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 discolouration. 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 occuring 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.

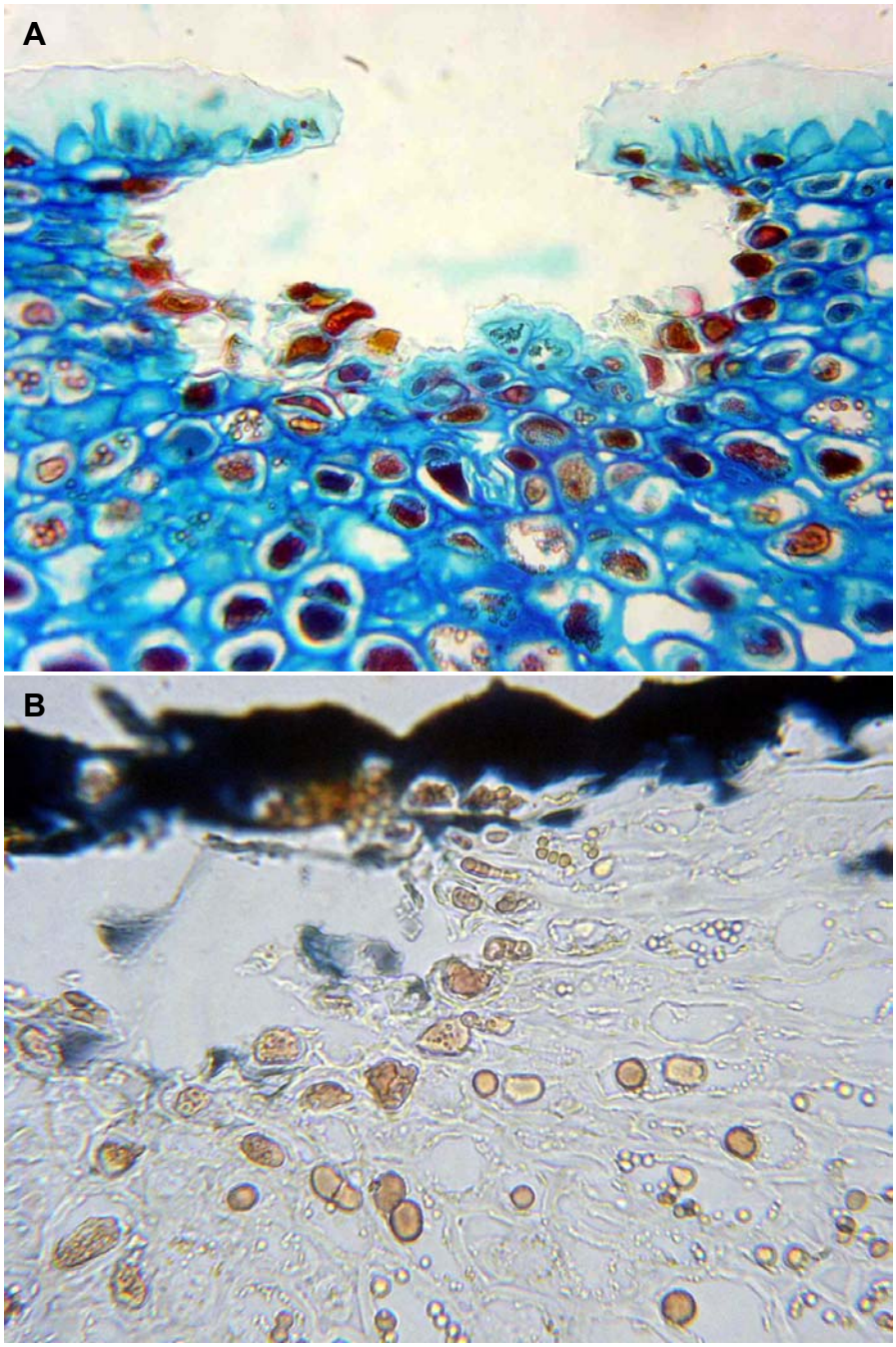

Figure 4.2 (A) Transverse sections of exocarp of mature 'TA' fruit showing lenticels subtended by cells containing pigments that stained red with Safranin A. (B) Staining of cuticle using Sudan Black and natural colour of pigments in vacuoles.

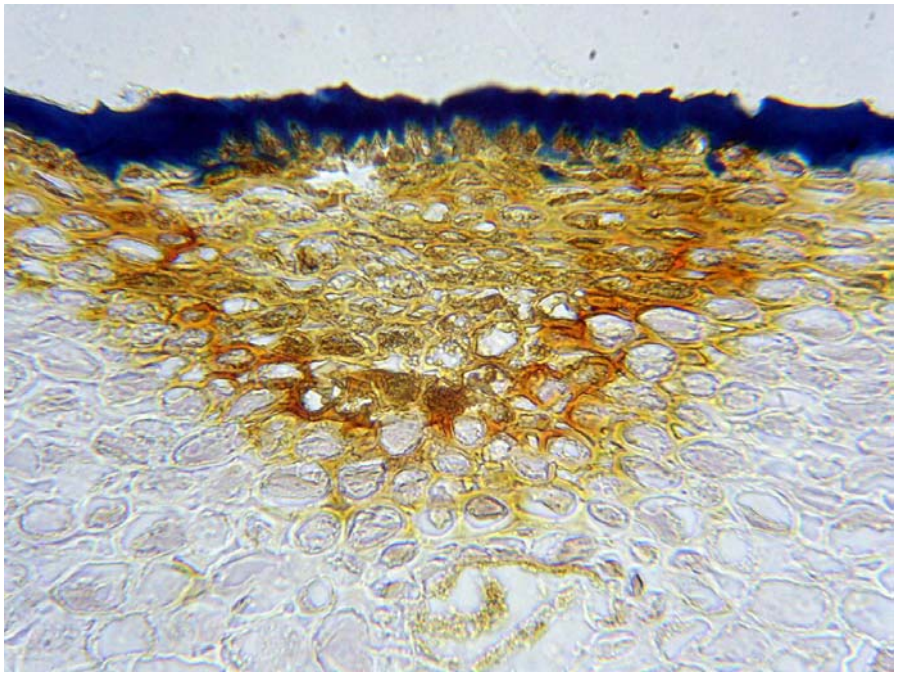

Figure 4.3 Transverse section of lenticel showing natural pigmentation in a discoloured lenticel's cell walls in the absence of chemical staining. The cuticle stained black with Sudan Black B.

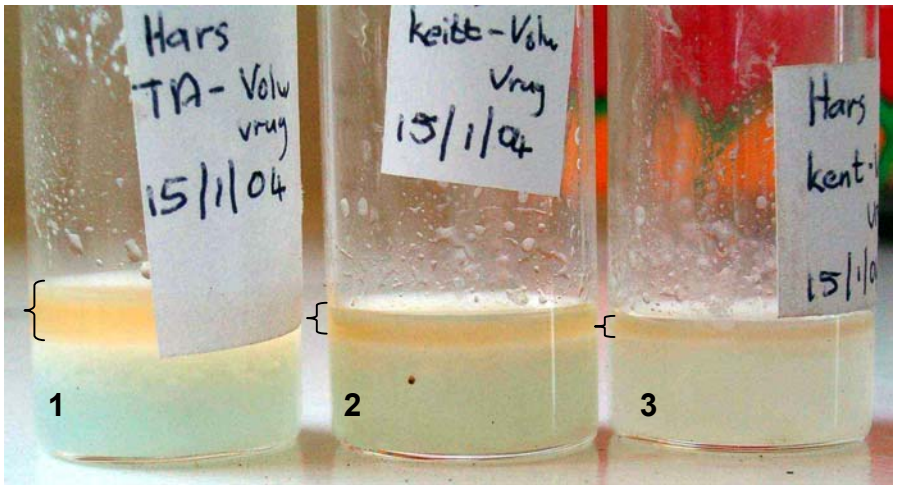

Figure 4.4 Collected resin from: 1 – 'TA', 2 – 'Keitt' and 3 – 'Kent'. The symbol { shows the oil fraction of the resin on top of the PPS fraction.

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 sublenticellular 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).

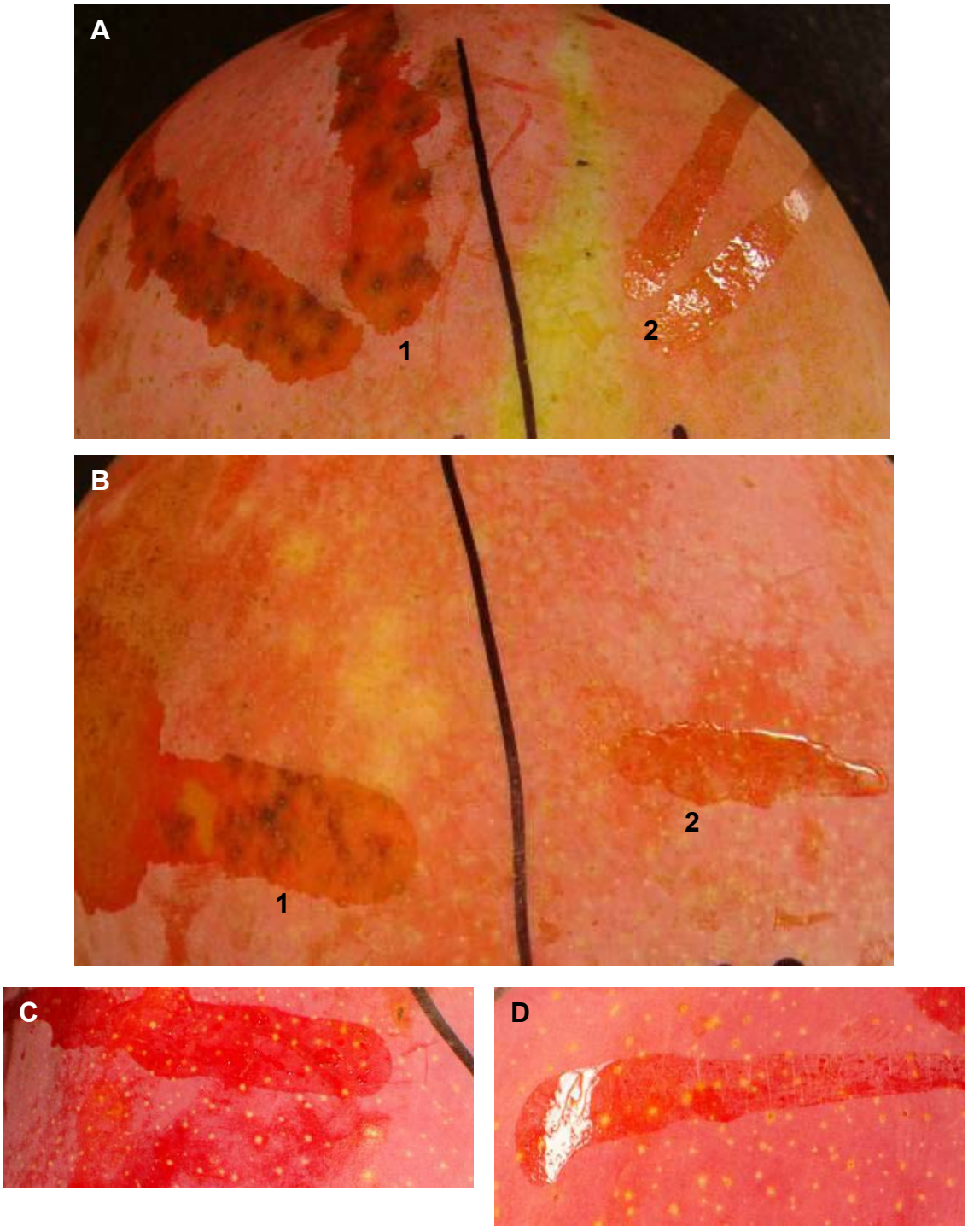

Figure 4.5 (A) 'Keitt' fruit showing the effect of the oil fraction (1) and the PPS fraction (2) of 'TA' resin. (B) 'Keitt' fruit showing the effect of the oil fraction (1) and PPS fraction (2) of 'Keitt' resin. 'TA' fruit treated with the oil fraction (C) and PPS fraction (D) of 'Kent' resin.

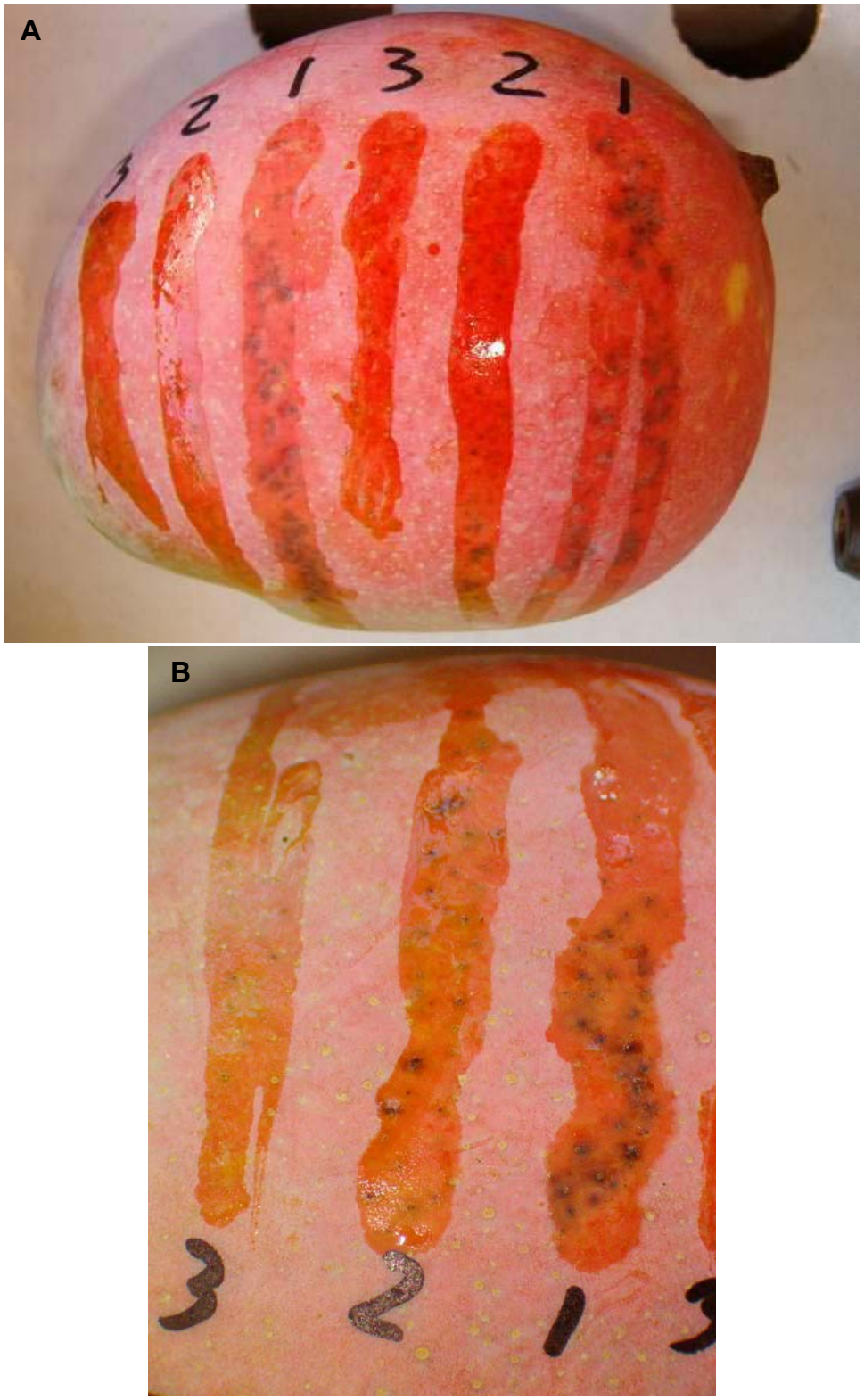

Figure 4.6 (A) Resin marks of the oil fraction of all three cultivars 'TA' (1), 'Keitt' (2) and Kent' (3) on a 'Keitt' fruit. (B) Close up a replication.

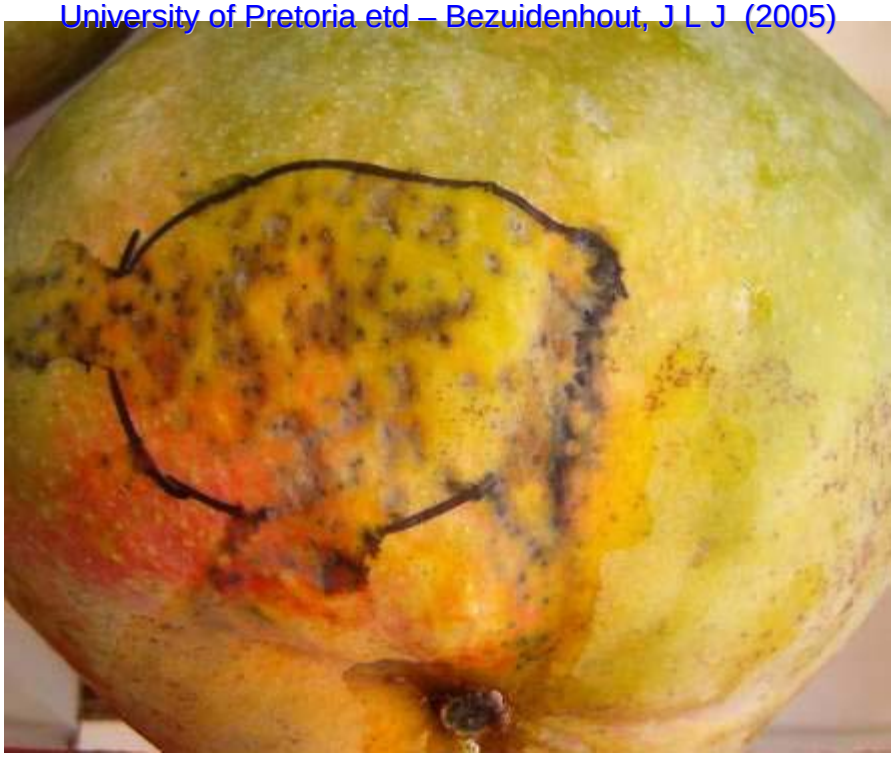

Figure. 4.7 Distinct damage on the surface of a 'Kent' fruit after the application of 'TA' resin.

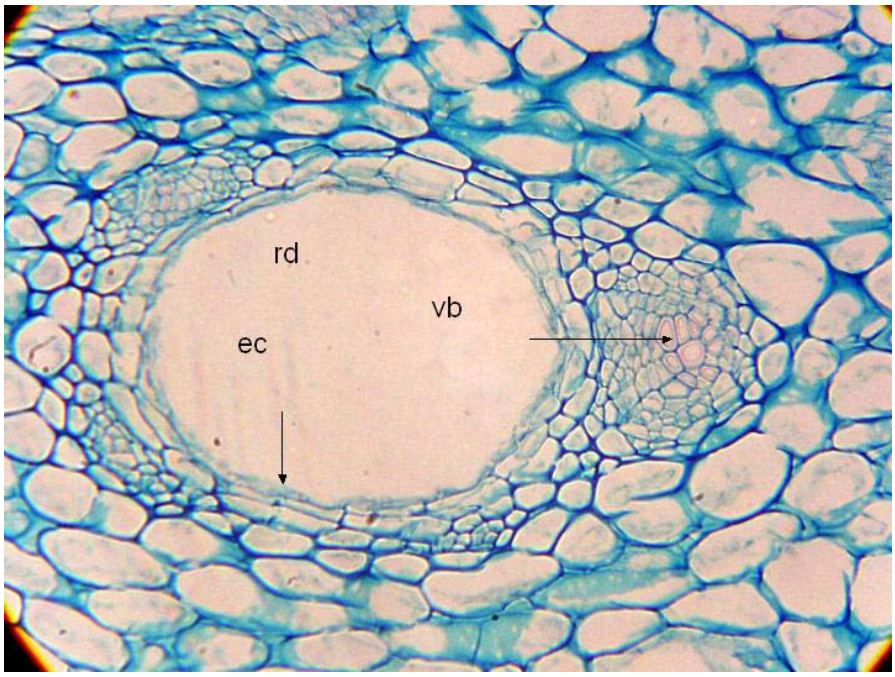

Figure 4.8 Transverse section through a resin duct (rd) in the wall of a mature 'TA' fruit. Resin duct lined epithelial cells (ec). Resin ducts are always are usually associated with adjacent vascular bundles (vb).

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.

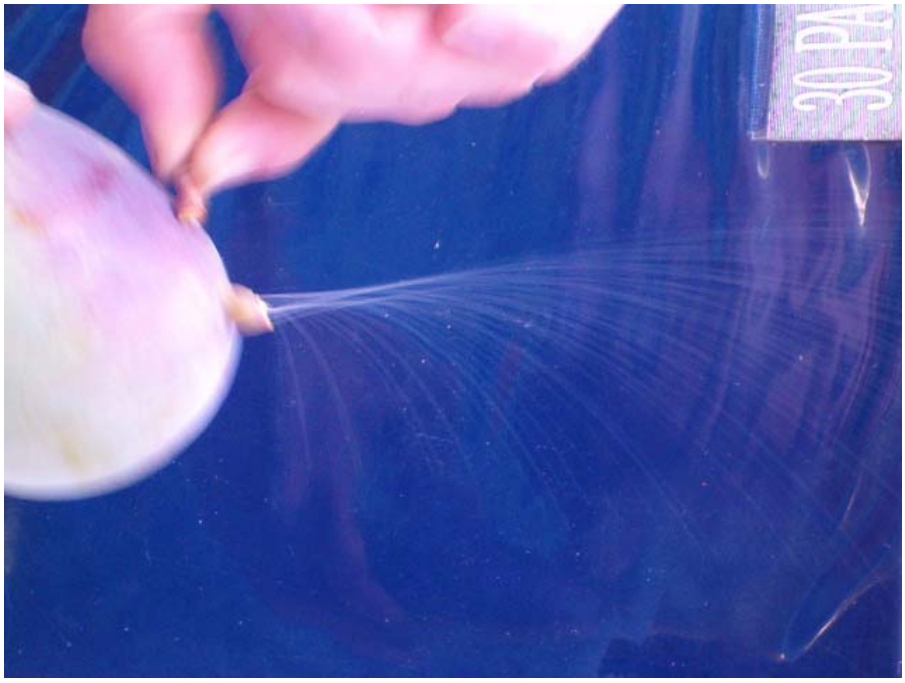

Figure 4.9 Resin squirting from the pedicel directly after removal of the inflorescence axis, indicating the pressure within the resin ducts.

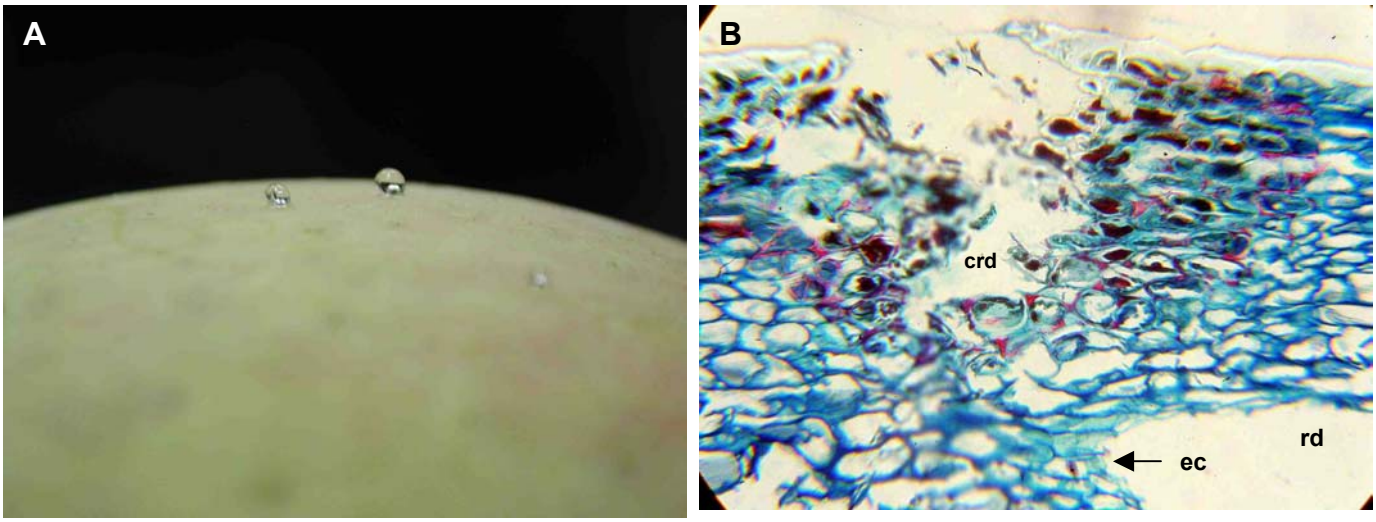

Figure 4.10 (A) 'Keitt' fruit where latex droplets had formed on the fruit surface prior to picking. (B) Collapsed resin duct (crd) adjacent to a discoloured lenticel. Surrounding cell walls were visibly discoloured (ec = epithelial cell; rd = resin duct).

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 ºC. 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), looses 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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