds develop, these stomata become lenticels (Hayward, 1974).

Lenticels often proliferate in wet soils, while in dry soils deposits of suberin and sometimes a cork barrier may form. These changes may affect the permeability of lenticels to gasses and their susceptibility to pathogens (Adams, 1975). They may have domed centres of loosely packed cells or a raised flat plateau in the centre. The centre of the lenticels is porous through which gaseous exchange takes place (Hayward, 1974).

Hayward (1974) also stated that wax structures in and around the pores of lenticels can be a contributing factor in the regulation of water loss from the tuber.

1.2.2 Lenticels on roots

FLOODED CONDITIONS

Exposure of roots to water-logged conditions results in the formation of hypertrophied lenticels on the submerged portions (Angeles *et al.*, 1986). Upon flooding, hypertrophic lenticels or pneumatodes can be observed on submerged stems, root collars or at the basis of adventitious roots of many tree species (Larson *et al.*, 1991). Langenfield-Heyser (1997) state that only flood-tolerant species are

able to form hypertrophied lenticels, but according to Aronen and Häggman (1994), Scot's pine (*Pinus sylvestris*), a flood-intolerant tree, also produces lenticels in water-saturated conditions. Roots of mangos grown in hydroponic systems also formed lenticels (Pers. comm; Robbertse, 2003*)

Hypertrophy of lenticels starts with swelling of filling tissue, an increased activity of lenticel phellogen, enlargement and loosening of complementary cells and an increment of lenticel phellogen. With extended flooding, enhanced activity of the phellogen goes beyond the lenticel, so that an excess of phellem is formed (Larson *et al.*, 1991), cells in the cortex enlarge and intercellular spaces grow and form a continuous network of wide intercellular spaces from stem to root (Aronen and Häggman, 1994).

Roots of certain trees occurring in estuaries, like those of mangrove trees, have pneumatophores. The roots of these trees are periodically exposed to flooded conditions due to the fluctuation of the tides, and therefore these pneumatophores or hypertrophic roots are essential. There are two physiological roles of hypertrophic lenticels. Hypoxia or anoxia leads to production of toxic metabolites through fermentation in flooded roots, like ethanol, acetaldehyde and ethylene. These compounds escape from the roots through the lenticels (Kawase, 1981). The second physiological role is to facilitate entry of atmospheric O₂ into flooded roots (Angeles *et al.*, 1986; Topa and McLoed, 1986).

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1.2.3 Lenticels on fruit

Pyrus malus

The structures on the pome of an apple can scarcely be regarded as lenticels, because it is seldom that periderm is associated with these structures.

Pome lenticels are derived in various ways: breaking of stomata caused by the stretching of the epidermis; breaks in the epidermis caused by complete removal of trichomes associated with young fruit; other breaks in the epidermis where the epidermis can't keep track with the development and expansion of the inner tissue (Clements, 1935).

Some lenticels can be regarded as open lenticels and others as closed lenticels (Clements, 1935). Closing of lenticels may be brought about in one of the following ways: 1) A cuticle may seal over the lenticel opening and thus seal the lenticel. 2) A cuticle develops over sublenticellular cells. 3) No cuticle develops in the lenticel, but sublenticellular cells become suberized. 4) A phellogen develop that results in a suberized layer. Open lenticels can likewise develop in different ways: 1) Stoma remains open with substomatal cells incompletely modified or completely modified, but with large intercellular spaces. 2) Lenticel has been firmly closed, but has been broken by the tension of the developing fruit, often the tear may extend into parenchymatous cells of storage tissue. 3) Those that are in various stages of repair. Breaks in young fruit may be repaired quite rapidly, but the older the fruit such tears are less easily covered.

These structures are therefore not similar to the lenticels of woody stems. It is only an occasional pome lenticel, that shows the development of a distinct phellogen (Clements, 1935)

Prunus avium L.

The epidermis of the 'Royal Ann' cherry is covered with a continuous cuticle which is interrupted by stomata (Wilson *et al.*, 1972). In the sour cherry, stomata are fully differentiated 18 days before full bloom and no stomata differentiate thereafter (Tukey and Young, 1939). According to Wilson *et al.* (1972) stretching and rupturing of the stomata due to fruit growth and enlargement can be the beginning of lenticel development, though, the lenticels observed was not "true" lenticels.

Cucumis melo L.

The surface network of tissue commonly referred to as the "net" in the fruit, is an elaborate system of interconnected lenticels (Webster and Craig, 1976). Phelloderm and complementary tissue, derived from a subepidermal periderm, are visible 14 days after anthesis and are well-developed 30 days after anthesis (Combrink *et al.*, 2001). Cork cells protrude through the surface fissures as the fruit enlarges.

Lenticels develop from stomata that have been covered with an extensive cuticle, thus rendering the stomata nonfunctional (Webster and Craig, 1976) or from cracks in the epidermis caused by rapid fruit growth. This may explain why fruit, developed under slow growth rates, tends to be poorly netted (Combrink *et al.*, 2001).

Mangifera indica L.

Dietz *et al.* (1988) maintain that mango fruit lenticels might originate in one of two ways: from preformed stomata or by shearing of the fruit epidermis as a result of rapid fruit growth. Tamjinda *et al.* (1992)

found that cells directly below the lenticel were smaller than surrounding cells and had larger intercellular spaces.

1.3 DISORDERS

Physiological discolouration occurring directly around apple lenticels is called lenticel spots. These spots often appear at harvest-time and seem to be favoured by high nitrogen applications, early harvest, high humidity and temperatures of 21–27°C after harvesting (Richmond and Dewey, 1969). Susceptibility of the fruit to lenticel spot also seems to vary widely from year to year and from orchard to orchard. Pathogens such as *Alternaria* sp. can penetrate through such a lenticel (Richmond and Dewey, 1969).

It is well known in the mango fruit industry worldwide that, by one way or another, mango lenticels may turn black and consequently depreciate the economic value of the fruit. Much research on the causal aspects has already been done, but no clear answer has been found to elucidate the problem (O'Hare and Prasad, 1992; Shorter and Joyce, 1998; Tamjinda *et al.*, 1992).

1.4 PHYSIOLOGY

1.4.1 Entrance of water

Intercellular spaces of lenticels can be infiltrated with water (Schönherr and Ziegler, 1980). However, under normal circumstances, they are not filled with water (Kleban, 1884) and are not wettable (Schönherr and Ziegler, 1980). Reasons for non-wettability of intercellular spaces could be suberization (Ish-Shalom-Gordon and Dubinsky, 1992) and/or a lining with wax crystals (Hayward, 1974).

For gas exchange, it is hugely advantageous that lenticellular intercellular spaces cannot be filled with water (rain, floodwater); the entrance of O₂ through lenticels to the interior of plant tissue would

otherwise be reduced, since migration of O₂ in water is about 300 000 times slower than in air (Langenfield-Heyser, 1997).

1.4.2 Gas exchange

Gaseous transport through lenticels depends on the number and area of lenticels on a given organ surface, on their degree of opening, developmental stage of the lenticel, species (type of lenticel and non-lenticellular periderm), season and environment (Langenfield-Heyser, 1997). Klebahn (1884) postulated that diffusive resistance of lenticels to gasses depends on the width of the intercellular spaces, on the path length through complementary cell layers and on their structure.

Carbon dioxide produced during respiration can be transported by the transpiration stream (Martin *et al.*, 1994), however, a considerable amount can leave the stems *via* lenticels. Diffusion of CO₂ through stem periderm with lenticels, open or sealed, was measured after chemical absorption (KOH) (Klebahn, 1884) or by means of an infrared gas analyzer (Langenfield-Heyser, 1997).

By its metabolic activity, lenticel chlorenchyma could reduce the loss of CO₂ from the stem and thus improve CO₂ refixation (Langenfield-Heyser *et al.*, 1996). This is especially important at times of high respiration rates such as spring, when lenticels must be open to facilitate transport of O₂ to the metabolic active tissues of the stem interior (Langenfield-Heyser, 1997).

1.4.3 Lenticellular transpiration

Usually lenticels are more permeable to water than the rest of the periderm (Groh *et al.*, 2002). Transpiration rate through older lenticels is lower than through younger ones. In water-saturated atmospheres, lenticellular transpiration is reduced to 16.5%, rising to

50% when the periderm dries (Langenfield-Heyser, 1997). Klebahn (1884) also observed that lenticel transpiration differed seasonally, with higher transpiration rates in summer than in winter.

It is suggested that the rate of lenticellular transpiration is not only dependant on the structure of the lenticel, but also on the type of non-lenticellular periderm, its permeability, its longevity and its influence on the structure of long lasting lenticels (Langenfield-Heyser, 1997).

1.5 SUMMARY

Lenticels are usually formed below stomata, usually on the surface of organs where the epidermis is replaced by a periderm. Cells below the substomatal cavity divide into different planes until the phellogen is formed. The phellogen gives rise to the phelloderm and complementary tissue, with intercellular spaces to allow gas exchange. Lenticels do not have any regulating mechanism like guard cells in the stomata regulating gas exchange and transpiration, but the complementary tissue protects the interior from excess transpiration and also from pathogens (Kuo-Huang and Hung, 1995). Because of suberization and/or lining of wax crystals of the complementary cells, lenticels cannot be filled with water under normal conditions.

Resultant upon the absence of phellogen, subsequent phelloderm and complementary cells in mango fruit lenticels, they are different from the lenticels described above or from typical lenticels. They have a rather ineffective physical barrier to protect the interior of the fruit from unwanted factors like pathogens, chemicals and water.

The reason for lenticel discolouration is still unknown and the purpose of this study is to determine the mechanism of mango lenticel discolouration in an effort to find a solution, which may enable us to prevent or reduce its

occurrence. The lack of literature on mango lenticel development and structure (Tamjinda *et al.*, 1992) inspired us to study the ontogeny of mango lenticels in detail, in order to understand and relate structure to discolouration.

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CHAPTER 2

FLOWER DEVELOPMENT WITH REFERENCE TO INFLORESCENCE DEVELOPMENT

ABSTRACT

Development of hermaphrodite flowers of 'Kent' was examined. Petal and sepal primordia are first to differentiate from the base of the apical dome of the flower initial, followed acropetally by primordia of the staminodes, the fertile anther and ovary. Signs of nectar gland primordia are only visible later in the development of the flower. There are no signs of adnation between the different floral parts, and the style, undergoing rapid elongation just before anthesis, ends in a single stigma. The fertile anther develops faster than any of the other floral parts, while the four to nine staminodes remain inconspicuous.

2.1 INTRODUCTION

In this study, the emphasis was on the ontogeny of the perfect (hermaphrodite) flower in order to determine at what stage of ovary development stomata start developing. Flower ontogeny is, however, an integral part of inflorescence development and is therefore discussed in more detail, mainly based on the paper by Robbertse *et al.* (2001). Figures of the latter paper are supplied in the appendix to make it more convenient for the reader to follow. Robbertse *et al.* (2001) did not include flower ontogeny.

Growth of mango branches is rhythmic or episodic and occurs in growth flushes. These flushes can be either vegetative or reproductive. Each flush starts in a resting bud (terminal or lateral), which consists of an apical dome (meristem) and a number of leaf primordia enveloped by protective bud scales (Mallik, 1957 and Robbertse *et al.* 2001).

Flushing involves two distinctly different components. The first event, common for vegetative and reproductive flushes involves the activation of the resting bud. Bud activation can be either triggered by phytohormones produced by the roots during active root growth or by pruning to activate lateral buds. The second component can be described as induction/differentiation (Pimental *et al.*, 1984; Robbertse *et al.*, 2001). Prevailing conditions during the induction/differentiation period will determine whether the differentiating bud will become a vegetative or reproductive shoot. Temperature is the most important factor where night temperature below 15 °C and day temperature below 20 °C will induce inflorescence development (Davenport and Núñez-Elisea, 1997 and Robbertse *et al.*, 2001).

The primary mango inflorescence is monopodial, which bears secondary and tertiary axes in bract axils. Each secondary inflorescent axis bears opposite bracts subtending sympodial tertiary inflorescence axes. All branches, primary, secondary and tertiary are determinate, ending in a flower (Davenport and Núñez-Elisea, 1997 and Robbertse *et al.*, 2001) (Appendix Fig. A6).

Night/day temperatures below 15/25 °C is crucial from mid April to July for the first five stages of floral bud differentiation to complete, taking about 40 to 60 days. Any change in the temperature regime during this period can have an influence on the morphology of the developing shoots.

Joubert (1995) divided early floral bud development into 5 developmental stages. Figure 1A (appendix) shows a transverse section and figure 1C (appendix), a SEM micrograph of a resting stage 1 bud and figure 1B (appendix) a section of an activated stage 1 bud.

The resting inflorescence bud has a flattened apical part with bract and leaf primordia, situated more or less at the same level as the apical dome.

Axillary bud meristems in the bract axils appear to be aborted while no prominent bud meristems are present in the leaf primordia axils (Appendix Fig. 1A). The elongation of the terminal bud axis (primary inflorescence axis) is the first microscopic sign of bud activation (Appendix Fig. 1B). Thereafter the appearance of bud meristems (Stage 2) in the axils of the preformed leaf primordia follows (Appendix Fig. 2A, B and C). These bud meristems are the precursors of the secondary inflorescence axes bearing the flowers (Appendix Fig. 4 and A5).

During stages 2 to 4 the terminal meristem continue to initiate new leaf primordia at its secondary axillary axis meristem (Appendix Fig. A3 and A4), before ending in a terminal flower bud primordia. The preformed and neoformed secondary axis meristems in turn produce their own leaf (bract) primordia, each subtending the terminal flowers of the sympodial, tertiary inflorescence axis (Appendix Fig. 4B and 5B), stage 5. Stage 5 depicts bud break and is followed by further axes elongation, differentiation of flower buds, unfolding of the inflorescence and anthesis. After bud development has progressed up to stage 5, the fate of the bud to become an inflorescence has been finalised since primordia of all the essential parts have been initiated (Robbertse *et al.*, 2001).

The inflorescence branches terminate in dichasia, consisting of a central (first order) perfect flower followed by lateral staminate or perfect flowers and third order staminate flowers (Appendix Fig. 6). In most instances, the apical portion of the inflorescence has the highest percentage of perfect flowers due to less third order flowers in comparison with the middle and basal part with more third order flowers (Pimental *et al.*, 1984).

The calyx of both staminate and hermaphrodite flowers has five free deciduous, ovate-oblong, concave, yellowish green to light green and densely pubescent sepals. The corolla, inserted at the base of a fleshy

disk, consists of five pale yellow petals, twice as long as the sepals with five red tinted ridges on the ventral sides. As the petals mature, they become yellowish to pinkish in colour. Each flower has only one functional stamen, opposite to the dorsal side of the ovary, and four to nine sterile stamens (staminodes). Perfect flowers contain one sessile ovary (Juliano and Cuevas, 1932 and Pimental *et al.*, 1984).

In spite of the amount of literature available on the mango inflorescence, very little information is available on flower ontogeny and will therefore this chapter reports on a study of the flower ontogeny, mainly to determine at which stage stomata start developing on the ovary.

2.2 MATERIALS AND METHODS

Flower buds and ovaries were obtained from fully bearing 9-year-old 'Tommy Atkins' (TA') mango (*Mangifera indica* L.) trees grafted onto 'Sabre' seedling rootstocks, from commercial blocks at Bavaria Estate, Hoedspruit (24°22'32"S, 30°53'26"E). Perfect flowers were obtained from the apical portion of the inflorescence as mentioned by Pimental *et al.* (1984).

Material was fixed in 2.5 % glutaraldehyde 0.1 M NaPO₄ buffer (pH 7.4), followed by three rinses (10 minutes each) with the same buffer. Postfixation was done with 1 % OsO₄ for two hours and were 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 (SEM), operated at 5 kV. Images were recorded digitally.

The material for resin embedding was fixed in paraformaldehyde (4% formaldehyde in 0.15 M phosphate buffer). 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) and preparations were stained with Toluidine blue. Preparations were viewed under a Leitz Biomed microscope and photographs were taken with an Olympus Camedia C-4000 Zoom digital camera.

2.3 RESULTS AND DISCUSSION

The flower bud primordia of the mango are subtended by bud scales (Fig. 2.1B and 2.2A), protecting them from the environment. Initiation and differentiation of the different organs are readily distinguishable. The axillary bud meristem starts by increasing in width (Fig. 2.1A), allowing space for the different primordia of the flower parts to initiate and develop.

The whorl of five sepals are already differentiated on the rim of the receptacle, while the other organs are still rather undifferentiated. The single fertile stamen is already distinguishable by the characteristic two-lobed anther while only small domes from which the staminodes and carpel will develop are visible (Fig 2.1A). All the primordia are separate without any adnation.

In a further stage of development, the elongating, arcing petals are starting to enclose the other juvenile flower parts (Fig. 2.1B). The fertile anther lobes are in an advanced stage of development, while, only the meristematic domes of the carpel and the staminodes are apparent. Primordia of the nectar glands are not yet distinguishable.

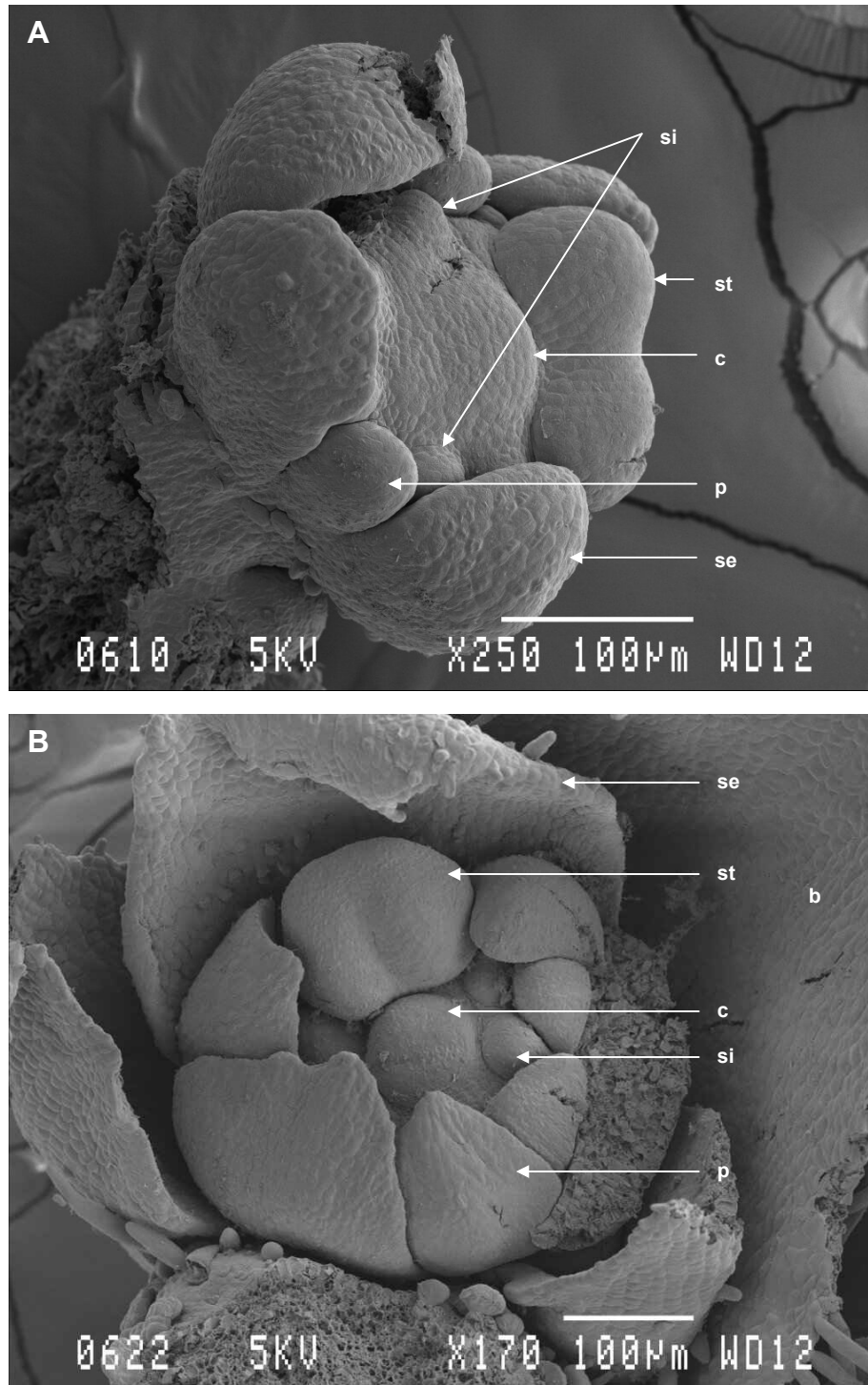


Figure 2.1 (A) Oblique view of a hermaphrodite 'Kent' flower bud primordium. Sepal (se), stamen (st), carpel (c), petal (p) and staminode primordia are visible. (B) Polar view of a more advanced stage of (A). b – bud scale.

The carpel in (Fig. 2.2A) started to differentiate, showing the depression of its single locule. The staminodes are visible as small stubs and the petals are starting to grow over, covering the different flower organs.

Figure 2.2B shows the carpel opening getting narrower as the carpel enlarges. First signs of nectar gland primordia are situated adjacent to the staminodes on the rim of the flower bud. Four larger staminode primordia are alternated by five smaller staminode primordia. An indentation caused by the removed fertile anther is visible on the ovary surface.

Style starts to elongate on top of the ovary that is still showing the unfused margins of carpel (Fig. 2.3A). The fusion of the carpel margins progress in an acropetal direction. The indentation caused by the fertile anther on the ovary is also visible in this figure. Nectar gland development caught up with the development of the rest of the organs. The filament of the fertile anther (removed) is visible, situated on the dorsal side of the carpel. This is consistent in all the flowers and flower buds investigated.

The four lobes of the fertile anther is visible in figure 2.3B, dominating the smaller two lobed staminodes surrounding the carpel. The style is still elongating. The fissure is closing acropetally by the confluence of the adjacent tissue (Fig. 2.3B - arrow).

An excised carpel is showed in figure 2.4A. The style straightened and ends in a relative unspecialised stigma. The development of the floral organs of the hermaphrodite flower of the 'Kent' mango is, therefore acropetal.

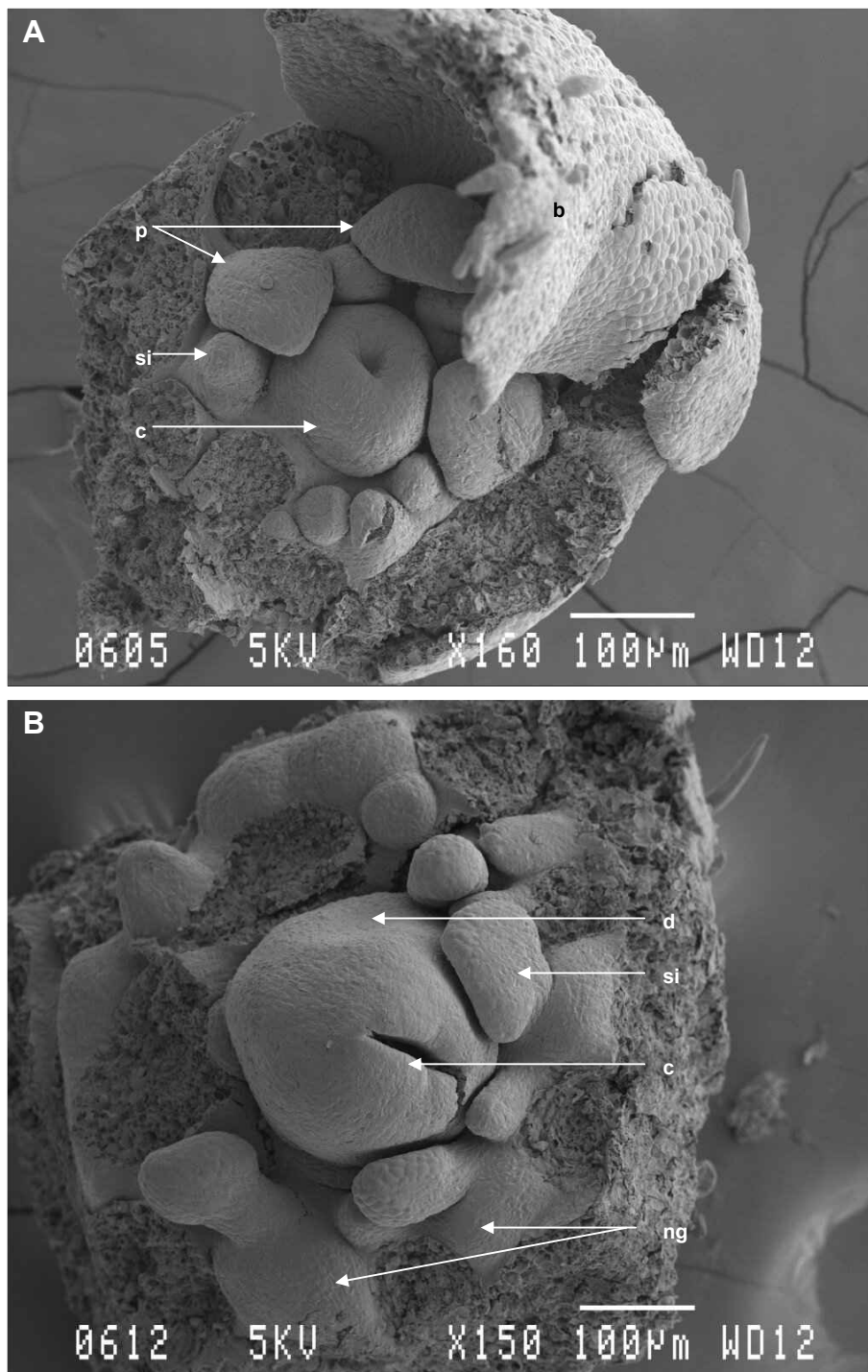


Figure 2.2 Flower buds with petals and sepals removed. (A) Differentiating carpel (c) with indentation of locule. Enlarging petal (p) and staminode primordia (si) visible. (B) Showing folded carpel. Further developed staminodes (si) with filament and first indications of nectar gland primordia (ng) are visible. d – indentation, b – bud scale.

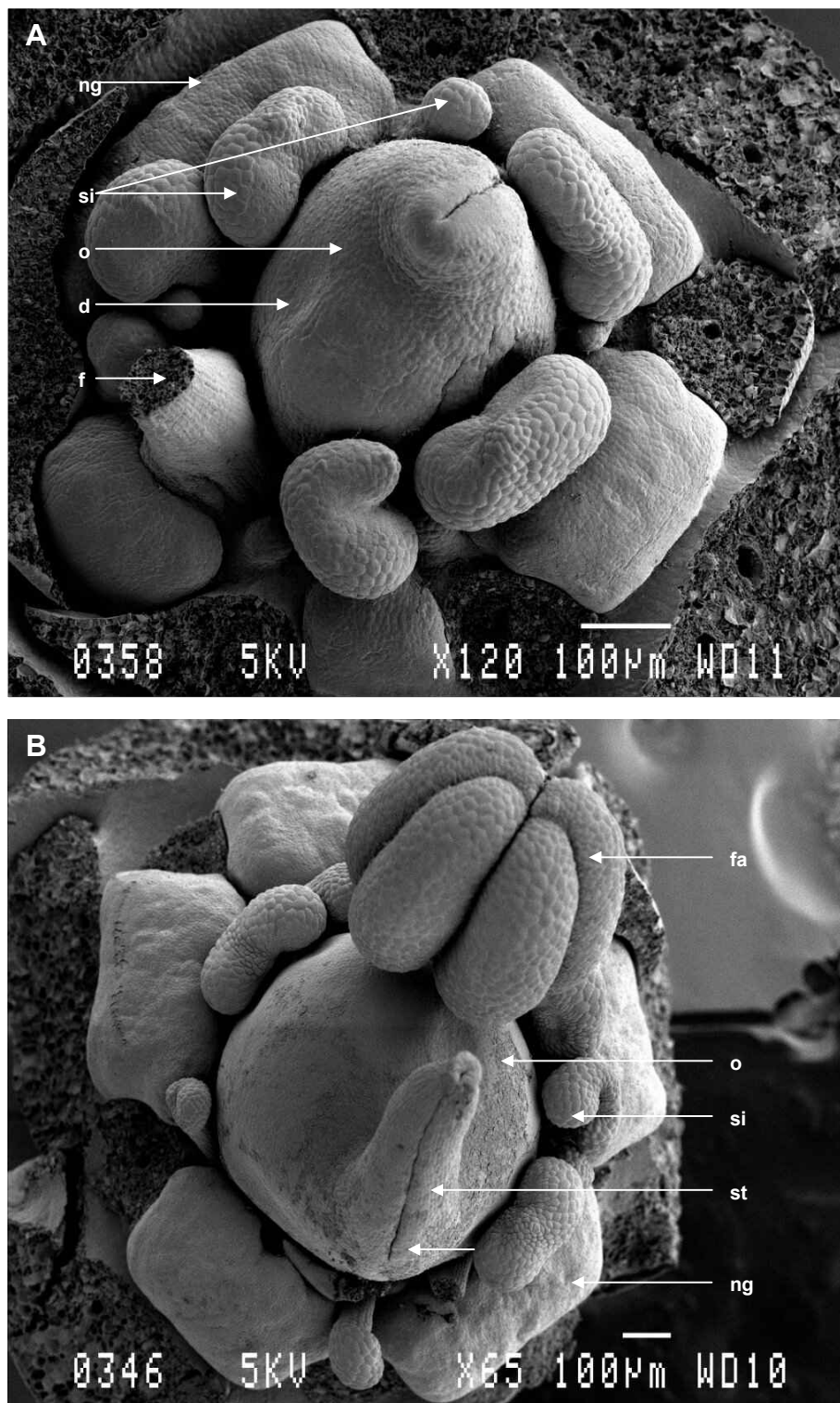


Figure 2.3 Flower buds with petals and sepals removed. (A) Style starts to elongate from ovary (o). Filament (f) of fertile stamen (anther removed) and nectar glands (ng) are visible. (B) More advanced stage of (A), showing fertile anther (fa) and elongated style (st). d – indentation.

Figure 2.4B shows part of a flower at anthesis. The nectar glands took on a strange reticulate appearance. Five nectar glands are situated on the rim of the receptacle. Clearly, the staminodes lost their integrity, shrivelled and died off.

2.4.1 Stomatal development

Stomata in dicotyledons may originate by cell division resulting in an oblique wall in the epidermis. The smaller cell resulting from this division functions as the guard cell mother cell or meristemoid (Cutter, 1978). Meristemoids were visible on the ovary surface prior to anthesis (Fig. 2.5A). They appeared as enlarged, spherical cells of which the cytoplasm stained dark blue with Toluidine blue (Fig. 2.5A). Figure 2.5B shows two guard cells of the developing stoma with the nuclei visible in the two cells. Subsidiary cells are absent. Guard cells took on their characteristic bean-shaped form at the time of anthesis (Fig. 2.5C), but seemed to be non-functional, as the schizogenic stomatal opening was still sealed with cutin and wax (arrow and Fig. 2.5E). Figure 2.5D shows a stoma of a fruitlet shortly after anthesis, clearly functional due to the opening visible between the two adjacent guard cells. The stomatal guard cells on mango fruit, therefore develops directly from a single differentiated cell in the epidermis. The absence of subsidiary cells means that their stomata correspond with the anomocytic type at anthesis (Cutter, 1978).

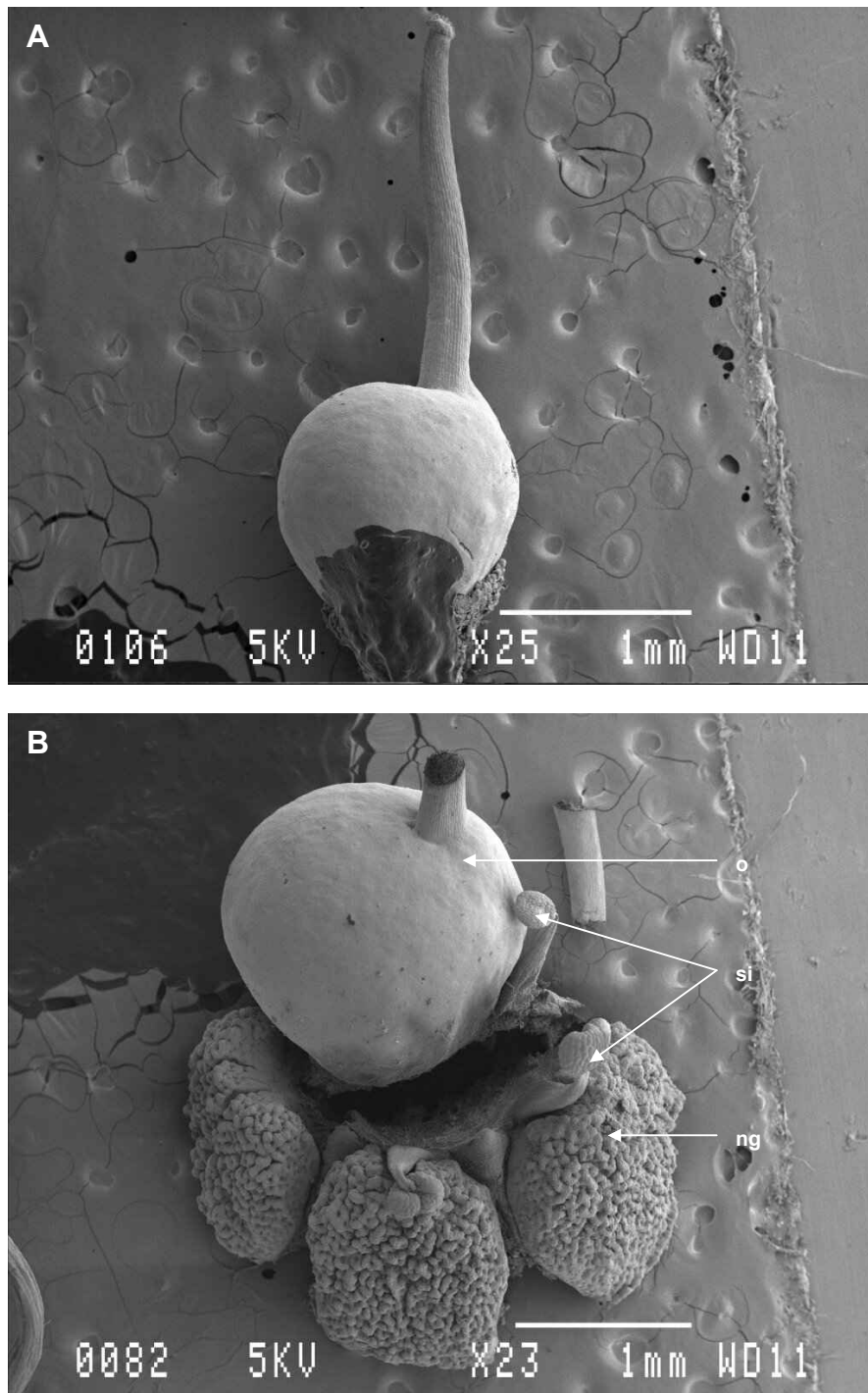


Figure 2.4 (A) Excised pistil prior to anthesis. (B) Showing a flower during anthesis. Staminodes (si) and nectar glands (ng) are visible.

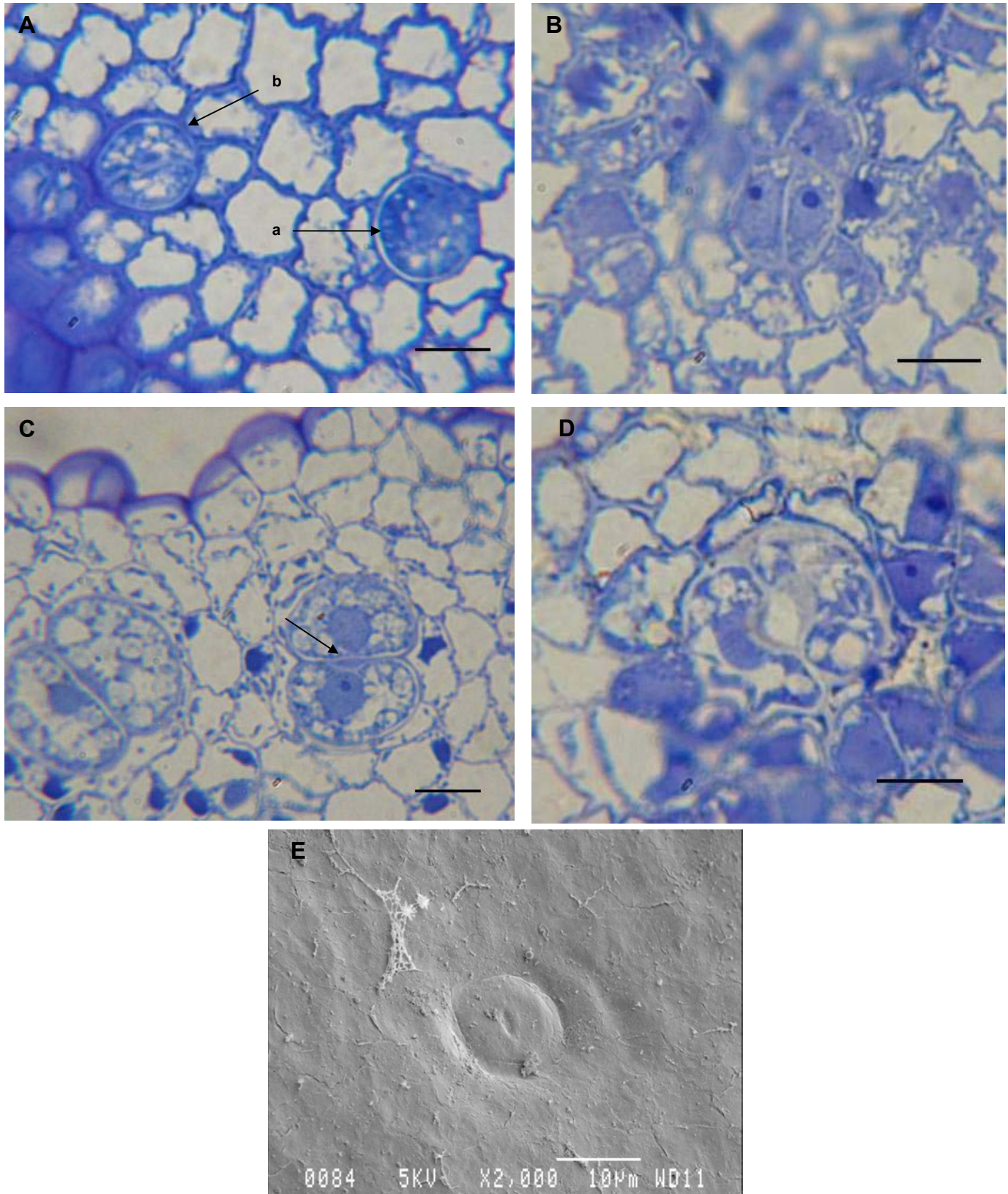


Figure 2.5 Paradermal sections of mango ovary surfaces of different ages. (A) Meristemoid (a) and early development of guard cells (b). (B) Nucleoli visible in cells just after division. (C) Later stage of development of guard cells. (D) Functional stoma shortly after anthesis. (E) Same stage as C, stomatal opening still covered with cutin, therefore not functional yet. Scale = 10µm

REFERENCE LIST

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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 be 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 chamber 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 were 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 NaPO₄ buffer (pH 7.4), followed by three rinses (10 minutes each) with the same buffer. Postfixation was done with 1 % OsO₄ 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'.

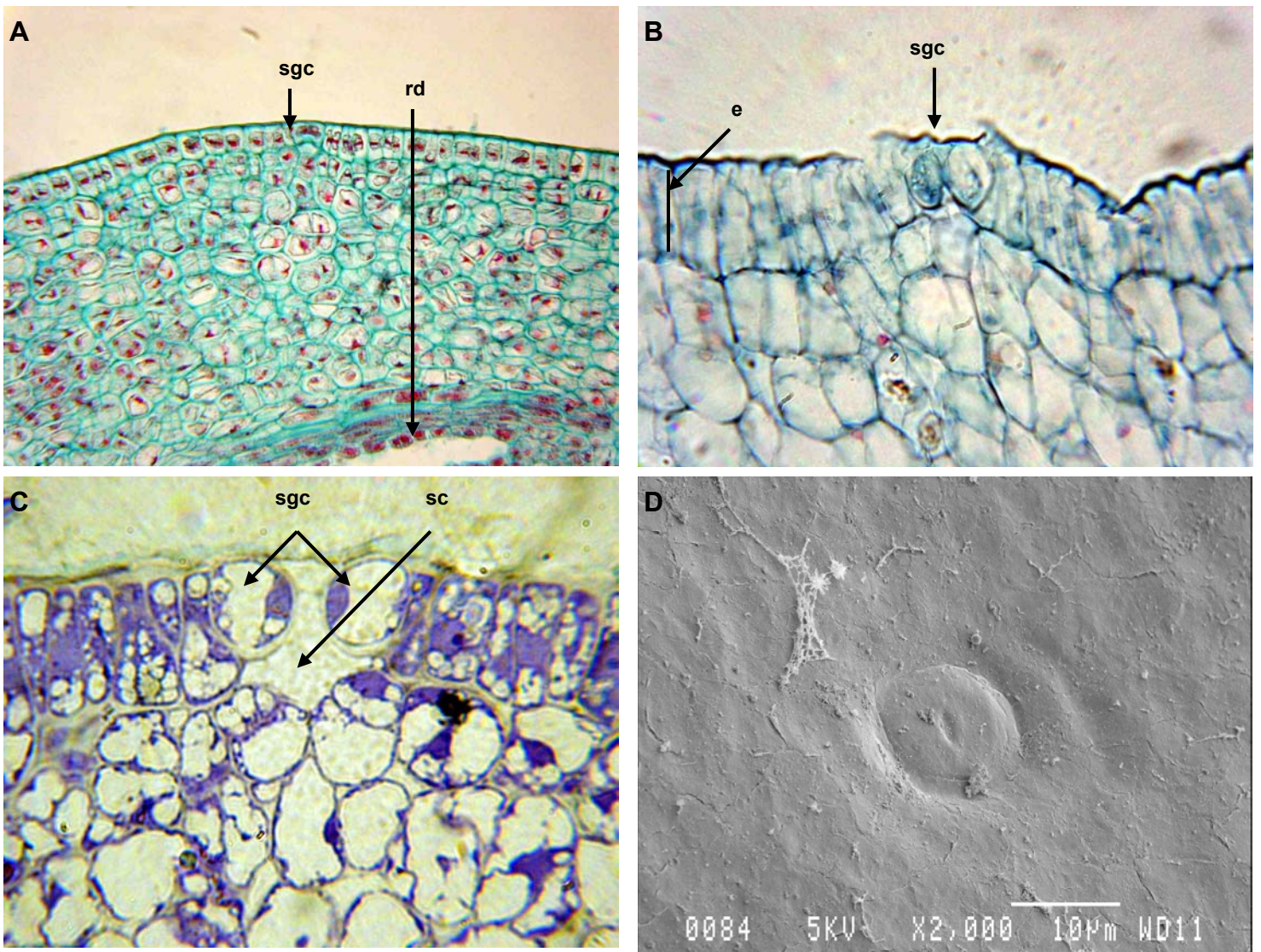


Figure 3.1 Sections of: (A) 1 mm 'TA' ovary, showing differentiating stomatal guard cells (sgc) and resin ducts (rd) already formed (at anthesis); (B) 2 mm 'Kent' fruitlet with differentiated guard cells; (C) 3 mm 'TA' fruitlet showing differentiated guard cells and substomatal cavity. Active cell divisions 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.

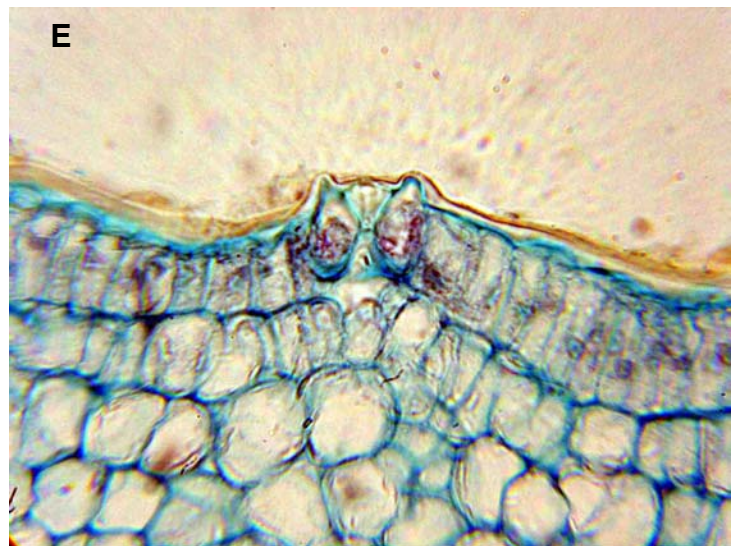
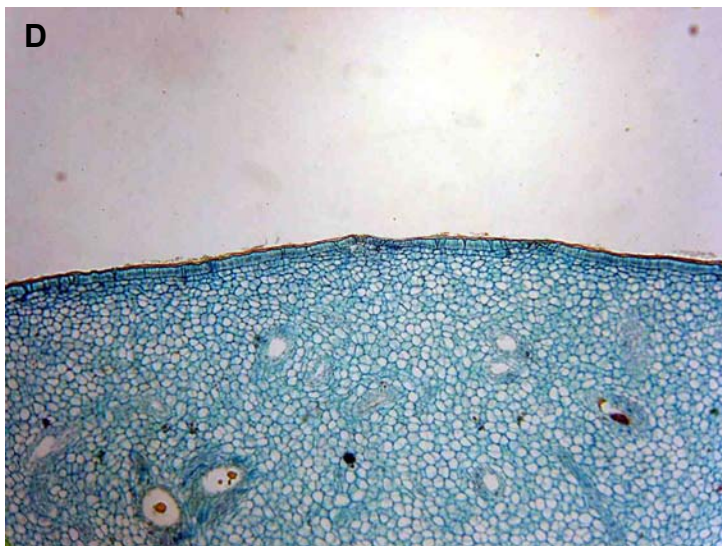
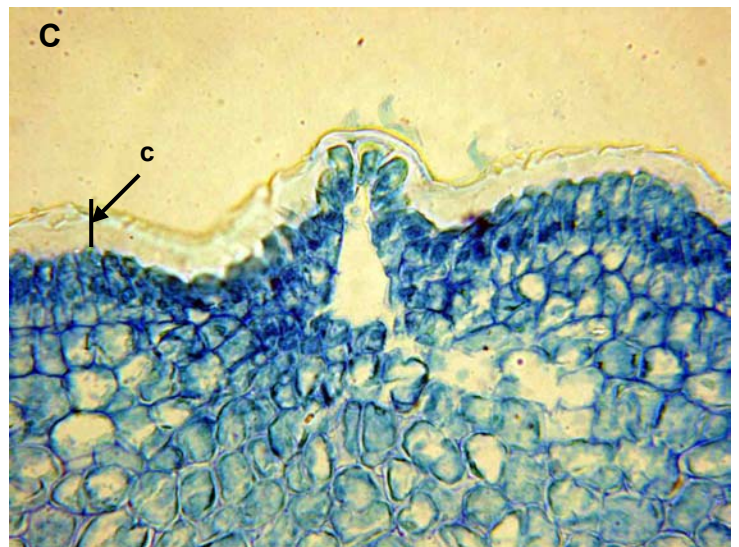


Figure 3.2 (A) 13 mm 'TA' fruit showing undulating epidermis; (B and C) Stomatal guard cells being forced upward. (D) 12 mm 'Kent' fruit with smooth surface. (E) Stomatal guard cells of a 14 mm 'Kent' fruit seem to be still functional. Note the abundance of resin ducts in (A, B and D). c = cuticle

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).

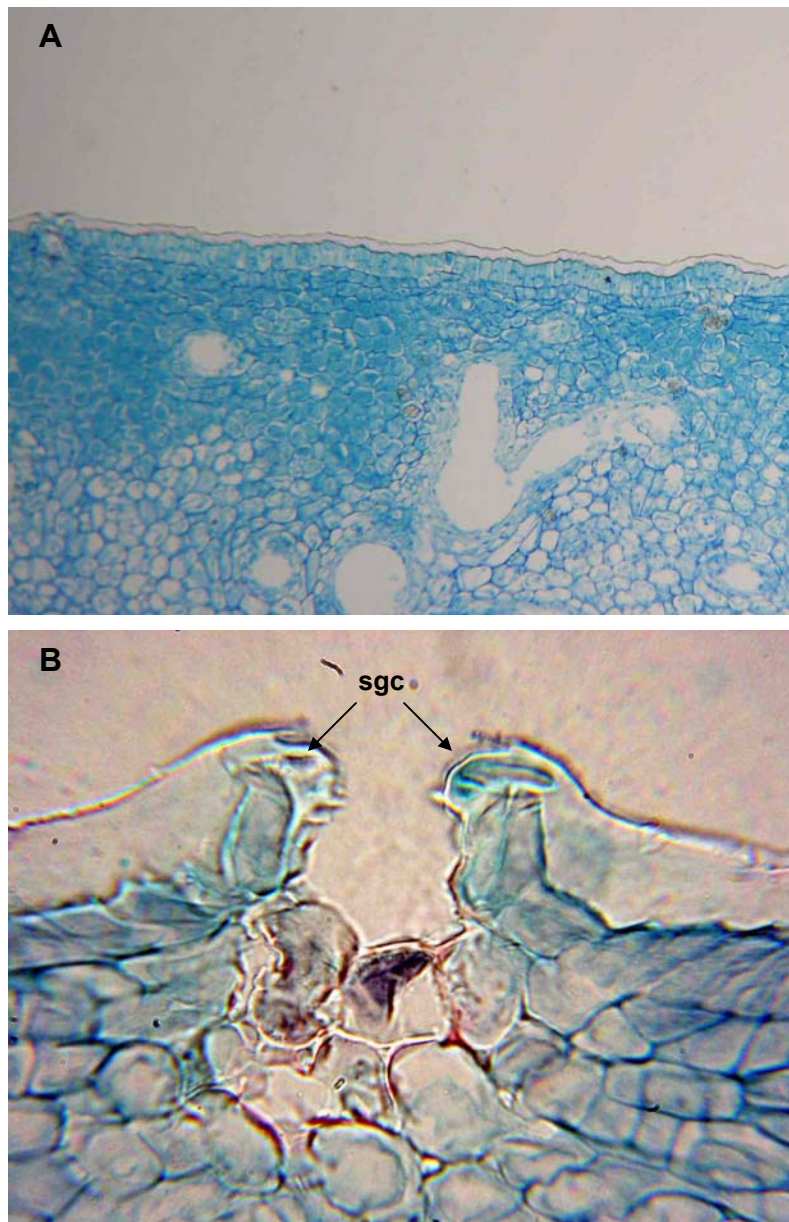


Figure 3.3 (A) Epidermis of 20 mm 'TA' fruit lost its undulating appearance. Resin ducts close to the fruit surface are also visible in this figure. (B) Stomatal guard cells (sgc) elevated above the now smooth epidermis.

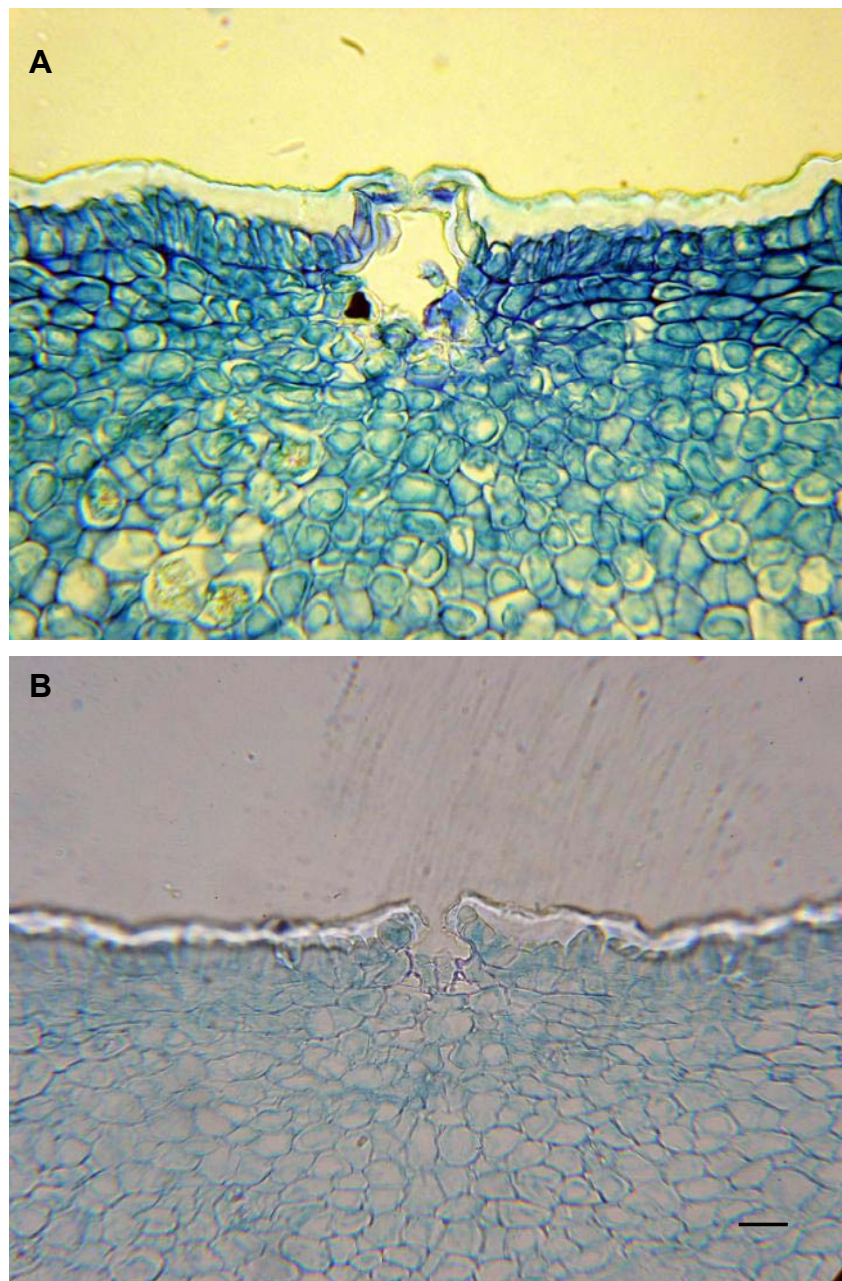


Figure 3.4 35 mm 'TA' fruit with stomatal guard cells still raised above epidermis (A) and 40 mm 'Kent' fruit (B). Enlarging lenticel cavity only protected by a thin cuticle. Scale - 0.02 mm.

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).

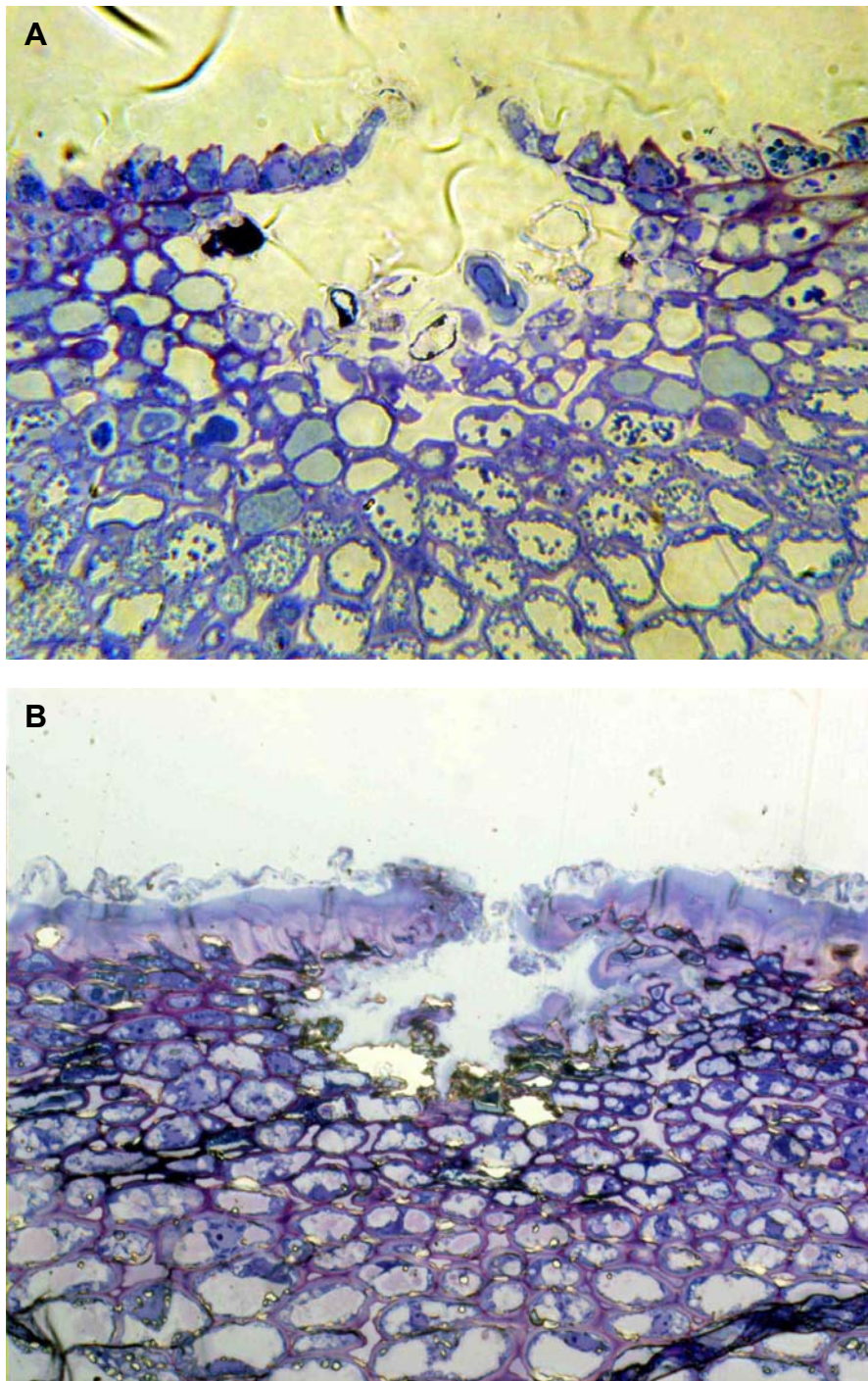


Figure 3.5 Lenticels of (A) 90 'TA' and (B) 70 mm 'Keitt' fruit. Lenticel cavity contains dead, loose cells and it is clear that a periderm is absent.

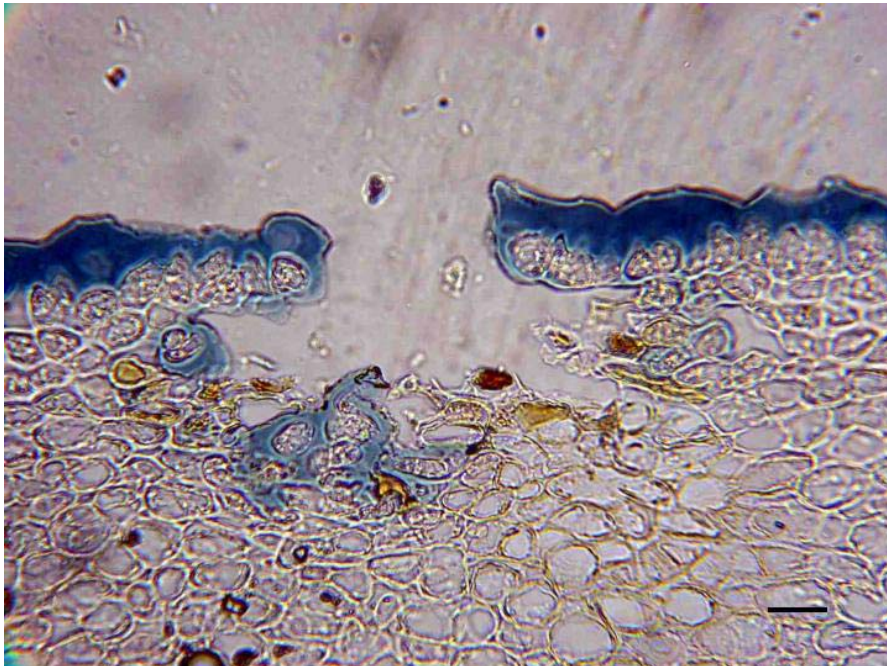


Figure 3.6 Section of a 100 mm 'TA' fruit. Lenticel cavity is only partially covered with cutin (staining black with Sudan Black B), making it more susceptible for penetration of foreign objects. (Scale = 0.02 mm)

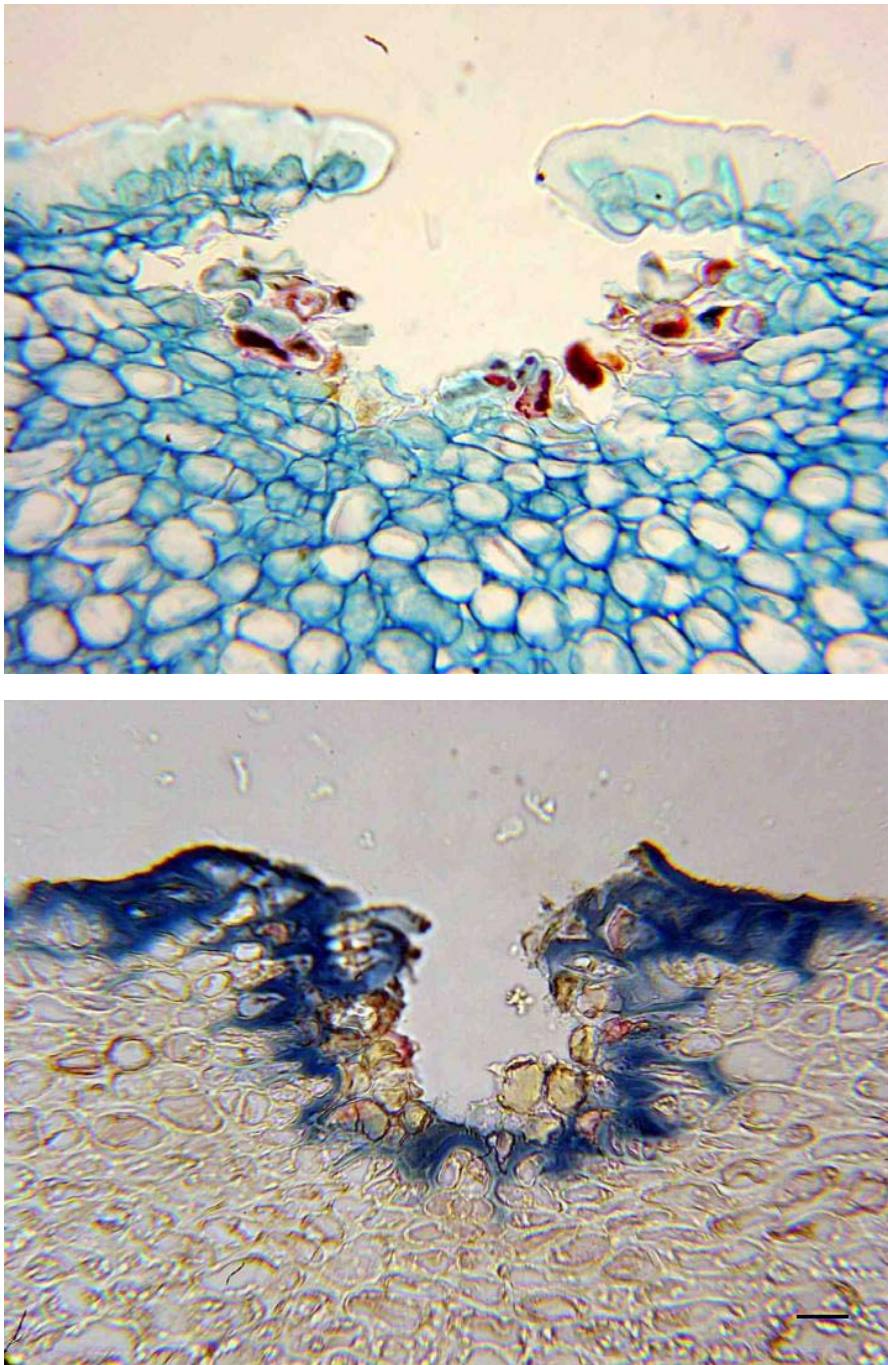


Figure 3.7 (A) 100 mm 'TA' fruit lenticel with pigments in vacuoles in sublenticular cells. (B) 100 mm 'Kent fruit. Continuous cuticle, stained black with Sudan Black B are not interrupted at the lenticel. Scale - 0.02 mm.

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.

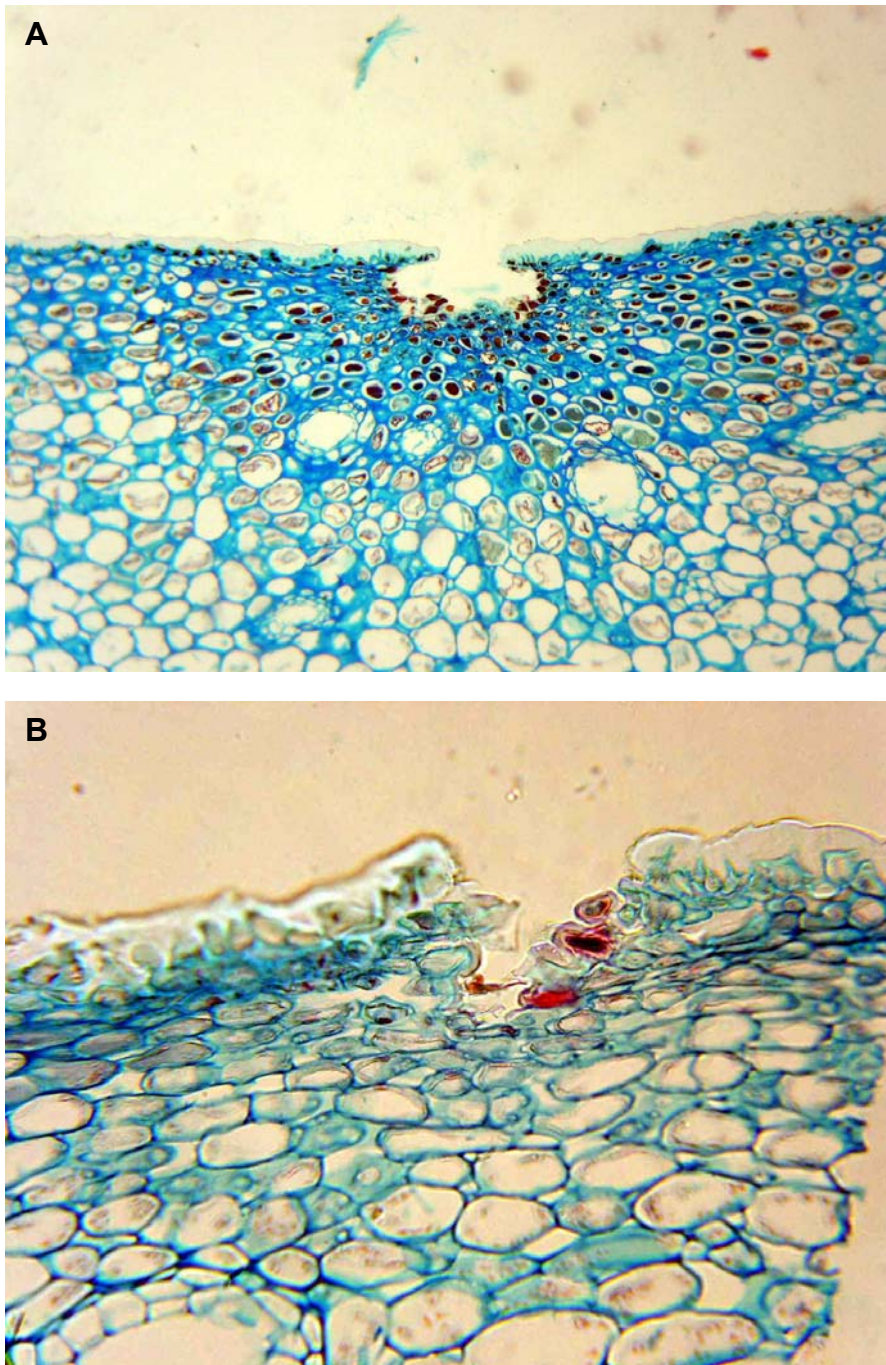


Figure 3.8 Lenticels of mature (A) 'TA' and (B) 'Kent' fruit. Note the abundance of pigments around 'TA' lenticel in contrast to the absence thereof in 'Kent'. It is also marked how closely situated resin ducts are to the lenticel.

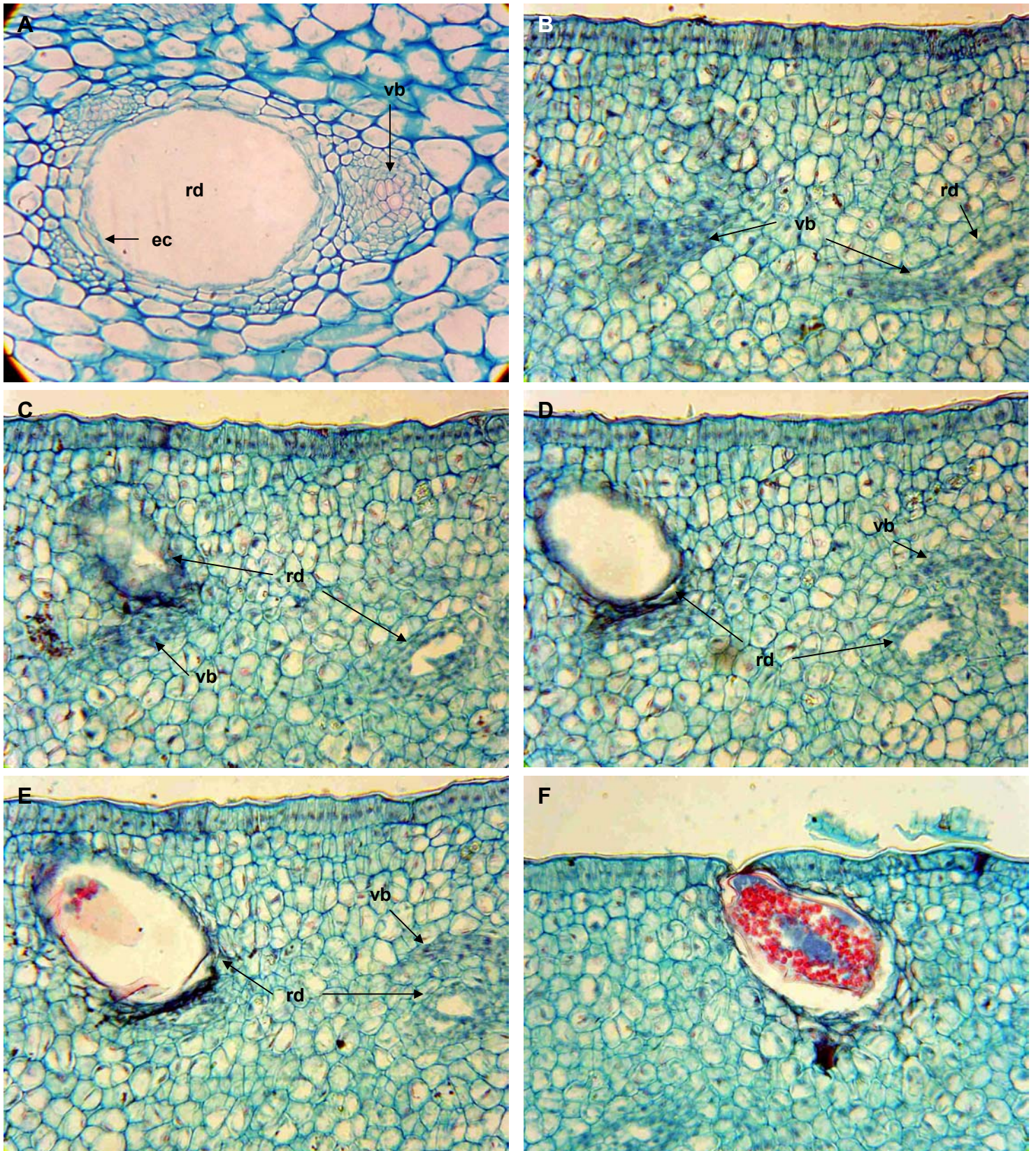


Figure 3.9 (A) Part of mature fruit skin of 'TA' showing a resin duct (rd) subtended with epithelial cells (ec) and bordering vascular bundle (vb), always associated with a resin duct. (B – F) Sequential sections of a 6 mm 'Kent' epidermis showing a resin duct breaking through the epidermis.

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.

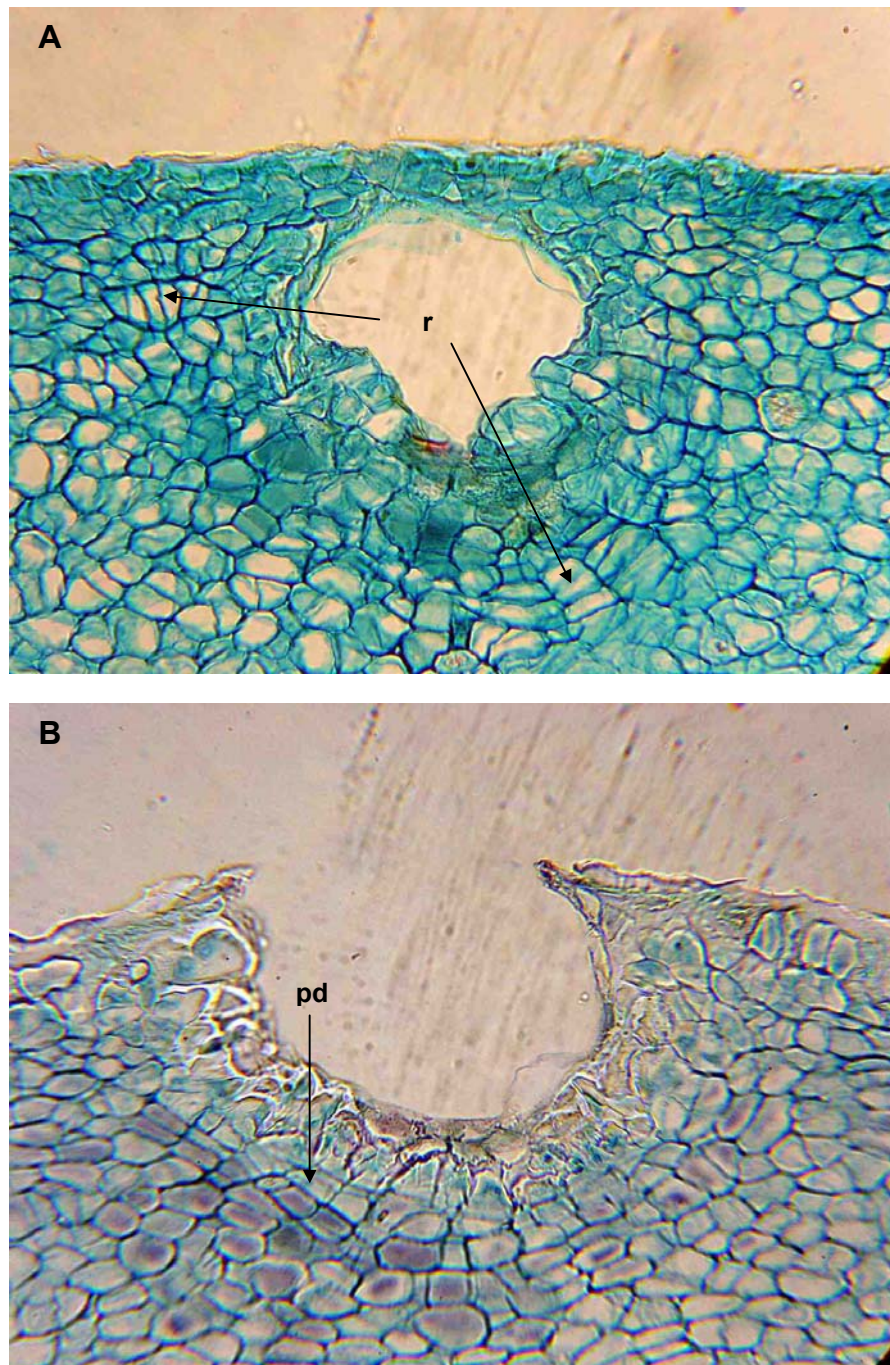


Figure 3.10 Sections of A) 30 mm 'Kent' fruit showing rays (r) of cells where a phelloderm (pd) are starting to develop; (B) 40mm fruit lenticel showing a well-developed phelloderm around the lenticel cavity as well as a cuticle present in the lenticel cavity, continuous with the epidermis.

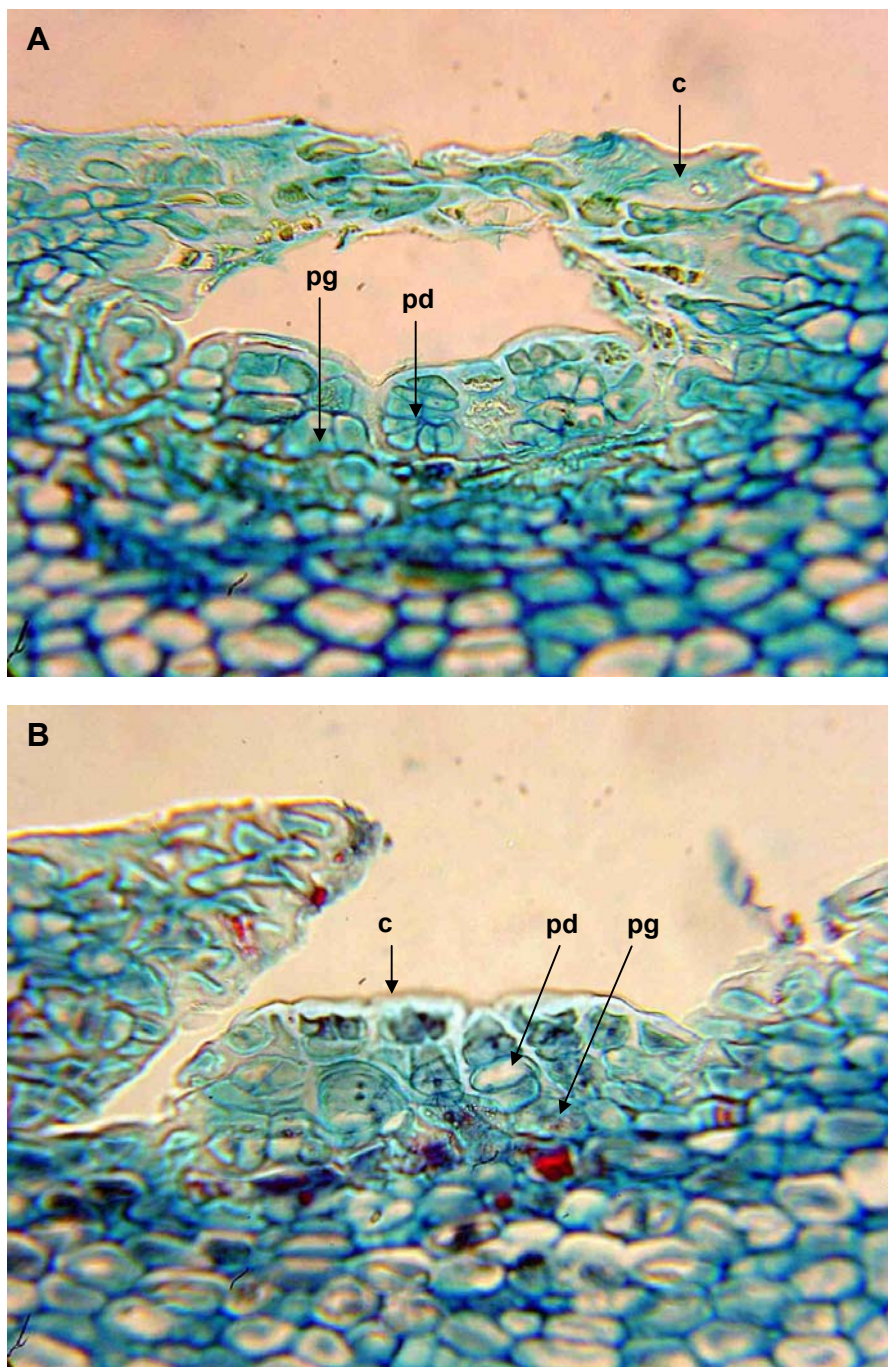


Figure 3.11 (A and B) Lenticels originated from resin ducts of mature 'Kent' fruit. In both lenticels phelloderm (pd) are clearly visible, filling the lenticel cavity with living cells. Lenticel cavities are also covered with the characteristic thick cuticle (c), extending into intercellular spaces.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

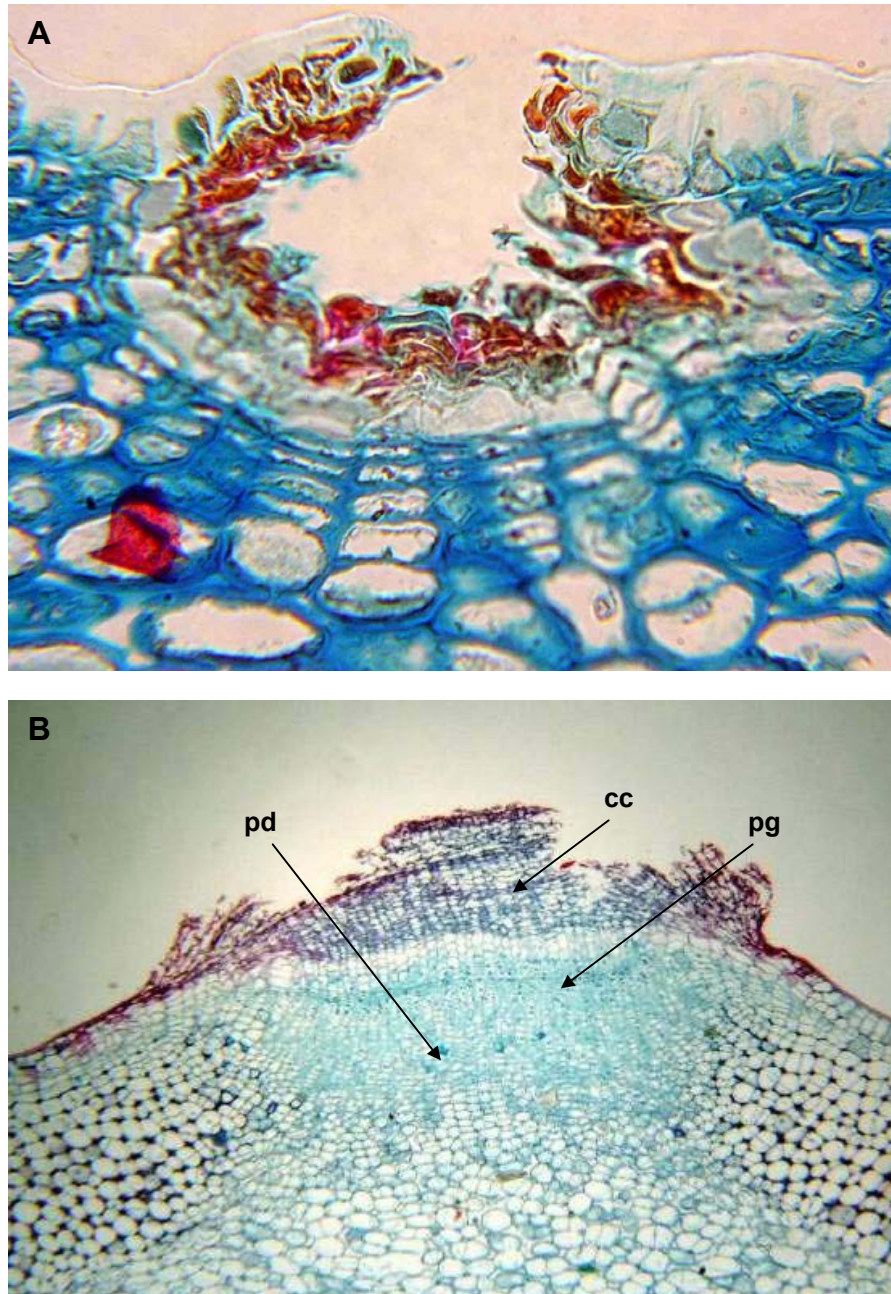


Figure 3.12 (A) Lenticel of mature Marula fruit. Radial cells of phelloderm (pd) are neatly arranged to the outside. The lenticel cavity is covered by a phellem (cork cells). (B) Petiole lenticel of *Phytolacca dioica* with a very active phellogen (pg). cc - complementary cells, pg – phellogen.

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 sublenticular 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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CHAPTER 4

DISCOLOURATION OF MANGO LENTICELS

ABSTRACT

Volatile terpenes occurring in the mango resin can penetrate the fruit through the lenticels, causing their discoloration. Resin in 'Kent' fruit has a lower concentration of the terpenes than 'Tommy Atkins' and 'Keitt' fruit, resulting in less lenticel damage or discoloration in this cultivar. 'Tommy Atkins' have the highest incidence of lenticel discoloration. Lenticel discoloration is a natural process in plants, restricting the penetration of microorganisms and foreign materials into the plant tissue. Oxidized phenolic substances in the cell layers lining the lenticels, are more effective antimicrobial agents than non-oxidized phenols and serve as a barrier against the penetration of pathogens.

4.1 INTRODUCTION

It is familiar to all in the mango industry that resin, exuded from the pedicel after harvesting, causes browning and necroses on the skin of the fruit. Although the damage is superficial, it detracts from the aesthetic value of the fruit and consequently depreciates its economic value (O'Hare and Prasad, 1992; Robinson *et al.*, 1993; Saby *et al.*, 1999). The extent of damage varies between cultivars: 'Tommy Atkins' ('TA') and 'Keitt' are most susceptible whereas 'Kent' is much less susceptible and often displays no damage at all.

Resin, exuded from epithelial cells in the resin ducts of the fruit, consists of two fractions, namely, oil and protein polysaccharide (PPS) fraction. Resin usually remains largely segregated from the normal fruit tissue unless canal rupturing occurs (Joel and Fahn, 1980^a; Joel and Fahn, 1980^b). O'Hare and Prasad (1992) and Loveys *et al.* (1992) found that the oil fraction is responsible for the damage on the fruit skin, while the PPS fraction only

leaves a clear glaze (non-damaging) on the surface of the skin. Loveys *et al.* (1992) maintain that the enzyme, polyphenol oxidase (PPO), plays a role in the browning when the oil fraction comes into contact with the fruit skin. The browning first appears around the lenticels where the resin penetrates the skin. According to Robinson *et al.* (1993) plastid bound PPO is separated from its phenolic substrates, localized in vacuoles with cell membranes. Volatile terpenes, occurring in the oil fraction, disrupt the cell membranes. This causes the PPO to come into contact with the phenolic substrate and consequently initiates enzymatic browning (Robinson *et al.*, 1993). The above-mentioned reaction is similar that occurring during lenticel discolouration (Tamjinda *et al.*, 1992). Terpenes, abundant in the oil fraction of most mango cultivars, are primarily responsible for the typical taste and aroma of the fruit (Macleod *et al.*, 1988).

The ontogeny and function of the lenticels pertaining to the three cultivars have been discussed and compared in Chapter 3. The conclusions drawn from Chapter 3 will be used to further describe the mechanism of lenticel discolouration in relation to the resin of the different cultivars.

4.2 MATERIALS AND METHODS

Over two seasons, representative fruit samples were collected regularly from anthesis to fruit maturity, and at harvesting from 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).

Several sections of the exocarp tissue from the side of fruit, exposed to direct sunlight, were cut into 2 to 3 mm pieces to be embedded in LR white resin. Other pericarp pieces (5 - 21 mm) were cut to be embed in paraffin wax. The material for resin embedding was fixed in paraformaldehyde (4%

formaldehyde in 0.15 M phosphate buffer). 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) and preparations were stained with Toluidine blue. FAA (5% Formalin, 5% Acetic acid and 50% Ethanol) was used to fix material for wax embedding. 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. 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). Preparations were viewed under a Leitz Biomed microscope and photographs were taken with an Olympus Camedia C-4000 Zoom digital camera.

In the field, resin from 'Keitt' and 'Kent' fruit was applied reciprocally to fruit still attached to the tree. This was done on fruit of about 40, 50 and 60 cm in length and evaluated at intervals of two weeks thereafter.

For resin collection, mature fruit of the three cultivars was harvested with the pedicel and peduncle still attached. Collection of the resin was done by breaking the pedicel over a glass container, allowing the fruit to 'bleed' for about 30 seconds. The two fractions separated spontaneously within a few seconds (Loveys *et al.*, 1992). Both fractions of the three cultivars were applied reciprocally to each cultivar after the resin had separated.

4.3 RESULTS

4.3.1 Effect of resin on immature fruit

Resin of 'Keitt' fruit had a definite effect on the fruit surface of both 'Keitt' and 'Kent' cultivars. The most evident symptoms were brown necrotic lesions where the resin came into contact with the fruit surface (Fig. 4.1A). This was observed two weeks after application. The resin also had an effect on the fruit growth on the side where the resin had been applied. Growth on the side of treatment had been stunted when compared with the untreated side (Fig. 4.1B). 'Keitt' resin had an even worse effect on 'Kent' fruit (Fig. 4.1C) while 'Kent' resin had no effect on both 'Keitt' and 'Kent' fruit (Fig. 4.1D).

4.3.2 Lenticel discolouration

The lenticels did not discolour until fruit had reached maturity. Some pigment accumulation was observed in vacuoles of the sublenticellular cells during the maturing stages of the fruit, but this became more prominent as fruit matured. Initially, lenticel discolouration took place in the form of a light purple spot surrounding the lenticel and, in transverse sections, this particular type of lenticel showed increased vacuolar pigmentation (Fig. 4.2A and B). In black, discoloured lenticels, no vacuolar pigmentation was observed, but the cell walls of the sublenticellular cells were discoloured by natural pigmentation (Fig. 4.3), taking on a brown appearance.

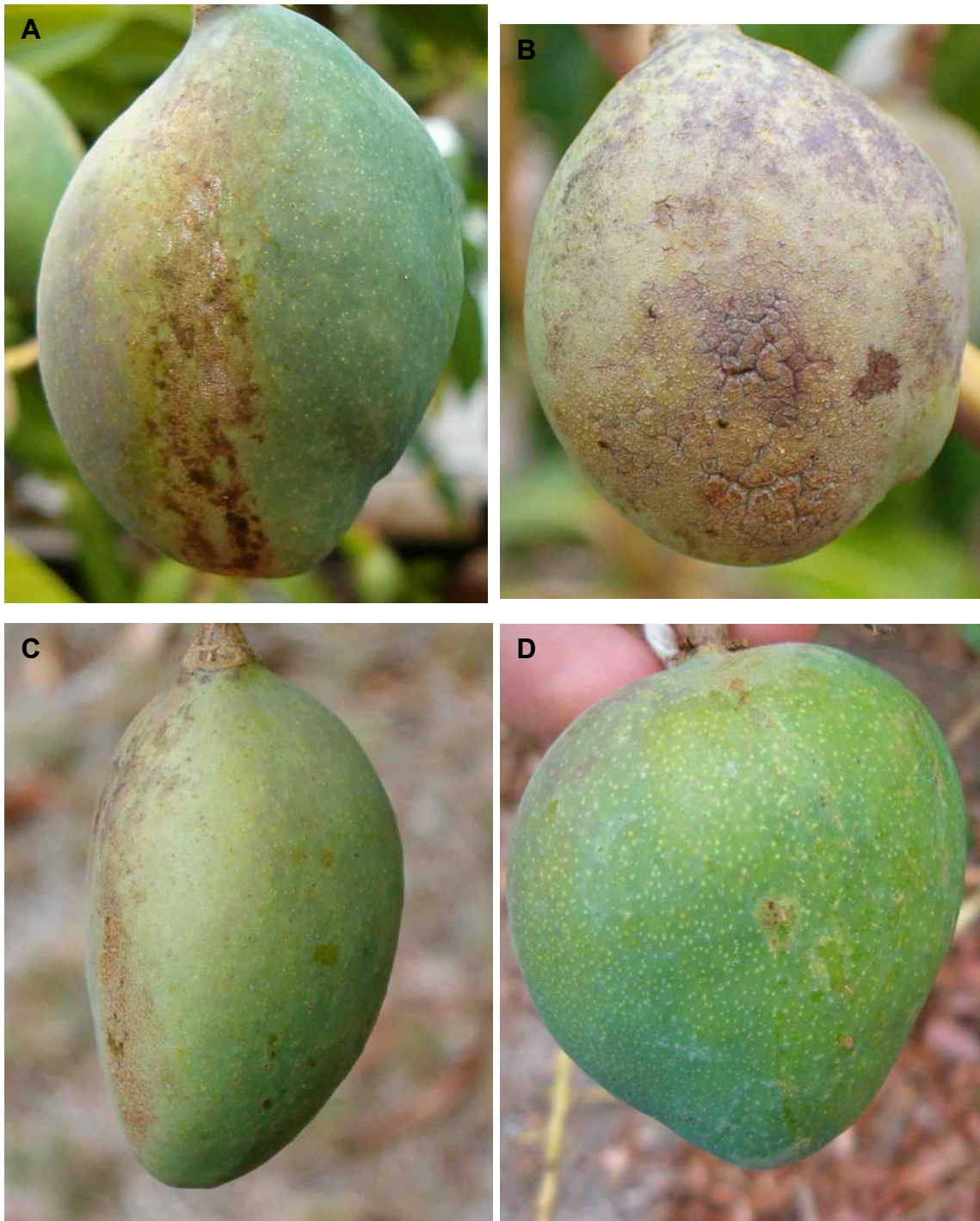


Figure 4.1 (A) An immature 'Keitt' fruit showing the damage caused after resin application from another 'Keitt' fruit. (B) An immature 'Kent' fruit also treated with 'Keitt' resin showing more severe damage. (C) An immature 'Keitt' fruit showing retarded growth on the side where 'Keitt' resin was applied. (D) 'Kent' fruit treated 'Kent' resin showing no damage.

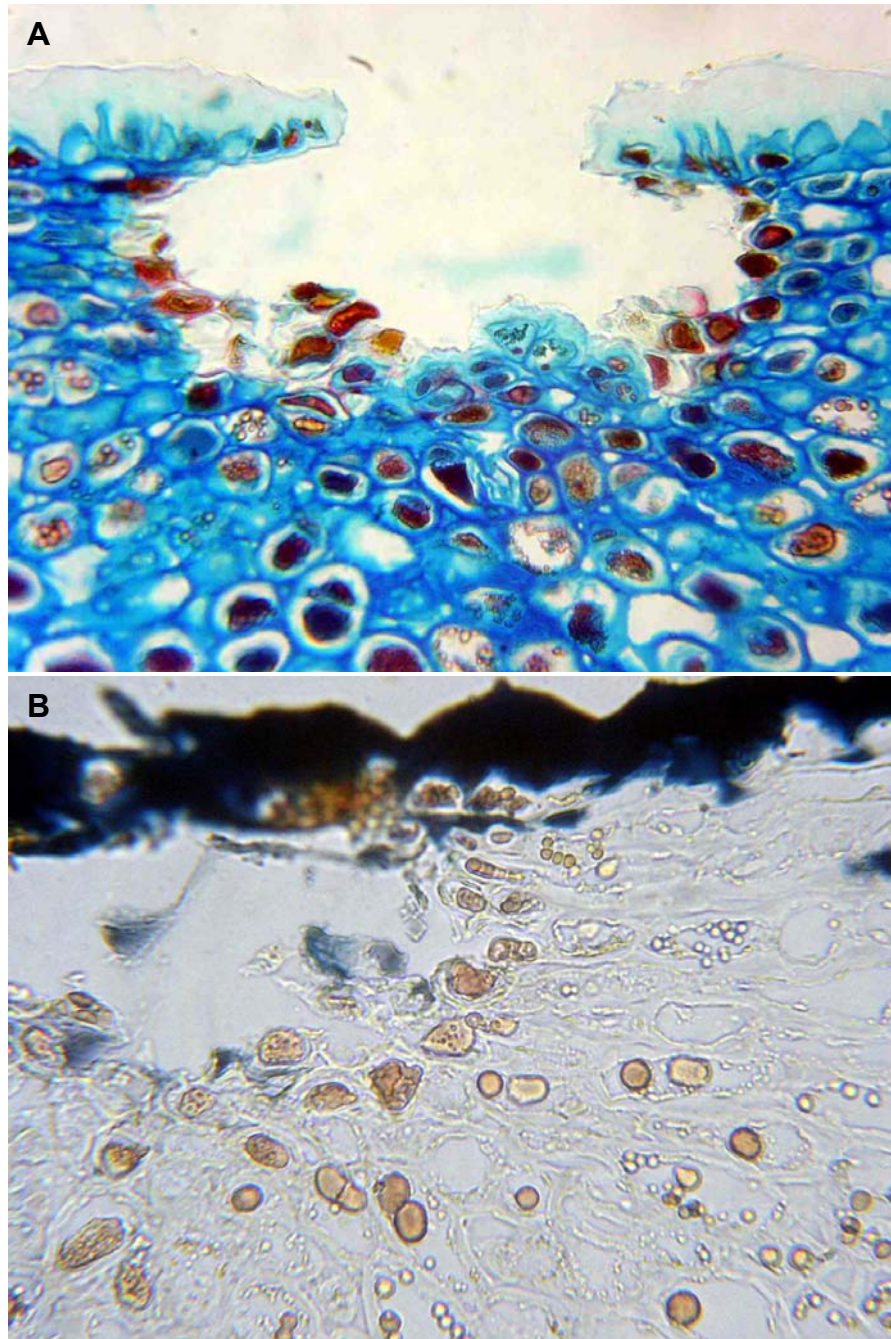


Figure 4.2 (A) Transverse sections of exocarp of mature 'TA' fruit showing lenticels subtended by cells containing pigments that stained red with Safranin A. (B) Staining of cuticle using Sudan Black and natural colour of pigments in vacuoles.

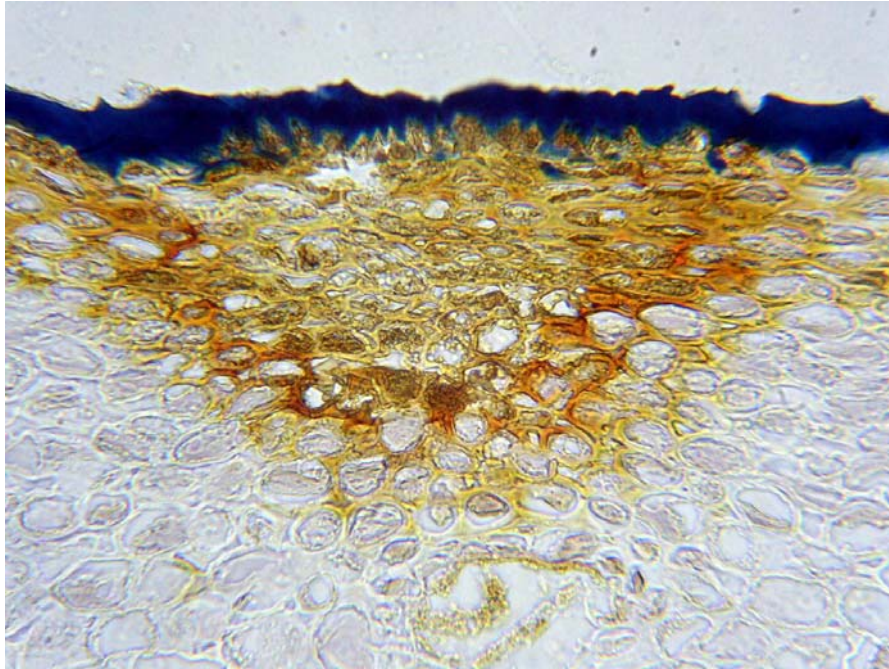


Figure 4.3 Transverse section of lenticel showing natural pigmentation in a discoloured lenticel's cell walls in the absence of chemical staining. The cuticle stained black with Sudan Black B.

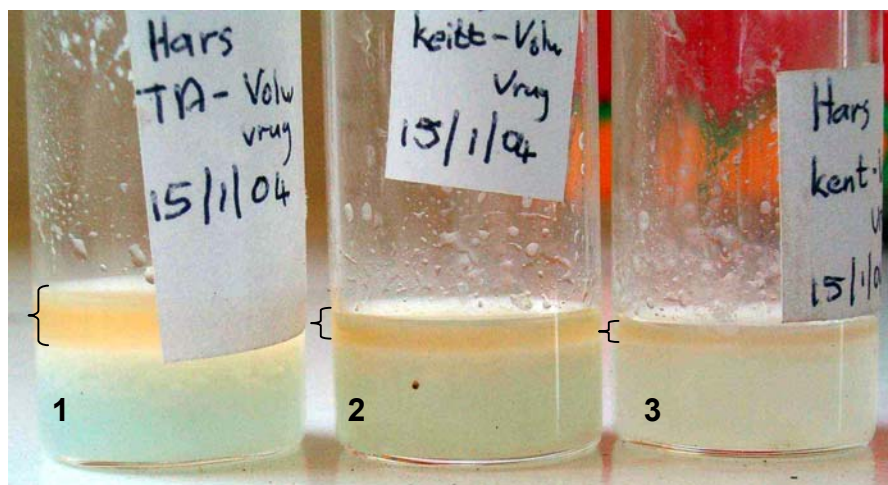


Figure 4.4 Collected resin from: 1 – 'TA', 2 – 'Keitt' and 3 – 'Kent'. The symbol { shows the oil fraction of the resin on top of the PPS fraction.

4.3.3 Effect of resin on mature fruit

Resin of all cultivars separated into the two fractions directly after collection (Fig. 4.4), which is in keeping with observations of Loveys *et al.* (1992). The oil fraction was brown in colour while the PPS fraction was white with a bluish shine. The ratio of the oil fraction to the PPS fraction varied between the three cultivars, with 'TA' the greatest (35%), 'Kent' the smallest (5%) and 'Keitt' (18%) an intermediate amount.

The PPS fraction of all three cultivars did not cause any visible effect in any treatment, which aligns with the observations of O'Hare en Prasad (1992) (Fig. 4.5A2, B2 and D). On the contrary, the degree of damage on the fruit surface caused by the oil fraction was different for the three cultivars (Fig. 4.6A and B). Areas around the lenticels started discolouring directly after resin application and gradually turned darker until large lesions were visible on the fruit surface. The oil fraction of 'TA' had the greatest effect (Fig. 4.7), especially on 'Kent' fruit. Lenticels appeared dark brown while areas between lenticels were light brown, causing a continuous brown lesion on areas where resin was applied. Depressions were visible around the lenticels and signify the probability that the sublenticular cells died off. The oil fraction of 'TA' also caused damage on 'TA' and 'Keitt' fruit (Fig. 4.5A), but the oil fraction of 'Keitt' was less destructive than that of 'TA' on all three cultivars and discolouration was limited to relatively small areas around the lenticels (Fig. 4.5B1 and 4.6B2). Oil fraction of 'Kent' resin caused virtually no discolouration on the fruit in any of the treatments (Fig. 4.5C and 4.6B3). It is clear, therefore, that resin of 'TA' caused the most severe damage. 'Keitt' damage was less and that of 'Kent' was practically null and void (Fig. 4.6A3 and B3).

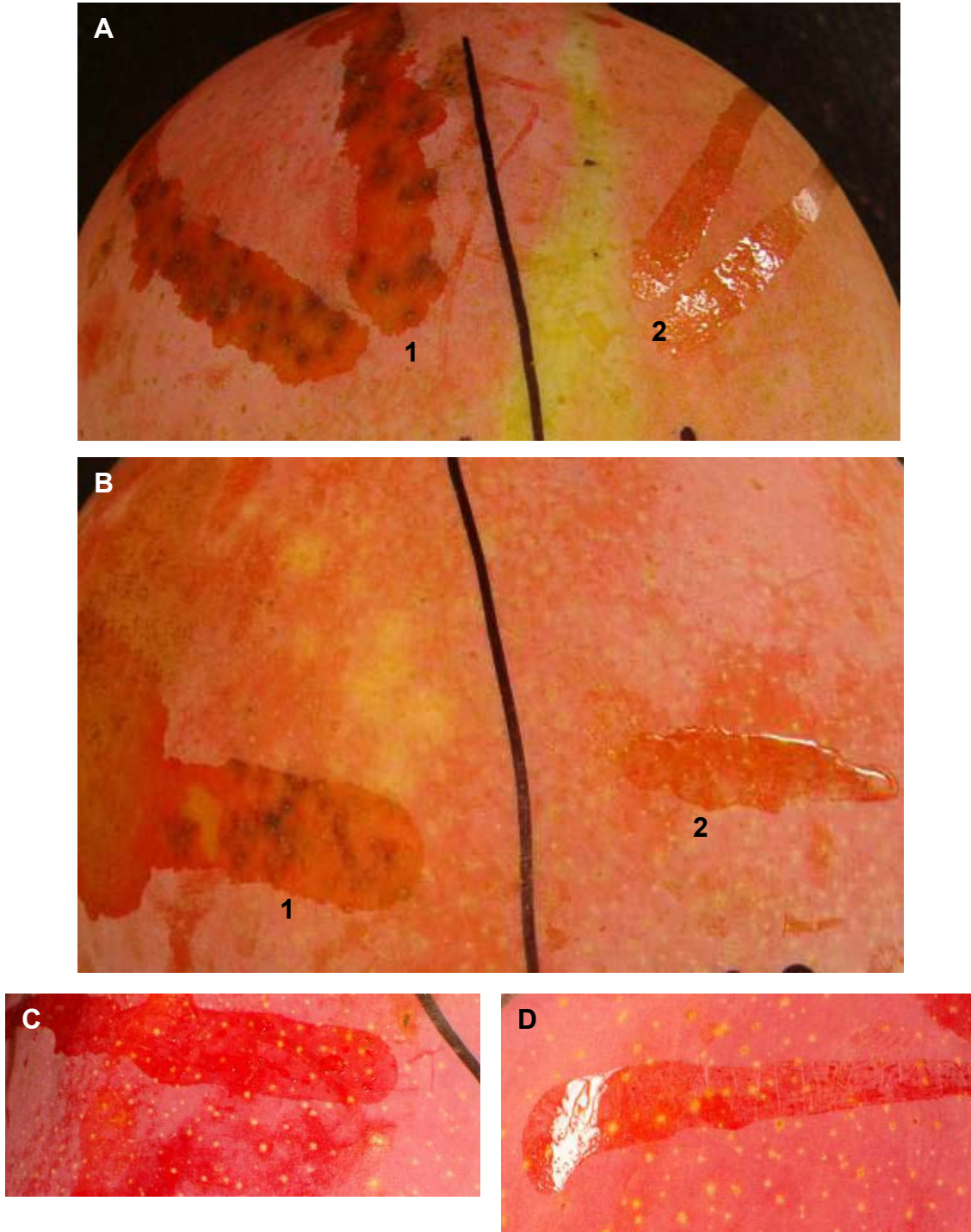


Figure 4.5 (A) 'Keitt' fruit showing the effect of the oil fraction (1) and the PPS fraction (2) of 'TA' resin. (B) 'Keitt' fruit showing the effect of the oil fraction (1) and PPS fraction (2) of 'Keitt' resin. 'TA' fruit treated with the oil fraction (C) and PPS fraction (D) of 'Kent' resin.

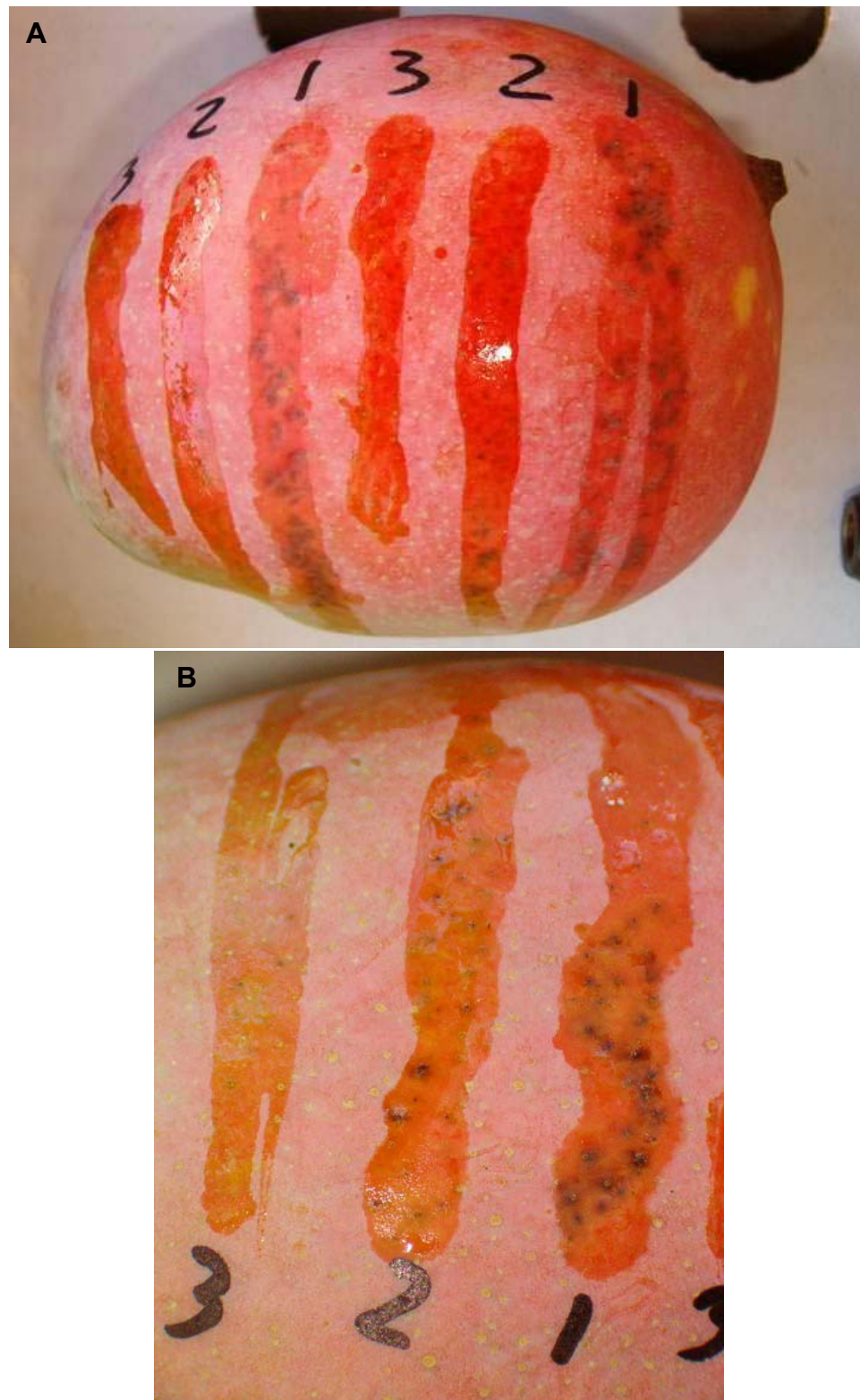


Figure 4.6 (A) Resin marks of the oil fraction of all three cultivars 'TA' (1), 'Keitt' (2) and Kent' (3) on a 'Keitt' fruit. (B) Close up a replication.



Figure. 4.7 Distinct damage on the surface of a 'Kent' fruit after the application of 'TA' resin.

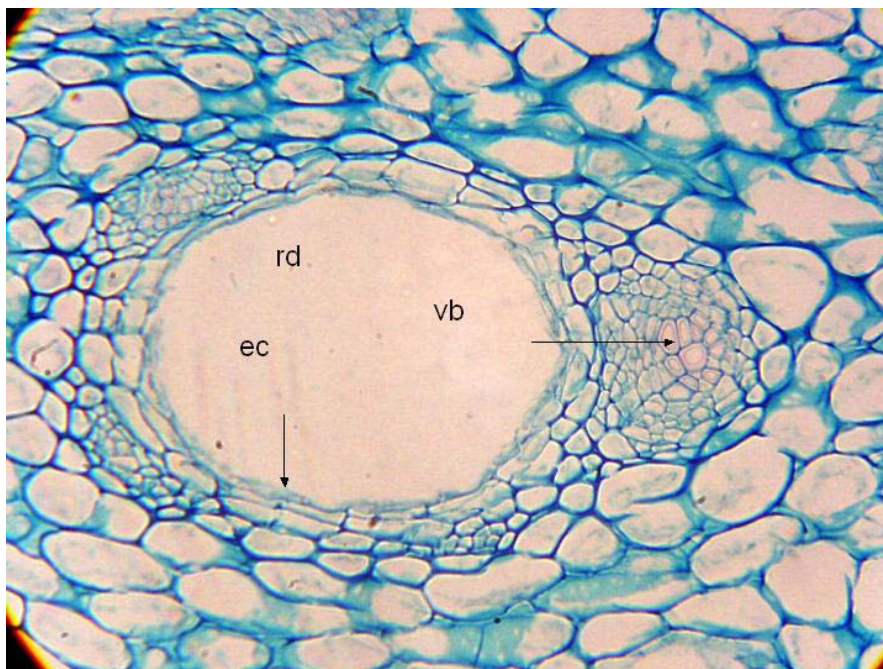


Figure 4.8 Transverse section through a resin duct (rd) in the wall of a mature 'TA' fruit. Resin duct lined epithelial cells (ec). Resin ducts are always associated with adjacent vascular bundles (vb).

4.3.4 Possible role of resin ducts

It is widely accepted that resin ducts occur throughout the mango exocarp (Joel, 1980) and can be seen in Fig. 4.1A and 4.2A,B and D. In the current study, resin ducts were usually closely associated with vascular bundles (Fig. 4.8), confirming the observations of Joel and Fahn (1980a). Resin ducts were already visible in 'TA' mango ovaries during anthesis (Fig. 4.1A) and these developed progressively closer to the fruit surface as the fruit developed and grew. Eventually, some even lay adjacent to the lenticels (Fig. 4.8A and 4.10B). Epithelial cells lining the resin duct are responsible for secretion of resin into the resin duct. The resin in the ducts are under high pressure and it is well-known that a fairly large amount of sap exudes from the pedicel after the fruit has been picked (Fig. 4.9). Indeed, during the collection of mature 'Keitt' fruit, it was observed that droplets of resin were present on the surface of some fruit above the lenticels (Fig. 4.10A). Further investigation of this phenomenon revealed that sap had leaked out of a resin duct through the lenticel (Fig. 4.10B). Microscopic examination of the relevant lenticel revealed a damaged resin duct immediately below the lenticel (Fig. 4.10B). Furthermore, the cell walls of the resin duct were also discoloured and appeared similar to those of the adjacent and other discoloured lenticels (Fig. 4.3). The findings of O'Hara and Prasad (1992), that mango sap is one of the major causes of lenticel discolouration, support further evidence of a link between resin ducts and lenticel discolouration.

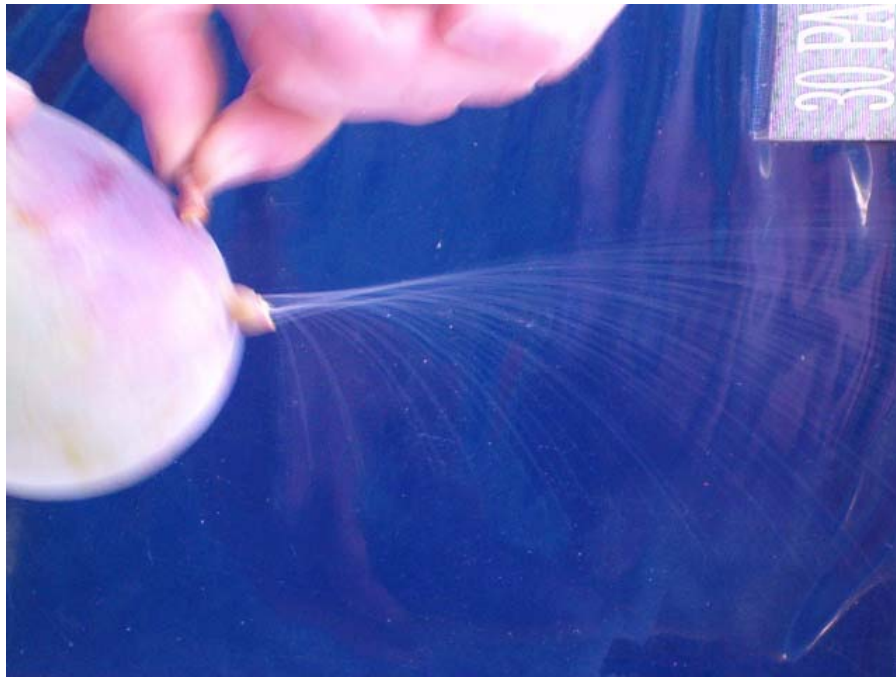


Figure 4.9 Resin squirting from the pedicel directly after removal of the inflorescence axis, indicating the pressure within the resin ducts.

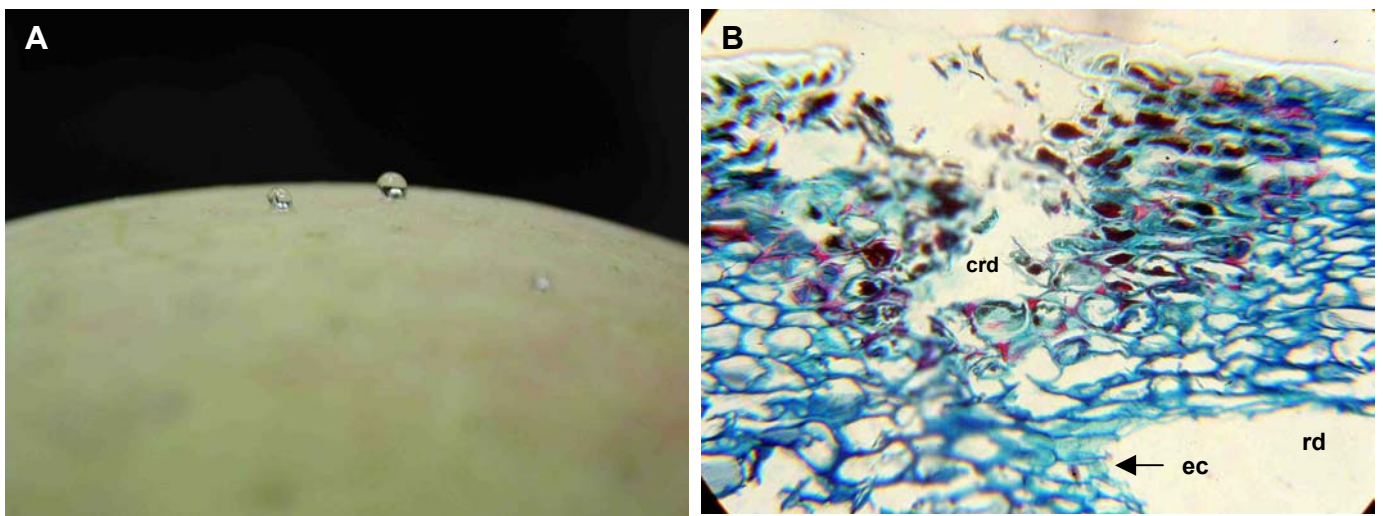


Figure 4.10 (A) 'Keitt' fruit where latex droplets had formed on the fruit surface prior to picking. (B) Collapsed resin duct (crd) adjacent to a discoloured lenticel. Surrounding cell walls were visibly discoloured (ec = epithelial cell; rd = resin duct).

4.4 DISCUSSION

In the packhouse, directly after the fruit is harvested, all the fruit is immersed in a cold-water bath situated at the start of the packline. Thereafter, the fruit is conveyed to a warm water bath of approximately 50 °C. In some commercial packhouses the same water will be used for the whole day, being topped up when necessary and replaced with fresh water the following day. The fruit will then be immersed in a fungicidal solution, waxed, dried, brushed and packed.

Based on the results obtained in this investigation regarding lenticel anatomy, as well as the composition and reaction of mango resin, it may be possible to explain lenticel discolouration according to the following scenarios:

4.4.1 Scenario 1

AS IN THE CASE OF 'TA' AND 'KEITT' FRUIT

During harvesting, resin inevitably contaminates parts of the fruit surface. The PPS fraction in the resin is soluble in water. After submersion of the fruit in the first water bath of the packline, the PPS fraction of the resin adhering to the fruit surface dissolves in the water, while the insoluble oil fraction accumulates on the water surface. It is now possible that the oil fraction may penetrate the skin of the fruit through the lenticels and consequently come in contact with the sublenticellular cells containing the phenolic pigments. Since 'TA' en 'Keitt' lenticels do not contain a protective phellem formed by the phellogen and the rupturing of lenticels, as a result of fruit growth (Fig. 4.2A), it is possible for the oil fraction to penetrate the cells unimpeded. As discussed in the introduction, it is possible

for the oil fraction to damage vacuole membranes containing the phenolic compounds. This will enable the PPO enzyme and phenolics to come into contact and react to form a brown oxidate, responsible for the discolouration of the cell walls (Fig. 4.3). As more fruit moves through the water bath, the concentration of oil in the water will increase and, consequently, one can expect that lenticel discolouration will increase. Supporting evidence for this hypothesis is the fact that when fruit is carefully harvested and not put through the packline, hardly any lenticel discolouration is observed.

It is known that the phenolics will be broken down as the fruit matures. This will leave less substrate available for the phenol oxidase process and, consequently, a reduction in the severity of lenticel discolouration (Gomes-Lim, 1997).

AS ON FRUIT OF 'KENT'

As indicated in the results, the resin in 'Kent' fruit has a smaller oil fraction while the lenticels contain a thick cuticle and suberized cells, preventing the lenticel floor from tearing open during fruit growth. This scenario is therefore less conducive to lenticel discolouration.

6.4.2 Scenario 2

AS ON FRUIT OF 'TA' AND 'KEITT'

The resin in the resin ducts is under high pressure, as demonstrated by the quick and violent spurt of resin from the pedicel during harvesting directly after the fruit has been picked (Fig. 4.9). When the resin ducts situated close to the surface of the fruit are being damaged in some way (e.g. rough handling of the fruit), the volatile

oil fraction will be forced through the weakened cell layers (situated between the resin duct and the lenticel) and move to the outside and lead to the discolouration of the cells as described above.

AS ON FRUIT OF 'KENT'

Despite of the equally high pressure evident in the resin ducts of 'Kent' fruit and the spontaneous eruption of the resin through the lenticels (Fig. 4.9), the resin contains less aggressive and a lower percentage of the oil fraction. The result is that browning of the lenticel tissue is isolated or even totally absent.

4.5 CONCLUSION

Lenticels are essential openings in the fruit epidermis, taking over the functions of respiration and transpiration when the stomata, due to fruit enlargement, are not functional any more. Unfortunately, it is possible for harmful pathogens to still penetrate the fruit through these openings. As a precautionary mechanism the lenticel cavity is lined with a cutin layer and/or a localized phellogen that forms underneath the lenticel cavity, giving rise to protective phellem cells. When the cuticle or phellem, lining the lenticel cavity (Fig. 4.7A and B), loses its functionality (as in the instance of 'TA' and 'Keitt'), it is necessary for another mechanism to take over, as is the case in the browning of lenticels due to the oxidation of phenolics. Browning of the lenticels in the plant is therefore a natural process to prevent, or at least limit, the chance of pathogens entering the fruit. Oxidized phenols in the cell walls (browned cells) have a higher anti-microbial potential than the non-oxidized phenols. When a wound is inflicted on the plant tissue, or an alien agent penetrates the tissue (as in the case of the oil fraction of the resin), the cell membranes are being damaged and the oxidation process will commence as described in the introduction.

This oxidation process causes the cell's wall to turn brown and the cells to die off, forming an effective protective layer (Saby *et al.*, 2003).

The best short-term strategy, in order to minimize the browning action, is to be aware of the factors promoting this process and to manage these factors effectively. In the long term, it will be necessary to find genetic material in breeding programs where the resin composition less damaging and the structure of the lenticels is such that the penetration of alien materials will be limited as effectively as possible.

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GENERAL DISCUSSION

Mango fruit lenticels originate from existing stomata on the fruit surface as also described by Dietz *et al.* (1988). The first guard cells are visible in the epidermis of ovaries shortly prior to anthesis, becoming functional shortly after anthesis.

Loss of stomatal integrity is due to increased cell division in the epidermis of 'TA' and 'Keitt' fruitlets 12 to 15 mm in size. The lenticels are pushed outwards, above the epidermis, looking like volcanic-like protuberances. Undulation of the epidermis is lost in fruit of about 20 mm, however, stomatal guard cells do not return to their original position, render it impossible for the guard cells to be still functional. 'Kent' stomata lose their integrity only in fruit of about 30 to 40 mm only due to stretching of the epidermis because of a rapid increase of fruit size. The lenticels can be regarded as atypical lenticels because of the absence of a periderm. In comparison with 'TA' and 'Keitt', lenticels of 'Kent' are considerably smaller and also contain a relative thick cuticle in the sublenticellular cavity, which is continuous with the cuticle of the epidermis. Suberized cells are also present in 'Kent' lenticels. Lenticels of 'Kent' fruit, therefore, can be regarded as well insulated. On the contrary, 'TA' and 'Keitt' lenticels consist of a rather large cavity, containing only a thin, inconspicuous cuticle as reported by Tamjinda *et al.* (1992). Loose dead cells are also common in 'TA' and 'Keitt' lenticels.

In 'Kent' fruit a second type of lenticel develop from a resin duct differentiating to close to the epidermis in fruitlets of about 6 mm. Rupturing of the epidermis occur above the resin duct, leaving a cup-like cavity. A phellogen, or rather a wound cambium, develops below this structure as fruit size increase. In a further developmental stage this structure will resemble a typical lenticel as observed in marula fruit. These lenticels also are well protected.

Resin, occurring in the resin ducts in the skin of the fruit, seem to play a major roll in the discolouration of the lenticels. As mentioned by O'Hare and Prasad

(1992) and Loveys *et al.* (1992), resin consist of two phases or fractions, namely a protein polysaccharide (PPS) fraction and an oil fraction. The ratio of the oil fraction to the PPS fraction varied between the three cultivars, with 'TA' the greatest (35%), 'Kent' the smallest (5%) and 'Keitt' (18%) an intermediate amount. When both fractions of the three cultivars were applied reciprocally to the skin of each cultivar after the resin had been separated, no effect could be observed in any trial concerning the PPS fraction. On the contrary, the degree of damage on the fruit surface caused by the oil fraction was different for the three cultivars. Oil fraction of 'TA' had the greatest effect, 'Keitt' was next and 'Kent' had almost no effect whatsoever. This results correlate with the normal manifestation of lenticel discolouration of the three different cultivars.

An explanation for spontaneous lenticel discolouration can be that the volatile terpenes present in the oil fraction volatilise, move out of the resin ducts through sublenticular cells, via the lenticels to the outside of the fruit. The moving of terpenes through sublenticular cells cause the loss of cell compartmentalization, letting plastid bound phenols to come into contact with polyphenol oxidase. The product of the resultant reaction taking place is a brownish quinone, accumulating in the cell walls and are visible as black markings on the fruit surface.

Lenticel discolouration may also occur due to maltreatment i.e. rough handling, to high temperatures in the warm water bath, extended period on brushes on packline, breaking of the cold chain and spilling of resin onto the surface of the fruit. This all may cause the sublenticular cells and even subepidermal cells to get damaged leading to the above mentioned reaction to take place.

Therefore, great care must be taken during the harvesting process for the fruit to be handled as careful as possible. All will depend on strict management practices to be applied during harvesting, packaging and the following handling of the fruit to minimize lenticel discolouration as much as possible.

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APPENDIX

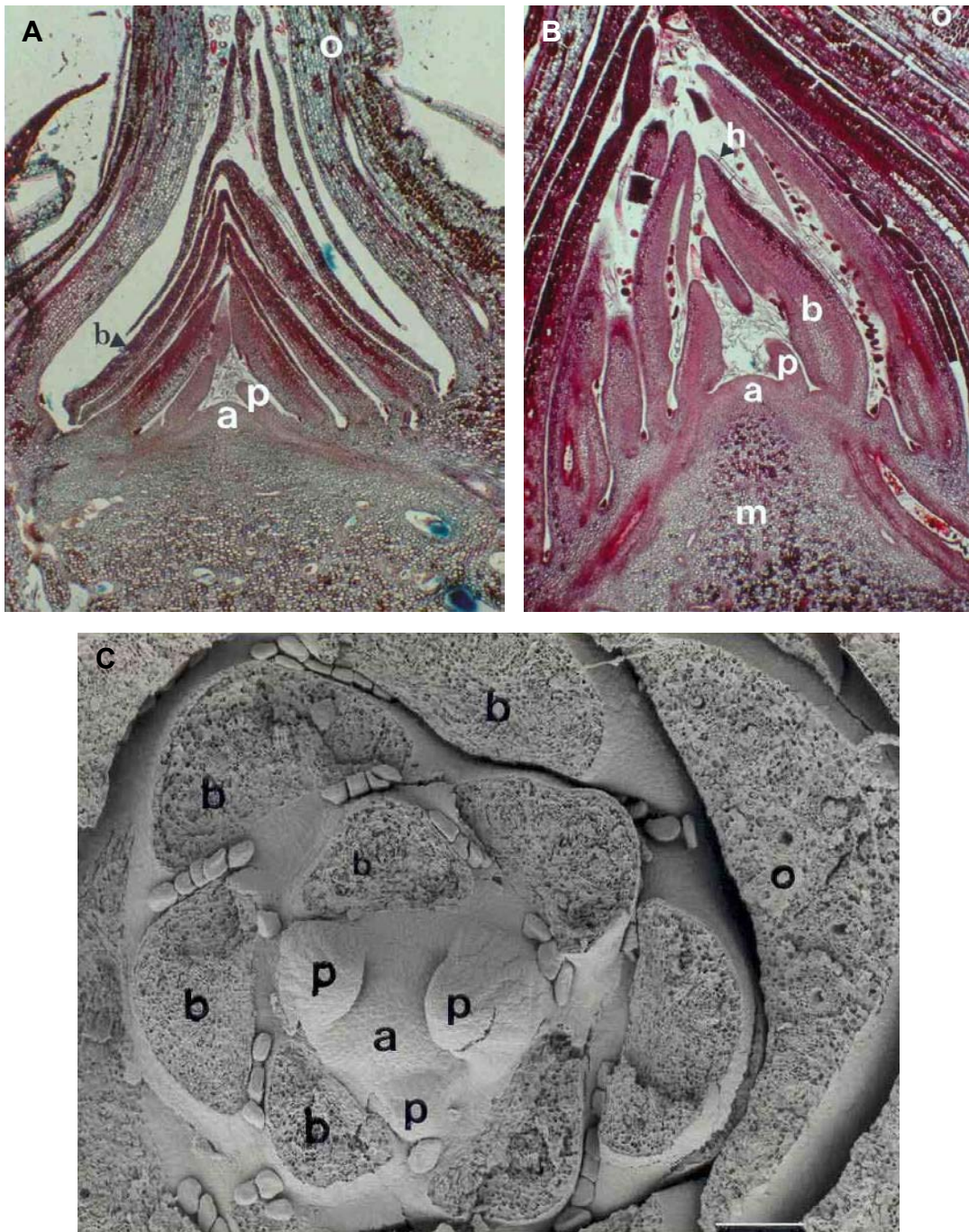


Figure 1 Light micrograph of (A) a “resting bud”. No meristematic activity in preformed leaf primordia axils (stage1). (B) an activated bud showing elongation of main axis (primary inflorescence axis) (late stage 1). (C) Scanning electron micrograph of apical buds with leaf (bract) primordia removed; same stage as A, showing resting bud with quiescent apical meristem (a), youngest leaf primordia (p) scars of outer bud scales (o) and inner bract scars (b). (Courtesy Robbertse, *et al.*, 2001)

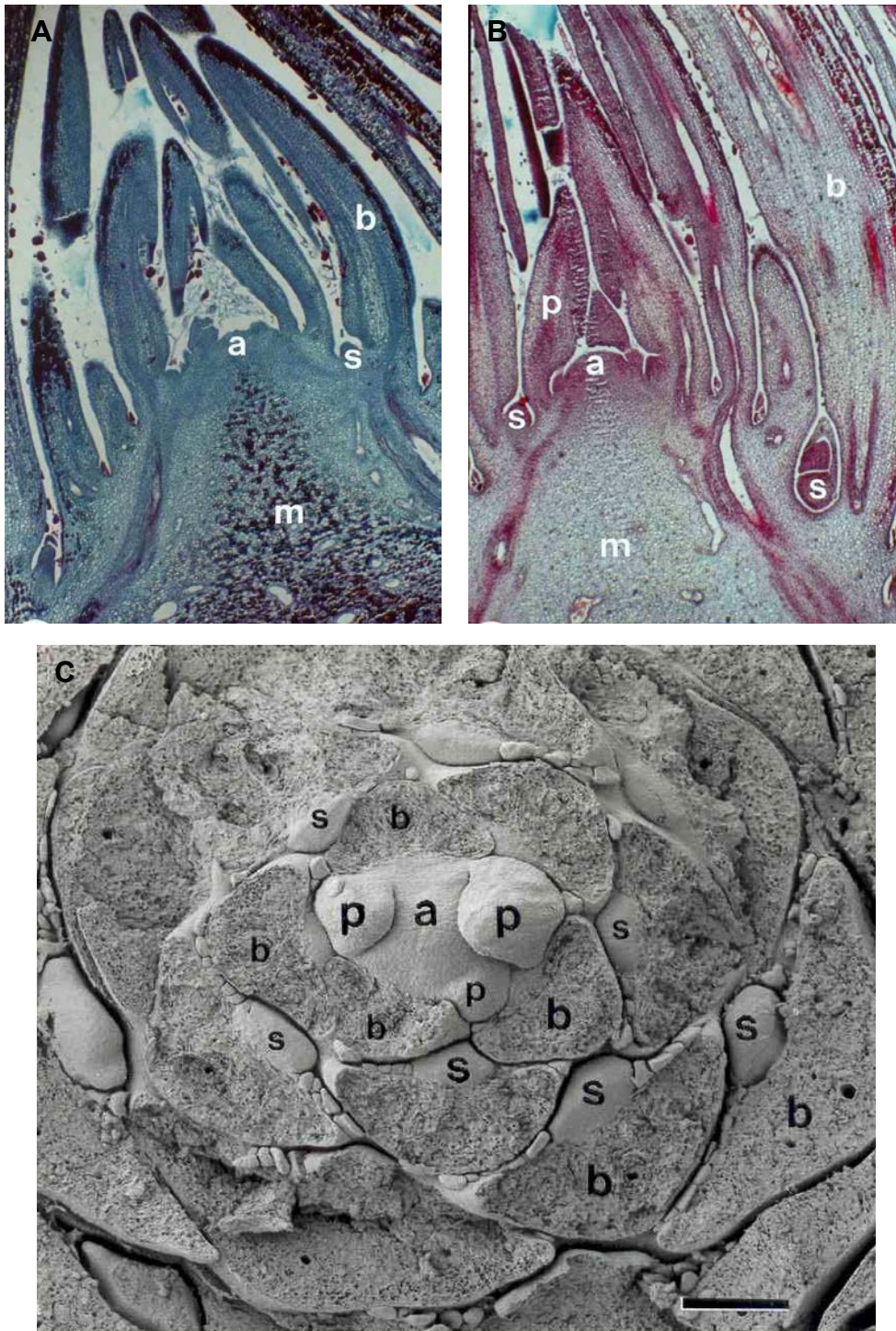


Figure 2 (A and B) Light micrograph of differentiating apical buds (stages 2 and 3) showing elongating primary inflorescence axis (m) and appearance of first lateral bud meristem (s) that will become secondary inflorescence axes apical meristems (s) producing new leaf (bract) primordia (p). (C) Scanning electron micrograph of activated bud induced to produce lateral inflorescence axis meristems (s) in the axils of the bracts (b). (Courtesy Robbertse, *et al.*, 2001)

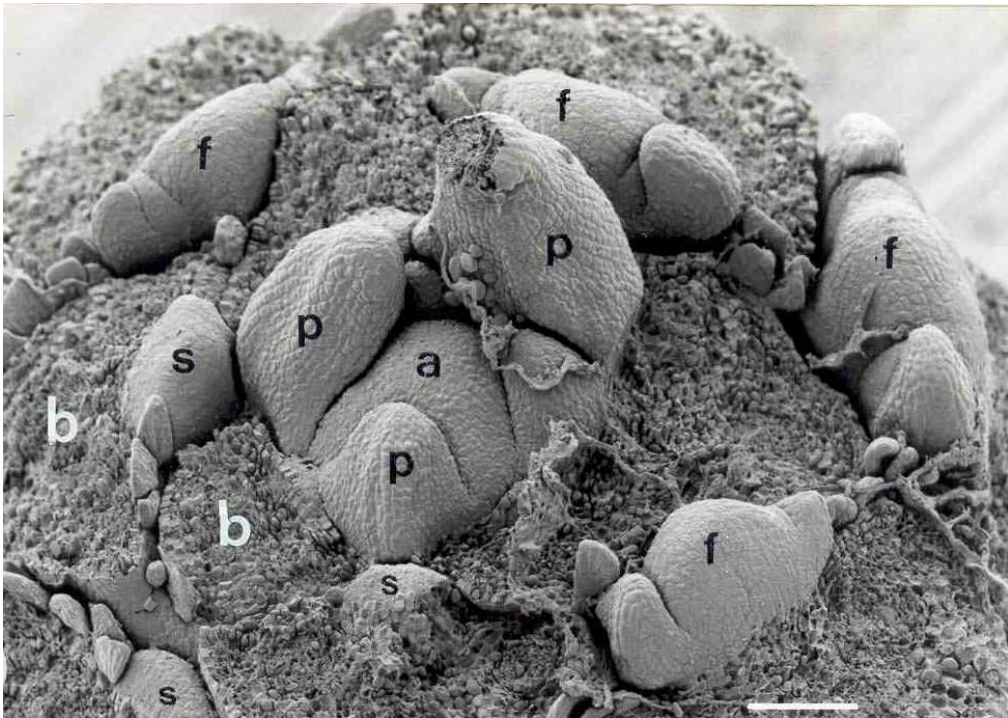


Figure 3 Scanning electron micrograph of stage 3 buds with preformed bracts removed to show secondary inflorescence axis buds (f). Note that the apical meristem (a) is producing new bract primordia (p) and secondary axis primordia (s). (Courtesy Robbertse, *et al.*, 2001)

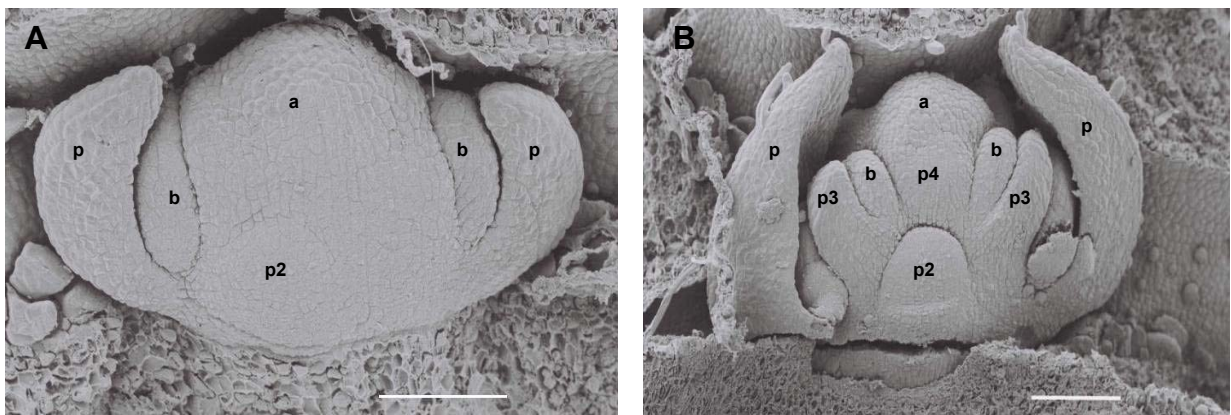


Figure 4 (A and B) Scanning electron micrographs of two stages of secondary axis differentiation. Note the apical meristem (a) producing opposite, decussate bracts (p-p4) each subtending a bud primordium (B) which are meristems producing tertiary inflorescence axis. (Courtesy Robbertse, *et al.*, 2001)

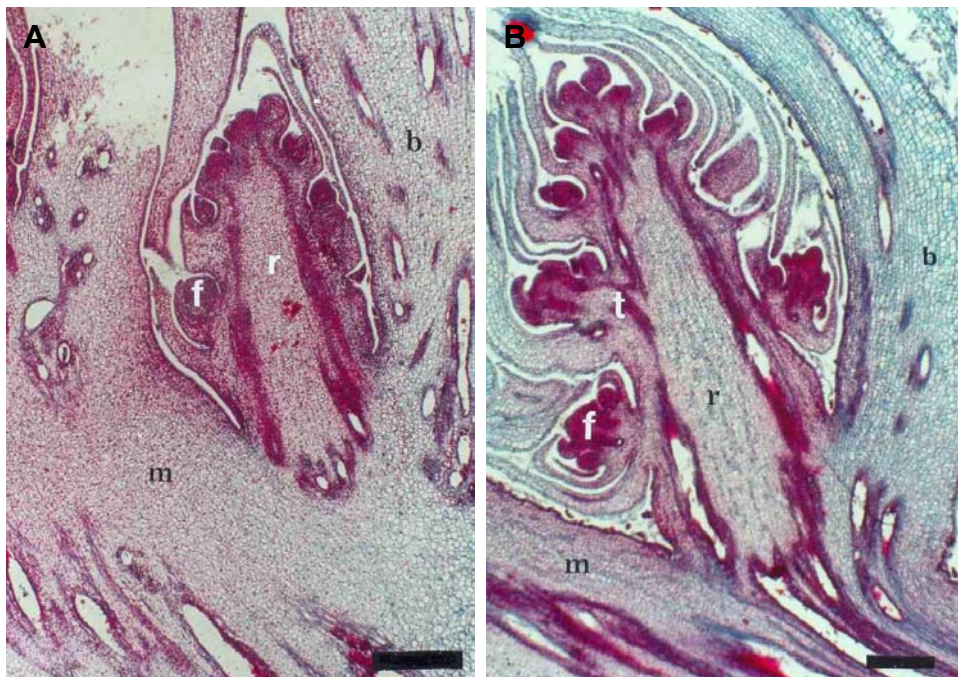


Figure 5 (A and B) Sections of two stages of lateral inflorescence axis differentiation; each forming a number of tertiary axes with terminal flower bud primordia (f). (Courtesy Robbertse, *et al.*, 2001.)

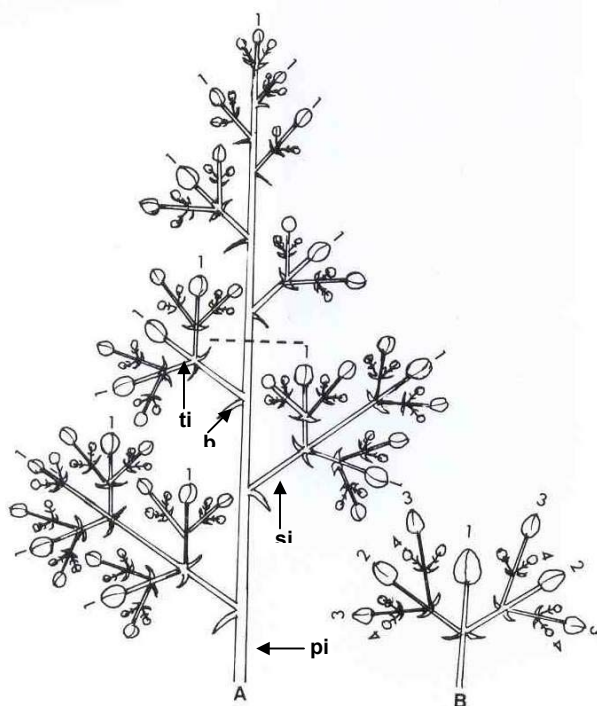


Figure 6 (A) Diagram of mango inflorescence. (B) shows one sympodial tertiary inflorescence branch. Numbers indicate the sequence of branching, ending in first, second third and fourth order branches. - Monopodial primary inflorescence axis (pi), secondary inflorescence axes (si), bract axil (b) and tertiary inflorescence axes (ti). (Courtesy Robbertse, *et al.*, 2001)