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Final Report

Reducing skin damage and improving postharvest efficiency of Calypso mango

Dr Peter Hofman
The Department of Agriculture, Fisheries and Forestry
Queensland

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Reducing skin damage and improving post-harvest efficiency of Calypso mango: Phase III

HAL Project Number: MG10008
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This is the final report for MG10008: “Reducing skin damage and improving post-harvest efficiency of Calypso mango: Phase III

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Contents

1. General introduction

'B74' mango (trading as Calypso^{TM}) was bred and developed at Childers, SE Queensland. It is the progeny of 'Sensation' and 'Kensington Pride' and carries the best attributes of both parents. The cultivar has reliable flowering and is highly productive, yielding more than 25 t.ha^{-1} from mature orchards. The fruit is highly coloured with a red blush overlaying a bright yellow skin when ripe. The flesh is fibre-free and firm with a distinctive mango flavour. The cultivar has good tolerance to flower and fruit diseases with extended retail shelf life. At least 80% of the crop reaches premium grade, conforming to the preferred colour and size required by Australian retailers. The cultivar has excellent export potential into Asian, Middle Eastern and European markets.

The owners of the cultivar realised that a whole of chain approach was required to maximise the genetic potential of the cultivar. This includes both the commercial aspects (the need for an extended market season, close teamwork between chain members etc) and the R&D support. Commercial production and distribution quickly identifies the genotypic weakness in a cultivar, which provides a focus for an integrated whole of chain R&D program over an extended period. Using this philosophy, the previous two 3-4 year research projects (FR02049 and MG06005) achieved considerable progress in understanding nutritional requirements, postharvest handling systems, maturity standards, ripening practices, and export practices of 'B74' mango across the main production environments, with a clear focus on profitability for the chain members (including growers) and delivering on customer and consumer preferences.

The projects and continued commercial experience identified new opportunities to further improve cultivar performance, which were the foci for the R&D program reported here. These included:

1. Improving external appearance for export markets requiring irradiation against fruit fly and other quarantine pests (seed weevil etc). The previous project and commercial experience confirmed that 'B74' fruit often develop extensive lenticel discolouration (LD) following irradiation, and even without irradiation LD can be a significant issue for the domestic market. Project MG06005 confirmed that exposure to water after harvest (e.g. in the desapping solutions used to improve harvest efficiency and prevent sapburn) significantly increases lenticel sensitivity, and overseas research and local commercial experience suggested excessive rain during latter fruit growth can have the same effect. Hence, this project studied the development of LD on 'B74' during growth and fruit ripening compared with other cultivars, and whether reducing irrigation or preventing contact with water before and after harvest can reduce LD.

   In addition, the discolouration is likely a browning reaction requiring oxygen, so research focussed on whether the reduction of oxygen around the fruit during irradiation and ripening, or the use of antioxidants, can reduce LD. A more controlled assessment of dose responses of 'B74' to irradiation was also undertaken.

2. In transit ripening. Current recommendations for Australian mangoes requiring more than 2-3 d transit time from farm to ripener is to cool on farm to 12-13°C within 24 h of harvest, and transport at this temperature. However, ripening in transit has several significant cost advantages. These include reducing on-farm precooling and in-market ripening room floor space, reducing the time from harvest to market allowing access to higher prices at the start of the season, and reducing energy requirements by not cooling the fruit as much on-farm, running the trucks at higher temperatures, and not requiring warm-up of fruit in market before ripening. Technical challenges and risks with this approach were researched in this project.

3. The harvest window. The short time between when the fruit are first mature and when they start dropping from the tree creates significant challenges with labour and

infrastructure. The potential to extend the harvest window was examined by looking at whether the maturity dates can be shifted by manipulating flowering time, and how long the fruit can hang on the tree.

4. Fruit movement in the field. Getting the field bins from the tree to the holding area on farm has traditionally been done with tractor-drawn bin runners carrying about four bins. On large farms this requires considerable tractor numbers. Alternative systems were investigated.

2. Technical summary

'B74' (marketed as Calypso™) mango (*Mangifera indica* L.) commercialisation in Australia has been done with a supply chain focus, with production being spread from tropical to sub-tropical latitudes to ensure consistent fruit supply over the mango season, and an R&D support program to maximise its genetic potential in each of these environments. This project is the third phase of the R&D program and focussed on the most promising opportunities for 'B74' chain development.

'B74' mango fruit have an attractive ripe skin colour but small spots from discoloured lenticels often appear on the skin, especially after harvest. This study found that 'B74' fruit have 3-4 times higher lenticel density than the other main mango cultivars, which is a characteristic inherited from the paternal parent ('Sensation'). This may explain why 'B74' is generally more susceptible to lenticel discolouration (LD) after harvest compared with 'Kensington Pride' and 'Honey Gold'. The discolouration appears to be due to accumulation of phenolics compounds in the cells around the cavity.

Trials indicated that LD varies significantly across locations and seasons. Fruit from the hotter farms had less LD at ripe, and LD in the ripe fruit was less in smaller and more mature fruit, fruit with more blush, and from trees that had more uniform flowering, and from trees with smaller canopy area. However, these parameters accounted for only 32-35%of the variance in LD. Also, more severe LD has been associated with rain before harvest. Withholding irrigation from Katherine-grown trees for 3-8 weeks before harvest showed little evidence of strong effects on tree and fruit water relations. The treatments had no effect on average tree yield or fruit size, and withholding irrigation for 3-4 weeks actually increased LD after irradiation. Other options were tested for reducing fruit contact with water during growth. Bagging the fruit with paper and spraying with a carnauba-based wax before harvest reduced LD in the ripe fruit but the wax treatment needs commercial testing.

The discolouration of the lenticels is likely due to an oxidation reaction. However, holding the fruit in plastic bags or using fruit coatings to reduce the oxygen concentration around the fruit during irradiation, or using antioxidant dips before ripening, did not reduce LD. The most promising approaches to reduce LD were eliminating water from the harvesting and packing procedures, and irradiating near ripe fruit. However, the impacts of these treatments on the whole chain needs to be considered. Higher ripening temperatures and longer treatment with ethylene did not overcome the delayed loss of green colour during ripening following irradiation.

Research to increase the harvest window focussed on flowering time and how long the fruit can hang on the tree. Ethephon® trials had potential but were suspended pending registration. Removing flowers in the Katherine environment to encourage later flowering resulted in significant crop loss. Late hanging trials in the major production districts suggested that the harvest window was about 10 days longer in the two farms in the NT, perhaps because the fruit attained the minimum maturity more quickly.

Several options for reducing machinery and labour requirements for transport of half ton bins with fruit from the field to the on-farm fruit store were tested, including in-field transfer points and flat bed trucks. The most promising alternatives are six bin runners and possibly larger harvest aids that can deposit the bin directly onto flat bed trucks.

The ability to ripen fruit in transit from farm to market will reduce energy costs and infrastructure requirements in market. Commercial tests indicated that the newer containers can retain 'B74' fruit temperatures at about 18ºC but issues such as loading warmer fruit, and poor loading practices that disrupt air circulation in the containers can affect temperature management. Carbon dioxide concentrations can be controlled with hydrated lime as long as fruit temperatures are controlled, and several systems for slow release ethylene in transit showed promise. These results justified further commercial development.

3. Media summary

'B74' mango (marketed as Calypso™) was bred at Childers in subtropical Queensland specifically to overcome the inconsistent production of the cultivar 'Kensington Pride'. Ongoing research is maximising its genetic potential in the main production regions.

'B74' mango fruit develops an attractive blush during growth and a full yellow skin colour when ripe, but small spots from damaged lenticels often appear on the skin. This affects the visual appeal but does not affect the flesh; nevertheless the value of the fruit is reduced.

The project confirmed that fruit can have less LD if they are grown in hotter production, are smaller and more mature, have more blush, and are from trees that had more uniform flowering or smaller canopy area. However, these parameters accounted for only 32-35% of the variation in LD. Not irrigating the trees for 3-8 weeks before harvest may "dry" the fruit out and make them more resistant to LD but this was not observed. 'B74' is more prone to this lenticel discolouration (LD) than most other Australian mango cultivars, most likely because it has 3-4 times higher lenticel density on the fruit surface at harvest.

LD is worse when the fruit are exposed to water either from rain or during harvesting. In order to reduce contact with water, the fruit were paper bagged (done commercially in apple and other fruit in Japan), or sprayed with a carnauba-based wax, two months or several days before harvest, respectively. Both treatments reduced LD in the ripe fruit. Bagging is labour-intensive but may be profitable for high value markets, and wax sprays need commercial testing with whole tree spraying.

LD is likely due to an oxidation reaction, similar to when cut apple turns brown. Reducing oxygen around or in the fruit, or using anti-oxidants may reduce the browning reaction. However, holding the fruit in plastic bags, fruit coatings and antioxidant dips before ripening had either no effect. The most promising approaches were eliminating water from the harvesting and packing procedures, and irradiating fruit for export when they were about three days from ripe. Both approaches add extra challenges to the harvest-to-consumer chain, but they may have application is certain circumstances.

Mango fruit need to be harvested within 2-4 weeks of reaching minimum maturity to prevent fruit from falling from the tree. This short harvest window results in challenges with picking teams and equipment. Trials attempted to induce earlier or later flowering the spread the harvest window in the hotter production areas. Trials with Ethephon® sprays indicated its potential to stimulate earlier flowering. Removing flowers in the Katherine environment to encourage the trees to re-flower was unsuccessful.

The ability to ripen fruit in transit from farm to market will reduce energy costs and infrastructure requirements in market. Commercial tests indicated that the newer 12 m rail containers can retain 'B74' fruit temperatures at about 18ºC, and several systems to manage carbon dioxide and ethylene concentrations (both important in fruit ripening) can be controlled.

4. Improving the external appearance of the fruit

4.1. Changes in lenticels during fruit growth

Minh Nguyen (PhD candidate), Peter Hofman, Daryl Joyce, Andrew Macnish, Madan Gupta

This chapter forms part of a current PhD program (Minh Nguyen). The results presented here have not yet been published. The chapter was drafted by the Ph.D. student, and reviewed by the project team.

4.1.1. Summary

Lenticels are small openings on the surface of some fruit, including mango. They are thought to originate from functional stomata on the young fruit that contribute to water and gas exchange. These stomata become non-functional as the fruit grows and develop into non-structured micro-pores in the mature fruit. These lenticels can become discoloured during late fruit growth and after harvest, resulting in lenticel discolouration (LD) that reduces value. More study is needed to fully understand how lenticels develop during fruit growth and ripening, especially for the main mango cultivars in Australia.

This study found that, unlike 'Kensington Pride' ('KP'), 'Honey Gold' ('HG') and 'R2E2' cultivars, the number of lenticels on 'B74' fruit continually increased until near fruit maturity, suggesting additional origins apart from the young fruit stomata. At harvest, 'B74' had 3-4 times more lenticels per fruit and lenticel density than 'Kensington Pride', 'Honey Gold' and 'R2E2', but less than 'Sensation'. Lenticel cavity size increased with expansion of the fruit surface, but a larger proportion of the lenticels in 'B74' had smaller cavities compared to 'KP' because of their later formation in fruit growth. There was a positive correlation between lenticel aperture diameter and total fruit surface area. Morphological examination indicated that phenolics accumulated in the cells around the lenticel cavity as LD developed. No cutin was seen covering the cells facing the lenticel cavity, and there was no obvious association between resin ducts and LD. Wax possibly plays an important role in protection of lenticels, but wax covering lenticels may shear and crack as lenticel cavities enlarge during fruit development, thereby reducing this protective effect.

4.1.2. Introduction

Lenticels are microscopic openings on the surface of some fruit. However, the lenticels can discolour in mature and ripe fruit in many mango cultivars, resulting in darkened areas around the aperture and reducing appearance and commercial value (Tamjinda *et al.*, 1992). Some research has been undertaken to understand the ontogeny and morphology of lenticels from early fruit growth until maturity. Most lenticels of 'Tommy Atkins' mango originate from existing stomata that become progressively non-functional and rupture when the fruit are about 20-30 mm diameter (Bezuidenhout *et al.*, 2005). Lenticels in 'Namdokmai' mango form under each stoma on the fruit skin (Tamjinda *et al.*, 1992). However, (Dietz *et al.*, 1988b) stated that lenticels may develop from stomata or cracks in the cuticle.

The nature and cause of lenticel discolouration (LD) is still not clear, including why LD effectively only develops in later maturity or as the fruit ripen. More study is needed to fully understand how lenticels originate and develop during fruit growth and ripening, especially for the main mango cultivars in Australia.

The expression of LD varies with mango cultivar. In studies by (du Plooy *et al.*, 2004) 'Keitt' mango exhibited the most LD and 'Kent' mango the least, with 'Tommy Atkins' being intermediate. 'Keitt' had large lenticel cavities and less or no cutin covering the cells near the bottom of the lenticel cavity, while 'Kent' had smaller cavities and generally continuous cutin

and wax layer lining the cavity. Cell wall thickness may also play a role in LD (Tamjinda *et al.*, 1992). For example, the walls of the cells lining the lenticel cavity in LD-sensitive 'Namdokmai' mango were thinner than those of the more LD resistant 'Falan' cultivar (Tamjinda *et al.*, 1992). (Bezuidenhout *et al.*, 2005) suggested that the absence of a cork cambium and cork cells in the cavity contributed to LD because of the inability to replace damaged cells. Also lenticel aperture, dimensions, density and distribution may influence LD severity.

Previous research indicated that dipping 'B74' fruit in deionised water for 2 min after harvest was sufficient to increase LD on the ripe fruit, and especially following irradiation (Hofman *et al.*, 2010a). Thus, evaluating the potential for water uptake into lenticels and the surrounding cells, for example immersing the fruit in a dye solution, may indicate the potential for LD. For example, studies showed that lenticels with micro-cracks in the wax layer of the lenticel cells increased the potential for LD (Curry and Kupferman, 2004). This effect may be via water increasing lenticel cell turgidity (Cronje, 2009a). The potential for water movement into a small cavity (e.g. the stomate or lenticel) is related to the wetting angle of the surface (Mexal *et al.*, 1975). An aqueous solution containing a surfactant can spread completely on the fruit surface and easily infiltrate into stomata or lenticel (Peschel *et al.*, 2003). The fruit cuticle layer can change during fruit growth (Bally, 1999) and likely varies with cultivar, so studying the fruit surface wetting angle may indicate differences in the potential for water entry into lenticels.

To provide a better understanding for lenticel development, the reasons for differing LD sensitivity among Australian mango cultivars, and potential measures to reduce LD in 'B74', a chronological morphology and comparative study of 'B74', 'Kensington Pride' 'Honey Gold', 'R2E2' and 'Sensation' was undertaken. Fruit were examined at one month after full bloom until harvest at intervals of 1-2 months.

4.1.3. Materials and methods

4.1.3.1. Fruits

During 2011/2012, fruit samples were collected from commercial 'B74' mango trees at Childers, south east Queensland (25°14'S, 152°37'E). Three other cultivars, 'KP', 'HG' and 'R2E2' were collected from a commercial farm in Bundaberg, south east Queensland (24°98'S, 152°09'E), as comparators. In 2012/2013, 'B74' and 'KP' fruit were collected from the Childers farm and 'HG' and 'R2E2' were collected at the green mature stage from the Bundaberg farm. In 2013-2014, 'B74', 'KP' and 'Sensation' fruit were collected from an orchard near Mareeba, north Queensland (17°10'S, 145°46'E).

4.1.3.2. Sampling

From one month after full flowering through to maturity, fruits were randomly harvested at intervals of approximately one month in the first two seasons, and at intervals of two months in the last season. Five mango trees of each cultivar were used. The fruit were sampled from approximately 1-1.5 m height from all four aspects on each tree. When fruits were immature, 30 fruit from the five trees were harvested at each collection date. For wetting angle assessment, fruits were placed upside down into a nail board in order to prevent surface damage. Fruits were then transferred by plane or car that day to the Maroochy Research Facility (MRF), Nambour, Queensland for assessment the following day.

4.1.3.3. Assessments

Fruit surface area

Fifteen fruit were used to estimate the fruit surface area from the polar (a cm) and equatorial (b cm) radii. The fruit surface area based on an elliptical shape was calculated using the following formula (Khanal *et al.*, 2011):

Fruit surface area (cm²) = $2\pi b[(b + a^2/(a^2 - b^2)^{1/2}) \times \arcsin((a^2-b^2)^{1/2}/a)]$

where π = 3.1415; a: polar radius (cm); b: equatorial radius (cm).

Dye uptake

Ten fruit were used to assess dye uptake characteristics using a temperature differential approach. The fruit and a food dye solution of "Brilliant Blue" (1 $g.L^{-1}$ in tap water) were equilibrated at 25°C and the solution and fruit core temperatures monitored. The fruits were submerged into the dye and placed for 16 h at 12°C. Upon removal, the fruits were rinsed three times in running tap water and then blotted with paper towel. The dye uptake was recorded using a spotty dye (diameter of dyed spot <1 mm) uptake rating scale with calibration images as following:

1 = No spotty dye uptake;
2 = Minor spotty dye uptake by lenticels (<10% of fruit surface area);
3 = Spotty dye uptake by lenticels on 11-30% of fruit surface area;
4 = Spotty dye uptake by lenticels on 31-50% of fruit surface area;
5 = Spotty dye uptake by lenticels on 51-70% of fruit surface area; and
6 = Spotty dye uptake by lenticels on > 70% of fruit surface area.

The proportion (%) of dyed lenticels was calculated as the percentage of dyed lenticels/total lenticels in four circles (2 cm diam.) on the fruit equator. Dyed and non-dyed lenticels were counted using a magnification glass (Maggylamp model M.L. Apppro. No. 2141; Newbound Balmain).

Wetting angle

Ten fruit were used to measure wetting angle with 5 µL DI water droplets (Lamour and Hamraoui, 2010). The contact angles were measured in the middle of the cheeks at eight points around the equator of each fruit. Fruits were firmly positioned, with the selected test point in the horizontal direction and the droplet carefully placed on the fruit. At equilibrium, the contact angle between the droplet and the fruit surface (θ_c in Plate 1) was photographed with a Canon DOS40D camera fitted with a Canon macro-lens EF-S 60 mm. The contact angles from the images were measured using Image-J software.

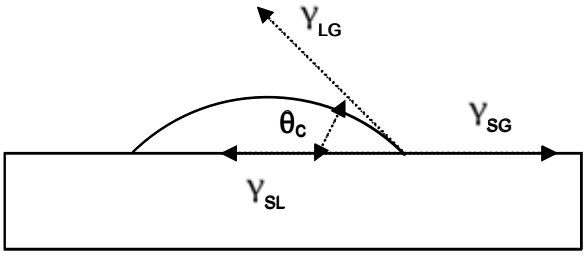


Plate 1 The wetting angle between a water droplet and the surface, with the wetting angle shown as θc

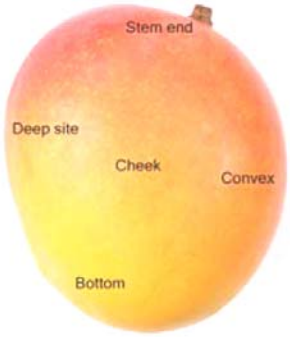


Plate 2 Locations on the fruit surface used to examine lenticel distribution around the fruit

Lenticel density and distribution

Five fruit were assessed for lenticel density and distribution on five parts of the mango fruit; top (proximal), bottom (distal), cheek and two equidistant around the equator of the fruit (Plate 2). For fruits at an early stage, lenticels were counted using the nail polish method. Each part of the fruits was coated with nail polish and allowed to dry for 30 min. The film was

peeled and placed on a slide. Lenticel counts were made at random in five fields of view per nail polish strip under the light complex microscope at x 400 magnification (Kakani *et al.*, 2003). For mature fruits, the lenticel number in each 3 cm^2 circle was counted using a magnifying glass, and then checked using a dissecting microscope (TYP 376788 Wild Heerbrugg, Switzerland). Image-J software was also used to check lenticel counts with a Canon DOS40D camera and a Canon macro-lens EF-S 60 mm. The lenticel density was determined as the total number of lenticels per unit surface area (Schotsmans *et al.*, 2004).

Diameter of lenticel openings on the fruit surface

The diameter of the lenticel opening was quantified by excising skin sections from five positions (cheek, stem end, bottom, convex and deepsite) using a razor blade. The sections were incubated for 2 min in 0.1% acridine orange which fluorescence stains cell walls, RNA and DNA, then blotted and transferred to the stage of a Olympus BH-2 epifluorescence microscope with a Olympus HBO 103 W/2 burner, and viewed at 100-200x magnification. Lenticel opening dimensions were measured using an eye piece micrometer which was calibrated using a haemocytometer (Kakani *et al.*, 2003; Khanal *et al.*, 2011).

Lenticel morphology

Five fruit were used for lenticel morphology. From each fruit, five 2 x 3 x 3 mm sub-samples were cut. Sections were either fresh by hand, or after serial fixation in FAA solution (95% ethyl alcohol, 50 mL: glacial acetic acid, 5 mL: 37% formaldehyde, 10 mL: distilled water, 35 mL), dehydration in an ethanol series (twice in 50%, then once in 70%, 90%, 95% and 100% twice; 2h in each step), infiltration (100% xylene with paraffin pieces added one by one until saturation), embedding in paraffin wax (Paraplast Plus, Sigma), and sectioning on a microtome (Interlaps) at 5-7 µm. The sections were stained with either “Toluidine Blue” which stains polyphenols blue-green or “Sudan IV” which stains lipids in the fruit cuticle red. Sections were examined under a light compound microscope (Nikon Eclipse TS100) and photographed using a Canon DOS40D camera. About 20 lenticels were examined per treatment to estimate typical treatment effects.

Lenticel discolouration assessment

Ten fruits from each of the five trees were used to evaluate lenticel discolouration at harvest and during ripening using the 0-5 score of (Hofman *et al.*, 2010a) as described in Table 1.

Table 1 Rating scales for lenticel discolouration on the skin of ‘B74’ mango

Rating	Lenticel discolouration*
0	Nil
1	Light spots on not more than 25% of the surface or dense pronounced spots on not more than 5% of the surface; not cracked
2	Light spots on not more than 50% of the surface or dense pronounced spots on not more than 10% of the surface; not cracked
3	Scattered pronounced spots on not more than 50% of the surface, or dense pronounced spots on not more than 25% of the surface; not cracked
4	Dense pronounced spots on not more than 50% of the surface
5	Dense pronounced spots on more than 50% of the surface

*The rating refers to the percentage of the overall area of skin affected by lenticel discolouration. Dense = spots no more than 5mm apart. Light = ‘pinprick’ size. Pronounced = more than half pinhead size, dark coloured.

4.1.3.4. Statistical analysis

Statistical analyses were performed by Genstat® 14 for Windows™ (VSN International Ltd., UK). Analysis of variance (ANOVA) used the ‘General Analysis of Variance’ model, with cultivar as ‘treatment’ structure. The protected least significant difference (LSD) procedure at

P=0.05 was used to test for differences between treatment means (LD, wetting angle and dye uptake).

4.1.4. Results

4.1.4.1. Stomatal/lenticel number

The total number of lenticels on ‘B74’ fruit increased rapidly from fruit set until about three months after full flowering in all three seasons, then either slowed or increased very little (Figure 1). In contrast, total lenticel numbers on ‘KP’, ‘HG’ and ‘R2E2’ were considerably less than ‘B74’, which increased from fruit set until two months after full flowering, and thereafter decreased slightly (2011/12) or changed little. Lenticel number in ‘Sensation’ increased continually to maturity and was almost twice the number as ‘B74’.

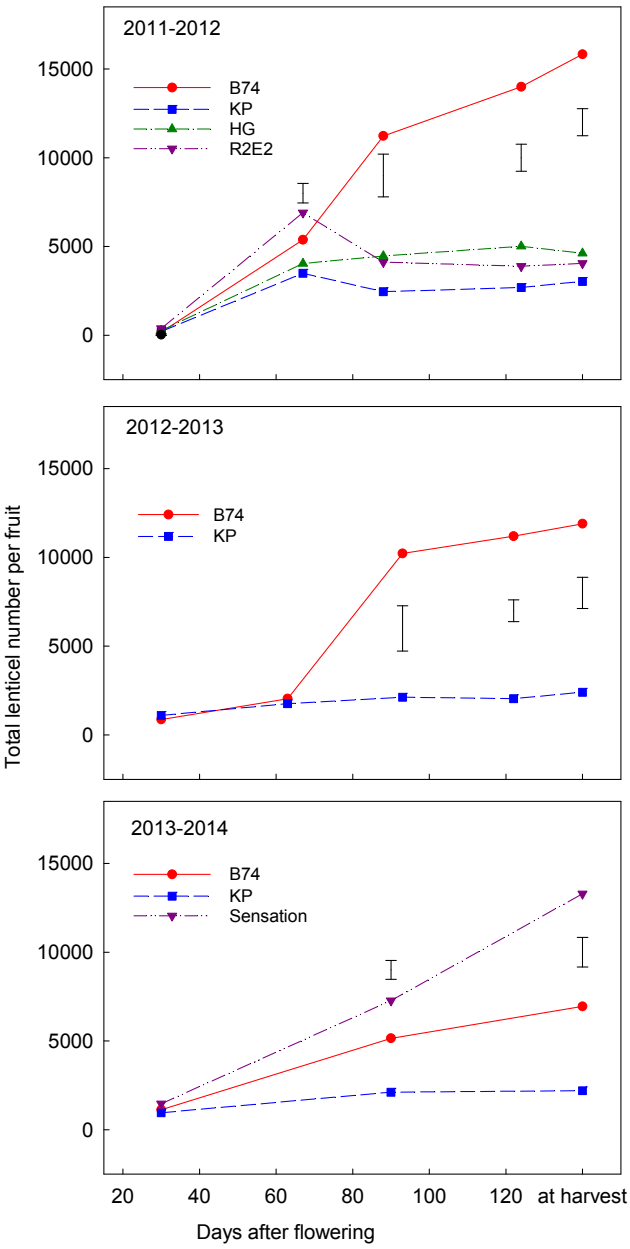


Figure 1 Total number of stomata/lenticel on the fruit surface of ‘B74’, ‘KP’, ‘HG’, ‘R2E2’ and ‘Sensation’ mango during the sequential development stages. LSD bar at each collecting time as tested by LSD (P=0.05).

4.1.4.2. Stomatal/lenticel density

Lenticel density of 'KP', 'HG' and 'R2E2' at harvest was similar, and varied from 5-15 lenticels per cm² (Figure 2). The lenticel density of 'B74' fruit was consistently higher than the above cultivars in all three years. In 2013/14, 'Sensation' had considerably higher lenticel density than 'B74' from the same farm.

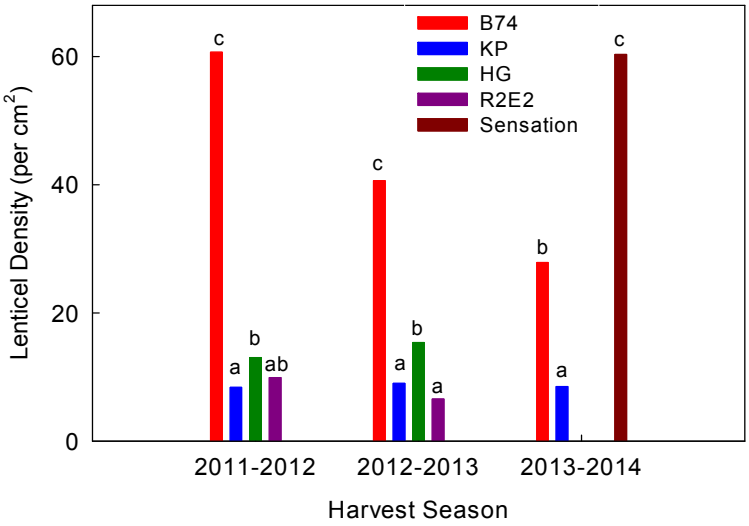


Figure 2 Lenticel density of 'B74', 'KP', 'HG', 'R2E2' and 'Sensation' mango at harvest. Means in year group with the same letters are not significantly different (P=0.05) as tested by LSD.

4.1.4.3. Lenticel aperture diameter and fruit surface area

Fruit surface expanded rapidly during development (Figure 3). Between 2-3 months after full flowering, the fruit surface area doubled for both 'B74' and 'KP' and continued to increase from three to four months. The lenticel aperture diameter increased slowly between two and three months and more rapidly between three and four months for both 'B74' and 'KP'.

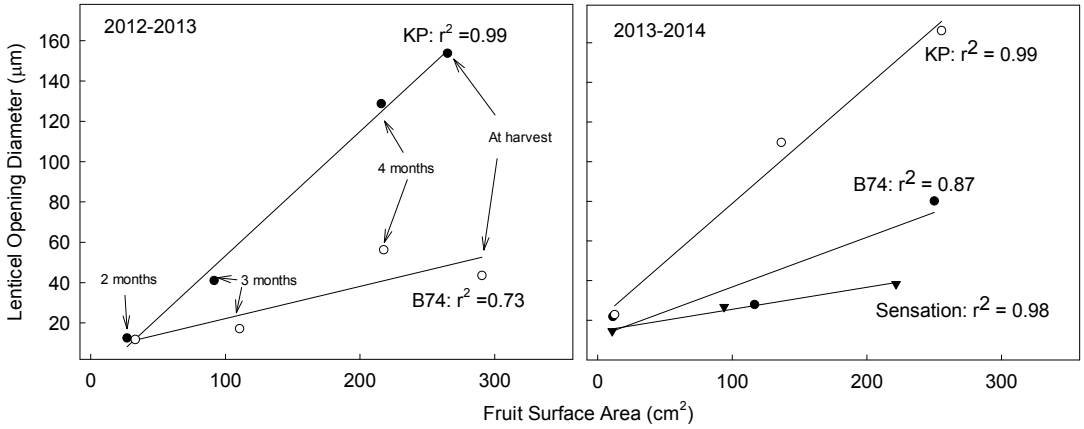


Figure 3 Relationship between lenticel opening diameter and fruit surface area during fruit development. The means are for the two, three and four months and at the green mature (left) and one and three months and at green mature (right).

There were strong positive correlations between fruit surface area and lenticel aperture diameter for 'KP', 'B74' and 'Sensation' (Figure 3). At green mature, 'KP' had the highest lenticel opening diameter while 'Sensation' had the lowest.

4.1.4.4. Lenticel distribution

There were some significant differences in lenticel distribution in the four cultivars (Table 2). In 'B74', the highest density of lenticels occurred in the convex area. In 'KP' and 'HG' fruit, there was a higher density at the bottom compared with the stem end. 'B74' again had considerably higher density that the other cultivars.

Table 2 The distribution of stomata/lenticels on the surface of mature green 'B74', 'KP', 'HG' and 'R2E2' mango fruit in 2011/12. The fruit locations are described in Plate 2.

	Lenticel distribution (number/ cm^2)			
	'B74'	KP	HG	R2E2
Stem end	54.2 [a]	6.8 [a]	9.4 [a]	6.6 [a]
Cheek	60.5 [a]	8.4 [a]	13.9 [ab]	8.5 [a]
Convex	74.7 [b]	7.9 [a]	13.9 [ab]	8.2 [a]
Deep side	61.5 [a]	8.3 [a]	13.2 [ab]	15.9 [c]
Bottom	66.9 [ab]	12.7 [b]	17.9 [b]	12.8 [b]
P value 5%	0.037	0.049	0.028	<.001

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD

4.1.4.5. Wetting angle

During fruit development, the average wetting angle varied from 120° to 151° (Table 3). Large wetting angles indicate that the fruit skin had very poor wetting capacity. The wetting angle of the skin of 'B74' and 'KP' increased during fruit growth, while in 'HG' the wetting angle decreased and did not change for 'R2E2'.

Table 3 Wetting angle of the skin of developing 'B74', 'KP', 'HG', and 'R2E2' mango fruit during fruit growth

Days after full flowering	Wetting angle (º)			
	'B74'	'Kensington Pride'	'Honey Gold'	''R2E2''
2 months	129.2 [a]	135.1 [a]	132.6 [b]	130.2
3 months	133.6 [b]	134.8 [a]	133.8 [b]	151.5
4 months	136.7 [bc]	136.9 [ab]	130.5 [b]	139.7
Green mature	137.4 [c]	139.2 [b]	124.8 [a]	134.5
P value	<.001	0.008	<.001	0.09

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD

4.1.4.6. Dye uptake

There was limited food dye uptake by fruit harvested 1-2 months after full bloom (Figure 4) but dye uptake into fruit increased at the third month post-flowering. 'B74' mango had the highest density of dyed lenticels, while the other three cultivars had approx. three times lower density (Table 4). However, in both years 'B74' had the lowest proportion of dyed lenticels. These findings are in line with our previous observations of LD in 'B74'.

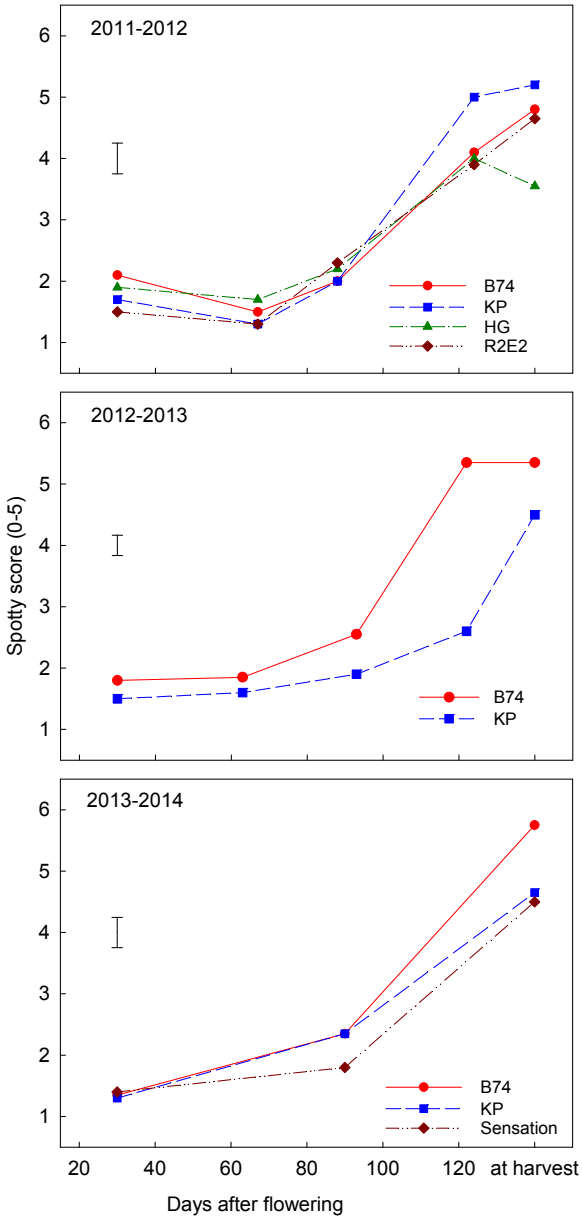


Figure 4 Dye uptake capacity, as evidenced by spotty dyed lenticels, of 'B74', KP, HG, R2E2 and Sensation in 3 consecutive seasons.

Table 4 The total number of lenticels, dyed lenticels and the proportion of dyed lenticels per area on mature green 'B74', 'KP', 'HG' and 'R2E2' mango fruit

Cultivar	2011/12		2012/13	
	Dyed lenticels /cm²	Proportion of dyed lenticels (%)	Dyed lenticels /cm²	Proportion of dyed lenticels (%)
'B74'	19.6 [b]	49.5 [a]	18.3 [b]	53.0 [a]
'KP'	5.4 [a]	90.2 [c]	6.2 [a]	56.9 [ab]
'HG'	6.1 [a]	66.5 [b]	6.7 [a]	63.8 [b]
'R2E2'	5.6 [a]	77.0 [b]	7.8 [a]	94.8 [c]

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD

4.1.4.7. Lenticel discolouration

'R2E2' had the highest LD in two of the three years. 'B74' LD was higher than 'KP' and 'HG' in 2012/13 (Table 5). 'Sensation' had the lowest LD in 2013/14.

Table 5 Lenticel discolouration (0-5) for 'B74', 'KP', 'HG' and 'R2E2' mango fruit at harvest and at full yellow skin colour (ripe). The fruit were harvested without exposure to water.

Cultivar	Lenticel discolouration (0-5)	
	At harvest	At full yellow
2011/12		
'B74'	0.4 [a]	0.3 [a]
KP	0.6 [a]	1 [b]
HG	1 [b]	1.1 [b]
'R2E2'	2 [c]	2.4 [c]
2012/13		
'B74'	1.3 [c]	2.5 [c]
KP	0.7 [b]	1.8 [b]
HG	0.2 [a]	0.4 [a]
'R2E2'	2.2 [d]	3.4 [d]
2013/14		
'B74'	0.8 [b]	1.2 [b]
KP	0.7 [b]	1.1 [b]
'Sensation'	0.1 [a]	0.3 [a]

For each year, means in columns with the same letters are not significantly different (P=0.05) as tested by LSD

4.1.4.8. Lenticel morphology

Using replica techniques (Plate 3A), stomata were evident on the fruit surface of all four cultivars at one month after flowering. At this stage there were no obvious cultivar differences. At three months, new crystalline waxy platelets covered the cuticle and the lenticel aperture (Plate 3B). The dissecting and fluorescence microscopy micrographs show the lenticel aperture (Plate 3C,D). When using 0.1% arcidine orange, the green halo of the lenticel was observed because of the stained cell walls and cell organelles.

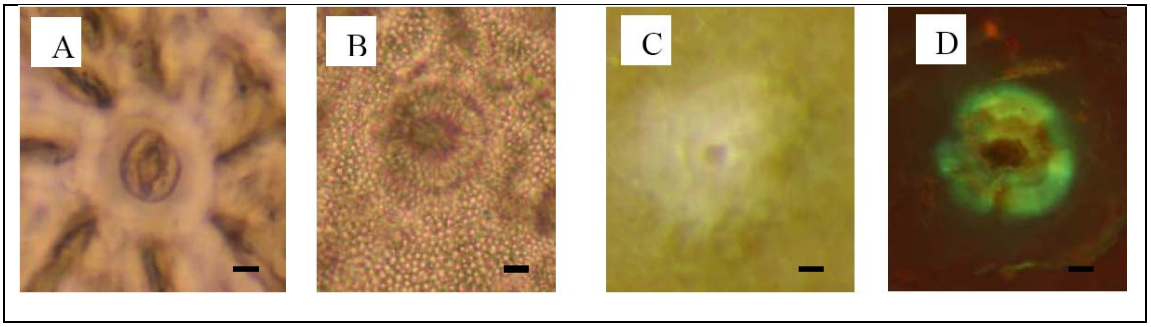


Plate 3 External features of typical stomata/lenticels (A, B: lenticel image using the replica technique observed under light microscopy; C: lenticel image using dissecting light microscopy; and D: lenticel image of green mature fruit using fluorescence microscopy. Scale bar:10 µm.

Lenticels are evident on the 'B74' fruit surface three months after flowering (Plate 4). There was no cuticle observed on the cells lining the lenticel cavity, and no cambium layer was present. There were no links observed between resin canals and LD. Cells inside lenticels of

‘B74’ fruit hanging on tree started browning. No cracking of the cuticle was observed green on mature fruit with fluorescence microscopy.

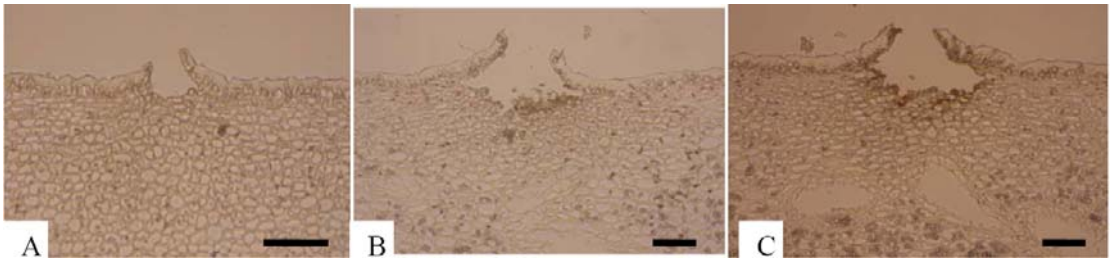


Plate 4 Unstained transverse sections of lenticels of ‘B74’ mango at three months (A), four months (B) and at the green mature stage (C). Scale bar: 50 µm.

When stained with “Toluidine Blue”, the cell walls around the undamaged lenticels of immature fruit were purple, while the cell walls around the discoloured lenticel of green mature fruit were blue (Plate 5). The blue colour indicated high phenolics concentrations in these cells.

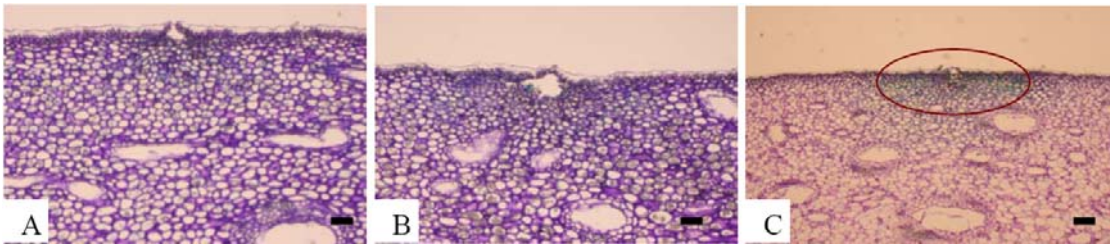


Plate 5 Stained transverse sections of lenticels of ‘B74’ at three months (A), four months (B) and at the green mature stage (C). Sections were stained with “Toluidine Blue” dye. The blue colour inside the circle suggests high phenolics concentrations around discoloured lenticels. Scale bar: 50 µm.

There were possible differences in lenticel morphology among the four mango cultivars (‘B74’, ‘KP’, ‘HG’ and ‘R2E2’) at maturity (Plate 6). Lenticels often showed blue coloured walls of the cells lining the lenticel cavity when stained with Toluidine Blue, which suggested high phenolics concentrations. More lenticel morphology examination will be done to understand the lenticel structure of the ‘B74’, KP, HG, ‘R2E2’ and ‘Sensation’ mango cultivars.

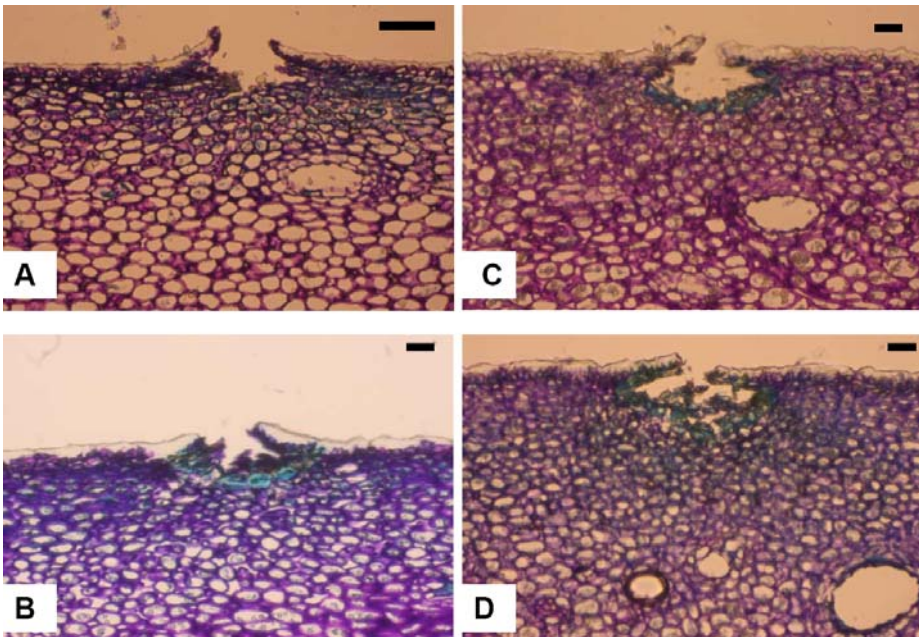


Plate 6 Transverse sections of lenticels of 'B74' (A), KP (B), HG (C), R2E2 (D) stained with "Toluidine Blue" dye. Scale bar: 50 µm.

'B74' fruit exhibit four typical lenticel "types" (Plate 7). Non-discoloured 'B74' lenticels show no abnormal pigments in the lenticel cells, while increasing intensity and extent of pigmentation is obvious in more discoloured lenticels.

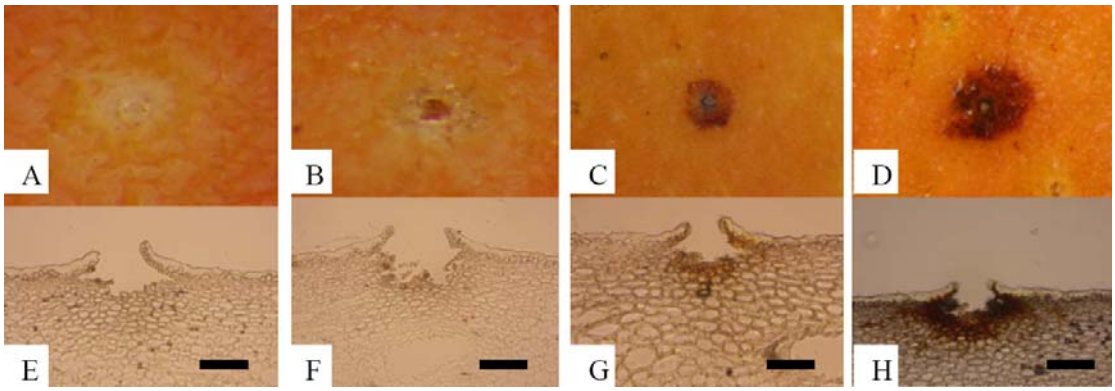


Plate 7 External features and respective transverse sections of four typical types of lenticels on 'B74' fruit at full yellow colour stage. A, E: undamaged lenticel; slightly damaged lenticel: B, F; small dark spot (C, G); and severe damaged lenticels: D, H. Scale bar: 100 µm.

4.1.5. Discussion

This study may have identified the distinct 'B74' characteristics that explain its commercially significant sensitivity to LD after harvest. Unlike 'KP', 'HG' and 'R2E2', 'B74' lenticel density continued to increase to maturity in most years, resulting in considerably higher lenticel density at harvest. Lenticels in mango are generally thought to form from stomata in the very young fruit that become dysfunctional as the fruit expands and matures. For example (Bezuidenhout *et al.*, 2005) stated that the stomata of 'Tommy Atkins' mango became ruptured when mango fruits reached 20-30 mm in diameter, and the lenticels on three month old fruitlets of 'Namdokmai' mango comprised of a small hole with deformed and degenerated cells around the hole (Tamjinda *et al.*, 1992). It is unlikely that stomata continue

to form after about two months since no functional stomata were observed on the fruit after this stage. Hence the lenticels formed after this period likely form from cracks in the cuticle as the fruit expands from about two months on. This agrees with the suggestions of Dietz *et al.* (1988b). However, in 'B74' fruit collected in 2011/12 and 2012/13, there was no cracking of the cuticle when examined under fluorescence microscopy, but this may also suggest that cuticle cracks progressed very quickly into lenticels.

The results also indicated that 'B74' lenticel aperture diameter was less than 'KP' from an early fruit age. The increase in surface area with time appeared similar, so it is possible that a significant proportion of the lenticels formed from cracks that appeared during the rapid fruit expansion phase, with less fruit expansion from then to maturity to allow large lenticel apertures.

At the green mature stage, 'B74' had three to four times the number and density of lenticels than 'KP', 'HG' and 'R2E2' fruit but much lower than 'Sensation'. Difference in total number of lenticels in five mango cultivars including 'Mallika', 'Alphonso', 'Dashehari', 'Pairi' and 'Totapuri' have also been observed, and water loss increased with the increase of lenticel number on the fruit surface (Dietz *et al.*, 1988a). 'B74' is a hybrid cultivar between 'KP' and 'Sensation'. This study indicated that the recorded characteristics of 'B74' lenticels, such as lenticel density during fruit growth and at maturity and aperture diameter, were intermediate between the two parents. There was no evidence of higher stomatal density in these cultivars at one month after flowering, so it is likely the higher density resulted from more cracking of the cuticle during rapid fruit expansion, possibly because of a weaker or thinner cuticle.

Lenticels with a cambium layer can regenerate new cells as the fruit expands thus retaining structure and organisation, and resistance to damage (Bezuidenhout *et al.*, 2005), and mango cultivars with a smaller lenticel cavity and cuticle lining the cells surrounding the cavity may also increase LD resistance (du Plooy *et al.*, 2004). These differences were not observed in the present study. The main observed cultivar distinction was 2-3x greater lenticel density in 'B74', which may explain its generally greater sensitivity to develop commercially significant LD, especially after irradiation and in ripe fruit (see section 5.2). The smaller aperture of 'B74' lenticels may explain why this cultivar is often less susceptible to the larger, sometimes cracking lenticel damage that occurs while the fruit are still on the tree.

In conclusion, this study identified important differences between the main Australian mango cultivars that may explain varying sensitivity to LD both before and after harvest. Further investigation could look at production practices to minimise cracking of the cuticle during fruit growth, thereby reducing lenticel density on 'B74' at harvest.

4.2. Lenticel changes during harvesting and ripening

Minh Nguyen (PhD candidate), Peter Hofman, Daryl Joyce, Andrew Macnish, Madan Gupta

This chapter forms part of a current PhD program (Minh Nguyen). The results presented here have not yet been published. The chapter was drafted by the Ph.D. student, and reviewed by the project team.

4.2.1. Summary

Lenticels are microscopic cavities on the surface of mango fruit. The cells around the lenticels can become discoloured before and after harvest, reducing external appearance and value many mango cultivars including 'B74' mango. Several postharvest practices such as exposure to water and chemicals, and irradiation, often increase the risk of significant lenticel discolouration (LD), especially when the fruit reaches the ripe stage. This study showed that lenticel number and density did not change during the ripening process, but the percentage of discoloured lenticels increased depending on the postharvest practices. Postharvest handling decreased the wetting angle of fruit skin, which possibly facilitates water entry into lenticel cavities. There was often an accumulation of brown pigments in the cells surrounding the lenticel cavity during ripening.

4.2.2. Introduction

In mango fruit, lenticels originate from stomata on the young fruit that become dysfunctional as the fruit expands, resulting in microscopic cavities with little organised structure and function. Lenticels can likely also form from cracks in the cuticle that expand as the fruit grows, which explains the continually increasing number of lenticels on the 'B74' fruit, and one of its parents ('Sensation') during most of fruit growth and maturation (see section 4.1).Several postharvest practices, such as exposure to water and chemicals and irradiation, cause discolouration of the lenticel cells, and especially as the fruit reaches the ripe stage (Cronje, 2009a; Hofman *et al.*, 2010c). Lenticel number and density may play a significant role in LD severity, and the significantly higher lenticel density in 'B74' compared with 'Kensington Pride' ('KP', 'Honey Gold' ('HG') and 'R2E2' may partly explain the relatively greater 'B74' sensitivity to LD. Also there is still no indication as to why some lenticels show discolouration while others on the same fruit do not.

The surface of mango fruit is covered by a cuticle which consists of a cutin layer and epicuticular wax (Hess and Foy, 2000), which may play an important role in reducing LD. For example, 'Keitt' mango exhibited the most discoloration possibly because of a reduced cutin layer over the cells near the base of the lenticel cavity, and relatively large lenticel cavities compared with more tolerant cultivars (du Plooy *et al.*, 2004).

To better clarify LD during the postharvest stage, lenticel characteristics and morphology changes after harvest were examined. Histochemical examination was used to investigate lenticel structure among cultivars at different periods from fruit harvesting to ripe. The results may lead to a better understanding of the development of lenticel disorders.

4.2.3. Materials and methods

4.2.3.1. Fruit and sampling

'B74' mango trees were selected at a commercial orchard at Childers, Queensland (25°14'S, 152°37'E). Fruits were all harvested at about 14% dry matter (minimum commercial maturity). Ten 'B74' fruits were harvested from each tree, the fruit held upside down and the stems removed and desapped for about 30 min. The fruit were then dipped in 0.25% Mango Wash® for 1 min and put in the shade to dry. They were then carefully placed into single layer trays with a plastic insert and transferred by car to the Maroochy Research Facility (MRF)

laboratories, Nambour, Queensland. The fruit were treated with fungicide at 0.2% v/v (Sportak®, Bayer CropScience Pty Ltd) for 30 s and brushed using a Lenze AC Tech brushing unit with soft brushes with no water for 1 min at 84 revolutions.min^{-1} to mimic commercial practice. Lenticel discolouration, lenticel density, and the percentage of discoloured lenticels were recorded at the green mature stage. Fruit were then treated with 10 µL.L^{-1} ethylene at 20°C for 2 d then ripened at 20°C until fully ripe. Fruit were reassessed for lenticel discolouration, lenticel density and percentage with LD as described below.

For examination of wetting angle and lenticel morphology changes along the commercial packing line, 32 fruit were sampled at four points (eight fruit per sample) from harvest directly off the tree, after Mango Wash (see above), after brushing, and after packing. The fruit were placed into single layer trays and transported to the MRF laboratories to measure the wetting angle at harvest (see below). Fruit were then treated with 10 µL.L-1 ethylene at 20°C for 2 d then ripened at 20°C until fully ripe. Fruit were rated for LD and sampled for lenticel morphology as described below.

Ten fruit were collected at the end of commercial packing-line to study lenticel external features and morphology changes during ripening. The fruit were transported to the MRF laboratories then treated with 10 µL.L^{-1} ethylene at 20°C for 2 d then ripened at 20°C until fully ripe. 100 similar green lenticels (10 lenticels per fruit) were selected and marked on the fruit surface. During ripening, external pictures of lenticels were recorded over time using a dissecting light microscope and lenticel morphology were examined as described below.

4.2.3.2. Assessments

Lenticel density and proportion of damaged lenticels

To assess lenticel density and discoloured lenticel percentage, four circles of 3 cm² each were marked on the two cheeks and two sides on the fruit equator. The total lenticel number and the number of discoloured lenticels in each circle were recorded at harvest and at 8 d after full yellow. The lenticel density per cm^2 and proportion (%) of damaged lenticels was calculated (Schotsmans *et al.*, 2004).

Lenticel discolouration

Lenticel discolouration for each fruit was rated as described section 4.1.3.3.

Wetting angle

Eight fruit were used to measure the wetting angle on four points on the fruit equator with 5 µL droplets of distilled water (Lamour and Hamraoui, 2010). The contact angles were measured at one point on either cheek and one in between on both sides. Fruits were firmly positioned with the selected site in the horizontal direction. The droplets were carefully applied onto the selected site and photographs of the droplets on the fruit skin taken with a Canon DOS40D camera fitted with a Canon macro-lens EF-S 60 mm. The contact angles from the images were measured using Image-J software.

Lenticel external appearance and morphology

One hundred typical lenticels on each of five mature, non-ripe ‘B74’ fruits were marked. During ripening, micrographs were taken with a dissecting microscope fitted with a Canon D40 camera.

Five fruit were used at each of 2 d after harvest and 8 d after full yellow skin colour (ripe) and morphology studied using the methods described in section 4.1.3.3.

4.2.3.3. Statistical analysis

Statistical analyses were performed by Genstat® 14 for Windows™ (VSN International Ltd., UK). Analysis of variance (ANOVA) used the ‘General Analysis of Variance’ model. The

protected least significant difference (LSD) procedure at P=0.05 was used to test for differences between means (lenticel density, proportion of damaged lenticels and wetting angle).

4.2.4. Results

4.2.4.1. Harvest and packing effects

Lenticel discolouration increased from 2 d after harvest to 8 d after full colour (Table 6). The densities of lenticels were similar at both assessment times but the % of discoloured lenticels almost doubled during ripening (Plate 8).

Table 6 Lenticel discolouration severity (0-5), density of lenticels, and the proportion of discoloured lenticels on the same area of each 'B74' mango fruit 2 d after harvest and 8 d after full colour (in the same position).

Assessment time	Lenticel discolouration	Density per cm^2	Proportion of discoloured lenticels (%)
At 2 d after harvest	0.9 [a]	44.8	9.8 [a]
8 d after full colour	1.9 [b]	44.1	17.1 [b]
P value at 0.05	<0.001	0.814	<0.001

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD, (n=10 fruit x 4 positions)

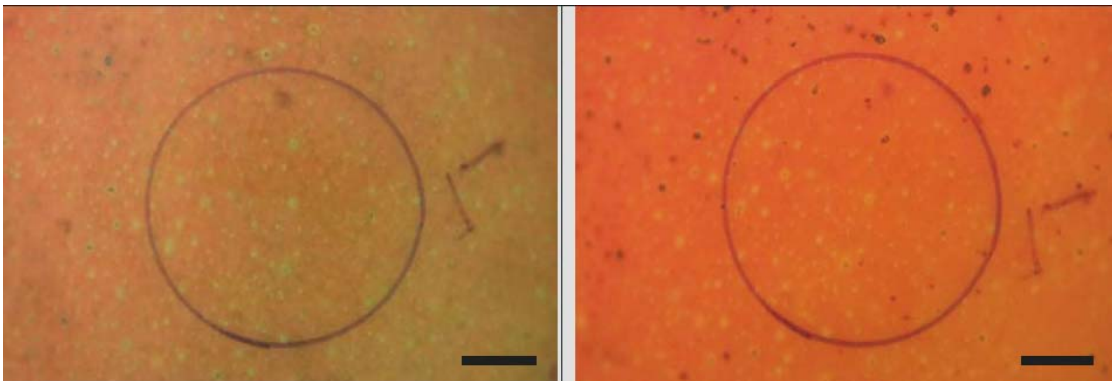


Plate 8 Pictures of one of the lenticel assessment areas at 2 d after harvest (left) and 8 d after full colour (right). Scale bar = 1 cm.

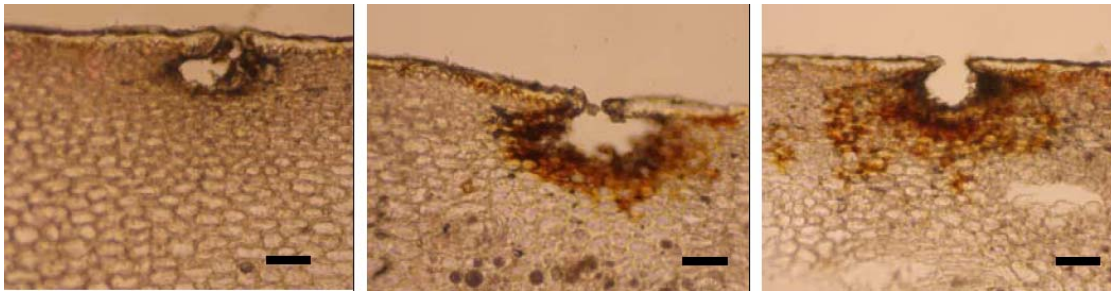
Wetting angles decreased significantly during harvesting and packing, and the LD severity at full yellow increased from after harvest to after packing (Table 7).

Table 7 Wetting angle of DI water on the 'B74' fruit surface at harvest and LD at full yellow, fruit were sampled at off the tree, after mango wash, after brushing and at end of pack-line

Fruit sampling point	Wetting angle (°)	LD at full yellow (0-5)
Off-tree	117.4 [d]	0.6 [a]
After harvest	105.1 [c]	0.7 [ab]
After brushing	100.1 [b]	1.1 [b]
End of the packing line	91.9 [a]	1.7 [c]

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD, (n=8 fruit x 4 positions)

Typical micrographs confirmed the increase in LD from the tree to after packing (Plate 9).



Off tree fruit | After brushing | End-packing line

Plate 9 Unstained lenticel morphology from fruit sampled directly off the tree (left), after brushing (middle) and after packing (right). The lenticels were sampled once the fruit had reached full yellow skin colour. Scale bar = 50 µm.

4.2.4.2. Changes during ripening

Two days after harvest most of the lenticels showed no signs of discolouration (Plate 10). As the fruit ripened, a significant proportion of the lenticels developed brown pigment around the lenticel.

Sectioning of typical lenticels on the ripening fruit indicated almost no brown pigment in the cells surrounding the lenticel 2 d after harvest (Plate 11). As the fruit ripened, an increasing area around the lenticels expressed brown pigmentation.

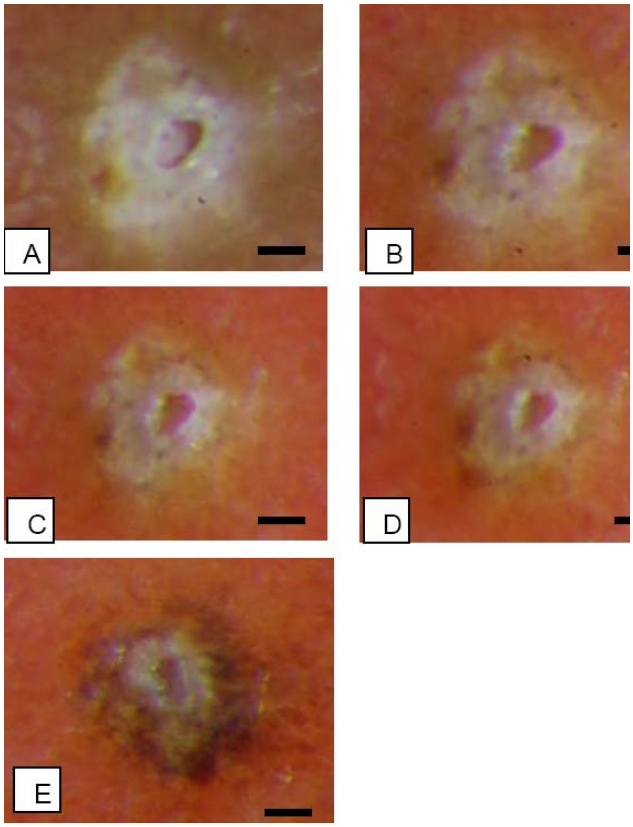


Plate 10 Change in appearance of the same lenticel from 2 d after harvest to 6 d after full yellow on a typical 'B74' mango fruit picked and packed under commercial conditions and ripened at 20ºC. Lenticel at 2 d (A); 7 d (B); 9 d (full yellow) (C); 12 d and (D); 15 d after harvest (E). Scale bar = 50 µm.

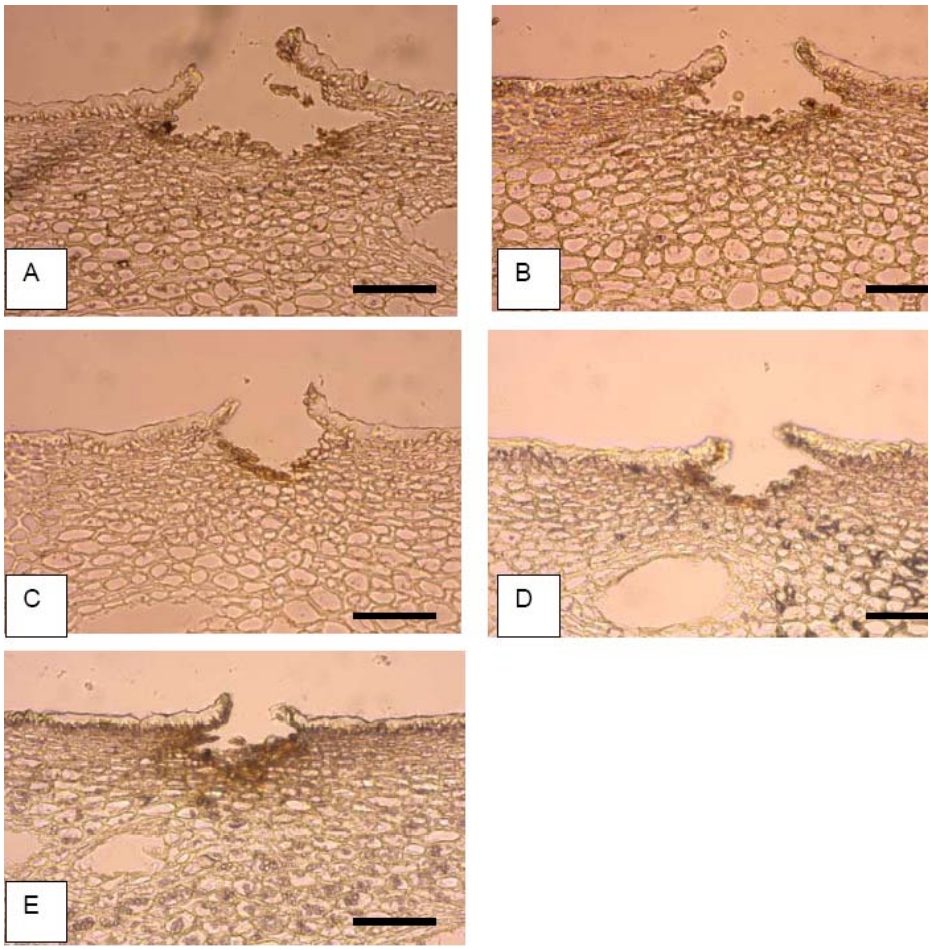


Plate 11 Change in appearance of typical lenticels from 2 d after harvest to 6 d after full yellow on a 'B74' mango fruit picked and packed under commercial conditions and ripened at 20ºC with 2 d of 10 µL.L-1 ethylene; Lenticel at 2 d (A); 7 d (B); 9 d (full yellow) (C); 12 d and (D); 15 d after harvest (E). Scale bar = 50 µm.

4.2.5. Discussion

There was no evidence of changes in lenticel density after harvest, suggesting no formation of new lenticels after harvest. However, the proportion of discoloured lenticels increased during ripening, and this was likely the main cause of the increased LD.

Postharvest treatments such as solutions (detergents etc) used during desapping (O'Hare and Prasad, 1992; Willis and Duvenhage, 2002; Whiley *et al.*, 2006) and packhouse operations such as brushing packing increase LD (Dietz *et al.*, 1988b; Cronje, 2009a; Hofman *et al.*, 2010c). Decreased wetting angle is often associated with alternations to the wax structure on the plant surfaces, which was also observed in this trial. These changes may indicate greater potential for water to enter the lenticel, which may be one of the mechanisms involved in LD.

The micrographs indicated that LD developed from gradual pigmentation of the cells surrounding the lenticel cavity, and postharvest handling contributed to LD severity in an accumulative way (Self *et al.*, 2006). The dark pigmentation is likely from the accumulation of of polyphenols in these cells (du Plooy *et al.*, 2006), which can act as a protective barrier against pathogen invasion (Tamjinda *et al.*, 1992; du Plooy *et al.*, 2004).

4.3. Effects of production factors

("Survey of production factors influencing lenticel discolouration")

Roberto Marques, Peter Hofman, Jonathan Smith, Justin Davies, Connie Banos, Barbara Stubbings

4.3.1. Summary

Previous work showed that lenticel discolouration (LD) in 'B74' fruit can vary with season, production location and farm, but there was little understanding of the factors contributing to these differences. To identify those factors affecting lenticel sensitivity of 'B74' mango, and help develop predictive tools to reduce the problem, trials were established on five representative commercial farms in the main production areas in the Northern Territory, north Queensland and south-east Queensland. Weather stations were set up and 30 trees marked at each site. Growing conditions, tree flowering and flushing patterns, and tree and fruit characteristics at harvest were monitored in these locations. Fruit samples were taken from 15 marked trees, with half of the fruit irradiated at the Lucas Heights facility, Sydney. Fruit responses to irradiation during fruit ripening and ageing were related to these parameters. The trial was repeated over three seasons to provide sufficient data to understand seasonal effects and provide more robust results.

Field temperatures generally varied moderately across the three seasons and across the five locations during 28 and 56 d before harvest, while rainfall varied markedly across years and locations at periods of 7-56 d before harvest. In general, temperatures were higher and rainfall was lower in the Northern Territory sites compared to Queensland ones, while relative humidity was higher in the north Queensland sites compared to Katherine and Childers. It is likely such climatic variation was a contributing factor to the large variation between seasons and locations observed in fruit characteristics at harvest and LD severity at ripe. There were significant negative correlations (r=0.60-0.69) between the mean temperatures during the period of 56 d before harvest and LD at full yellow in both non-irradiated and irradiated fruit.

Characteristics varied considerably across seasons and farms for most of the tree and fruit characteristics, including canopy volume, yield, yield efficiency, trunk difference above and below the graft union, flowering and flushing characteristics at full flowering and at harvest, fruit dry matter, flesh colour, average fruit weight and fruit blush at harvest. At each farm, there was also large variation among trees for these parameters.

In general, LD in 'B74' mango fruit varied significantly across locations and seasons for both non-irradiated and irradiated fruit. Compared to non-irradiated fruit, irradiated fruit from all locations and seasons had more severe LD during fruit ripening and aging, as assessed 1 d after treatment, at the full yellow (ripe) stage, and 7 d later. Skin browning (SB) severity increased in irradiated fruit 7 d after full colour compared to control fruit for most locations and seasons. Irradiation treatment also slowed down the loss of green skin colour by 1-4 d, depending on the location and year. As a result, irradiated fruit were generally softer at full yellow than control fruit in most locations and years.

Multivariate analysis showed that multiple regression on the combined data across farms and years was the most useful approach. All the tree and fruit characteristics were tested (explanatory variables), with LD severity (in both non-irradiated and irradiated fruit data sets) at full yellow as the dependant variable. The best final model from the forward stepwise regression suggested that across all farms and years, less severe LD at full yellow in fruit that had been irradiated was associated with smaller and more mature fruit from trees that had more uniform flowering and smaller trunk differences. For the non-irradiated fruit, less severe LD at full yellow was associated with fruit that were smaller, more mature (higher % DM) and with more blush, from trees with smaller canopy area and with more uniform

flowering. However, this model accounts for only 35% and 32% of the variance in LD for irradiated and non-irradiated fruit, respectively.

4.3.2. Introduction

Skin defects, including undesirable changes in the appearance of fruit lenticels after harvest and packing, are a common issue in mango producing countries (du Plooy *et al.*, 2009; Rymbai *et al.*, 2012). Lenticel discolouration (LD; also called lenticel spotting or lenticel damage) often appears as darkening of the cells in surrounding tissues, producing a brown or black spot, or as a red or green halo around the lenticel, with or without the black or brown spot in the centre (Bezuidenhout *et al.*, 2005; du Plooy *et al.*, 2006). It can markedly reduce the visual appeal of fruit leading to a downgrade of their commercial value, especially in export markets (Johnson and Hofman, 2009; Rymbai *et al.*, 2012). The appearance and severity of the condition can vary depending on cultivar (Cronje, 2009b; du Plooy *et al.*, 2009). In cultivars such as 'Tommy Atkins', 'Keitt', 'Kent', and 'Kensington Pride' ('KP'), LD is associated with a number of production factors, such as irrigation, leaf:fruit ratios on the canopy, relative humidity and rain conditions at harvest, fruit maturity at harvest (e.g. time of harvest during the season), harvest method (including de-sapping method and detergent used) and several postharvest handling operations during the packing process, including delays between picking and packing, fruit brushing, and hot water/air and chemical treatments (Simmons *et al.*, 1995; Bally *et al.*, 1997; Oosthuyse, 1998; Simmons *et al.*, 1998; Self *et al.*, 2006; Cronje, 2009b; Cronje, 2009a; Feygenberg *et al.*, 2014). To a lesser degree, skin browning is another type of discolouration in the mango skin that can cause product downgrade and rejection on Australian markets (Bally *et al.*, 1997). It is thought to be caused by and number of factors, including extreme temperatures, moisture, and sap or skin abrasion (O'Hare *et al.*, 1999).

Results from projects FR02049 and MG06005 showed that LD on 'B74' (trading as 'Calypso'^{TM}) fruit can vary with season, production location and farm (Whiley *et al.*, 2006; Hofman *et al.*, 2010b). However, there was little understanding of the factors contributing to these differences. To identify production factors affecting lenticel sensitivity of 'B74' mango to irradiation, and help develop predictive tools to reduce the problem, trials were established on five representative commercial farms in the main production areas in Australia: two farms in the Northern Territory (NT; at Darwin and Katherine), two farms in north Queensland (NQld; at Dimbulah and Mareeba) and one farm in south-east QLD (SEQld; at Childers). Growing conditions and tree and fruit characteristics in these locations were monitored. Fruit responses to irradiation during fruit ripening and ageing were related to these parameters. The trial was repeated over three seasons to provide sufficient data to understand seasonal effects and provide more robust results.

4.3.3. Materials and methods

4.3.3.1. Sites

Five field trials were established in July 2010 at representative 'B74' blocks in the main production areas in the Northern Territory (NT), north Queensland (NQld) and south east Queensland (SEQld) as shown in Table 8. Fifteen adjacent trees in each of these blocks were clearly marked to prevent early harvesting and to allow the same trees to be used for three years.

4.3.3.2. Production characteristics

Climactic conditions

Weather stations were established at each site to record air temperature, relative humidity and rainfall volume from the start of flowering until harvest. The following loggers were used:

- Tinytag temperature/RH loggers (in a HDL Datamate datalogger shelter)

- Tinytag count input data logger attached to a CS700 Rain Gauge (one count = 5 mm rain)

Table 8 Selected field sites used for the trial 'Survey of production factors influencing lenticel discolouration' from 2010 to 2013

Location	Farm	Block
Darwin (NT)	Acacia Hills	Starbucks
Katherine (NT)	Oolloo Katherine (K1)	1
Dimbulah (NQld)	Oolloo Dimbulah	52
Mareeba (NQld)	Willbi	B1
Childers (SEQld)	Simpson (Goodwood)	Home 1

Flowering assessments

The marked trees were assessed twice during flowering (about two to three weeks apart) to determine when each tree reached full flowering, if there were multiple flowerings, when the first 5% and the last 5% of the panicles flowered, the average stage of development of panicles on each tree, and the range (i.e. the spread or variation of flowering).

The following visual ratings were used on opposite sides of the tree along the row (the results for each tree were then averaged):

Percentage of terminals flowering: by looking at the tree canopy as a whole, each side of the row/canopy was visually rated as the % of panicles that showed any signs of flowering compared to the total number of panicles on the tree.

Stage of flowering based on the visual scale: 0=nil; 1=1-5 cm panicle; 2=up to full extension but very few of the flowers had opened; 3= full flowering (or full bloom), when most of the flowers opened except the last 1-3 cm of the end of the panicle; 4= all flowers on the panicle opened, and some of the bottom flowers have set very small fruit; 5=up to pea size fruit on the panicles; 6=up to large marble size; 7=larger than marble size.

Full flowering stage: the date when at least 50% of the panicles on the tree reached full flower (on average the stage of flowering 3 – most of the flowers opened, except for the last 1-3 cm of the end of the panicle (Plate 12).

Plate 12 Panicle of a 'B74' mango tree at a typical stage 3 of flowering ('full flowering').

Spread of flowering: the average flowering stage for the 5% of the least (latest flowering) advanced panicles, and flowering stage for the 5% of the most advanced panicles (earliest flowering) was estimated. The greater the difference in the stage of flowering between the least and most advanced stage, the greater the spread of flowering time. The stage of the least advanced was subtracted from the most advanced to give a flowering range. This was repeated for the different assessment times.

In the first season, assessments of spread of flowering were not done in the NT farms, as the flowering on the trees was already too advanced by the time the project was approved and the trial established.

Vegetative growth assessments

The potential competition between vegetative and reproductive growth was also determined by recording the percentage of terminals with new vegetative flush at full flowering, and the flushing vigour at the end of flowering and at harvest. The following visual ratings were used on opposite sides of the marked trees as above:

- % of terminals with vegetative flush.
- Flushing vigour based on the visual scale: 0=no flushing; 1= less than 5 cm; 2= up to 20 cm; 3= up to 50 cm; 4=up to 100 cm; 5=more than 100 cm.

Tree characteristics at harvest

To allow correlations to be done between tree characteristics, flowering, flushing and fruit maturity, digital photographs were taken on two sides (along the rows) of the tree canopy at harvest using a reference board (0.28 x 0.22 m). Canopy area was then estimated with SigmaScan (Systat Software Inc., Chicago, USA).

The vegetative flushing was recorded as described above.

Canopy density was estimated by a visual rating: 1 = open, 2 = average, 3 = dense.

Trunk diameter about 5 cm below and 5 cm above the graft union was recorded using a measuring tape.

4.3.3.3. Harvest procedure

The total number of fruit on each tree was carefully counted and recorded using a hand counter. The data was used to estimate yield per tree, together with average fruit weight per tree from the sampled fruit.

Whenever possible, fruit samples were harvested just before commercial picking started at each location and block, aiming at a target fruit maturity of 14-16% dry matter (DM). The dates are given in Table 9.

Table 9 Harvest dates at the selected field sites used for the trial 'Survey of production factors influencing lenticel discolouration' from 2010 to 2013

Location	2010-11	2011-12	2012-13
Darwin (NT)	19/10/10	12/10/11	22/10/12
Katherine (NT)	29/10/10	22/10/11	14/11/12
Dimbulah (NQld)	07/12/10	12/12/11	10/12/12
Mareeba (NQld)	14/12/10	13/12/11	11/12/12
Childers (SEQld)	08/02/11	06/02/12	04/02/13

- Two trays of count 18 or 20 (total of about 36-40 fruit) per tree (depending on fruit size) were picked with long stems and carefully placed on the ground. Sampled fruit were sound (with no cuts or open wounds, not severely sunburnt and no yellow skin), of average size for the tree and proportionally representing all aspects of the tree, including sun-exposed fruit and those further inside the canopy.
- An extra five fruit per tree were sampled for fruit maturity assessments at harvest. Fruit were snapped and placed on the ground (stem end down) away from the sun to de-sap. Fruit were then placed in small labelled plastic bags, kept in the shade and taken to the local research station. Dry matter (using the percent dry matter maturity test) and flesh colour (using the ''B74'™Picking Guide') per tree were determined as described in the

"'B74' Best Practice Guide" (Hofman and Whiley, 2010). Whenever possible, assessments were done on the same day as harvest. If next day, fruit were kept in the plastic bags overnight in a coldroom at 12-13°C to reduce weight loss.

- To reduce variability, and allow more uniform harvesting and packing operations across farms and seasons, commercial de-sapping was simulated in the fruit picked with long stems by removing the stem and holding fruit for two seconds (with stem end down) to remove spurt sap. All 36-40 fruit per tree were then placed in a de-sapping solution of (75 g of 'Mango Wash' powder (Septone) in 30L of water for 4 min using a 50-60 L plastic container. The solution was replaced after each tree.
- Fruit from the de-sapping solution were then removed and placed into labelled perforated plastic crates (one per tree). The crates were taken to the packhouse or the local research station and dipped into Sportak (Bayer - active ingredient: 450g/L prochloraz) (55 ml/100 L) for 30 seconds. Fruit were then allowed to dry.
- Fruit were packed into labelled P-84 trays with inserts. The trays were separated into two lots (one tray from each tree going to each lot): not irradiated and irradiated. One temperature logger was inserted into a representative tray of each lot. Trays from each lot were stacked into bundles of two trays, covered with a lid, before being wrapped with duct tape.

4.3.3.4. Fruit handling after harvest

Fruit handling before irradiation

To stimulate LD, and further investigate the effects of irradiation on lenticel sensitivity, as well as the processes taking place within the lenticels following irradiation, half of the sampled fruit were air-freighted to Sydney to be irradiated within 48 h of harvest. The remaining fruit were air-freighted (except fruit from Childers which were transported by car) to the DAFF postharvest laboratory at Maroochy Research Facility (MRF) Nambour and held as controls (not-irradiated).

Irradiation treatment

Fruit were irradiated at the Gamma Technology Research Irradiator (GATRI) at the Australian Nuclear Science and Technology Organisation (ANSTO) facility at Lucas Heights, Sydney. Gamma radiation from a cobalt-60 source was used at a target dose of 350 Gy and a dose rate of approx. 8-10 $Gy.min^{-1}$. With each batch, the trays were randomly divided into two lots, eight trays for the first run and six to eight trays for the second run.

Fricke dosimeters were placed throughout the array at the expected minimum and maximum dose zones, taking into account locations of inhomogeneous product distribution. About four dosimeters were placed on the top and bottom of fruit in each try (Plate 13), and two dosimeters were attached to the outside of one tray to provide a reference to the minimum and maximum doses (monitoring position). For each run, the trays were positioned on a rig parallel to the plaque source (Plate 14). Since the dosimeters used are calibrated for reading 50-350 Gy, it was necessary to irradiate in two increments targeting a dose of 350 Gy (actual doses in Table 10), with a change of dosimeters after the first increment. The samples from the first run were used to carry out a dose mapping exercise at about 150 Gy. The trays were then irradiated to bring the cumulative average dose to 350 Gy. This dose mapping information was used to process the remaining trays which were irradiated in two equal increments of 175 Gy.

Plate 13 Dosimeters placed in 'B74' mango trays before irradiation treatment at ANSTO (Sydney).

Plate 14 'B74' mango trays positioned for irradiation treatment at ANSTO (Sydney).

The doses absorbed by the fruit complied with required specifications for all locations and years (Table 10).

Fruit quality assessments

Fruit were individually assessed based on the rating systems in the "'B74' Quality Assessment Manual" (Hofman *et al.*, 2010a).

Fruit were air freighted to MRF as soon as possible after irradiation. Together with non-irradiated fruit, all fruit were ripened at 20ºC. In the first 2 d of ripening, fruit were exposed to 10 µl.L-1 of ethylene. Fruit were assessed for external quality 1 d after irradiation, at the full yellow (ripe) stage, and 7d after as described below.

Skin colour and fruit firmness

Assessments were done when more than 80% of fruit in each tray reached the full yellow stage (90% or more of yellow colour on the skin). Fruit firmness was also assessed at that time.

The background skin colour (non-red area) was rated using the scale: 1=0-10% yellow; 2=10-30% yellow; 3=30-50% yellow; 4=50-70% yellow; 5=70-90% yellow; 6=90-100% yellow. Fruit firmness was rated using hand pressure as follows: 0=hard (no 'give' in the fruit); 1=rubbery (slight 'give' in the fruit); 2=sprung (flesh deforms by 2-3 mm with extreme thumb pressure); 3=firm soft (whole fruit deforms with moderate hand pressure); 4=soft (whole fruit deforms with slight hand pressure).

Table 10 Irradiation doses received by 'B74' mango fruit in 2010 -2013 (the target dose was 350 Gy)

Location	Irradiation date	Minimum dose (Gy)	Maximum dose (Gy)	Average dose (Gy)
Darwin (NT)				
2010-11	22/10/10	329 ± 11	372 ± 21	350 ± 12
2011-12	14/10/11	333 ± 6	367 ± 7	350 ± 5
2012-13	24/10/12	329 ± 6	358 ± 7	343 ± 5
Katherine (NT)				
2010-11	01/11/10	327 ± 10	365 ± 9	346 ± 7
2011-12	24/11/11	330 ± 6	362 ± 7	347 ± 5
2012-13	16/11/12	331 ± 6	360 ± 7	346 ± 5
Dimbulah (NQld)				
2010-11	09/12/10	324 ± 12	350 ± 13	337 ± 9
2011-12	14/12/11	327 ± 6	363 ± 7	345 ± 5
2012-13	12/12/12	332 ± 6	361 ± 7	346 ± 5
Mareeba (NQld)				
2010-11	14/12/10	316 ± 12	372 ± 13	344 ± 9
2011-12	15/12/11	329 ± 6	360 ± 7	344 ± 5
2012-13	13/12/12	331 ± 6	360 ± 7	346 ± 5
Childers (SEQld)				
2011-12	08/02/12	328 ± 6	359 ± 7	344 ± 5
2012-13	06/02/13	332 ± 6	362 ± 7	346 ± 5

Note: fruit from Childers not irradiated in 2010-11 due to insufficient yield.

Lenticel discolouration

Each tray was rated for LD (section 4.1.3.3) when on average more than 80% of the fruit in each tray were at the following stages:

- Non-irradiated fruit: at full yellow (no green remaining on the fruit) and 7 d later.
- Irradiated fruit: at the same time as when the non-irradiated fruit reached full yellow, then when the irradiated fruit reached full yellow (since the irradiated fruit often lost green colour more slowly than the non-irradiated fruit), then 7 d later .

Skin browning and disease

Assessments were based on the scale: 0=nil; 1=<1cm^2; 2=1-3 cm^2 (approx 3%); 3=3-12 cm^2 (about 10%); 4=12 cm^2 (about 10%) to 25%; 5=> 25%. The diseases were described based on the appearance and location of the lesions on the fruit (Hofman *et al.*, 2010a).

The proportion (%) of acceptable fruit was calculated as number of fruit with a severity rating lower than 3 for either LD or SB, and a combined rating lower than 5 for both defects, in relation to the total number of fruit examined per treatment.

4.3.3.5. Statistical analysis

Statistical analyses were performed by Genstat® 11 for Windows™ (VSN International Ltd., UK). For the fruit quality data, analysis of variance used the 'General Analysis of Variance' model for each year, with farm by irradiation treatment as 'treatment' structure and tree/fruit as 'block' structure. The protected least significant difference (LSD) procedure at P=0.05 was used to test for differences between treatment means. The relationships between production characteristics (including tree yield, canopy assessments during flowering, vegetative growth and at harvest, fruit dry matter and flesh colour), climactic data, and fruit quality attributes were established using correlation analysis on the means for each tree or farm (for climactic data) or individual fruit (for fruit quality assessments). The significance of the correlations was determined by linear regression analysis (P = 0.05), and the strength by the correlation coefficient (r).

Aiming at identifying variables that may predict LD in 'B74' fruit, a number of multivariate and regression approaches were performed on the combined data sets across farms and years, including multiple linear regression, canonical variates analysis, principal components analysis, and regression/classification trees. Multiple regression used a forward stepwise procedure, with LD at full yellow as the dependent variable (and both the non-irradiated and the irradiated data sets analysed separately), and all the assessed tree and fruit characteristics as the explanatory variables.

4.3.4. Results and discussion

4.3.4.1. Climatic conditions during fruit growth

Field temperature (ranging from 24.1 to 30.7°C) and relative humidity (ranging from 56 to 88%) generally varied moderately across the three seasons and across the five locations during 28 and 56 d before harvest (Table 11). In contrast, rainfall varied markedly across years and locations at different periods (from 7 to 56 d) before harvest (Table 11). In general, temperatures were higher and rainfall was lower in the NT sites compared to Qld ones, while relative humidity was higher in the NQld sites compared to Katherine and Childers.

Table 11 Total rainfall (mm), average air temperature (°C) and relative humidity (%) at the five 'B74' mango trial sites in 2010/11, 2011-12 and 2012-13 up to 56 days before harvest.

Location	Harvest date	Total rainfall (mm)				Mean Temp. (°C)		Mean RH (%)	
		Days before harvest							
		7	14	28	56	28	56	28	56
Darwin (NT)									
2010-11	19/10/10	20	104	151	175	28.5	27.2	77	70
2011-12	12/10/11	49	49	49	49	27.7	26.1	74	68
2012-13	22/10/12	0	42	42	42	27.8	26.7	73	71
Mean		23	65	81	89	28.0	26.7	75	70
Katherine (NT)									
2010-11	29/10/10	5	31	78	82	29.5	30.0	58	52
2011-12	22/11/11	86	142	176	181	29.6	30.1	70	62
2012-13	14/11/12	0	33	35	65	30.7	28.0	61	56
Mean		30	69	96	109	29.9	29.4	63	57
Dimbulah (NQld)									
2010-11	7/12/10	32	32	183	228	25.3	24.9	88	80
2011-12	12/12/11	3	3	3	14	26.1	25.1	71	72
2012-13	10/12/12	3	3	27	27	25.6	24.6	60	61
Mean		13	13	71	90	25.7	24.9	73	71
Mareeba (NQld)									
2010-11	14/12/10	65	79	281	357	25.1	24.4	88	85
2011-12	13/12/11	54	93	100	145	25.2	24.1	67	65
2012-13	11/12/12	0	0	36	36	24.5	23.6	77	77
Mean		40	57	139	179	24.9	24.0	77	76
Childers (SEQld)									
2010-11	8/2/11	1	5	59	135	25.8	25.3	69	73
2011-12	6/2/12	3	227	312	510	24.6	24.1	67	65
2012-13	4/2/13	0	469	471	476	26.0	25.6	67	63
Mean		1	234	281	374	25.5	25.0	68	67

It is likely that the above climatic variation was a contributing factor to the large variation between seasons and locations observed in this trial in fruit characteristics at harvest (Table 14), as well as in fruit quality (particularly LD severity) at ripe (Table 15). Linear regression analysis of the climate data collected from each farm showed significant negative correlations between the mean temperatures during the period of 56 d before harvest shown

in Table 11 and LD at full yellow in both non-irradiated fruit (correlation coefficient, r=0.60) and irradiated fruit (r=0.69). The correlation was also significant between the period of 28 d before harvest and LD at full yellow in irradiated fruit (r=0.60). In contrast, the correlations between LD (in either non-irradiated or irradiated fruit) and the other parameters shown in Table 11 (rainfall and relative humidity) were not significant (data not shown).

In 'Tommy Atkins' mango, strong negative correlations were reported between LD and maximum and minimum temperature and class A pan evaporation, and strong positive correlations with maximum humidity and rain at harvest (Oosthuyse, 1998). These results suggest that cool, humid and wet conditions around harvest may increase the risk of LD, possibly due to relatively high water (turgor) pressure in the fruit in these conditions. This is supported by reports of increased LD after excess irrigation in the latter stages of 'Kensington Pride' fruit growth (Simmons *et al.*, 1998) and reduced LD with withholding irrigation for periods of 1.5-3 wk before harvest in 'Tommy Atkins' and 'Keitt' mangoes in South Africa (Cronje, 2009b).

4.3.4.2. Tree growing characteristics

Means per tree for most of the tree characteristics assessed varied considerably across seasons and farms (Table 12 and Table 13). At each farm, there was also large variation among trees in tree and in flowering and flushing characteristics, as shown by the tree range data.

Generally, yield per tree and yield efficiency in the QLD farms increased considerably from the first to the second and third seasons compared to the NT farms, likely due to the younger and smaller trees in QLD (Table 12). Yield per tree was more uniform across seasons in Darwin and Mareeba compared to the other three farms. Trunk differences above and below the graft union were generally higher in trees in Mareeba and lower in Childers (larger differences may indicate stronger graft incompatibilities between rootstock and scion).

In general, the difference between the least and the most advanced flower stage (an indication of flowering variation) increased from the first season (or the second for the NT farms, as the assessments were not done in the first season on those farms) to the third season in all farms (Table 13). A higher % of terminals flushing at full bloom were generally associated with low tree yield (e.g. Dimbulah and Childers in 2010-11, Katherine in 2011-12; Table 12 and Table 13).

Table 12 Tree characteristics of 'B74' mango grown at the five 'B74' mango trial sites in 2010/11, 2011-12 and 2012-13 (data are the means of 15 selected trees per farm).

Tree characteristic	Season	Darwin (NT)	Katherine (NT)	Dimbulah (NQld)	Mareeba (NQld)	Childers (SEQld)
Yield per tree (Kg)	2010-11	59	70	23	41	15
	2011-12	56	36	57	61	49
	2012-13	57	66	42	59	51
	Tree range (all seasons)	34-121	1-96	7-79	9-98	7-73
Yield efficiency (Kg/m^2)	2010-11	13.9	16.6	14.1	12.7	10.1
	2011-12	14.9	6.6	28.5	20.0	16.9
	2012-13	13.6	16.8	27.6	24.4	13.8
	Tree range (all seasons)	7.3-26.2	0.2-26	5.0-45.8	2.6-40.7	4.8-23.1
Canopy area (m^2)	2010-11	4.2	4.2	1.6	3.2	1.5
	2011-12	3.8	5.8	2.0	3.1	2.9
	2012-13	4.2	3.9	1.5	2.4	3.7
	Tree range (all seasons)	3.2-5.8	2.8-7.0	1.1-2.9	1.3-4.6	0.8-4.7
Canopy density (1-3)	2010-11	2.0	2.0	1.9	2.1	2.0
	2011-12	1.9	2.0	1.8	2.0	2.1
	2012-13	1.9	2.0	2.1	2.0	2.1
	Tree range (all seasons)	1.5-3	1.5-2.5	1.0-2.5	1.5-2.5	1.0-3.0
Trunk difference (cm)	2010-11	2.6	7.9	8.5	15.7	2.6
	2011-12	3.6	4.8	6.8	12.4	3.2
	2012-13	5.9	6.6	6.8	9.2	2.8
	Tree range (all seasons)	0-12	2.5-11.0	0.5-12.1	2.5-24.0	0-6

4.3.4.3. Fruit characteristics at harvest

As with tree characteristics, there was considerable variation between seasons and farms in the characteristics of fruit sampled from the sample trees around commercial harvest (Table 14). At each farm, there was also large variation among trees, as shown by the tree range data.

The variation in both flesh dry matter and flesh colour (two key indicators of fruit maturity in 'B74') highlights the challenges faced by commercial farms in developing effective harvesting schedules that ensure fruit is harvested within commercial maturity and in time before excessive drop occurs (section 7.2). The fruit from the NQld farms were generally picked below the targeted maturity due to the need to accommodate logistical requirements to allow the fruit to be irradiated before the closure of the facilities in Sydney during the Christmas and New Year break.

Average fruit weight varied among locations and years by up to 164 g, while fruit blush varied by up to 1.4 rating units. Generally, fruit from Katherine were the smallest, and from Childers the largest.

4.3.4.4. Lenticel discolouration during ripening

As the interactions between location and irradiation treatment for LD severity were significant for all seasons, the results are presented as a factorial analysis.

Lenticel discolouration severity in 'B74' mango was significantly higher in irradiated fruit at the full yellow (ripe) stage and 7 d later compared to non-irradiated fruit from all farms and in all seasons (Figure 5 and Figure 6). Compared to non-irradiated fruit, the severity of LD in irradiated fruit increased as early as 1 d after irradiation in most farms and across the two

last seasons (fruit were not assessed in the first season; Figure 5). Fruit were also initially assessed for LD when the control fruit reached full colour (about 1-2 d earlier than when the irradiated fruit reached full yellow), but there was generally little increase in LD between both assessments (data not shown), so only the data when the irradiated fruit reached full yellow is presented. In general, LD varied significantly also across locations and seasons for both not irradiated and irradiated fruit. Similar results of increase in LD severity in 'B74' mango fruit with irradiation treatment were noted in previous trials (Hofman *et al.*, 2010b). Likewise, irradiation of mangoes in Australia at doses varying from 300-1200 Gy resulted in increased severity of LD in several other cultivars, including 'KP, 'Kent', and 'Irwin' (Johnson *et al.*, 1990; McLauchlan *et al.*, 1990).

Table 13 Flowering and flushing characteristics of 'B74' mango grown at the five 'B74' mango trial sites in 2010/11, 2011-12 and 2012-13 (data are the means of 15 selected trees per farm).

Flowering / flushing characteristic	Season	Darwin (NT)	Katherine (NT)	Dimbulah (NQld)	Mareeba (NQld)	Childers (SEQld)
Terminals flowering 2 weeks before full bloom (%)	2010-11	n.a.	91	76	71	74
	2011-12	46	28	82	64	89
	2012-13	n.a.	91	76	71	74
	Tree range (all seasons)	8-85	0-98	18-98	3-100	33-100
Terminal flowering at full bloom (%)	2010-11	73	90	60	49	72
	2011-12	94	34	92	82	91
	2012-13	78	95	87	75	84
	Tree range (all seasons)	58-95	0-100	25-100	6-100	13-100
Least-most flowering stage difference (0-7)	2010-11	n.a.	n.a.	1.1	1.7	1.5
	2011-12	1.9	0.3	1.9	2.5	3.0
	2012-13	2.7	3.7	3.2	3.2	2.7
	Tree range (all seasons)	1.0-3.0	0-4.0	0-3.5	0.5-3.5	0.5-3.8
Terminals flushing at full bloom (%)	2010-11	8	2	15	5	16
	2011-12	2	41	0.4	1	3
	2012-13	1	3	5	4	8
	Tree range (all seasons)	0-26	0-85	0-47	0-50	0-60
Flushing vigour at full bloom (0-5)	2010-11	1.7	1.7	n.a.	1.7	1.5
	2011-12	1.2	2.9	0.4	0.7	1.1
	2012-13	1.3	1.2	0.9	1.0	2.0
	Tree range (all seasons)	1.0-2.5	0.5-4.0	0-1.5	0.5-2.0	0.5-3.0
Terminals flushing at harvest (%)	2010-11	0	0	0	3	5
	2011-12	5	18	8	5	4
	2012-13	4	6	12	5	1
	Tree range (all seasons)	0-25	0-70	0-30	0-30	0-60
Flushing vigour at harvest (0-5)	2010-11	Nil	Nil	Nil	0.9	2.2
	2011-12	1.3	1.8	1.6	1.4	1.1
	2012-13	1.4	1.6	2.0	1.5	1.4
	Tree range (all seasons)	0.5-2.0	1.0-2.5	1.0-2.5	0-2.5	0.8-3.0

n.a. = not assessed

Table 14 Fruit characteristics of 'B74' mango grown at the five 'B74' mango trial sites in 2010/11, 2011-12 and 2012-13 (data are the means of 15 selected trees per farm).

Fruit characteristic at harvest	Season	Darwin (NT)	Katherine (NT)	Dimbulah (NQld)	Mareeba (NQld)	Childers (SEQld)
Flesh dry matter (%)	2010-11	15.4	18.8	15.5	14.5	17.2
	2011-12	13.7	16.4	13.6	13.1	14.4
	2012-13	17.2	17.3	14.0	13.2	16.0
	All (tree range)	12.7-17.8	15.1-20.0	12.4-16.3	11.7-15.9	13.3-18.8
Flesh colour (1-11)	2010-11	7.6	n.a.	8.6	7.3	10.7
	2011-12	5.8	6.9	6.6	5.4	8.8
	2012-13	n.a.	8.1	6.2	6.4	9.8
	All (tree range)	4.0-9.0	5.4-9.4	5.0-9.8	3.6-10.4	7.6-11.0
Average fruit weight (g)	2010-11	420	365	470	414	516
	2011-12	381	373	408	399	443
	2012-13	379	369	352	379	422
	All (tree range)	351-452	287-491	307-565	352-471	368-706
Fruit blush (1-6)	2010-11	3.5	4.2	4.4	4.1	3.7
	2011-12	4.0	3.0	3.8	3.7	3.9
	2012-13	3.8	3.4	3.9	3.4	3.5
	All (tree range)	2.9-4.6	1.8-4.6	3.2-4.9	2.8-4.7	2.4-5.3

4.3.4.5. Ripening time and other fruit quality characteristics

As the interactions between location and irradiation treatment for time between irradiation treatment (ripening time), firmness and SB were significant (except for the SB data in 2010-11). The results are presented as a factorial analysis (Table 15).

Irradiation treatment significantly slowed down the loss of green skin colour in 'B74'mango fruit by approx. 1-4 d, depending on the location and year (Table 15). A similar pattern was noted in previous trials (Hofman *et al.*, 2010b). There was considerable variation in days to full yellow between seasons and locations, which in part probably reflect the variation in fruit maturity reported in section 4.3.4.3. Delays in skin de-greening after irradiation treatment have also been reported in 'Kensington Pride' mango (Jessup *et al.*, 1988; Boag *et al.*, 1990; McLauchlan *et al.*, 1990). As a result of the delayed degreening, irradiated fruit were generally softer at full yellow than control fruit for most locations and years (Table 15). This suggests that irradiation retards the loss of green colour more than fruit softening.

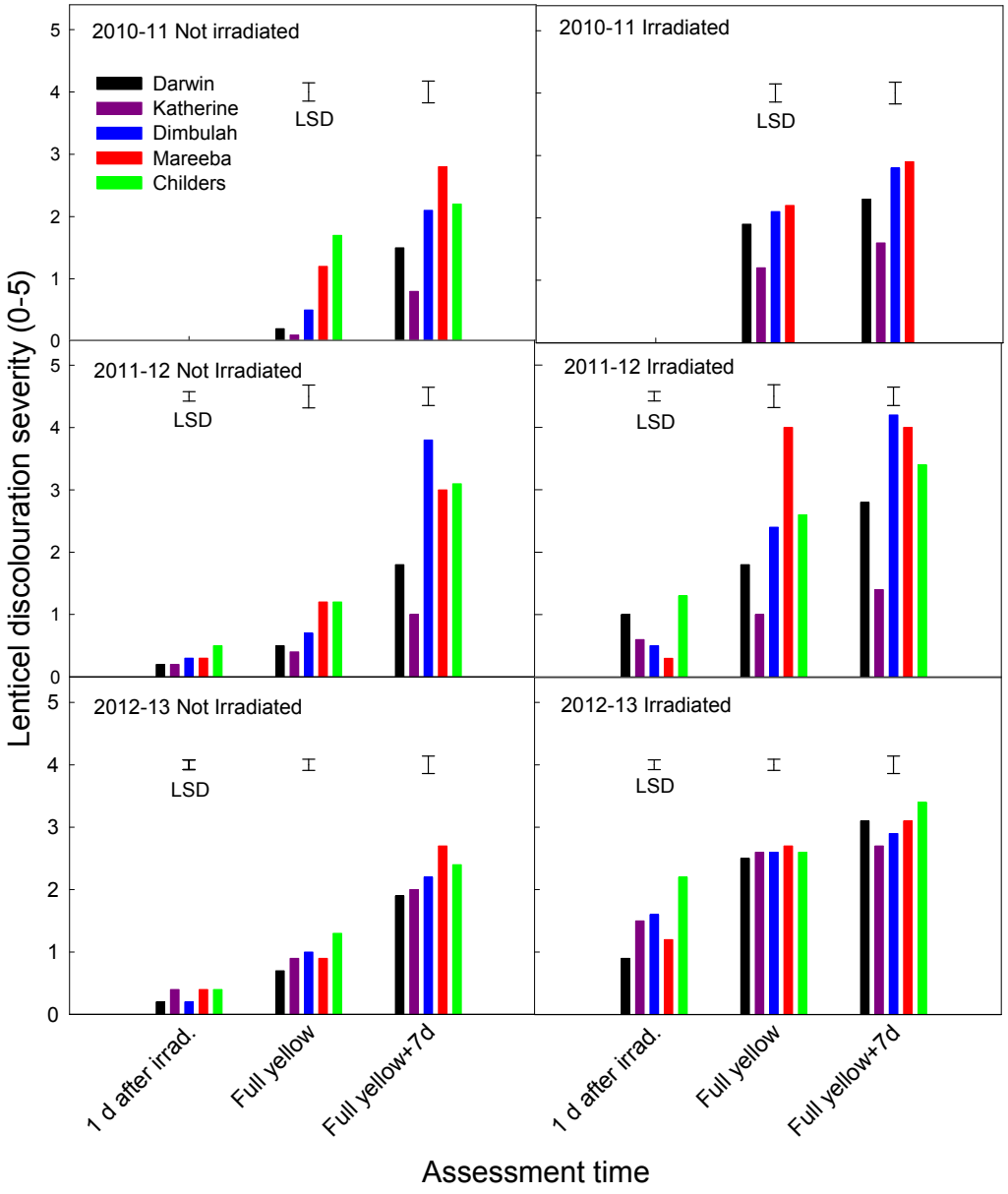


Figure 5 Lenticel discolouration severity in 'B74' mango fruit during ripening at 20°C as affected by growing location and irradiation in the 2010/11, 2011-12 and 2012-13 seasons. Fruit were not irradiated, or irradiated at 350 Gy. Ratings are based on visual assessment of the skin surface area affected (0=nil, 5=more than 50%) assessed 1 d after irradiation, at full yellow and 7 d after. LSD bars indicate the least significant difference at P=0.05 considering both not irradiated and irradiated fruit combined for each season. Each data point in the graph is the mean of 18-20 fruit from 15 selected trees (total of 270-300 fruit). In 2010-11, assessments were not done 1 d after irradiation. Fruit from Childers in 2010-11 were not irradiated due to insufficient fruit number (poor yield).

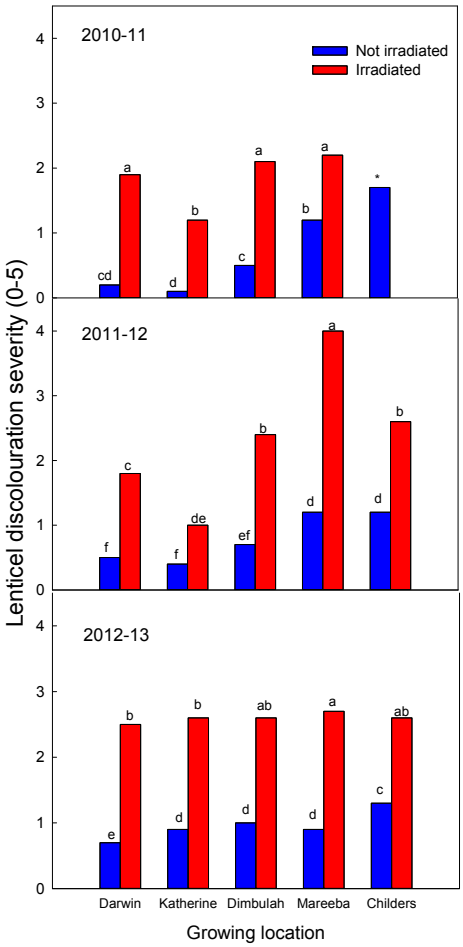


Figure 6 At full yellow: lenticel discolouration severity (0-5) in 'B74' mango fruit at the full yellow (ripe) stage as affected by growing location and irradiation in the 2010/11, 2011-12 and 2012-13 seasons. Fruit were not irradiated or irradiated at 350 Gy. Ratings are based on visual assessment of the skin surface area affected (0=nil, 5=more than 50%). For each season, bars with the same letter are not significantly different at P=0.05 as tested by LSD. Each bar represents the mean of 18-20 fruit from 15 selected trees (total of 270-300 fruit).

Skin browning severity also increased in irradiated fruit 7 d after full yellow compared to control fruit for most locations and seasons (Table 15). Skin browning severity at full yellow was almost nil in all locations and years, for both not-irradiated and irradiated fruit (data not shown). As with LD, similar results of increase in SB severity in 'B74' mango fruit with irradiation treatment were noted in previous trials (Hofman *et al.*, 2010b).

There were generally no diseases in fruit at full yellow across all locations and seasons, and on most farms 7 d after (data not shown).

4.3.4.6. Multivariate analysis

The analysis of the data outputs from multiple linear regression, canonical variates analysis, principal components analysis, and regression/classification trees, showed that multiple regression on the combined data across farms and years was the most useful approach to be adopted in this study, and its key results are presented below. The canonical variates analysis, which relates the set of explanatory variables with groupings of the LD data, was not as appropriate or useful as the multivariate regression, mainly because the LD data is a

continuous variable rather than just groups. The principal components analysis found linear combinations of the traits that explain the maximum variance in the data (explanatory variables), but does not have any direct connection with the LD trait, so are likely irrelevant. The regression tree can be used to predict the LD of new fruit based on the data that have been collected, but it is not easily reported. Outputs from all approaches are presented in Appendix 1.

Table 15 The effects of growing location and irradiation on days to full yellow, firmness, and severity (0-5) of lenticel discolouration (LD) in 'B74' mango fruit at the full yellow (ripe) stage and 7 d after. Data are the means of 18-20 fruit from 15 selected trees (total of 270-300 fruit) per farm per year.

Season	Irradiation	Darwin (NT)	Katherine (NT)	Dimbulah (NQld)	Mareeba (NQld)	Childers (SEQld)
Days to full yellow after irradiation						
2010-11	No	2.4 ^{c}	4.0 ^{b}	2.3 ^{c}	2.5 ^{c}	5.1
	Yes	4.0 ^{b}	5.0 ^{a}	4.9 ^{a}	4.9 ^{a}	n.a.[2]
2011-12	No	8.2 ^{c}	6.5 ^{d}	6.7 ^{d}	6.5 ^{d}	5.3 ^{e}
	Yes	12.1 ^{a}	9.3 ^{b}	9.7 ^{b}	9.7 ^{b}	7.0 ^{d}
2012-13	No	3.9 ^{g}	3.4 ^{g}	5.9 ^{f}	6.9 ^{de}	7.4 ^{cd}
	Yes	6.7 ^{e}	7.5 ^{c}	11.0 ^{a}	10.9 ^{a}	8.9 ^{b}
Firmness at full yellow (0-4)						
2010-11	No	2.5 ^{c}	2.1 ^{d}	2.0 ^{d}	2.0 ^{d}	2.1
	Yes	2.5 ^{c}	3.0 ^{a}	2.7 ^{ab}	2.0 ^{d}	n.a.[2]
2011-12	No	1.9 ^{ef}	2.5 ^{c}	2.7 ^{bc}	1.9 ^{f}	2.0 ^{de}
	Yes	2.8 ^{b}	2.7 ^{b}	3.0 ^{a}	3.1 ^{a}	2.1 ^{d}
2012-13	No	2.2 ^{d}	1.7 ^{e}	1.7 ^{e}	1.8 ^{e}	2.3 ^{d}
	Yes	3.3 ^{a}	2.7 ^{c}	3.1 ^{b}	3.3 ^{a}	2.6 ^{c}
Skin browning severity 7 d after full yellow (0-5) [1]						
2010-11	No	0.0	0.0	0.2	1.7	0.2[2]
	Yes	0.2	0.5	0.8	1.9	n.a.[2]
2011-12	No	0.2 ^{g}	0.7 ^{f}	1.0 ^{ef}	1.1 ^{e}	1.4 ^{e}
	Yes	4.3 ^{b}	1.9 ^{d}	4.6 ^{a}	4.3 ^{ab}	3.1 ^{c}
2012-13	No	0.5 ^{gh}	0.2 ^{h}	0.3 ^{h}	0.8 ^{f}	0.6 ^{fg}
	Yes	4.4 ^{b}	3.3 ^{d}	4.8 ^{a}	4.1 ^{c}	2.9 ^{e}

For each quality attribute and season, means (n= 270-300) with the same letters are not significantly different (P=0.05) as tested by LSD. The absence of letters indicate that the interaction between location and irradiation treatment was not significant.

[1]Each fruit were visually assessed using a severity rating scale of 0 = no LD or skin browning symptoms to 5 = dense pronounced spots on more than 50% of the skin surface area affected.

[2]n.a.= not assessed (only 4 farms were analysed in 2010-11, as fruit from Childers were not irradiated due to insufficient fruit numbers/poor yield in that season).

In the multiple regression analysis, all the tree and fruit characteristics variables (refer to Table 12, Table 13and Table 14) were tested, with LD severity (in both non-irradiated and irradiated fruit data sets) at full yellow as the dependant variable. The final model from the forward stepwise regression included the following terms as the most important ones in the regression equations (refer to Appendix 1): flowering spread (least to most flowering stage difference), trunk difference (above and below the graft union), fruit size and %DM at harvest (fruit maturity). The regression was positive for the first three terms and negative for %DM. That suggests that across all farms and years, less severe LD at full yellow in fruit that had been irradiated was associated with smaller and more mature (higher %DM) fruit from trees that had more uniform flowering and smaller trunk differences. However, this model accounts for only 35% of the variance in LD.

For the non-irradiated fruit, the final model included the following terms as the most important ones in the regression equations: canopy area, flowering spread, fruit size, fruit blush, and %DM. The correlation was positive for the first three terms and negative for the last two. That

suggests that across all farms and years, less severe LD at full yellow in fruit was associated with fruit that were smaller, more mature (higher %DM) and with more blush, from trees with smaller canopy area and with more uniform flowering. However, this model accounts for only 32% of the variance in LD.

In cultivars such as ‘Tommy Atkins’, ‘Keitt’, ‘Kent’, and ‘KP’, LD has been associated with a number of production factors such as irrigation, leaf:fruit ratios, relative humidity and rain conditions at harvest, fruit maturity at harvest (e.g. time of harvest during the season) and harvest method, including de-sapping method and detergent used (Simmons *et al.*, 1995; Bally *et al.*, 1997; Oosthuyse, 1998; Simmons *et al.*, 1998; Self *et al.*, 2006; Cronje, 2009b; Cronje, 2009a; Feygenberg *et al.*, 2014).

4.4. Reducing lenticel discolouration with irrigation

Minh Nguyen (PhD candidate), Peter Hofman, Daryl Joyce, Andrew Macnish, Medan Gupta

This chapter forms part of a current PhD program (Minh Nguyen). The results presented here have not yet been published. The chapter was drafted by the Ph.D. student, and reviewed by the project team.

4.4.1. Summary

Lenticel discolouration (LD) can reduce visual appeal and loss of value in mango. More severe LD has been associated with rain before harvest, and exposure to water after harvest. To test the potential of deficit irrigation to reduce fruit water status at harvest, and thereby reduce LD in ripe 'B74' mango, irrigation was withheld for 3-8 weeks before harvest over two seasons on a commercial farm in the Katherine area of northern Australia (hot, dry tropics). The results indicated that, even with eight weeks withholding, there was little evidence of strong effects on tree and fruit water relations. The treatments had no effect on average tree yield, fruit size or fruit % dry matter, and withholding irrigation for 3-4 weeks increased LD after irradiation. These results indicate good tolerance of 'B74' mango to reduced irrigation, and may provide a cost saving strategy with relatively minor effect on fruit yield will quality. Further research is required to confirm these results, as well as the impact of significant deficit irrigation on subsequent years' tree performance.

4.4.2. Introduction

Lenticels are macropores on the fruit surface that play a role in gas exchange in many fruits such as apple, avocado and mango (Curry *et al.*, 2008; Everett *et al.*, 2008; Hofman *et al.*, 2010b). In mango, lenticels often become discoloured after harvest, and their discolouration manifests as dark tissues around the lenticel cavity. This disorder may be affected by water on fruit before and after harvest from rainfall and water associated with postharvest dipping (O'Hare *et al.*, 1999; Self *et al.*, 2006). Fruit water status possibly plays an important role in lenticel discolouration (LD) as it affects cell turgor and cell function. The water status of fruit can be affected by many factors, such as climate, harvesting and handing techniques and especially irrigation regimes.

Withholding irrigation may have an effect on LD by reducing soil, leaf and fruit water status. Reduction in soil moisture (at -50 and -70 kPa) during the three weeks prior to harvest significantly reduced LD of 'Keitt' and 'Tommy Atkins' mango (Cronje, 2009b). Withholding irrigation for 7.5 weeks or 1.5 weeks before harvest slightly reduced LD of 'Kensington pride' ('KP') mango fruit as compared to control ((Simmons, 1998). However other studies have shown that withholding irrigation in mangoes before harvest had no significant effect on yield, fruit size, fruit quality or postharvest ripening (Lechaudel *et al.*, 2002; Spreer *et al.*, 2009).

Major indicators of soil water status are soil water content and soil water potential (Meurant *et al.*, 1999). Soil type and water table characteristics may also play a key role in tree water status. Trees grown in sandy soils can produce higher water stress than clay soil during withholding irrigation due to the lower water holding capacity of those soils. In drying soil conditions, fruit trees have to adapt to reduced water availability, which then affects the water status of leaves, stems and fruits (Swati *et al.*, 2000; Jongdee *et al.*, 2002; Cronje, 2009b; Al-Yahyai, 2012).

Irradiation is required for phytosanitary treatment when mangoes are exported to some countries such as New Zealand and the USA. Irradiation can reduce mango fruit quality by causing uneven ripening and skin damage (Sivakumar *et al.*, 2011). Irradiation delayed ripening of 'Tommy Atkins' mango (Durigan *et al.*, 2004). Irradiation at 300-900 Gy resulted in LD on 'Tommy Atkins' and 'Keitt' mango fruit, but did not affect LD on 'Kent' mango (Grové

et al., 2004). The carotene content of 'P' mango was not significantly affected when the fruit had been irradiated at 75, 300 and 600 Gy (Mitchell *et al.*, 1990).

The water status of fruit may contribute to LD when fruit is treated with irradiation, and fruit with low cell turgor may be less sensitive to irradiation. Fruit with low water status may have lower activity of enzymes associated with browning reactions in plants, such as polyphenol oxidase (PPO) which oxidises phenolic compounds into brown-coloured polymeric compounds. In addition, withholding irrigation, particularly in the latter stages of fruit growth, may reduce excessive fruit expansion, which may be a contributing factor to changes to the lenticels that make them more susceptible to damage after harvest (Simmons, 1998).

Water potential can be measured by several methods such as the pressure chamber, vapour pressure withholding, and "equilibrium" methods. The last method is non-destructive and has been applied to fruits and vegetables such as carrot and cherries (Jobling *et al.*, 1997). The gape size that forms between the cut surfaces of the fruit is affected by the fruit water status and can be expressed as a gape measurement (Hatfield and Knee, 1988). Soil and tree water status can be estimated using soil water content and potential, leaf stomatal conductance, leaf and fruit water potential and relative water content.

The objective of the present experiments was to study the effect of water withholding on LD of 'B74' mango. We hypothesise that reducing water availability during later fruit growth can minimise LD on the ripe fruit, especially when fruit is irradiated.0 Irrigation was withheld from trees grown on a commercial orchard in the dry, hot tropics of Katherine (Northern Territory) in two seasons. The above tests for soil, tree and fruit water status were applied. Additional fruit were irradiated at the commercial phytosanitary dose, the fruit ripened and ripe fruit quality assessed. Histochemical studies were also undertaken.

4.4.3. Materials and methods

4.4.3.1. Fruits and treatments

2012

The trials were conducted on a commercial 'B74' mango farm in Katherine, Northern Territory (NT) (14°595"S; 132°002"E) in 2012 and 2013. There is usually minimal rainfall in the catchment area before fruit harvest, however to minimise the effects of any rainfall, black plastic (4 m width) was placed under trees of the withholding irrigation treatments to minimise any rain effects on soil and tree water status. Weather data (rainfall, temperature and relative humidity) were recorded from a weather station in the orchard.

In 2012, two irrigation treatments were applied on K1 farm; nil irrigation (no irrigation from three weeks before harvest) and standard commercial irrigation by one micro-sprinkler under each tree and irrigating once per day. The irrigation treatments were applied to separate, but adjacent rows. The control treatment row received the standard commercial irrigation as above. For the nil irrigation row the main irrigation line was cut and plugged at the start of the 20 trees. These trees had plastic placed on the ground extending to the drip zone of each tree. The 8-10 m spacing between rows minimised the interaction between the control and the no irrigation treatments.

Each irrigation treatment included a total of 20 trees, with 10 datum trees selected with similar tree size and fruit load. One day before harvest, stomatal conductance of leaves was measured for both irrigation treatments (see below). Fruit were harvested at commercial harvest time at dry matter of approx. 15%. Sixteen fruit of typical size per datum tree were harvested, placed in the shade in crates (without exposure to water or solutions). Fruit were marked with tree number and treatment name, and then transported to the research laboratory, Katherine, Northern Territory on the same day of harvest for postharvest treatments as described in Table 16.

A total of 320 fruit were harvested from the two irrigation regimes (16 fruit per tree). Four fruit per tree were either treated with water at harvest or not, or irradiated or not (Table 16). There

were a total of eight treatments (Table 1) and 40 fruits per treatment (four fruit per tree per treatment). For postharvest water treatments, fruit were dipped in DI water for 2 min within 2 h of harvest to determine the effects of water after harvest.

Table 16 Treatments applied to 'B74' mango trees and fruit for the irrigation trial in 2012.

Treatments	Description
Withholding Irri, No Water, No Irra	Fruits from withholding irrigation were not dipped in deionised (DI) water at harvest or irradiated
Withholding Irri, Water, No Irra	Fruits from withholding irrigation were dipped in DI water for 2 min and were not irradiated
Withholding Irri, No Water, Irra	Fruits from withholding irrigation were not dipped in DI water, but were irradiated
Withholding Irri, Water, Irra	Fruits from withholding irrigation were dipped in DI water for 2 min and were irradiated
Standard Irri, No Water, No Irra	Fruits from standard irrigation were not dipped in DI water or irradiated.
Standard Irri, Water, No Irra	Fruits from standard irrigation were dipped in DI water for 2 min and were not irradiated
Standard Irri, No Water, Irra	Fruits from standard irrigation were not dipped in DI water, but irradiated
Standard Irri, Water, Irra	Fruits from standard irrigation were dipped in DI water for 2 min and irradiated.

Forty fruits (four fruit per tree) of the no water, and forty fruit (four fruit per tree) of the water treatment (no irradiation), were assessed at the research laboratory in Katherine for fruit water content, relative water content index, water potential, water uptake and gape. The remaining 80 fruits from four non-irradiated treatments were placed in 7 kg single layer trays and transported to Darwin, air-freighted to Brisbane, and then transported by car to the Maroochy Research Facility (MRF) laboratories at Nambour, Queensland for ripening and assessment.

Fruit from the treatments with irradiation (160 fruits) were transported by air conditioned car to Steritech Pty Ltd (a commercial irradiation facility near Brisbane, about 60 min from MRF). The fruit were randomly assigned to 10 kg single layer cardboard trays. A dosimeter (Opti-chromic detectors FWT-70-40 Min) was positioned in the middle of each tray so as to be covered by the fruit and measure irradiation doses. Trays were covered with 4 mm plywood lids with a 15 mm low density foam lining to prevent fruit movement. Trays were placed on their side in a rack designed to fit on 1 m high bins used for irradiating other products. This ensured more consistent doses between trays of the same treatment. Fruit in cardboard trays were exposed to a dose aimed at achieving approximately 550 Gy (commercial rate) of gamma irradiation from a Cobalt 60 source at 20°C. To achieve this approximate dose each rack occupied 12 positions within the chamber at 1 min 40 s in each position. The average received dose was 494 Gy (min.-max. of 408-629 Gy). After irradiation, the fruits were transported by car to the MRF laboratories. Forty fruits (four fruit per tree) of the no water, and forty fruit (four fruit per tree) of the water treatment (irradiation), were assessed for fruit water content, relative water content index, water potential and gape. The remaining 80 fruits from four irradiated treatments were ripened with 10 $\mu L.L^{-1}$ ethylene at 20°C for 2 d, and then ripened at 20°C. During ripening, skin colour, firmness and LD were assessed every 3 d.

2013

In the second year (2013), two growing sites with differing soil type were selected in the same farm as the previous year: Site 1 (K1- block 16) was the same site as used in 2012 and had relatively higher water holding capacity soil. Site 2 (K2 - block 43) had potentially lower water holding capacity. At Site 1 (K1) three irrigation regimes were applied (Table 17):

- standard irrigation (similar to commercial irrigation as above; row 26)
- withholding irrigation for four weeks before harvest (row 27)
- withholding irrigation for eight weeks before harvest at (row 28).

Two irrigation treatments were applied at site 2 (K2)

- standard irrigation as above (row 27)
- withholding irrigation for four weeks before harvest (row 28).

Tree numbers per treatment and harvest procedures were similar to those used in 2012. A total of 250 fruit were harvested from the five irrigation regimes (five fruit per tree).

One day and two days before harvest, stomatal conductance of leaves was measured (see below). At harvest, 20 fruits from each treatment (2 fruit per tree, 100 fruit for the five irrigation treatments) were assessed at the research laboratory in Katherine for water content, relative water content index, water potential, and gape.

Table 17 Treatments applied to 'B74' mango trees for the irrigation trial in 2013

Treatments	Description
K1-I (Site 1, Commercial Irrigation)	Fruit trees from the 2012 trial site, with relatively higher water holding capacity and following commercial irrigation
K1-4 (Site 1, Withholding 4 wk)	As above but withholding all irrigation from four weeks before harvest
K1-8 (Site 1, Withholding 8 wk)	As above but withholding all irrigation from eight weeks before harvest
K2-I (Site 2, Commercial Irrigation)	Fruit trees from a new site with relatively lower water holding capacity; commercial irrigation
K2-4 (Site 2, Withholding 4 wk)	As above but withholding all irrigation from four weeks before harvest

The remaining 150 fruits from five irrigation treatments were transported by car to Darwin, and air-freighted to Brisbane. The fruit were transported by car and irradiated at Steritech (Narangba) with the average dose of 409 Gy (min.-max. of 358-489 Gy). After irradiation, the fruits were transported by car to MRF. The fruit were ripened with 10 $\mu L.L^{-1}$ ethylene at 20°C for 2 d, and then ripened at 20°C. During ripening, skin colour, firmness and LD were assessed every 3 d.

4.4.3.2. Assessments

Tree yield and fruit size

The day before harvest, the total fruit number per tree and the number of fallen fruit were recorded. At harvest the fruit required for postharvest assessment were harvested (average fruit size for each tree) and weighed. The average fruit yield (kg) per tree was calculated from the fruit number per tree and the average fruit weight.

Soil water content

In 2012, soil was sampled at 3, 2, 1 and 0 weeks before harvest at 0-15, 15-30, and 30-60 cm depth, with approximately 50 g soil per sample. The samples were taken from four sampling points (replications) along each treatment row, with 3-4 core samples taken per sampling point. In 2013, at site K1, soil samples were taken with a soil corer at 8, 6, 4, 2, 1 and 0 weeks, and at site K2 at 4, 2, 1 and 0 weeks before harvest. The samples were taken from three sampling points along the treatment row, with 3-4 core samples taken per sampling point, and from 0-10 cm, 10-20 cm and 20-30 cm depth (about 50 g per sample). Samples were weighed and recorded for wet weight (WW) and dried at 105-110 C° until constant weight. The samples were then weighed immediately for dry weight (DW) and soil moisture (SM) content calculated by the formula: SM (%) = 100 x (WW – DM)/WW.

Soil water potential

Soil water potential was monitored at a depth of 30 cm using tensiometers (Meurant *et al.*, 1999). Three 30 cm tensiometers (Soilspect Tensimometer Systems) were used per row

(treatment) and were placed in an active root zone towards the edge of the canopy (one near the start of the row, one in the middle trees of the row and one near the end of the row). Tensiometers were assembled and filled with good quality water to which algaecide was added. All tensiometers were left to stand in a bucket of water for 1 d before installation and were taken to the site with the tips in water or wrapped in wet rags. A suitable auger and a hammer were used to make a hole to the required depth. The 30 cm tensiometers were carefully pushed into the soil. If the soil was too sandy, clay was packed around the ceramic tensiometer tips to provide an adequate contact continuum. The tensiometers were checked every 2-3 d and topped up with more algaecide-treated water if necessary. The tensiometer soil water potential was recorded before 8 am at 8, 6, 4, 2, 1 and 0 weeks before harvest.

Leaf stomatal conductance

Stomatal conductance was measured for each treatment using a portable steady state porometer (Decagon Devices, SC-1 Leaf Porometer). For each tree, three fully expanded upper canopy leaves, adjacent to fruit, were selected. The measurements were recorded from the lower side of the leaves attached on the tree. Thirty leaves (30 replications) were selected per treatment. Measurements were made every two hours between 6.00 and 18.00 during the day before harvest.

Leaf relative water content and water content

At harvest, ten healthy, mature leaves per tree (fully developed topmost leaves) on the shoots bearing fruits were harvested from each tree and placed in plastic bags and in a covered tray, and then transported to the laboratories in Katherine, Northern Territory for assessing within 1 h of harvest. Leaves were cleaned with soft paper towel. For each leaf, the section in the middle of the leaf was sampled with a 1 cm diam. cork borer. Fresh leaf disks were weighed (FW) using analytical scales (0.001 g). The leaf discs with abaxial sides down were placed on filter paper (Whatman®), pre-moistened with distilled water and equilibrated in a covered petri dish, then held in the covered dish for 3 to 4 h at room temperature with laboratory lighting. After quickly blotting the leaf discs with tissue paper, the discs were reweighed and recorded as turgid weight (TW). Samples were dried at 65°C for about 72 h and weighed (dry weight -DW) (Boyer *et al.*, 2008; Elsheery and Cao, 2008). Leaf relative water content (RWC %) and water content (WC %) were calculated from the formulas: RWC (%) = 100 x (FW – DW)/(TW-DW); WC (%) = 100 x (FW – DM)/FW.

Fruit dry matter (%)

For each treatment, 10 fruits (one per tree, 10 replications) were selected and flesh sample taken from the cheek of the fruit to a depth of 3 cm. Each sample was weighed and recorded (FW). The % dry matter (DM) of the fruit pulp was determined by drying at 65°C to constant weight (Lechaudel *et al.*, 2002; Hassan *et al.*, 2009), weighing the dried sample (DM) and using the formula: DM (%) = 100 x DM/FW (%).

Fruit relative water content (RWC %)

Thin pericarp tissue slices were excised to measure the relative water content. For each treatment, one flesh sample per fruit (10 flesh samples from 10 individual fruit) at the middle of a cheek was cut by a knife. Five discs (approx 1 cm diameter and 5 mm thick) from each mango flesh sample were cut using a 1 cm diam cork borer. Five discs from each mango were weighed (FW). The discs, with skin down, were placed on filter paper (Whatman®), soaked in distilled water for 2 h in a covered petri dish at 20°C in the dark to rehydrate. After 2 h, samples were blotted dry, reweighed and recorded as turgid weight (TW). They were then placed in an oven at 65°C for 2-3 d to determine DW. Relative water content index was calculated from the formula: RWC (%) = 100 x (FW – DW)/(TW-DW) (%) (Burdon and Clark, 2001).

Fruit water potential

The tissue water potential was determined using the method of Burdon and Clark (2001), with modification. Five disks (each disc of 10 mm diameter and 2 mm thick) of the outer pericarp from the middle of a cheek of each of 10 fruit per treatment were removed and immersed in a series of polyethylene glycol (PEG) 6000 solutions (0, 100, 200, 300, 400 $g.L^{-1}$ H_2O) at 25°C for 3 h. Disks were weighed before and after dipping using analytical scales. The water potential at which there was neither gain nor loss of weight was recorded.

Fruit gape

Gape is the width of the slit that develops after placing a small cut in the fruit (Hatfield and Knee, 1988; Shaftner and Conway, 1988). Twenty green mature mango fruits per treatment (two per tree; total of 20 replications) were longitudinally cut in the cheek area (one cut per fruit) to a depth of 1 cm and 2 cm long with a sharp, thin, narrow blade. After thirty minutes, the length and width of the slit at the widest point was measured with digital verniers to within 0.01 mm.

Fruit quality

Fruit quality at harvest and during ripening was assessed on 20 fruit per treatment (two fruit per tree). Fruit firmness was assessed using the Aweta Acoustic Firmness Tester (Aweta, the Netherlands). This is a non-destructive system based on the analysis of resonance frequencies when the fruit surface is tapped. The reading decreases as the fruit soften.

Skin colour (1-6), hand firmness (0-4) and LD (0-5) were rated as described in Hofman *et al.* (2010a), as outlined in section 4.1.3.3.

Lenticel morphology by light microscopy

Skin samples were obtained from five fruit per treatment and typical morphology observed using the methods described in section 4.1.3.3.

4.4.3.3. 4.2.4. Statistical analysis

Statistical analyses were performed by Genstat® 14 for Windows™ (VSN International Ltd., UK). Analysis of variance (ANOVA) used the 'General Analysis of Variance' model, with irrigation as the 'treatment' structure. The protected least significant difference (LSD) procedure at P=0.05 was used to test for differences between treatment means.

4.4.4. Results

4.4.4.1. 2012

Temperature, humidity and rainfall

There was no rain from flowering (May) to August, and little rain from three months before harvest (Table 18).

Soil moisture content

The irrigation treatments had no significant effect on soil moisture content at 2-3 weeks before harvest (Table 19). During the last week before harvest, soil moisture content for withholding irrigation were lower than for standard irrigation at all three soil depths. In the standard irrigation treatment, the 0-15 cm layer had higher water content than the 15-60 cm layers one week before harvest. With withholding irrigation, the differences in soil water content for the three soil depths were not significant.

Table 18 Average day and night temperature and relative humidity (RH), and the total monthly rainfall at the irrigation trial sites in 2012 and 2013.

Month	Average temperature		Average RH (%)		Rainfall (mm)
	Day	Night	Day	Night	
2012					
May	25.4	21.1	52.7	69.4	0
June	22.8	17.2	47.3	63.5	0
July	23.9	19.4	43.3	54.0	0
August	25.6	18.9	40.1	50.6	0
September	30.5	24.7	40.4	47.0	38
October	32.6	28.2	45.4	52.3	1
November	32.7	28.2	60.4	75.6	138
2013					
September	32.4	27.9	50.0	56.6	5*
October	33.8	29.0	47.0	55.7	10
November	32.2	27.9	53.2	60.5	3**

*20-30 September
**1-14 November

Table 19 Change in soil moisture content (%) at three soil depths at 3, 2, 1, 0 weeks before harvest, from an irrigation trail on a commercial 'B74' mango farm, where the trees were either given standard commercial irrigation, or no irrigation from three weeks before commercial harvest in 2012.

Soil depth (cm)	Soil moisture content (%)	
	Standard irrigation	Withholding irrigation *
3 wk before harvest		
0-15	9.7	7.8
15-30	7.8	6.5
30-60	7.6	6.9
2 wk before harvest		
0-15	8.9	4.7
15-30	7.2	4.4
30-60	7.0	5.7
1 wk before harvest		
0-15	10.1 [a]	6.0 [c]
15-30	8.0 [b]	5.5 [c]
30-60	7.6 [b]	5.9 [c]
At harvest		
0-15	8.7 [a]	4.7 [c]
15-30	7.4 [b]	4.6 [c]
30-60	7.3 [b]	5.1 [c]

* Irrigation withheld for three weeks before harvest.
Means (n=4) for each date without letters or with the same letters are not significantly different (P=0.05) as tested by LSD.

Soil water potential

There were no irrigation treatment effects on soil water potential (Table 20).

Leaf stomatal conductance

The pattern of stomatal conductance over the day was similar for both irrigation treatments, with the maximum conductance recorded between 1200-1400 h (Figure 7). Differences in stomatal conductance between the two treatments were not significant from 6.30 am to 12.30 pm. However, in the afternoon until 1830 hr, 'B74' trees subjected to withholding irrigation had significantly lower stomatal conductance compared to trees under normal irrigation.

Table 20 Change in soil water potential (%) at two soil depths at 2, 1, 0 weeks before harvest, from an irrigation trail on a commercial 'B74' mango farm, where the trees were either given standard commercial irrigation, or no irrigation from three weeks before commercial harvest in 2012.

Soil depth (cm)	Soil water potential (KPa)	
	Standard irrigation	Withholding irrigation
2 weeks before harvest		
0-15	-6.5 [b]	-5.5 [b]
15-30	-7.0 [ab]	-10.5 [a]
1 week before harvest		
0-15	-7.0	-9.0
15-30	-7.5	-9.5
At harvest		
0-15	-9.0	-9.5
15-30	-9.5	-10.0

Means (n=3) for each time without letters or with the same letters are not significantly different (P=0.05) as tested by LSD.

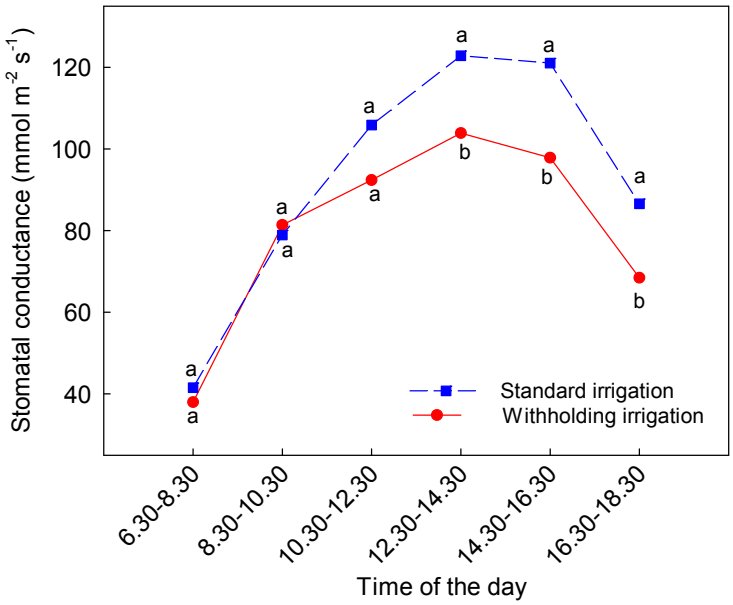


Figure 7 Stomatal conductance of the leaves of 'B74' mango trees receiving standard irrigation or no irrigation, from three weeks before harvest. Means at the same time measurement with the same letters are not significantly different (P=0.05) as tested by LSD.

Leaf relative water content and water content

Neither the leaf relative water content nor the leaf water content showed significant treatment differences. Across the two treatments, the trees had an average relative leaf water content of 66.8 and 39.0% leaf water content.

Fruit gape and fruit water status

Table 21 indicates that fruit from the withholding irrigation treatment developed a lower gape, as expected from fruit with lower water status. This difference was not observed after dipping fruit in water for 2 min soon after harvest, suggesting a fairly rapid uptake of water. The irradiation fruit produced significantly smaller gapes because the test was applied 2-3 d later,

following transport to south-east Queensland and irradiation. Even so, the results suggested that the withholding irrigation fruit still had lower water status after this time.

Table 21 The gape (mm) of a cut (1cm deep x 2 cm long) in 'B74' mango fruit after 30 min. The fruit were harvested from an irrigation trail on a commercial 'B74' mango farm, where the trees were either given standard commercial irrigation or no irrigation from three weeks before commercial harvest in 2012. The test was done within 1 d of harvest (no irradiation), or after irradiation (about 4 d after harvest).

Irrigation treatment	No water		Water	
	No irradiation	Irradiation	No irradiation	Irradiation
Standard irrigation	1.1 ^{e}	0.4 ^{b}	1.0 ^{d}	0.4 ^{b}
Withholding irrigation	0.9 ^{c}	0.3 ^{a}	1.0 ^{cd}	0.3 ^{a}

Means (n=20) with the same letters are not significantly different (P=0.05) as tested by LSD.

Fruit water potential

'B74' fruit water potential varied from -0.5 to -1.2 MPa across treatments (Table 22). There was little significant irrigation or postharvest water dip treatment effect on fruit water potential.

The water potential of irradiated treatments was significantly more negative than that of non-irradiated treatments, most likely because of the 2-3 d delay in water potential measurement.

Table 22 Fruit water potential (MPa) of 'B74' mango fruits from an irrigation trail on a commercial 'B74' mango farm, where the trees were either given standard commercial irrigation or no irrigation from three weeks before commercial harvest in 2012. Fruit were either dipped in water for 2 min after harvest, or not. The test was done within 1 d of harvest (no irradiation), or after irradiation (about 4 d after harvest).

Irrigation treatment	No water		Water	
	Irradiation		Irradiation	
	No	Yes	No	Yes
Standard irrigation	-0.6 ^{cd}	-1.1 ^{ab}	-0.5 ^{d}	-1.0 ^{b}
Withholding irrigation	-0.8 ^{c}	-1.2 ^{a}	-0.6 ^{d}	-1.2 ^{a}

Means (n=10) with the same letters are not significantly different (P=0.05) as tested by LSD.

Fruit relative water content

Fruit relative water content values varied from 94.1 to 95.2%, however there were no significant treatment effects.

Dry matter, firmness, fruit yield and fruit size

There were no irrigation treatment effects on fruit % DM at harvest (average of 15.7%, fruit number per tree (average of 189), average fruit weight (311 gm) or average tree yield (58.8 kg per tree).

Fruit quality

Irrigation treatment had no significant effect on LD when the fruit had reached full yellow skin colour (Table 23). The only significant irrigation effect was when withholding irrigation increased LD at 7 d after full yellow from no irrigation following water dipping and irradiation. Irradiation consistently increased LD across all treatments except standard irrigation, no water 7 d full yellow. The water treatment had no effect on LD at full yellow but increased LD 7 d after full yellow in most instances.

There were no treatment effects on fruit firmness at full yellow, or on SB (data not presented).

Table 23 Lenticel discolouration (0-5) at full yellow and 7 d after full yellow of 'B74' mango fruit from an irrigation trail on a commercial 'B74' mango farm, where the trees were either given standard commercial irrigation or no irrigation from three weeks before commercial harvest in 2012.

Irrigation treatment	No water		Water	
	Irradiation		Irradiation	
	No	Yes	No	Yes
At full yellow				
Standard irrigation	0.4 [a]	0.9 [bc]	0.5 [ab]	1.0 [c]
Withholding irrigation	0.3 [a]	1.1 [c]	0.3 [a]	1.1 [c]
7 d after full yellow				
Standard irrigation	0.9 [a]	1.5 [abc]	1.8 [c]	2.5 [d]
Withholding irrigation	1.1 [ab]	3.2 [e]	1.6 [bc]	2.9 [de]

Means (n=20) with the same letters are not significantly different (P=0.05) as tested by LSD.

4.4.4.2. Lenticel morphology

The typical lenticel morphology of 'B74' fruits at full yellow was affected by irradiation (Plate 15). More extensive browning in the cells around the lenticel cavity was observed in irradiated compared to non-irradiated fruit. Neither irrigation nor water treatment had any obvious effect on lenticel morphology.

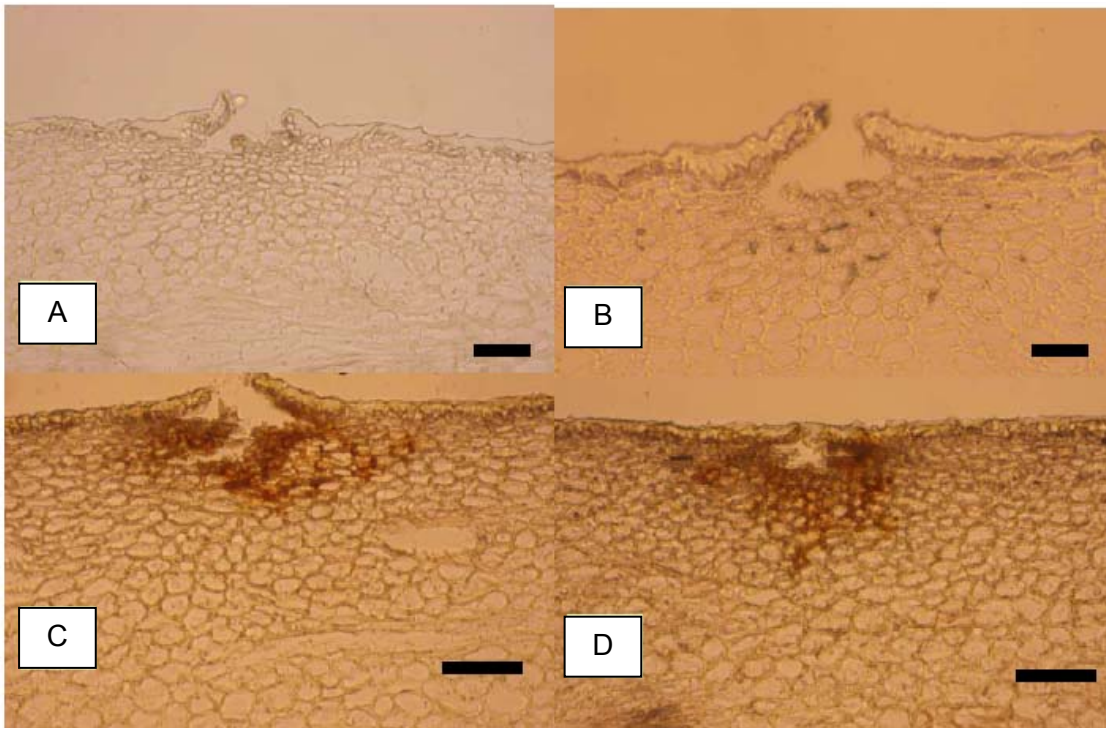


Plate 15 Unstained transverse sections of 'B74' at full yellow; withholding irrigation + no water + no irradiation (A); withholding irrigation +water + no irradiation (B); withholding irrigation + no water + irradiation (C); withholding irrigation + water + irradiation (D). Scale bar = 100 µm.

4.4.4.3. 2013

The temperature and rainfall

There was relatively little rain during the fruit growth period in 2013 (Table 18).

Soil moisture content

The soil water content at both sites was significantly affected by irrigation treatment (Figure 8). At K1, withholding irrigation from eight weeks before harvest significantly reduced soil moisture content at all measured depths by week four, while withholding irrigation for four weeks resulted in significant lower soil water content differences by two weeks before harvest compared with the control. Therefore, the eight week treatment provided at least four weeks of significant soil moisture content difference, compared to only two weeks for the four week withholding treatment. In K2, a significant irrigation effect was noticed by two weeks before harvest.

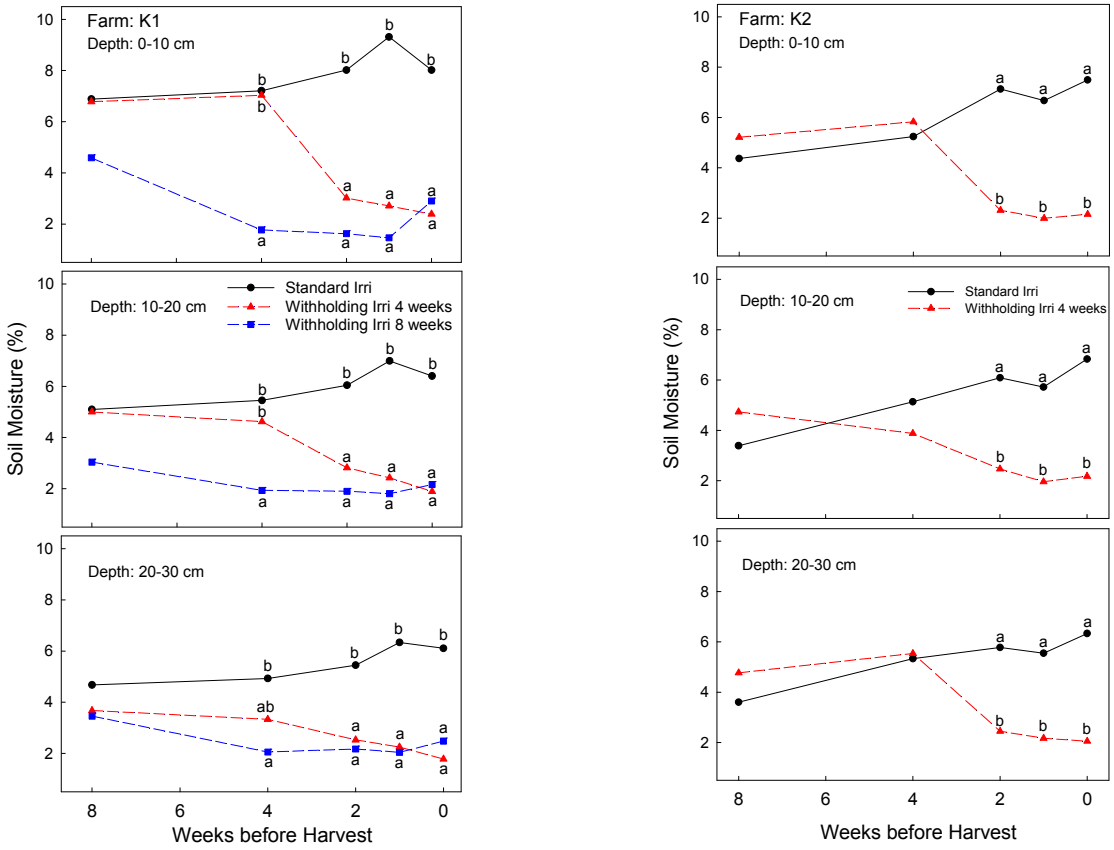


Figure 8 Soil water content (%) at three soil depths at 8-0 weeks before harvest, from irrigation trials on two blocks two sites (K1 and K2) on a commercial 'B74' mango farm. The trees were either given standard commercial irrigation, or no irrigation from either eight of four (K1) or four (K2) weeks before commercial harvest in 2013. The same letters at the same sample week are not significantly different (P=0.05).

Soil water potential

Soil water potentials at 30 cm depth increased gradually during irrigation withholding (Figure 9). The increasing trends were similar for both sites, K1 and K2. There was a significant difference at one week before harvest at K1, but the difference was not significant at harvest

because of increasing soil water potential in the irrigated treatment. At K2, there were no significant differences between the commercial irrigation and withholding irrigation at any assessment time.

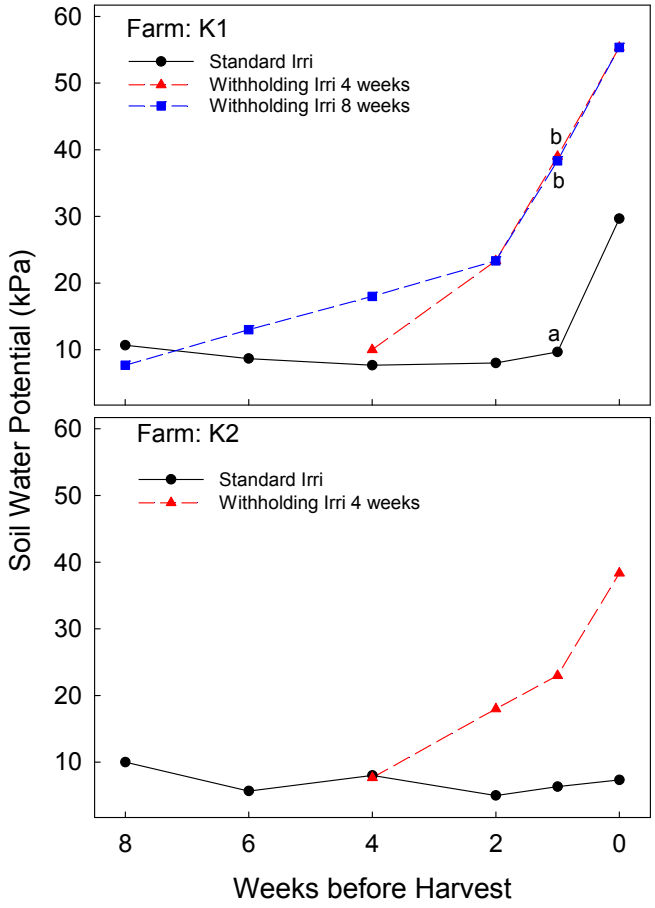


Figure 9 Soil water potential at 8-0 weeks before harvest, from irrigation trials on two sites (K1 and K2) on a commercial 'B74' mango farm. The trees were either given standard commercial irrigation or no irrigation, from either eight or four weeks before commercial harvest in 2013. Means with the same letters for the same assessment time are not significantly different (P=0.05).

Stomatal conductance

K1 leaves from the no irrigation trees had significantly lower stomatal conductance between 900-1500 h compared with irrigated trees (Figure 10). The lack of increase between 1100-1300 h suggested sufficient water stress to restrict further opening of the stomata. The similar pattern in the irrigated treatment suggests some level of water stress in this treatment also. There was no difference in stomatal conductance between the two withholding irrigation treatments.

In K2, the lower stomatal conductance with no irrigation again suggests an irrigation treatment response on tree water status, while the pattern for the irrigated treatment suggests relatively little effect of tree water status on stomatal conductance (Figure 10).

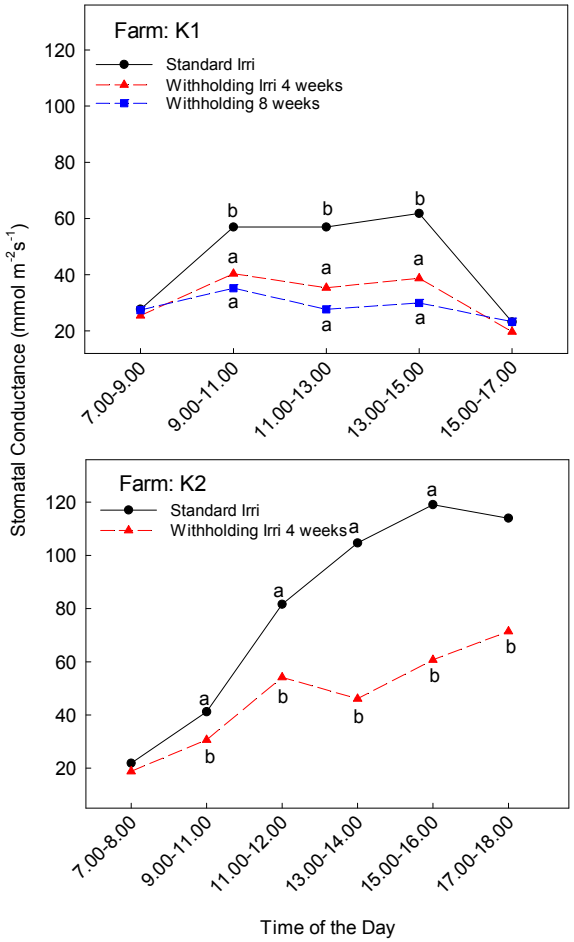


Figure 10 Stomatal conductance 1-2 days before harvest, of the leaves of 'B74' mango trees from irrigation trials on two sites (K1 and K2) on a commercial 'B74' mango farm. The trees were either given standard commercial irrigation or no irrigation from either eight or four weeks before commercial harvest in 2013. Means with the same letters for the same assessment time are not significantly different (P=0.05).

Fruit water potential and gape

The eight week treatment at K1 had a less negative water potential compared with the irrigation treatment (Table 24), contrary to expectations. There was no effect from the four week treatment at K1 or the irrigation treatments at K2. However, the smaller gapes from the four week K1 and K2 treatments suggested lower water status in these fruit.

Fruit dry matter, tree yield and fruit size

There were no treatment effects on % dry matter or total tree yield at either K1 or K2.

Fruit quality

The eight week K1 treatment lost significantly less weight during the middle and later stages of ripening compared to the four week and irrigation treatments (Figure 11). A similar response was observed with the no irrigation treatment on K2. This suggests that these treatments had some effect on fruit physiology, possibly through fruit water relations.

Table 24 Fruit water potential (MPa) of 'B74' fruit from irrigation trials on two sites (K1 and K2) on a commercial 'B74' mango farm. The trees were either given standard commercial irrigation or no irrigation from either eight or four weeks before commercial harvest in 2013. Means from the same site and column with the same letters are not significantly different (P=0.05).

Site/Treatment	Water potential	Gape (mm)
K1		
K1- Standard	-1.5 ^{ab}	0.73 ^{b}
K1-4 weeks withholding	-1.6 ^{a}	0.57 ^{a}
K1-8 weeks withholding	-1.3 ^{b}	0.63 ^{ab}
K2		
K2-Standard	-1.2	1.11 ^{b}
K2-4 weeks withholding	-1.2	0.89 ^{a}
	ns	

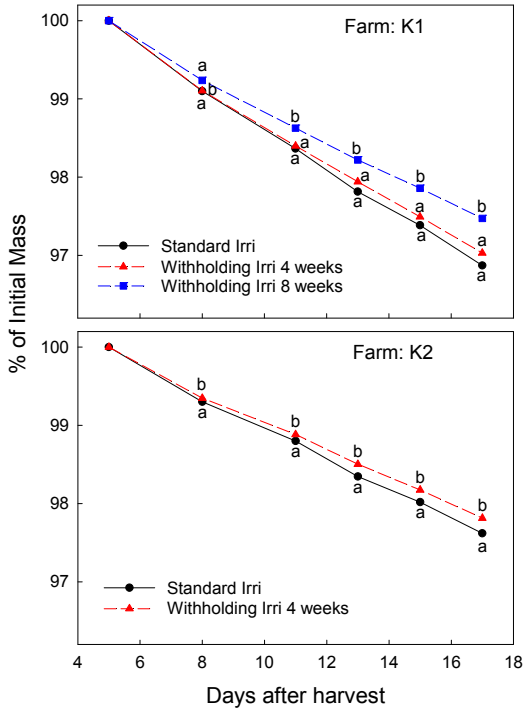


Figure 11 Weight loss of 'B74' fruit from irrigation trials on two sites (K1 and K2) on a commercial 'B74' mango farm. The trees were either given standard commercial irrigation or no irrigation, from either eight or four weeks before commercial harvest in 2013. The fruit were ripened at 20ºC. Means at the same assessment time with the same letters are not significantly different (P=0.05).

Fruit firmness declined during ripening, but there were no significant treatment effects on fruit firmness on any of the days measured during ripening (data not presented).

Lenticel discolouration was significantly higher in the four week treatments at both sites, at full yellow and 7 d later (Table 25). Discolouration with eight weeks withholding at K1 was similar to the irrigated treatment.

Table 25 Lenticel discolouration of 'B74' fruit from irrigation trials on two sites (K1 and K2) on a commercial 'B74' mango farm. The trees were either given standard commercial irrigation or no irrigation, from either eight or four weeks before commercial harvest in 2013. The fruit were ripened at 20ºC.

Site/treatment	Lenticel discolouration (0-5)	
	At full yellow	7 d after full yellow
K1		
K1- Standard	3.2 [a]	3.1 [bc]
K1-4 weeks withholding	4.1 [b]	4.0 [d]
K1-8 weeks withholding	3.4 [a]	3.4 [c]
K2		
K2-Standard	2.2 [a]	2.0 [a]
K2-4 weeks withholding	3.0 [b]	2.9 [b]

Means within each site and column with the same letters are not significantly different (P=0.05) as tested by LSD.

4.4.5. Discussion

Previous research suggested that rain during later fruit growth (Oosthuyse, 1998), and exposure to water after harvest (Hofman *et al.*, 2010b) increases LD on the ripe fruit. Also, withholding irrigation for six weeks before harvest can reduce LD in 'KP' (Simmons *et al.*, 1998). The present trials were designed to test the potential of withholding irrigation to reduce LD in 'B74' fruit grown in the hot, dry tropics through fruit water relations. However, there were few fruit or tree responses to the irrigation treatments.

The 2012 results suggested a reduction in soil moisture content during the last week before harvest, but the nil treatment effects on soil water potential suggests the lower moisture content would likely have little effect on the ability of the trees to extract water from the soil. The lower stomatal conductance in the non-irrigated trees implied some treatment effect on tree performance, but it was likely relatively mild, since under stronger water stress the stomatal conductance declines during the hotter parts of the day to reduce excessive water loss. The other measures of tree and fruit water status did not indicate a strong treatment effect on water status.

Small treatment effects were also obtained in 2013, despite longer periods with no irrigation. With K1, the results indicated irrigation treatment effects on soil water potential at two weeks before harvest, but no significant difference at harvest primarily because of the more negative water potential in the irrigated treatment. This was reflected in the absence of increasing stomatal conductance from mid morning to early/mid afternoon in the irrigated trees, indicating some reduction in water status resulting in stomatal closure. There was no evidence of statistically significant treatment effects on soil moisture potential, while stomatal conductance suggested a small treatment effect that was not reflected in leaf water status. Fruit water status measures were inconclusive, with the gape suggesting treatment responses that were not consistently reflected in the other water status measures.

The small treatment effects on tree and fruit water status not surprisingly resulted in small effects on fruit quality. Where treatment effects were noted, in both years deficit irrigation increased LD, suggesting that any beneficial effect of irrigation treatment on turgor of the cells around the lenticel cavity was counteracted by other stress responses increasing LD with deficit irrigation. This response was contrary to the findings of (Cronje, 2009b) and (Simmons, 1998), where deficit irrigation reduced LD in the ripe fruit, but may reflect differing treatment impacts on tree and fruit water status. The 2013 results indicated that eight weeks deficit can reduce LD compared with four weeks, which may indicate the potential for more severe treatments to reduce LD in the ripe fruit.

The results confirmed previous findings that fruit exposure to water and irradiation after harvest increases the potential for significant LD. These conditions were generally required to stimulate an irrigation treatment response.

While the results did not support the hypothesis of reducing irrigation to reduce LD, the nil effect of deficit irrigation on fruit yield may indicate a potential cost savings strategy. However, the results indicate that the fruit from deficit irrigation blocks should not be irradiated. Further research is required to confirm maximum tree and fruit water stress criteria to prevent crop and fruit quality loss, and negative effects on following season tree and fruit performance.

4.5. Pre-harvest bagging and coatings

Minh Nguyen (PhD candidate), Peter Hofman, Daryl Joyce, Andrew Macnish, Medan Gupta

This chapter forms part of a current PhD program (Minh Nguyen). The results presented here have not yet been published. The chapter was drafted by the Ph.D. student, and reviewed by the project team.

4.5.1. Summary

Lenticels are microscopic openings on the surface of fruits, including mango. These can become discoloured during late fruit growth and after harvest, resulting in reduced appearance and a loss of value. Previous research with 'B74' mango suggested that exposure of fruit to water before and after harvest increases the risk of lenticel discolouration (LD), and particularly after irradiation for disinfestation against insect pests. In order to reduce LD by manipulating fruit contact with water, the effects of bagging (white paper bags) and surface coatings to the fruit before harvest was evaluated over three years on a commercial property in south-east Queensland. The study showed that bagging the fruit about eight weeks before harvest reduced LD at full yellow colour (ripe) and 7 d later in most years tested, with little obvious effect on other aspects of fruit quality. Of the coatings tested (RainGard, Raynox and Natural Shine), 2.5% solution of Natural Shine TFC210 (a carnauba-based wax) applied 7 and 1 d before harvest was the most promising in reducing LD. There were no other obvious effects on fruit quality. Therefore, fruit bagging may be a viable option for high-value markets requiring irradiation. The full potential of Natural Shine needs to be evaluated through whole-tree sprays and under commercial conditions before wider adoption.

4.5.2. Introduction

Lenticels play a small role in gas exchange on the surface of some fruits, including mangoes. Lenticel discolouration on the fruit at and after harvest can reduce visual appeal and value (Hofman *et al.*, 2010b). Darkening of the tissue surrounding lenticels is due to air and water penetration through the lenticel cavity (Tamjinda *et al.*, 1992). Mango fruit are often exposed to water containing various contaminants during pre-harvest and postharvest from rain, desapping solutions, fungicides and insecticides, as well as condensation during and / or after cold storage. For example, 'Keitt' mango fruit harvested during wet periods often had more LD than those harvested during dry weather (Oosthuyse, 2002; Cronje, 2009b). 'B74' mango fruit treated with detergents expressed more LD when they reached the ripe stage than those that were not treated with detergents (Whiley *et al.*, 2006). Commercial mango desapping detergents and water caused LD on 'Kensington Pride' ('KP') mango fruit (O'Hare *et al.*, 1999) and hot water treatment increased black LD on 'Tommy Atkins' mango fruit (Self *et al.*, 2006).

Free water entering into the lenticel cavity could cause cell turgor and other changes resulting in the cells responding differently to external factors such as mechanical stress and irradiation. A delay after harvesting reduced LD due to lower fruit turgidity (Cronje, 2009b). This delay may enhance water evaporation from lenticels. Turgid lenticel cells of avocado fruit are also sensitive to physical damage (Everett *et al.*, 2008). Hence, treatments such as bagging and coatings that potentially reduce water ingress into the lenticels during fruit growth may help to reduce LD development at and after harvest.

Pre-harvest bagging and coatings have been researched on mango. Pre-harvest bagging creates a modified micro-environment around mango fruit with high humidity, along with changed lighting (Chonhenchob *et al.*, 2010) which can result in increased cell turgidity and increased the skin glossiness for mango. However, bagging with white paper bag did not

affect fruit quality attributes such as flesh colour, total soluble solids and eating quality in 'Keitt' mango (Hofman *et al.*, 1997).

Pre-harvest coatings to reduce water contact with the skin may reduce LD. A carnauba-based wax applied three weeks before harvest reduced LD on 'Gala' apples after three months cold storage (Curry *et al.*, 2008). Coatings on mango fruit can reduce water loss, while still maintaining gas exchange for respiration (Oosthuyse, 2007). Coatings can also minimise the risk of sub-cuticular cell damage of fruit by covering micro-cracks in the cuticle. During fruit development, rapid fruit expansion and enlargement may lead to micro-cracks. Cracking possibly increases with the increasing growing temperature or the amount of rainfall near harvest. Coating with a sunburn protectant such as Raynox® can reduce heat on the fruit surface by filtering UV, visible and IR light (International, 2011). Coating with RainGard® may heal the cracks and decrease water uptake by fruit (International, 2011). Coating cherry fruit with RainGard® significantly reduced cracking as compared to the untreated control, and four weekly applications before harvest with RainGard® at 5% was more efficient in minimising cracking than two applications at 5% or at 10% (Schrader and Sun, 2006). Also, postharvest coating with Natural Shine™ TFC210, an emulsifiable carnauba wax, reduced weight and firmness loss of 'Keitt' mango fruit (Baez-Sañudo *et al.*, 2005).

The potential for pre-harvest bagging and coatings to reduce LD at and after harvest on 'B74' mango fruit grown in southeast Queensland was tested over several seasons under commercial conditions. The fruit were harvested and assessed for fruit quality during ripening. Histochemical studies and dye uptake capacity was also used to assess cuticle and lenticel structure changes.

4.5.3. Materials and methods

4.5.3.1. Fruits

'B74' mango trees were selected at a commercial orchard at Childers, southeast Queensland (25°14'S, 152°37'E) where high rainfall was expected during late fruit growth and harvest. Fruit hanging in full sunlight were chosen for the pre-harvest treatments. Ten trees were selected in the two adjacent rows of the orchard and four fruits per tree were selected for each treatment. In each year, 6-7 treatments were applied to each tree. Care was taken to select fruits for the treatments with no skin damage or defects at two months before harvest.

4.5.3.2. Treatments

To test the hypothesis that bagging may reduce LD, and coating before harvest may create a barrier to prevent water penetration into lenticels, the treatments outlined in Table 26 were applied. The treatments which reduced LD in 2011/12 and/or 2012/13 were re-tested in the following year with additional treatments.

For bagging, fruits were enclosed with 22 cm x 31 cm commercial white fruit bagging paper bags as used in Japan, and bags were stapled close around fruit. Various coating materials were tested, including Natural Shine TFC210 (a carnauba-based postharvest coating), RainGard® (a cracking suppressant), and Raynox® (a carnauba based coating for UV protection) (International, 2011). These chemicals were supplied by Colin Campbell Chemical Pty Ltd. In 2013/14, Maxx (Maxx Organosilicone Surfactant™, Sumitomo Chemicals Australia Pty Ltd) was added to improve coating application. Coating was done by spraying the whole fruit surface to runoff with Raynox®, RainGard® or TFC using a low pressure handgun (International, 2011).

Fruits were all harvested at average maturity of approx. 14% dry matter (DM). Bags were removed from the fruit at harvest. All fruit were labelled with tree number and treatment name. The stems were removed and the fruits dipped in 0.25% Mango Wash® for 1 min to simulate commercial harvest, then placed in the shade to dry. Once dry they were carefully placed in single layer trays to prevent surface damage and transferred via car to the Maroochy Research Facility (MRF) laboratories, Nambour, Queensland. Fruits were dipped

in fungicide at 0.2% v/v (Sportak®, Bayer CropScience Pty Ltd) for 30 sec then brushed using a Lenze AC Tech brushing unit with soft brushes with no water for 1 min at 84 revolutions.min^{-1}.

Table 26 Treatments applied in 2011/12, 2012/13 and 2013/14 to test the effects of bagging at two months before harvest, and coatings applied 1-21 d before harvest, on harvest and post-harvest quality of 'B74' mango fruit.

Treatments	Description
2011/12	
Control	Control: No bagging or coating treatment
Bag	Bagging at 2 months before commercial harvest
5% TFC 3, 2, 1 weeks	Coating with Natural Shine™ TFC210, at 5%, 3 applications at 3 wk, 2 wk and 1 wk before harvest
5% TFC 3 weeks	Coating with Natural Shine™ TFC210, at 5% at 3 wk before harvest
5% TFC 1 d	Coating with Natural Shine™ TFC210, at 5%, at 1 d before harvest
2.5% TFC 1d	Coating with Natural Shine™ TFC210, at 2.5%, at 1 d before harvest
2012/13	
Control	Control: No bagging or coating treatment
Bag	Bagging at 2 mth before commercial harvest
RainGard	Coating with 5% RainGard, three applications at 2 mth, 1 mth and 1 d before commercial harvest without water immersion
RainGard + Water	Coating with 5% RainGard, three applications at 2 mth, 1 mth and 1 d before harvest. About 2 h after each spraying, fruit on the tree were covered with a polyethylene bag filled with deionised water for 15 min to simulate a rain event
Raynox	Coating with 5% Raynox, three applications, at 2 mth, 1 mth and 1 d before harvest
2.5% TFC 1 d	Coating with Natural Shine TFC210 at 2.5% at 1 d before harvest
2013/14	
Control	Control: No bagging or coating treatment
Bagging	Bagging at 2 mth before commercial harvest
2.5% TFC-surfactant	Coating with Natural Shine™ TFC210, two applications at 1 wk and 1 d before harvest
2.5% TFC+ surfactant	Coating with 2.5% Natural Shine™ TFC210 plus 0.1% Maxx, a surfactant, two applications at 1 wk and 1 d before harvest
5% TFC+ surfactant	Coating with 5% Natural Shine™ TFC210 plus 0.1% surfactant, two applications at 1 wk and 1 d before harvest
10% TFC+ surfactant	Coating with 10% Natural Shine™ TFC210 plus 0.1% surfactant, two applications at 1 wk and 1 d before harvest
20% TFC +surfactant	Coating with 20% Natural Shine™ TFC210 plus 0.1% surfactant, two applications at 1 wk and 1 d before harvest

Before ripening, a set of 20 fruits per treatment were used to assess at harvest quality including dye uptake, water potential and lenticel morphology. The other 15 -20 fruits / treatment were treated with 10 µL.L^{-1} ethylene at 20°C for 2 d then ripened at 20°C until fully ripe. Fruit were assessed for the parameters as described below.

4.5.3.3. Assessments

Dye uptake

Ten fruits were used to assess dye uptake characteristics using a temperature differential approach. Fruit and a dye solution of "Brilliant Blue" (1 g.L^{-1} in tap water) were equilibrated separately at 25°C. The solution and fruit core temperature were monitored to ensure consistent treatment application. The temperature trend was similar for the dye solution and

fruit core temperature had similar trend, but the fruit core temperature was about 30 to 60 min delayed compared with the dye solution.

The fruits were then submerged into the dye then placed for 16 h at 12°C. Upon removal, fruits were rinsed three times in running tap water then blotted with paper towel. Blotchy dye uptake and spotty dye uptake were subjectively rated, and the proportion of dyed lenticels per cm^2 recorded as described in section 4.1.3.3.

Water potential of fruit

The tissue water potential was assessed as described in section 4.4.3.2 .

Fruit quality: Lenticel discolouration and firmness

Lenticel discolouration and hand firmness for each fruit were rated as described in section 4.4.3.2 (Hofman *et al.*, 2010a). Fruit firmness was also assessed with the Aweta Acoustic Firmness Tester (Aweta, Nootdrop, the Netherlands) (Padda *et al.*, 2011).

Fruit flavour

A panel of nine staff at MRF were asked to rate the flesh samples on "how much do you like the flavour". Flesh from the cheeks of five fruits per treatment at 9 d after full yellow were diced and mixed. Samples of the seven treatments were placed in small plastic trays, coded and presented to the tasters. The replications were presented separately to the panel at about 2 h intervals on the one day. Fruit flavour was evaluated using the 9-points hedonic scale, where 1 = dislike very much; 2 = dislike a lot; 3 = moderately dislike; 4 = dislike a little; 5 = neither dislike nor like; 6 = like a little; 7 = moderately like; 8 = like a lot; 9 = like very much.

Lenticel morphology

Lenticel morphology was examined as described in section 4.4.3.2 .

4.5.3.4. Statistic analysis

Statistical analyses were performed by Genstat® 14 for Windows™ (VSN International Ltd., UK). Analysis of variance (ANOVA) used the 'General Analysis of Variance' model, with bagging and coatings as 'treatment' structures, and tree as 'block' structure. The protected least significant difference (LSD) procedure at P=0.05 was used to test for differences between treatment means (water potential, LD and dye uptake).

4.5.4. Results

4.5.4.1. 2011/12

Lenticel discolouration

In the 2011/12 season, LD in all treatments was very low (<1) at the full yellow stage (Table 27). There were significant differences in LD, but the differences were small and no treatment was significantly different to the control. Seven days after full colour, all bagging and coating treatments except coating at three, two and one week, and 5% 1 d before harvest reduced LD compared with the control. It is likely that these treatments increased LD because of the more frequent application and higher concentration.

Bagging and coating with 2.5% Natural Shine 1 d before harvest had the lowest LD.

Dye uptake

Bagging and coating three times before harvest decreased both blotchy and spotty ratings compared with the control (Table 28). This reduction with bagging agrees with the lower LD of the bag fruit 7 d after full yellow.

Table 27 Lenticel discolouration on 'B74' mango fruit at full yellow skin colour (ripe) and 7 d later, following bagging with paper bags two months before harvest, and treatment Natural ShineTM TFC210 three times at weekly intervals before harvest, or at three weeks, or at 1 d before harvest (2011/12).

Treatment	Lenticel discolouration (0-5)	
	Full yellow	7 d after full yellow
Control	0.77 abc	1.93 d
Bag	0.70 ab	1.00 a
5% TFC 3, 2, 1 weeks	0.60 a	1.60 cd
5% TFC 3 weeks	0.87 bc	1.30 abc
5% TFC 1 d	0.97 c	1.50 bcd
2.5% TFC 1d	0.90 bc	1.10 ab
P value	0.038	0.002

n=10. Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD.

Table 28 Blotchy and spotty degree of 'B74' fruit at harvest following bagging with paper bags two months before harvest, and treatment Natural ShineTM TFC210 three times at weekly intervals before harvest, or at three weeks, or at 1 d before harvest (2011/12).

Treatment	Dye rating (0-6)	
	Spotty	Blotchy
Control	5.05 c	3.50 b
Bag	4.25 b	2.60 a
5% TFC 3, 2, 1 weeks	3.15 a	2.55 a
5% TFC 3 weeks	4.40 bc	2.50 a
5% TFC 1 d	4.30 bc	2.80 a
2.5% TFC 1d	3.85 ab	2.95 ab
P value at 0.05	<.001	0.009

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD.

All coating treatments reduced the number and proportion of dyed lenticels compared with the control, while bagging reduced the number of dyed lenticels, but not the proportion (Table 29). These results mostly conform to the LD and spotty and blotchy rating scores, with the exception of bagging.

Firmness

There were no treatment effects on firmness at full yellow or 7 d later when assessed with Aweta (data not presented). The average firmness by Aweta was 35.5 at full yellow, and 31.3 at 7 d after full yellow.

Weight loss

The bagged fruit lost the most weight by 22 d after harvest compared with all other treatments (Figure 12). There was little difference in loss between control and the 5% treatments, while 2.5% 1 d before harvest was intermediate in loss between these treatments and the bag treatment.

Table 29 Proportion of dyed lenticels of 'B74' fruit at harvest, following bagging with paper bags two months before harvest, and treatment Natural ShineTM TFC210 three times at weekly intervals before harvest, or at three weeks, or at 1 d before harvest (2011/12).

Treatment	per cm^2		Proportion of dyed lenticels (%)
	Total lenticels	No. of dyed lenticels	
Control	36	16.5 ^{d}	49.3 ^{c}
Bag	37	13.7 ^{c}	40.8 ^{bc}
5% TFC 3, 2, 1 weeks	45	8.1 ^{a}	26.9 ^{a}
5% TFC 3 weeks	39	10.7 ^{ab}	30.2 ^{a}
5% TFC 1 d	43	9.5 ^{ab}	25.3 ^{a}
2.5% TFC 1d	42	12.2 ^{bc}	32.5 ^{ab}
P value at 0.05	0.056	<.001	<.001

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD.

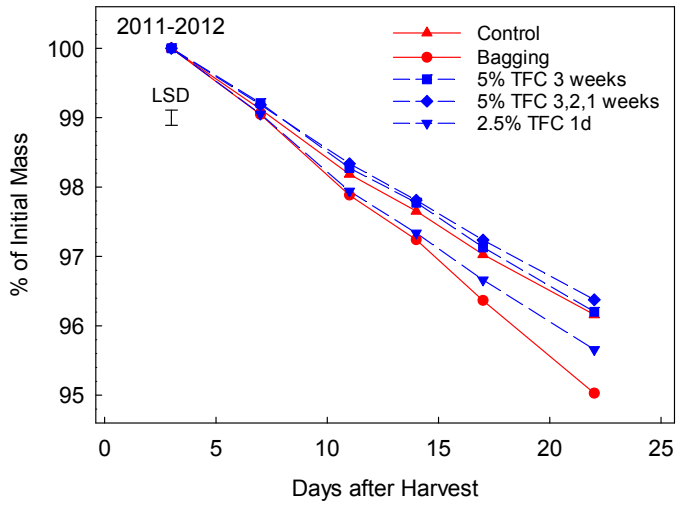


Figure 12 Changes in fruit weight loss during ripening. LSD for days =0.22 at harvest, following bagging with paper bags two months before harvest, and treatment Natural ShineTM TFC210 three times at weekly intervals before harvest, or at three weeks, or at 1 d before harvest (2011/12) (n=10).

Lenticel morphology

Lenticel structures of all six treatments were similar at 7 d after full yellow. Browning cells inside the lenticel chambers were observed for all treatments and control (Plate 16).

Overall, coating with TFC at 2.5%, and bagging were the best treatments. Coating with TFC at 2.5% reduced LD, dye absorption and water loss. These treatments were tested again in 2012/13.

4.5.4.2. 2012/13

Lenticel discolouration

At the green mature stage, bagging and coating with TFC had lower LD than the control (Table 30). At the full yellow stage, the LD of the bagging, Raynox®, TFC and RainGard® (no water) fruit were less than the control fruit. There were no significant treatment effects by one week after full yellow.

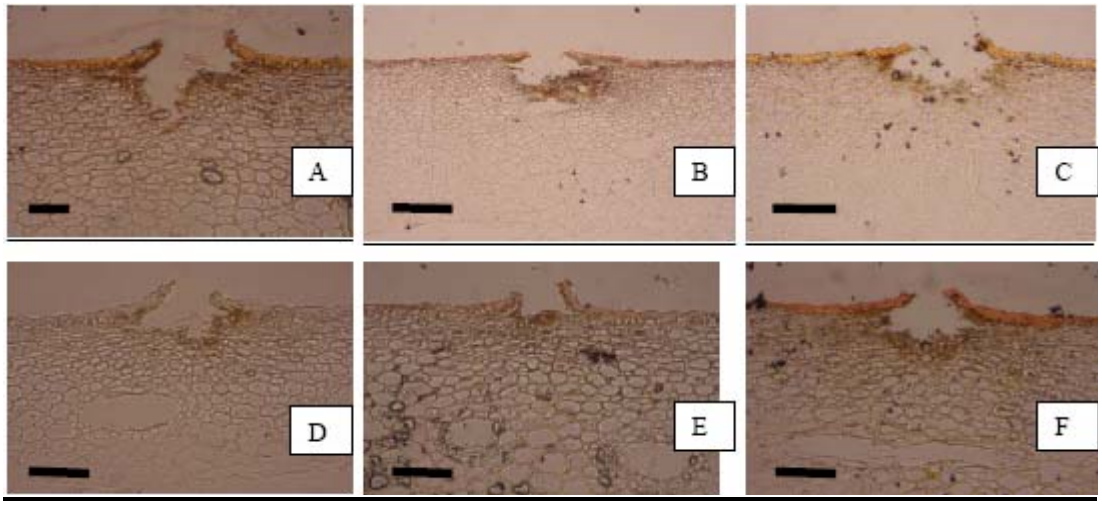


Plate 16 "Sudan IV" stained transverse sections of 'B74' at a wk after full yellow Control (A); bag (B); 1+2+3 wks (C); 3 wks before harvest (D); 5% TFC (E) and 2.5% TFC (F). scale bar 100µm.

Table 30 Lenticel discolouration on 'B74' mango fruit at harvest, full yellow skin colour (ripe) and 7 d later, following bagging with paper bags two months before harvest, and coating with RainGard ± water or Raynox, three applications, at 2 mth, 1 mth and 1 d before harvest; and coating with Natural Shine TFC at 1 d before harvest (2012/13)

Treatment	Lenticel discolouration (0-5)		
	At harvest	Full yellow	7 d after full yellow
Control	2.0 ^{c}	2.8 ^{c}	3.5
Bagging	0.9 ^{a}	1.6 ^{a}	2.9
RainGard	1.7 ^{bc}	1.6 ^{a}	2.4
RainGard Water	1.9 ^{bc}	2.5 ^{bc}	3.1
Raynox	1.5 ^{bc}	2.0 ^{ab}	2.4
2.5% TFC 1 d	1.4 ^{ab}	1.7 ^{a}	2.2
LSD (P = 0.05)			ns

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD. ns = not significant.

Dye uptake

There were no significant treatment effects on spotting or blotchy dye ratings (data not presented). The average rating across all treatments was 4.9 for spotty and 4.4 for blotchy ratings.

Fruit firmness

There was also no significant treatment effect on fruit firmness as measured by hand or Aweta (data not presented). The average firmness by Aweta at full yellow was 33.1 and 24.8 Aweta 7 d after full colour.

Weight loss during ripening

Figure 13 indicates that RainGard® (no water) and Raynox® resulted in less weight loss during the later stages of ripening compared with control, bagging and TFC. There was no difference in weight loss between the bagging and control fruit, which was contrary to the results of 2011/12 with the same bags. There was no effect of water after RainGard®, compared with RainGard® alone.

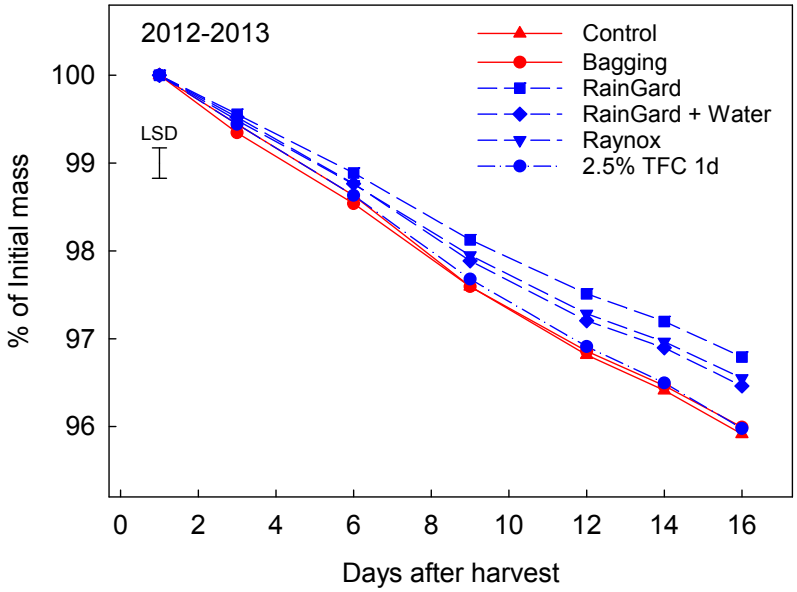


Figure 13 Changes in fruit weight loss during ripening on 'B74' mango fruit at harvest, full yellow skin colour (ripe) and 7 d later, following bagging with paper bags two months before harvest, and coating with RainGard ± water or Raynox®, three applications, at 2 mth, 1 mth and 1 d before harvest; and coating with Natural Shine TFC at 1 d before harvest (2012/13).

Water potential

Because of heavy rain and flooding one week before harvest, there were insufficient bagged fruit to test water potential. However, there was no significant treatment effect on water potential with the remaining five treatments. The average water potential for these treatments was -0.6 MPa.

Lenticel morphology

There was little evidence of treatment effects on lenticel structure and morphology (Plate 17). Discoloured lenticels had distinct discolouration of the cells surrounding the lenticel cavity.

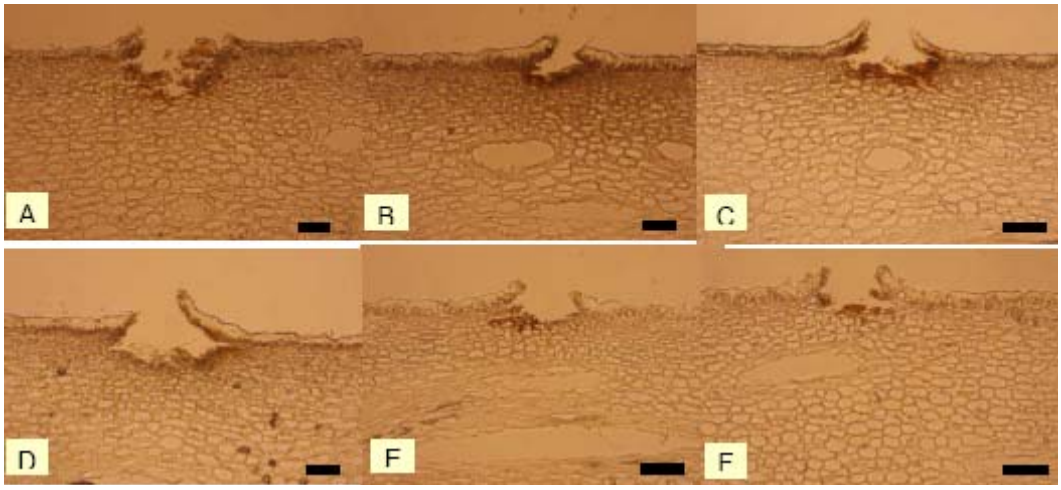


Plate 17 Unstained transverse sections of 'B74' at full yellow colour of Control (A); RainGard (B); RainGard + Water (C); Raynox (D); TFC (E) and bagging (F); scale bar 100µm.

2013/14

Lenticel discolouration

All treatments resulted in lower LD at full colour and 9 d after full colour, compared with the control (Table 31). Bagging, 2.5% TFC with no surfactant, and 10-20% TFC with surfactant resulted in the lowest LD at both assessment times.

Table 31 Lenticel discolouration on 'B74' mango fruit at full yellow and full yellow + 9 d at harvest, following bagging with paper bags two months before harvest, and coating with 2.5% Natural Shine TFC ± surfactant, and 5%, 10% or 20% Natural Shine TFC + surfactant, two applications, at 1 week and 1 d before harvest (2013/14).

Treatment	LD at full yellow	LD at 9 d after full yellow
Control	1.9 [c]	3.2 [d]
Bagging	0.8 [a]	2.2 [ab]
2.5% TFC-surfactant	0.7 [a]	2.1 [ab]
2.5% TFC+surfactant	1.4 [b]	2.7 [c]
5% TFC+surfactant	1.4 [b]	2.7 [c]
10% TFC+surfactant	0.9 [a]	2.3 [bc]
20% TFC +surfactant	0.9 [a]	1.8 [a]

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD.

Percentage dry matter at harvest

There was no treatment effect on the % DM at harvest (data not presented). The average DM was 17.8%.

Dye uptake

Bagging and 2.5% TFC-surfactant produced the lowest spotty score rating (Table 32). The other treatments resulted in a higher spotty score compared with the control. The lower bagging and 2.5% TFC results are similar to those observed for LD, but this was not the case for the other treatments where they also had lower LD but higher spotty scores. Coatings with TFC + surfactant increased botchy score as compare to the control, bagging and TFC without surfactant.

Table 32 Dye test spotty and blotchy score on 'B74' mango fruit at harvest, following bagging with paper bags two months before harvest, and coating with 2.5% Natural Shine TFC ± surfactant, and 5%, 10% or 20% Natural Shine TFC + surfactant, 2 applications, at 1 week and 1 d before harvest (2013/14).

Treatment	Spotty score at harvest (1-6)	Blotchy score at harvest (1-6)
Control	4.5 [b]	3.2 [a]
Bagging	4.0 [a]	2.8 [a]
2.5% TFC-surfactant	4.1 [a]	3.2 [a]
2.5 TFC+surfactant	5.4 [c]	4.4 [bc]
5% TFC+surfactant	5.4 [c]	4.5 [c]
10% TFC+surfactant	5.6 [c]	4.6 [c]
20% TFC +surfactant	5.5 [c]	3.9 [b]

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD

Skin colour

Fruit coated with TFC+surfactant appeared to have delayed yellow colour expression (data not analysed; Table 33). At 4 d after gassing with ethylene gas, the skin colour of fruit treated with 5%TFC had 95% of fruit reaching the full yellow stage, while the 20% surfactant treatment had only 70% of fruit reaching full yellow. At 7 d after gassing, all treatments had 100% full yellow colour, except the treatment with 20% TFC. However, the green part on the fruit of the treatment with 20%TFC was small, as the skin colour score was 5.9.

Table 33 Skin colour at 7, 10 and 13 d on 'B74' mango fruit after harvest, following bagging with paper bags two months before harvest, and coating with 2.5% Natural Shine TFC ± surfactant, and 5%, 10% or 20% Natural Shine TFC + surfactant, two applications, at 1 week and 1 d before harvest (2013/14). Data not statistically analysed by ANOVA because of no mean variance

Treatment	Percentage of full yellow fruits (%)			Average skin colour score (1-6)		
	7 d	10 d	13 d	7 d	10 d	13 d
Control	100	100	100	6.0	6.0	6.0
Bagging	100	100	100	6.0	6.0	6.0
2.5% TFC-surfactant	100	100	100	6.0	6.0	6.0
2.5 TFC+surfactant	90	100	100	6.0	6.0	6.0
5% TFC+surfactant	95	100	100	6.0	6.0	6.0
10% TFC+surfactant	80	100	100	5.9	6.0	6.0
20% TFC+surfactant	70	80	95	5.5	5.9	6.0

Hand firmness

Bagging had no effect on firmness at full yellow compared with control (Table 34). All coating treatments except 2.5%-5% TFC+surfactant were more firm (lower firmness rating) than control at full yellow suggesting a delay in the softening process normally associated with ripening. This confirms the skin colour data above.

No significant treatment effects on firmness were noted 7 d after full yellow with Aweta (data not presented). The average Aweta reading was 29.4.

Weight loss

There was little difference in weight loss during ripening between control and the bagged fruit (Figure 14). However, all other treatments resulted in significantly less weight loss in the latter stages of ripening compared with control, with the higher concentrations of TFC resulting in less loss.

Table 34 Hand firmness ratings at full yellow on 'B74' mango fruit, following bagging with paper bags two months before harvest, and coating with 2.5% Natural Shine TFC ± surfactant, and 5%, 10% or 20% Natural Shine TFC + surfactant, 2 applications, at 1 week and 1 d before harvest (2013/14).

Name of treatments	Hand firmness (0-4) at full yellow
Control	1.6 ^{c}
Bagging	1.6 ^{c}
2.5% TFC-surfactant	1.3 ^{ab}
2.5 TFC+surfactant	1.5 ^{bc}
5% TFC+surfactant	1.8 ^{d}
10% TFC+surfactant	1.4 ^{ab}
20% TFC +surfactant	1.2 ^{a}

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD.

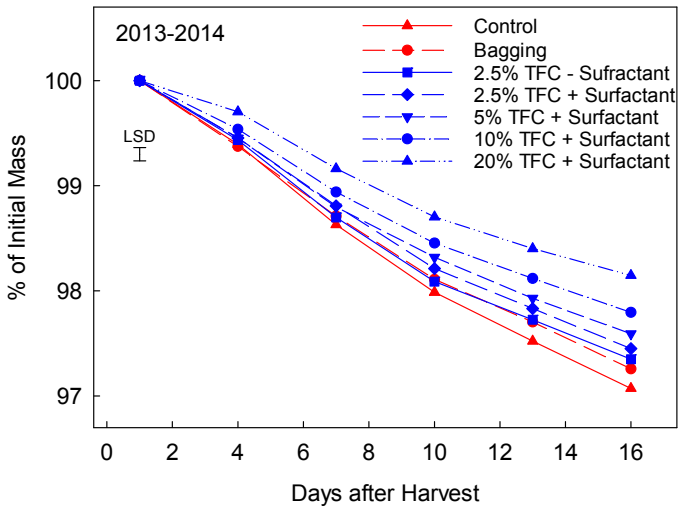


Figure 14 Changes in fruit weight loss of 'B74' mango fruit after harvest, following bagging with paper bags two months before harvest, and coating with 2.5% Natural Shine TFC ± surfactant, and 5%, 10% or 20% Natural Shine TFC + surfactant, two applications, at 1 week and 1 d before harvest (2013/14). (n=20); LSD bar for days after harvest (P=0.05).

Flavour

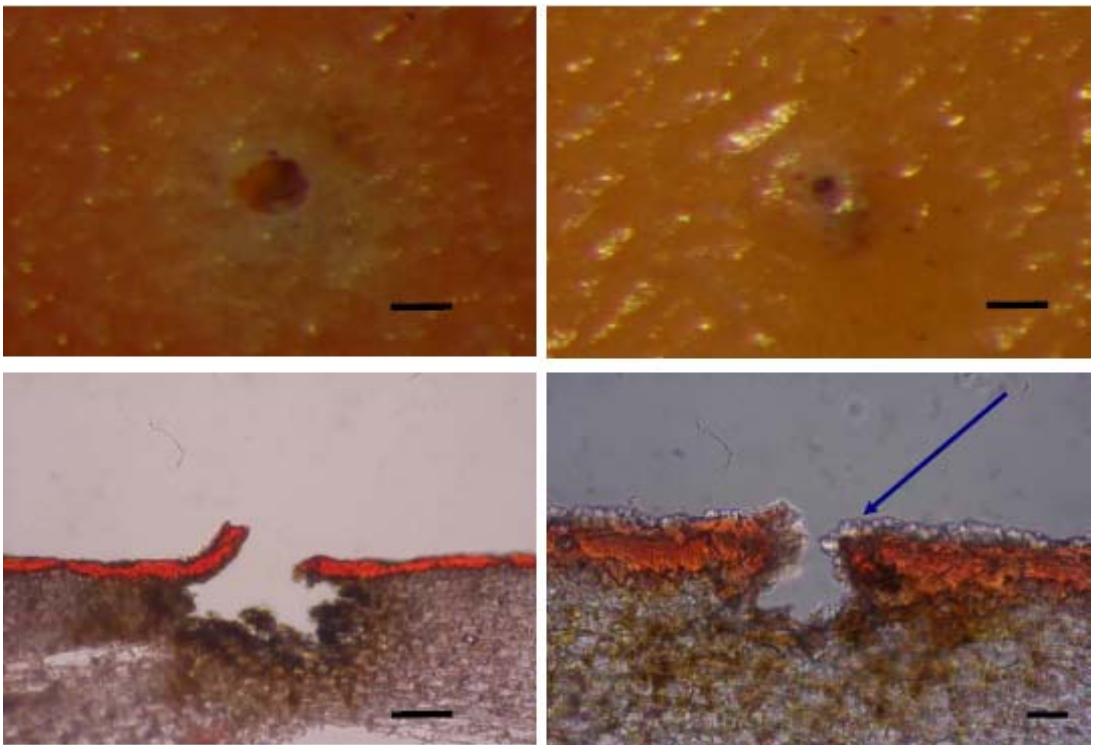
Sensorial evaluation (flavour) was assessed at the full yellow (ripe) stage. There were no significant treatment effects on flavour, and no off-flavours were noted.

Lenticel external features and morphology

Wax was observed inside the lenticel cavity (blue arrow) in the 20%TFC treatment (Plate 18) while no wax was obvious inside the lenticel chamber in the control. Thus, it is possible that a protective wax coating from the TFC treatment reduced water and other substance entry into the lenticel cavity and minimised stress/disruption to the function of the surrounding cells.

The visual observation on fruit appearance based on the fruit photos (Plate 19) showed that 20% TFC had much less dark spots on the fruit surface as compared to the control.

Pre-harvest coatings also reduced LD on the ripe fruit. Natural Shine (TFC 210) is a carnauba-based product designed specifically for postharvest application on mango (International, 2011). A 2.5% treatment without surfactant provided the most promising option. The addition of surfactant increased LD possibly because of a direct surfactant effect on the lenticels. Higher concentrations of Natural Shine+surfactant gave similar results to the 2.5% treatment, possibly because the higher concentrations counteracted the negative effects of surfactant. Further trials using whole-tree spray applications are warranted, including monitoring tree performance after wax application and placing the fruit through the commercial picking and packing process.



Control (no wax was observed inside the lenticel chamber)

Coating with 20% TFC + surfactant (wax was observed inside the lenticel chamber)

Plate 18 External features and transverse sections stained with Sudan IV of control (left) and 20% TFC (right); Sudan IV stained with lipid of cuticle layer turning on red colour, green arrow showed TFX wax covered the fruit surface and entered lenticel chamber at full colour stage. Scale bar 50µm

Plate 19 Control (left) with high LD and 20% TFC (right) with low LD at 7 d after full yellow

The dye uptake results largely mirrored the LD results, suggesting that the ability for water entry into the lenticels, as indicated by dye uptake, may be a factor in LD. These results suggest that the dye treatment may provide a commercial indicator test for consignments prone to significant LD after commercial harvesting and packing, and potentially irradiation. In 2013/14, TFC coating +surfactant increased dye uptake possibly because surfactant facilitated dye entry into lenticel cavities.

4.5.5. Discussion

Previous research on ‘B74’ mango showed that fruit harvested directly from the trees into trays without exposure to at harvest or postharvest water or solutions (e.g. desapping

detergents and pesticides) developed very little LD on the ripe fruit, even after irradiation (Hofman *et al.,* 2010). However, dipping in deionised water after harvest increased LD both without and with irradiation. Hence water exposure is a significant trigger for LD. The results of the present trials indicate that pre-harvest treatments aimed at reducing contact of the lenticels with water can reduce LD after simulated commercial picking and packing.

Bagging the fruit with white paper bags (similar to those used commercially in Japan) significantly reduced LD at full yellow and 7 d after in most seasons tested. (Joyce *et al.*, 1997) and (Hofman *et al.*, 1997) evaluated the effects of bagging on 'Keitt', 'Sensation' and 'KP' mango but did not document treatment effects on LD. However, they observed significant reductions in postharvest disease with longer bagging times, more yellow colour of the skin at eating soft, and potentially less blush with longer bagging times. Ripening time can be reduced by bagging but there was little effect on eating quality. Therefore, paper bagging may be beneficial for high value markets, particularly where irradiation for market access is required.

The bagging treatments had inconsistent effects across the seasons on weight loss during ripening, although in two out of three seasons weight loss was no different to the control. Plastic bagging of 'Sensation' fruit from seven weeks prior to harvest significantly increased weight loss during ripening, causing shrivelling and an inability to ripen (Joyce *et al.*, 1997). It is likely that the plastic bags significantly increased relative humidity around the fruit, which potentially reduced the ability of the cuticle to minimise water loss after harvest. Paper bags likely have a lesser effect on gas concentrations around the fruit compared with plastic, although the paper bags did increase water loss during ripening in 2011/12.

4.6. Impact of crop load on lenticel discolouration

Roberto Marques, Peter Hofman

4.6.1. Summary

To investigate the potential of reduced crop load on 'B74' trees to reduce lenticel discolouration (LD) at harvest and at ripe, fruitlets and panicles from the western side of the trees were removed about three to four weeks after fruit set in a commercial farm in Katherine, NT. The treatment reduced the % of sunburnt fruit, but had little impact on the total number of fruit per tree, average fruit size, and fruit maturity at harvest. There was also unsurprisingly little treatment effect on the severity of lenticel discolouration at the full yellow (ripe) stage.

4.6.2. Introduction

Lenticel discolouration in 'Kensington Pride' ('KP') mango is reportedly more severe in larger fruit from branches with higher leaf:fruit ratios than smaller fruit, possibly because of greater disruption to lenticel structure during fruit growth (Simmons *et al.*, 1998). In 'Sensation' mango, fruit thinning shortly after full flowering increased fruit size at harvest (Yeshitela *et al.*, 2004). To test if a similar response would occur in 'B74', fruit was sampled from a crop load trial conducted by Oolloo Farms at their farm in Katherine in 2013. Fruit were removed from the western side of the tree canopy at about three to four weeks after fruit set. The aim was to increase the size of remaining fruit, reduce the proportion of commercially unacceptable fruit (particularly due to sunburn) and evaluate possible treatment effects on LD following irradiation.

4.6.3. Materials and methods

4.6.3.1. Field treatments and harvesting

About 100 'B74' trees from two adjacent rows were selected and labelled (with different coloured ribbons placed at the beginning and end of each row) in a commercial farm (Oolloo Farms, K1) in the Katherine area, Northern Territory in 2013. At about three to four weeks after fruit set, fruitlets (mostly at about 1.5 cm diameter), together with panicles, were removed from the western side of one of the selected rows. Trees on the other row were not thinned (used as control). About a week before typical commercial harvest, the total number of fruit on each of 10 adjacent trees per selected row (total of 20 trees) was counted and recorded using a hand counter.

At typical commercial harvest maturity, 18 fruit from each of the 10 adjacent trees per selected row (total of 360 fruit from 20 trees) were picked on 19/11/2013. Sound fruit (with no cuts or open wounds) were randomly picked with short stems (4-6 cm) from all positions around the canopy. Fruit were carefully placed into labelled (tree number) perforated plastic crates and immediately taken to the local Katherine Research Station (KRS). Fruit were de-sapped by removing the stem and holding fruit for two seconds (with stem end down) to remove the spurt sap. Fruit were then dipped for 4 min in a de-sapping solution of 75 g of Mango Wash® powder in 30 L of water using a 50-60 L plastic container (about three crates were dipped together each time). The solution was replaced after each dip.

An extra 10 fruit per selected row were sampled from 10 trees (two fruit per tree, total of 20 fruit) for fruit maturity assessments at harvest. Fruit were snapped and placed on the ground (away from sun) to de-sap (stem end down). On arrival at KRS, dry matter was determined as described in the 'B74' Best Practice Guide' (Hofman and Whiley, 2010).

Fruit were then randomly placed into crates and allowed to dry before being packed into labelled P-84 trays with inserts. Wet fruit were gently dried with paper towel as required.

Trays were stacked into bundles of two trays, covered with a lid, labelled on top, and wrapped with duct tape before being air-freighted to Brisbane the next day.

4.6.3.2. Irradiation treatment

After arrival in Brisbane, fruit were transported by car to the Maroochy Research Facility (MRF), Nambour and randomly re-packed into Mod-6 trays, so as to ensure that each tray contained fruit from both treatments and from a number of trees. The next day, fruit were transported by car and irradiated at the commercial Steritech facilities at Narangba (Brisbane) using a cobalt 60 source. The target dose was 550 Gy and two dosimeters (Opti-chromic detectors FWT-70-40 M) were placed in each tray to monitor doses. Trays were covered with 4 mm plywood lids with a15 mm low density foam lining to prevent fruit movement. Trays were placed on their side in a rack designed to fit on 1 m high bins used for irradiating other products to ensure more consistent doses between trays. The next morning, the fruit were transferred back to MRF by car.

4.6.3.3. Fruit quality assessments

On arrival at MRF after irradiation, fruit were sorted by trees, re-packed into P-84 trays (18 fruit per tray), and the fruit in each tray (tree) weighed. Fruit were visually rated for sunburn. Fruit were considered sunburnt if the rating was higher than one (yellow breaching on more than 5% of the surface area of the fruit skin). The fruit were then treated with 10 $\mu L.L^{-1}$ ethylene for 2 d at 20ºC, then ripened at 20ºC. Fruit were individually assessed for skin colour, fruit firmness and the severity of LD and skin browning (see section 4.1.3.3) based on the rating systems in the B74 Quality Assessment Manual (Hofman *et al.*, 2010a). Most of the fruit reached full yellow skin colour stage by 12 d after harvest.

4.6.3.4. Statistical analysis

Statistical analyses were performed by Genstat® 16 for Windows™ (VSN International Ltd., UK). A completely randomised design was used with 10 single replicates (trees) per treatment (total of 20 trees) and 18 fruit per tree (total of 360 fruit). The 'General Analysis of Variance' model was used to analyse the data, with crop load as 'treatment' factor and no block. The least significant difference (LSD) procedure at P = 0.05 was used to test for differences between treatment means.

4.6.4. Results and discussion

The mean irradiation dose applied was 706 Gy, ranging from 537 to 801 Gy. Although considerably variable and higher than the target, the impact of the high variation was likely reduced by the fact that fruit from both treatments were randomly allocated within each tray.

As expected, the removal of fruitlets and panicles from the western side of the trees about 3-4 weeks after fruit set significantly reduced the % of sunburnt fruit (Table 35). In contrast, there was little treatment impact on the total number of fruit per tree, average fruit size, and fruit maturity (as measured by % dry matter) at harvest. The reasons for little treatment effect are unknown, but it may be that the number of fruit removed was insufficient to affect the crop load of a relatively productive tree like 'B74'. It is also possible that the thinned trees 'compensated' for the loss of fruit by retaining more fruit on the other parts of the tree, which has been reported in 'Sensation' mango (Yeshitela *et al.*, 2004). In addition, the intensity of fruit thinning affected treatment responses to fruit size in 'Sensation' mango (Yeshitela *et al.*, 2003). It is also possible that fruit removal in the present trial was not done at the right time relative to full flowering, which is reported to be an important factor in the effectiveness of fruit removal in 'Sensation' mango (Yeshitela *et al.*, 2004). As neither crop load nor fruit size was affected by fruit removal, there was unsurprisingly little treatment effect on the severity of LD at the full yellow (ripe) stage. Thus, a possible impact of a reduced crop load on LD in 'B74' mango fruit was not confirmed.

Table 35 The effect of fruit removal from the western side of the tree on the % of sunburnt fruit, fruit % dry matter, average fruit weight and the severity of lenticel discolouration (0-5) of the ripe 'B74' mango fruit.

Fruit removal on the western side of the tree	At harvest				At full yellow
	Total number of fruit per tree	Average fruit weight (g)	% Dry matter	% Sunburnt fruit	Lenticel discolouration severity (0-5)
No	93	361	18.9	16 [a]	2.1
Yes	97	362	18.7	6 [b]	2.0

Means (n=180 for lenticel data or n=10 for the other parameters) in each column without letters are not significantly different (P=0.05) as tested by LSD.

4.7. Harvesting effects

Roberto Marques, Minh Nguyen, Peter Hofman

4.7.1. Summary

Lenticel discolouration (LD) on the skin of 'B74' mango fruit can reduce consumer appeal and value. Previous results showed that harvesting practices can affect LD, both without and after irradiation. Exposure to water during harvesting and packing is a major contributor to lenticel sensitivity, but there is little understanding of the mechanisms involved. Cultivar variation in lenticel sensitivity may help to identify the underlying causes by comparing fruit characteristics in relation to lenticels.

The present work reports observations on cultivar differences, and the use of a dye solution to indicate the potential for water uptake through the lenticels. The effects of key postharvest practices known to have a significant effect on lenticel sensitivity were tested in relation to dye uptake. The key results were:

- In 'B74' fruit, exposure to water increased LD for both off tree (not exposed to commercial harvesting or packing practices) and end of packline fruit, and this response was further increased by exposure to dye solution.
- In 'Honey Gold' fruit, only the exposure to dye solution (but not water alone) increased LD.
- In both cultivars, LD was more severe in fruit from the end of packline than in off tree fruit.
- In both cultivars, fruit from the end of the packing line had more lenticels that took up dye, and a higher dye uptake score than off-tree fruit, reflecting the increased LD on the packline fruit.
- 'B74' had a higher density of lenticels per unit area compared with 'Honey Gold'.

These results suggest that harvesting/packing procedures increase LD severity in 'B74' and to a lesser extent in 'Honey Gold'. This likely occurs by increasing the severity of damage in susceptible 'B74' lenticels, but not in 'Honey Gold'. However, the most likely cause for greater LD sensitivity of 'B74' mango is because of its considerably greater density of lenticels, even though a greater percentage of the 'Honey Gold' lenticels have the potential to take up water as evidenced by the dye treatment. The dye treatment may be a suitable indicator of significant LD development in packed / irradiated fruit.

4.7.2. Introduction

Discolouration of the lenticels on the skin of 'B74' mango fruit can be a significant cause of quality downgrade. Harvesting practices can affect LD in 'Tommy Atkins' and 'Keitt' mangoes (Self *et al.*, 2006; Cronje, 2009b). Previous project results confirmed that harvesting practices can affect LD in 'B74' mango, both without and after irradiation (Whiley *et al.*, 2006; Hofman *et al.*, 2010b). Exposure to water during harvesting and packing was a major contributor to lenticel sensitivity, but there is little understanding of the mechanisms involved. The current hypothesis for LD suggests three key processes (Bezuidenhout, 2005; du Plooy *et al.*, 2006; Rymbai *et al.*, 2012):

- water entry into the lenticel cavity, then
- water entry into the cells surrounding the lenticel cavity, then
- expression of stress responses within the cells, potentially including cell death and pigment formation associated with enzymatic browning.

Each of these steps needs to be understood to identify preventative measures. In addition, the significant cultivar variation in lenticel sensitivity provides a good model for identifying underlying causes by comparing cultivar characteristics in relation to lenticels.

The work reported here provides preliminary observations on cultivar differences, and the use of a dye solution to indicate water uptake through the lenticels. The effects of some key postharvest practices known to have a significant effect on lenticel sensitivity were tested in relation to dye uptake.

4.7.3. Materials and methods

'B74' and 'Honey Gold' fruit were sampled from commercial farms in the Bundaberg area at typical commercial harvest maturity on 1/2/2011 ('B74') and 10/2/2011 ('Honey Gold'). The fruit were transferred to the Maroochy Research Facility (MRF), Nambour by car within 4 h of harvest and the following treatments applied in a factorial design:

Treatment:

- Not water or dye treatment.
- Water treatment only; as per the dye treatment but with tap water only.
- Dye treatment (see following).

Handling:

- Off tree: fruit harvested directly from the tree into trays without any exposure to water or further handling.
- End of packline: fruit exposed to Mango Wash® (Septone Products Australia) in the field during commercial harvesting, and to water, fungicides and brushing during packing.

Irradiation:

- No irradiation.
- Irradiated (see following).

Representative fruit were given a dye infiltration treatment using a temperature gradient approach. This consisted of equilibrating fruit pulp temperature to about 22°C, then placing in the dye solution pre-cooled to 12°C, and holding at 12°C for about 14 h. The dye solution consisted of 'Brilliant Blue' food dye made up at 1 $g.L^{-1}$ in tap water (Clements, 1935). On removal, the fruit were rinsed three times in tap water then dried with paper towel.

Half of the 'B74' fruit were irradiated at the commercial Steritech facilities at Narangba (Brisbane) using a cobalt 60 source. Four dosimeters (Opti-chromic detectors FWT-70-40 M) were placed in each tray one fruit in from the corners to monitor doses. Trays were covered with four mm plywood lids with a15 mm low density foam lining to prevent fruit movement. They were then placed on their side in a rack designed to fit on 1 m high bins used for irradiating other products to ensure more consistent doses between trays of the same treatment. The fruit were divided into four batches and received the following average doses: 666 Gy (batch 1), 575 Gy (batch 2), 492 Gy (batch 3) and 556 Gt (batch 4). 'Honey Gold' fruit were not irradiated.

4.7.3.1. Fruit quality assessments

The fruit were ripened at 20°C and rated for LD at full yellow skin colour. Fruit were individually assessed for skin colour and LD (Hofman *et al.*, 2010a) and dye uptake as summarised in section 4.1.3.3.

At each stage, each fruit was also rated for the percentage of visible lenticels that could be characterised as light or pronounced spots.

The extent of dye uptake was rated at full colour using the following scale:

1 = No dye uptake;

2 = Minor dye uptake by lenticels (i.e. infrequent / occasional in numbers and distribution on fruit surface);
3 = Dye uptake by lenticels on <50% of fruit surface area;
4 = As above, but also blotchy areas of dye uptake (each dyed area larger than 1 mm in diameter);
5 = Dye uptake on >51% of fruit surface area; and,
6 = As above, but also blotchy areas of dye uptake (Plate 20).

Plate 20 Blotchy areas of dye update on 'B74' mango fruit. Fruit pulp temperature was calibrated to approximately 22°C. Fruit were then placed in the dye solution pre-cooled to 12°C, and holding at 12°C for about 14 h. The dye solution consisted of 'Brilliant Blue' food dye made up at 1 $g.L^{-1}$ in tap water. On removal, fruit were rinsed three times in tap water, dried with paper towel, and assessed.

The density of lenticels per unit area, and the percentage of dyed lenticels were recorded by counting the number of lenticels within four, five cent piece-sized (3 cm^2) areas around the equator of each fruit. Each area was in one of four quadrants; either 1) the front face directly above the beak; 2) right-hand side cheek face; 3) rear face behind the beak; and 4) left-hand side cheek face. In addition, the % surface area dyed within each quadrant was recorded using the following scale:

1 = less than 20% of the surface area with dye;
2 = 21-40%;
3 = 41-80%;
4 = 81-100%.

4.7.3.2. Statistical analysis

Statistical analyses were performed by Genstat® 11 for Windows™ (VSN International Ltd., UK). On both trials each treatment consisted of 10 fruit, with each fruit considered a single replication, and the 'General Analysis of Variance' model was used. In the effect of harvesting procedures and cultivar trial, a factorial design was used involving 'harvest procedures' times 'water/dye treatments' or 'harvesting procedure' times 'irradiation treatment' for each cultivar as 'treatment' structures and no 'block' structure. The least significant difference (LSD) procedure at P = 0.05 was used to test for differences between treatment means.

4.7.4. Results and discussion

In 'B74' fruit, the exposure to water significantly increased LD for both off tree and end of packline fruit compared to control fruit, and that effect was further increased by exposure to dye solution (Table 36). Similar results of increased LD in fruit washed in water were reported for 'KP' mango (O'Hare *et al.*, 1999).

Lenticel discolouration was also more severe in fruit from the end of packline (compared to off tree fruit) for all three treatments (Table 36). This confirms results from previous projects with 'B74' showing some impact of harvesting and packing operations in LD, including de-sapping with detergents (Whiley *et al.*, 2006) and fungicide treatment after harvest (Hofman *et al.*, 2010b). Likewise, in cultivars such as 'Tommy Atkins', 'Keitt', and 'Kensington Pride', harvest method (including de-sapping method and detergent used) and several postharvest handling operations (including delays between picking and packing, fruit brushing, and hot water/air treatments) increased LD in a cumulative way (Bally *et al.*, 1997; Self *et al.*, 2006; Cronje, 2009b; Cronje, 2009a).

In contrast, irradiation had little impact on LD of 'B74' in this trial most due to the high LD severity of the end packline fruit in the absence of irradiation. This was likely because of the unusually high rainfall in southeast Queensland in 2011, and the large fruit size due to poor flowering and fruit set.

Compared to control, exposure to water also increased the % of pronounced lenticels in irradiated fruit, while exposure to dye solution did so for both non-irradiated and irradiated fruit (Table 36). In contrast, the % of pronounced spotting was not affected by the harvesting and packing procedures (data not shown). This suggests that the increased LD severity following harvesting/packing was not due to an increased percentage of more pronounced spots, but more likely due to an increased number of visibly damaged lenticels. However, irradiation increases the LD severity partly increasing the percentage of the pronounced (more damaged) lenticels.

Table 36 Effects of water and dye treatments and either harvesting procedure (fruit sampled from the tree with no exposure to water or packing systems (off tree) or from the end of the packing line) or irradiation on lenticel discolouration severity and the percentage of pronounced lenticel spots in 'B74' mango. Fruit were rated at the full yellow (ripe) stage. The table presents the significant treatment interactions.

Factor	Treatment		
	Control	Water	Dye
Lenticel discolouration severity (0-5)			
Off tree	0.8 [d]	3.0 [c]	4.0 [b]
End of packline	3.3 [c]	4.0 [b]	4.8 [a]
Pronounced spotting (%)			
Non-irradiated	22 [b]	22 [b]	35 [a]
Irradiated	23 [b]	30 [a]	32 [a]

For either lenticel discolouration or pronounced spotting, means with the same letters are not significantly different (P=0.05) as tested by LSD.

In 'Honey Gold' fruit, the interactions between irradiation treatment and harvesting procedure were not significant for either LD or % of pronounced spots (data not shown). Across both harvesting procedure treatments combined, the exposure to water/dye increased the severity of LD compared to water and control fruit (Table 37). Across all three treatments combined, LD was more severe in fruit from the end of packline compared to off tree fruit, a response similar to that observed above with 'B74' mango. In contrast, the % of pronounced lenticels was not significantly affected by either treatment or harvesting procedure, suggesting that the increased LD severity was due mainly to an increasing number of obviously damaged lenticels rather than an increase in the more pronounced lenticels. This response was different to that observed with 'B74'.

'B74' fruit sampled from the end of the packing line had a significantly greater percentage of lenticels that had taken up dye, and a higher dye uptake score, compared with fruit sampled from the tree (Table 38; Plate 21). This reflects the increased LD of the packline fruit (Table 36). However, irradiation had no effect on the percent of dyed lenticels, despite irradiation increasing LD. Similar responses were observed with 'Honey Gold' in relation to off tree and end of packline, with increased percentage of dyed lenticels and dye uptake score with end

of packline fruit (Table 38). This also reflects the increased LD on the packline fruit (Table 37).

Table 37 Effects of the water and dye treatments and of harvesting procedure (fruit sampled from the tree with no exposure to water or packing systems or from the end of the packing line) on lenticel discolouration severity and the percentage of pronounced lenticel spots in 'Honey Gold' mango. Fruit were rated at full skin colour (ripe). The table presents the main factor effects; the treatment interactions were not significant.

Factor	Lenticel discolouration (0-5)	Pronounced lenticels (%)
Treatment		
Control	2.8 [b]	32
Water	3.0 [b]	31
Dye	3.8 [a]	34
Harvesting procedure		
Off tree	2.6 [b]	31
End of packline	4.0 [a]	34

For either treatment or harvesting procedure, means in columns with the same letters are not significantly different (P=0.05) as tested by LSD. The absence of letters indicates no significant differences.

'Honey Gold' had approximately three times less lenticels and less than half the number of dyed lenticels per unit area compared to 'B74' (Table 38). These results suggest that the density of lenticels per unit area in 'Honey Gold' may be one of the main reasons for this cultivar being less sensitive to developing significant LD following irradiation. This effect likely dominated the overall LD response, despite the observation that a greater percentage of the 'Honey Gold' lenticels had taken up dye compared with 'B74'.

Table 38 Effects of harvesting procedure (fruit sampled from the tree with no exposure to water or packing systems or from the end of the packing line), irradiation, and cultivar on the number of lenticels and the number of dyed lenticels per 12 cm^2, the percentage of dyed lenticels compared to the total, and dye uptake score in 'B74' and 'Honey Gold' mango. Fruit were rated at full skin colour (ripe). The table presents the main factor effects; the treatment interactions were not significant.

Cultivar/Factor	Total number of lenticels per 12cm^2	Number of dyed lenticels per 12cm^2	% dyed lenticels per 12cm^2	Dye uptake score (1-4)
'B74'				
Harvesting procedure				
Off tree	318	144	45 [b]	2.0 [b]
End of packline	297	186	62 [a]	2.8 [a]
Irradiation				
No	290	164	55	2.6
Yes	326	166	52	2.2
'Honey Gold'				
Harvesting procedure				
Off tree	89	65 [b]	74 [b]	1.8 [b]
End of packline	100	89 [a]	90 [a]	2.2 [a]
Cultivar				
'B74'	290 [a]	164 [a]	55 [b]	2.6 [a]
'Honey Gold'	94 [b]	77 [b]	82 [a]	2.0 [b]

For each cultivar/factor, means in columns with letters are significantly different (P=0.05) as tested by LSD. The absence of letters indicates no significant differences.

Off-tree End of packline

Plate 21 Effect of harvesting procedure (four fruit on the left were sampled from the tree with no exposure to water or packing systems, while the four fruit on the right were sampled from the end of the packing line) on dye uptake in 'B74' mango.

'B74' fruit had more lenticels in quadrant one, and also a higher dye uptake score compared with most of the other quadrants (Table 39). In contrast, there were no significant quadrant effects for any the parameters with 'Honey Gold'. These results again confirm the relationship between lenticel numbers, percent dyed lenticels and dye uptake score, and the significantly lower lenticel density in 'Honey Gold' compared with 'B74'.

Table 39 The number of lenticels and the number of dyed lenticels per 3 cm^2, the percentage of dyed lenticels compared to the total, and dye uptake score in 'B74' and 'Honey Gold' mango in the different quadrants around the fruit. Fruit were rated at the full yellow (ripe) stage. Results for each cultivar were added across all handling and irradiation treatments.

Quadrant	Total number of lenticels per 3 cm^2	Number of dyed lenticels per 3 cm^2	% dyed lenticels	Dye uptake score (1-4)
'B74'				
1	92 ^{a}	53 ^{a}	57	2.7 ^{a}
2	74 ^{bc}	39 ^{b}	52	2.4 ^{ab}
3	64 ^{c}	28 ^{c}	47	2.1 ^{b}
4	78 ^{b}	45 ^{ab}	58	2.4 ^{ab}
'Honey Gold'				
1	25	21	86	2.3
2	24	19	83	2.0
3	22	17	81	1.7
4	24	20	85	2.1

For each cultivar, means in columns with the same letters are not significantly different (P=0.05) as tested by LSD. The absence of letters indicates no significant differences.

4.8. Brushing and water effects

Minh Nguyen (PhD candidate), Peter Hofman, Daryl Joyce, Andrew Macnish, Medan Gupta

This chapter forms part of a current PhD program (Minh Nguyen). The results presented here have not yet been published. The chapter was drafted by the Ph.D. student, and reviewed by the project team.

4.8.1. Summary

Lenticel discolouration (LD) is a common postharvest disorder of mango fruits, caused by dark areas surrounding the lenticels. Commercial harvesting and packhouse practices increase LD, and brushing in the packing line to remove residues and improve appearance is a likely contributor. The contribution of liquids, solutes and brushing to LD in 'B74' mango fruit merited further investigation. 'B74' mango fruits were given typical commercial brushing treatments, and exposure to solutions of differing osmotic potential to determine effects on skin characteristics, lenticel morphology and LD. Polyethylene glycol 6000 (PEG6000) resulted in very high LD as the residual PEG6000 on the fruit surface absorbed moisture from the atmosphere or from the fruit during fruit ripening. Deionised water with gentle agitation increased LD in the ripe fruit, while adding surfactant or 1-3 % NaCl further increased LD. The effect of brushing on LD was unclear, but may play a role as a cumulative stress on lenticels, and its effect influenced by the fruit turgor status during brushing. However, brushing increased skin browning (SB) on the ripe fruit, so this treatment should be minimised to ensure adequate residue removal and "polishing" without increasing the risk of skin damage.

4.8.2. Introduction

Lenticel discolouration is a common postharvest disorder of mango fruits (du Plooy *et al.*, 2002; Self *et al.*, 2006). This discolouration often exhibits as a dark area surrounding the lenticels (Pesis *et al.*, 2000; Self *et al.*, 2006). Many packhouse practices cause LD (Cronje, 2009a). Stresses arise pre-harvest, at harvest, and postharvest in the packing-line, including contact with water and chemicals (such as rain at harvest, and water and chemicals used during harvesting and disease / pest control), physical challenges (for example brushing) and dehydration.

Exposure to treatments involving water and solutes during harvesting and packing can affect LD in 'B74' mango fruit (Hofman *et al.*, 2010b). Commercial solutions used during harvesting contributed to LD in several cultivars (Willis and Duvenhage, 2002). For example, 'B74' mango fruit treated with detergents expressed more LD when they reached the ripe stage (Whiley *et al.*, 2006). Commercial products used to reduce sapburn and skin browning (SB) during harvesting increased LD on 'Kensington Pride' mango fruit (O'Hare *et al.*, 1996). Also, hot water treatment increased black LD of 'Tommy Atkins' mango fruit (Self *et al.*, 2006). Vapour heat treatment, or a combination of vapour heat and hot water treatment (HWT), accelerated LD on 'Kensington Pride' ('KP') fruit (Jacobi and Giles, 1997) and 'Kent' mango fruit had slightly more LD after hot water treatment at 47°C for 5 min (du Plooy *et al.*, 2002). Postharvest treatment with 6% $CaCl_2$ caused damage to cells surrounding the lenticels on 'KP' fruit (Shorter and Joyce, 1998), and hot fungicide dip or spray treatments increased LD severity (Hofman *et al.*, 2010c). Turgid lenticel cells of avocado fruit, were more sensitive to physical damage (Everett *et al.*, 2008). Thus, liquids may infiltrate into the lenticel cavity, increase cell turgidity, and potentially increase sensitivity to discolouration. Also, reducing cell turgor may reduce LD, since a delay after harvesting and before packing reduced LD due to lower fruit turgidity (Cronje, 2009a).

Water contact is largely unavoidable in commercial mango packing in Australia because the high labour cost requires cost-effective and efficient harvesting and packing systems. One proposition to minimise the water effect on LD is to balance the water activities of treatment

liquids and fruit cells so that the cells in the lenticel cavities neither lose nor gain water. For example, Cronje (2009a) found that postharvest treatment with NaCl solution at 20 mS for 2 min followed by hot water dipping reduced LD. The water potential of this solute may reduce free water entry into cells of the lenticel cavity. Polyethylene glycol (PEG) has been used to create varying osmotic potential solutions. The PEG is not absorbed by the plant tissues being tested, as compared to sugars and salts (Mexal *et al.*, 1975). The osmotic potential of individual epidermal cells is about -0.8 MPa (Shackel, 1987), while the osmotic potential of PEG-6000 can vary from -0.19 MPa to -1.7 MPa at concentrations of 100-400 $g.kg^{-1}$ H_2O at 25^oC (Michel and Kaufmann, 1973). The correct balance between the osmotic potential of the surrounding liquids and fruit water potential may prevent water entry into lenticels and reduce the LD often associated with fruit treatment with water.

Brushing may accelerate LD, but is a significant step in commercial packing-lines to remove spray deposits and debris on the fruit surface. This reduces the waxy bloom and creates a shiny appearance. However, brushing affected the lenticel structure and modified the cuticular wax layer (du Plooy *et al.*, 2002), and brushing in commercial packing lines can accelerate LD (Oosthuyse, 2002). Likewise, brushing after HWT increased LD in 'Keitt' (Cronje, 2009a) and 'Carabao' mango cultivar (Esguerra *et al.*, 2004).The duration of brushing by itself for 0.5-5 min did not significantly affect LD of 'Keitt' mango but brushing with wax to form a fruit coating significantly reduced LD (Cronje, 2009a).

The mechanism/s by which liquids and brushing increase LD in mango fruit merits further investigation. The aim of these experiments was to characterise the effects of brushing and solutes with different osmotic potential (salts and PEG at different concentrations to vary the potential for the water to affect cell turgor) on skin appearance, cuticle and lenticels of 'B74' fruits. Histochemical observations were also used to characterise lenticel cavity changes.

4.8.3. Materials and methods

4.8.3.1. Fruits

Green mature 'B74' mango fruits were harvested from an orchard at Oolloo Farms, in Dimbulah, North Queensland (17°11'S, 145°10'E) on 12th December 2011. For solute treatments, fruit were harvested directly from 15 trees (10 fruit per tree) without exposure to water or solutes and 15 other fruit collected at the end of commercial packing line as the "end pack-line" control for the solute trial. For brushing, 225 fruit were collected from a field bin at a commercial packhouse (i.e. fruit had been commercially harvested and treated with 0.25% Mango Wash® for 1 min for sap removal, but had not entered the packing line for fungicide treatment, air drying, brushing, sorting and packing). Another 25 fruit were collected from the end of commercial packing line as the "end pack-line" control for the brushing experiment.

4.8.3.2. Treatments

Solutes

After harvest or after packing, fruit were placed into single layer trays with plastic inserts and driven by car to the research laboratories at Mareeba (45 min drive). The treatments outlined in Table 40 were applied within 1 h of harvest. There were 15 single fruit replications per treatment.

The shaking treatment consisted of gently shaking the bucket containing fruit and DI water for 2 min. No agitation was applied for the other treatments. PEG 6000 (class PG) and NaCl (AR) was supplied by Chem Supply Pty Ltd., and Agral® (non-ionic surfactant) supplied by Crop Care Australia Pty Ltd.

Following treatment, the fruit were transported by airplane from Cairns, north Queensland to Brisbane, south east Queensland. Fruit were exposed to 600 to 800 Gray (Gy) at the Steritech Pty Ltd facility in Brisbane as described in section 4.4.3.1. The fruit were irradiated

within 3 d of harvest. Following irradiation, the fruit were transported by car to MRF, Nambour for ripening and assessment. Fruit were treated with 10 $\mu L.L^{-1}$ ethylene at 20°C for 2 d, then ripened at 20°C until 7 d after full yellow skin colour (ripe). During ripening, 10 fruits per treatment were used for assessing LD and firmness. Five fruit were used for lenticel morphology examination.

Table 40 Solute treatments in 2011 in North Queensland to test the effect of DI water ± surfactant, NaCl at 1, 2, 3% and PEG 6000 at 150, 300, 400 $mg.L^{-1}$ (DI= deionised. OP = osmotic potential. PEG = polyethylene glycol) on harvest and post-harvest quality of 'B74' mango fruit.

Treatment	Description
Controls	
Off-tree control	Fruits were harvested directly from the tree into trays without any exposure to water or further handling
End pack-line control	Fruit were sampled at the end of the commercial pack-line (fruit exposed to Mango Wash® in the field during commercial harvesting, and to water, fungicides, brushing and packing
Treatments	
DI Water	Off-tree, dipped in DI water for 2 min
DI Water shaking	Off-tree, dipped in DI water plus shaking for 2 min
DI Water surfactant	Off-tree, dipped in DI water plus surfactant 0.1% Agral for 2 min
NaCl 1%	Off-tree, dipped in 1% NaCl for 2 min (osmotic potential- OP = -0.8 MPa)
NaCl 2%	Off-tree, dipped in 2% NaCl for 2 min (OP = -1.3 MPa)
NaCl 3%	Off-tree, dipped in 3% NaCl for 2 min (OP = -2.2MPa)
PEG150	Off-tree, dipped in 150 $mg.L^{-1}$ PEG 6000 for 2 min (OP = -0.3 MPa)
PEG300	Off-tree, dipped in 300 $mg.L^{-1}$ PEG 6000 for 2 min (OP = -0.9 MPa)
PEG400	Off-tree, dipped in 400 $mg.L^{-1}$ PEG 6000 for 2 min (OP = -1.7 MPa)

Brushing

After collection from the field bin and at the end of packing line, fruit were placed into single layer trays with plastic inserts and driven by car to Cairns airport (approx. 2 h drive). Fruit were air freighted to Brisbane and then by car to MRF for treatment. The fruit were divided equally into nine groups for treatment (Table 41).

Table 41 Brushing treatments for the 2011 trial to test the effect of brushing and water spray on the quality of 'B74' mango fruit.

Treatments	Description
Controls	Treatment
Field bin control	Fruit collected from the field bin after commercial picking without further treatment
End packing line control	Fruits from the same batch were placed over the commercial packing line, treated with fungicide, brushed, dried by forced air, packed and sampled from the end of the pack-line
Treatments	
Soft brush 1 min dry	Field bin fruit brushed with soft brush for 1 min with no water spray
Soft brush 2 min dry	Field bin fruit brushed with soft brush for 2 min with no water spray
Soft brush 1 min wet	Field bin fruit brushed with soft brush for 1 min with water spray
Soft brush 2 min wet	Field bin fruit brushed with soft brush for 2 min with water spray
Hard brush 1 min dry	Field bin fruit brushed with hard brush for 1 min with no water spray
Hard brush 2 min dry	Field bin fruit brushed with hard brush for 2 min with no water spray
Hard brush 1 min wet	Field bin fruit brushed with hard brush for 1 min with water spray
Hard brush 2 min Wet	Field bin fruit brushed with hard brush for 2 min with water spray

The soft brush was blue poly propylene bristles and the hard brush was black nylon (about 0.3 mm diam bristles). The brushing unit was manufactured by Adds Up Engineering Co.

(Bundaberg, Queensland), and consisted of five brush rollers rotating at 84 rpm. Each treatment comprised 25 single fruit replications.

Immediately after brushing, 10 fruits per treatment were used to measure wetting angle then assessed for skin gloss assessment and dye uptake characteristics. Another 10 fruit per treatment were treated with 10 $\mu L.L^{-1}$ ethylene at 20°C for 2 d then ripened at 20°C and assessed for quality. An additional five fruit were used for lenticel morphology examination.

4.8.3.3. Assessments

Each fruit in both experiments were individually assessed for skin colour based on the % of the non-blush skin area with yellow colour (0-6), hand firmness (0-4), and LD and skin browning (SB; 0-5) (Hofman *et al.*, 2010a) as described in section 4.1.3.3. Fruit firmness was also assessed with the Aweta Acoustic Firmness Tester.

In the brushing experiment, immediately after brushing 10 fruit per treatment were exposed to the dye test as described in section 4.1.3.3 then rated for the severity of spotty and blotchy dye uptake the day after brushing.

Wetting angle and lenticel morphology were examined as described in section 4.1.3.3.

For fruit gloss, the percentage of fruit surface area with gloss appearance was rated using a 1-6 scale similar to the skin colour scale but including the whole fruit. Thus 1=0-10% of the skin surface with gloss, 2=11-30%, 3=31-50%, 4=51-70%, 5=71-90% and 6=91-100%.

4.8.3.4. Statistical analysis

Statistical analyses were performed by Genstat® 14 for Windows™ (VSN International Ltd., UK). Analysis of variance (ANOVA) used the 'General Analysis of Variance' model, with bagging and coatings as 'treatment' structures, and tree as 'block' structure. The protected least significant difference (LSD) procedure at P=0.05 was used to test for differences between treatment means (gloss, skin colour, firmness, LD and dye uptake with 10 single fruit replications).

4.8.4. Results

4.8.4.1. Solutes

Lenticel discolouration

The off-tree control and DI water treatment had the lowest LD at both full yellow and 7 d later (

Table 42). However, DI water plus shaking increased LD at full yellow compared with no water and water with no agitation, while the addition of surfactant increased LD even more.

Fruit sampled after commercial picking and packing showed considerably higher LD at full yellow compared with fruit sampled directly from the tree (

Table 42). A solution of 1% NaCl resulted in similar LD compared with the DI water alone (with agitation), while increasing concentrations resulted in higher LD. All PEG treatments were associated with very high LD. The residual PEG on the skin appeared to maintain a moist layer on the fruit, thus resulting in consistently moistened skin.

The treatment effects at 7 d after full yellow were less, largely because of large increases in LD in the off tree control and DI treatments between full yellow and 7 d later (

Table 42). By this time, there were few treatment differences between the off tree control and the DI and and NaCl treatments, except for surfactant still being significantly higher than the other DI treatments.

Fruit firmness

There was no significant difference in hand firmness at full yellow, while at 7 d later the end of packing line fruit were the softest and the PEG 400 fruit the firmest (Table 43). When measured by Aweta, there were significant differences at full yellow but with little logical pattern.

Table 42 Lenticel discolouration on 'B74' mango fruit at full yellow skin colour (ripe) and 7 d later, following DI water without or with surfactant, sodium chloride (NaCl) at 1, 2, 3% and PEG 6000 at 150, 300, 400 mg.L^{-1}.

Treatments	Full colour	Full colour + 7 d
Off-tree control	0.8 ^{ab}	2.1 ^{a}
End packing line control	3.2 ^{f}	3.2 ^{b}
DI Water	0.6 ^{a}	1.6 ^{a}
DI Water + shaking	1.2 ^{c}	2.0 ^{a}
DI Water + surfactant	2.1 ^{e}	2.8 ^{b}
NaCl 1%	1.2 ^{bc}	2.0 ^{a}
NaCl 2%	1.7 ^{d}	2.1 ^{a}
NaCl 3%	2.0 ^{de}	2.1 ^{a}
PEG150	3.6 ^{g}	3.9 ^{c}
PEG300	4.0 ^{g}	4.3 ^{cd}
PEG400	4.0 ^{g}	4.5 ^{d}
P value at 0.05	<001	<001

Means (n=10) in columns with the same letters are not significantly different (P=0.05) as tested by LSD.

Table 43 Hand firmness and Aweta firmness reading (lower values reflect softer fruit) of 'B74' mango fruit at full yellow skin colour (ripe) and 7 d later, following DI water without or with surfactant, sodium chloride (NaCl) at 1, 2, 3% and PEG 6000 at 150, 300, 400 mg.L^{-1}. FC=full colour.

	Hand firmness (0-4)		Aweta	
Treatments	At FC	7 d after FC	At FC	9 d after FC
Off-tree control	2.5	3.8 ^{cd}	20.0 ^{abc}	13.6
End packing line control	2.8	3.9 ^{d}	22.3 ^{bc}	16.9
DI Water	2.6	3.8 ^{cd}	20.3 ^{abc}	15.4
DI Water + shaking	2.7	3.7 ^{bcd}	16.4 ^{a}	16.5
DI Water + surfactant	2.6	3.7 ^{bcd}	23.5 ^{c}	15.9
NaCl 1%	2.6	3.7 ^{bcd}	22.6 ^{bc}	17.0
NaCl 2%	2.8	3.5 ^{ab}	23.0 ^{c}	18.1
NaCl 3%	2.6	3.5 ^{ab}	18.8 ^{ab}	16.7
PEG150	2.5	3.6 ^{abc}	21.8 ^{bc}	20.3
PEG300	2.7	3.6 ^{abcd}	23.5 ^{c}	17.2
PEG400	2.6	3.5 ^{a}	21.8 ^{bc}	18.6
P value at 0.05	ns	0.03	0.02	ns

Means (n=10) in columns with the same letters are not significantly different (P=0.05) as tested by LSD.

Lenticel morphology

Lenticels from fruit sampled from the end of the packing line clearly had more discolouration than those from the off-tree control and water treatments (Plate 22). PEG treatment had severely discoloured lenticels. Surfactant increased the damaged cells compared to the off tree control. DI water, DI water + shaking and NaCl treatments slightly increased browned cells.

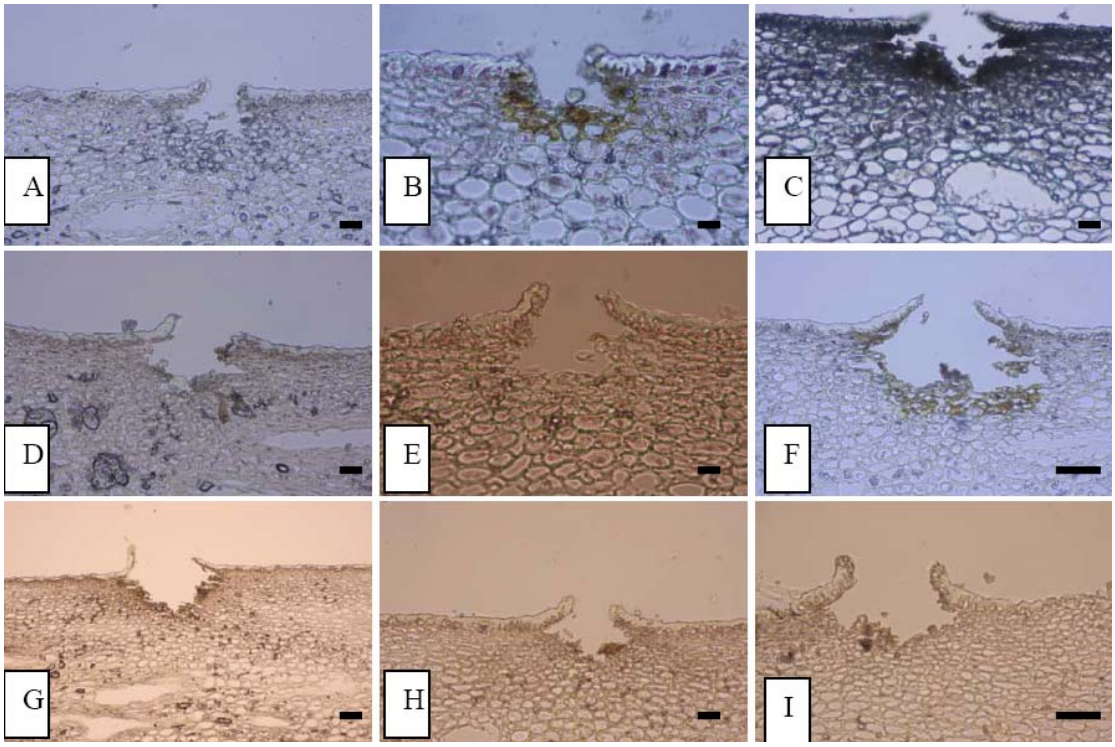


Plate 22 Unstained transverse sections of Calypso at full colour of Off tree control (A); End packline control (B); PEG 300mg.kg-1 H2O (C); DI Water (D); DI Water+shaking (E); Surfactant (F); NaCl 1% (G); NaCl 2% (H); NaCl 3% (I) Scale bar = 10 µm.

4.8.4.2. Brushing

Lenticel discolouration

At the full yellow stage, LD in all brushing treatments was similar to that of the field bin control, while LD in the end-packline fruit was considerably higher than all other treatments (Table 44). At 7 d after full yellow, most brushing treatments had similar LD as the field bin control, except for the soft brush 1 min dry and hard brush 2 min dry. Only hard brush 2 min wet had higher LD as compared to off-bin control. End-packline control fruit had the highest LD at both full yellow and 7 d after full yellow. There was little consistent evidence that the addition of water with brushing increased LD compared with brushing without water.

In general, there was no clear difference between brushing treatments and field bin control. Type of brushing and duration, or water did not contribute to LD.

Gloss

Off bin fruit had the lowest gloss because of minimal handling and no brushing (Table 44). Hard brushing increased the gloss compared with soft brushing in most cases. Longer brushing with soft brushes increased gloss, but there was no duration effect with hard brushes. The only water effect was reduced gloss with 2 min soft brushes.

Dye uptake

In general, all treatments had very high dye uptake (Table 45). The 2 min brushing generally resulted in higher blotchy and spotty ratings compared with 1 min brushing. Water increased the blotchy rating with hard brush 2 min, and similar trends were noted with the hard brush 1 min treatment.

Table 44 Lenticel discolouration of 'B74' fruit at full yellow and 7 d after full yellow, following brushing with soft or hard brushes without or with water spray during brushing for 1 or 2 min. FY = full yellow.

Treatments	Lenticel discolouration (0-5)		Gloss (1-6)
	At FY	7 d after FY	
Field bin	1.8 ab	3.1 bc	0.6 a
End packing line	3.2 c	4.6 e	2.7 b
Soft brush 1 min dry	1.3 a	2.5 a	2.9 b
Soft brush 2 min dry	1.8 ab	2.7 ab	4.6 cd
Soft brush 1 min wet	1.7 ab	3.1 bc	3.0 b
Soft brush 2 min wet	1.7 ab	3.0 abc	3.4 b
Hard brush 1 min dry	2.1 b	3.4 cd	4.6 cd
Hard brush 2 min dry	1.8 ab	2.5 a	5.1 d
Hard brush 1 min wet	1.9 ab	3.2 bcd	4.3 c
Hard brush 2 min wet	1.9 ab	3.7 d	4.5 cd
P value at 0.05	<001	<001	<001

Means (n=10) in columns with the same letters are not significantly different (P=0.05) as tested by LSD.

Table 45 Dye uptake of 'B74' fruit at full yellow and 7 d after full yellow, following brushing with soft or hard brushes without or with water spray during brushing for 1 or 2 min.

Treatment	Blotchy (1-6)	Spotty (1-6)
Field bin	4.7 cd	4.1 abc
End packing line	4.1 bc	4.9 def
Soft brush 1 min dry	3.4 a	4.3 bcd
Soft brush 2 min dry	5.4 e	5.2 ef
Soft brush 1 min wet	4.2 bc	3.6 ab
Soft brush 2 min wet	5.1 de	4.5 cde
Hard brush 1 min dry	3.7 ab	3.4 a
Hard brush 2 min dry	4.1 bc	4.6 cdef
Hard brush 1 min wet	4.3 bc	4.1 abc
Hard brush 2 min Wet	5.7 e	5.3 f
P value at 0.05	<001	<001

Means (n=10) in columns with the same letters are not significantly different (P=0.05) as tested by LSD.

Skin browning

Hard dry brushing resulted in the most severe SB of the treatments at full yellow, while water with hard brushes reduced SB to levels similar to the controls (Table 46). Soft brushes produced similar SB to the end packing line treatment. Skin browning of soft brush for 1 min was lower than that of other brushing treatments. These effects were not so obvious by 7 d after full yellow.

Wetting angle

All treatments resulted in lower wetting angles compared with the field bin control fruit (Table 47). Longer brushing time increased wetting angle in all cases except soft brushes without water. Water increased the wetting angle with hard brushes, and with the soft 2 min treatment.

Lenticel structure

In general, brushing did not change lenticel structure or appearance (Plate 23). Different microscopy techniques would be required to identify treatment effects on wax structures on the surface of the fruit.

Table 46 Skin browning severity of 'B74' fruit at full yellow and 7 d after full yellow, following brushing with soft or hard brushes without or with water spray during brushing for 1 or 2 min.

Treatments	Full yellow	7 d after full yellow
Field bin	0.5 [a]	1.6 [ab]
End packing line	0.8 [ab]	1.8 [abc]
Soft brush 1 min dry	1.1 [abc]	1.4 [a]
Soft brush 2 min dry	1.0 [abc]	1.8 [abc]
Soft brush 1 min wet	0.6 [a]	1.3 [a]
Soft brush 2 min wet	0.7 [ab]	2.5 [cd]
Hard brush 1 min dry	1.5 [c]	2.7 [d]
Hard brush 2 min dry	1.5 [c]	1.8 [abc]
Hard brush 1 min wet	0.8 [ab]	1.9 [abcd]
Hard brush 2 min wet	0.7 [ab]	2.3 [bcd]
P value at 0.05	0.006	0.018

Means (n=10) in columns with the same letters are not significantly different (P=0.05) as tested by LSD.

Table 47 Wetting angle of 'B74' fruit at full yellow and 7 d after full yellow, following brushing with soft or hard brushes without or with water spray during brushing for 1 or 2 min.

Treatments	Wetting angle after brushing
Field bin	104.20 [d]
End packing line	98.92 [c]
Soft brush 1 min dry	95.56 [b]
Soft brush 2 min dry	89.65 [a]
Soft brush 1 min wet	94.40 [b]
Soft brush 2 min wet	98.34 [c]
Hard brush 1 min dry	89.43 [a]
Hard brush 2 min dry	93.18 [b]
Hard brush 1 min wet	94.77 [b]
Hard brush 2 min wet	99.70 [c]

Means (n=80) in columns with the same letters are not significantly different (P=0.05) as tested by LSD.

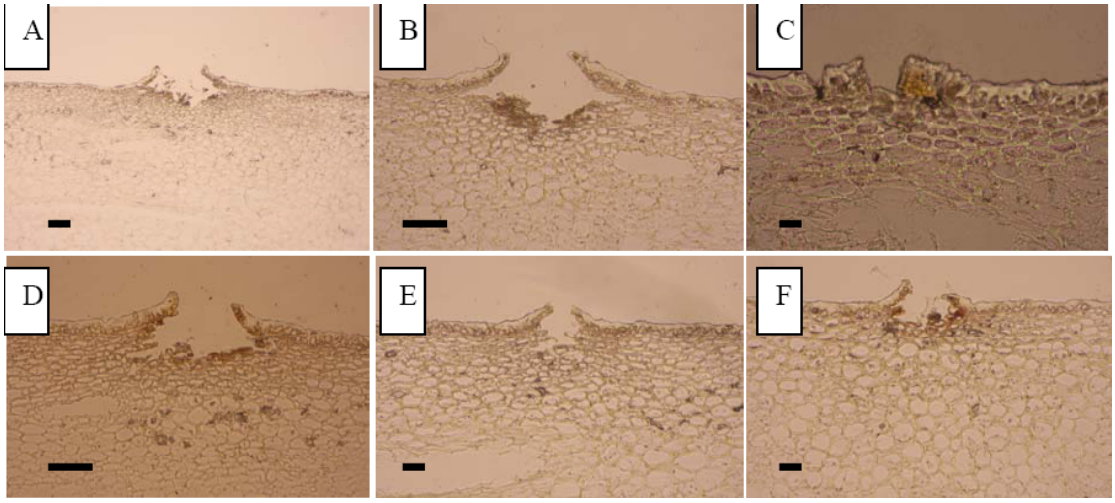


Plate 23 Unstained transverse sections of Calypso at full colour of Rough Dry 1 min Brush (A); Rough Dry 2 min Brush (B); Rough Wet 1 min Brush (C); Soft Dry 1 min Brush (D); Soft Dry 2 min Brush (E); Soft Wet 1 min Brush (F); scale bar = 10 µm

'B74' fruit picked from the tree and exposed only to brushing on a commercial packing line, had more LD than no brushing (Hofman *et al.*, 2010b). However, the current trial showed little effect of brushing. The brushing treatment, including brush type, brush rotation speed and duration were similar to those observed commercially. It is possible that brushes under typical industry conditions are more abrasive from excessive wear and other contaminants from the packing line. Also, all brushing treatments produced similar or more gloss to the commercial fruit, indicating that the brushing treatments used in this trial were sufficient to meet the industry expectations for gloss without increasing LD. However, there is increased risk of SB with brushing, especially with no water, so brushing in the packing line needs to be the minimum required to remove residues while polishing the fruit, especially when fruit are harvested during wet periods, and after HWT (Cronje, 2009a).

4.8.5. Discussion

Previous research has indicated that exposure of 'B74' mango fruit to water after harvest can increase LD (Hofman *et al.*, 2010b). The treatments that reduce the boundary layer effects between water and the fruit surface (for example gentle agitation and surfactants) were required to achieve this water effect. Also, imbibing 'Hass' avocado fruit with water for 12 h increased lenticel damage following simulated fruit movement/bumping (Everett *et al.*, 2008), presumably because increased cell turgor of the generally exposed lenticels (usually on the nodules of the lumpy skin) are more prone to damage from impacts. This is less so with mango.

None of the solute treatments reduced LD compared to the water controls. This is contrary to South African research which indicated that NaCl at 20mS can reduce LD on 'Tommy Atkins' (Cronje, 2009a). In the present trial, it is possible that higher concentrations of NaCl caused direct dysfunction of the cells around the lenticel cavity, thus resulting in increased LD. The PEG solutions can create osmotic potential pressure that prevents water entry into cells (Mexal *et al.*, 1975). In the current trails the PEG treatment likely increased LD significantly because of movement of water from the fruit toward the more negative water potential of the remaining PEG on the skin, and/or absorption of moisture from the air. Thus, it is uncertain whether the increased LD of the PEG treatments was from dehydration of the cells or from excess turgor.

4.9. Time from packing to retailer

Peter Hofman and Rob Gray

4.9.1. Summary

Fruit age (days between harvest and consumption) can significantly affect skin appearance as well as other characteristics such as flavour. Examination and analysis of the commercial Calypso™ ('B74') fruit movement data collected by The Harvest Company (THC) was undertaken to summarise fruit movement and durations and identify improvements to reduce quality loss through more efficient handling

The analysis showed that detailed records are collected of lot and batch movement from farm to retail chain distribution centre (DC) arrival. The days between packing and DC arrival were generally good and within acceptable limits given the distance between farm and market and the ripening times required. About 75% of consignments arrived at DC within 15 d of packing, and about 95% within 20 d. This provides about 2-7 d to buffer the imbalance between supply and delivery to retailer. Longer durations were usually associated with fruit from the Northern Territory (NT) sold in Perth and Darwin, which probably reflects both the longer distances from farm to ripener and ripener to DC. Longer durations were also associated with Dimbulah fruit.

Therefore, the commercial data recording batch movements from packing to retailer delivery were generally adequate to identify the need for improved practices. Recording the date of dispatch from the ripener would add value to the analysis. Improvements should target reducing the percentage of consignments that take longer than 15 d after packing to arrive at the DC, but with some tolerance for batches from remote farms.

4.9.2. Introduction

Poor external appearance of mango will reduce customer and consumer purchasing behaviour. Fruit with skin marks at harvest can be graded out during packing, while damage caused from harvesting onwards often will not become obvious until after packhouse dispatch. Skin damage after packing can occur by:

- transport and handling,
- excessive forced air cooling, particularly at low humidity, which can remove too much moisture from the fruit, and
- excessive holding before ripening or after ripening. Skin marking such as physical damage and lenticel discolouration (LD) become more obvious as the time between picking and consumption increases, and flavour can deteriorate.

Examination and analysis of the commercial Calypso fruit movement data collected by THC was undertaken to:

- advise on the detail of the data collected,
- summarise the data for fruit movement durations from packing to ripener dispatch,
- recommend improvements to reduce quality loss through more efficient handling, and
- suggest whether additional data collection will improve the ability to detect practices compromising quality.

Table 48 summarises the approximate days and conditions at each step from packing onwards for 'B74' mango, as understood by THC. To verify these figures the commercial THC data from the 2010/11 and 2011/12 seasons were summarised and recommend whether further efforts are required to reduce the time from packing to DC arrival to minimise quality loss.

Table 48 Estimated typical operations, temperatures and durations for most 'B74' consignments, based on discussions with THC. Most consignments in 2011/12 were part-ripened in transit by setting truck temperatures at 16-18°C.

Process	Temperature	Estimated days
Consolidating and cooling on farm	16-18°C	1
Transport to ripener	16-18°C	3-5
Ripening completed (ethylene as appropriate)[1]	18°C	3-5
Cooling to holding/dispatch temperature	13°C	1
Holding before dispatch	13°C	3-5, no more than 7-10
Transport to retailer	Usually 15°C	1-2
Summary		
Days from packing to ripener arrival		4-6
Days from ripener arrival to retailer arrival		8-18
Days from packing to DC arrival		12-24

4.9.3. Materials and methods

Commercial data of 'B74' mango lot movements were obtained from the THC files for each season. Calculations were based on the following:

- Only data for Woolworths and Coles were used because these dispatches recorded "Sale date" as the date fruit were received at the retail chain DC. This accounted for about 80% of lots.
- Multiple lot entries occurred when fruit from the lot were dispatched from the ripener on different days (called dispatches in this report). All Woolworths and Coles lot entries (except those with missing dates) were used in the analysis.
- It is possible that most of the lot was sold at the earlier sale date and that only a small proportion of the lot was held over for later sale, but these data were difficult to extract. Hence the analysis is not based on when e.g. 90% of the lot was sold, but on batch movement dates irrespective of trays in the batch.

More detailed qualitative analyses of the data were undertaken for those dispatches taking longer to transport to the packhouse or to arrive at DC to identify farm and sale location.

4.9.4. Results

Figure 15 presents the detailed results of the percentage of dispatches and days between packing and ripener arrival, days between ripener arrival and DC arrival, and packing and DC arrival. Figure 16 presents the cumulative percentage of lots or dispatches arriving at ripener or DC for the same categories. These data are further summarised below.

4.9.4.1. Average days

Table 49 indicates that, on average, fruit arrived at the ripener within about 5 d of packing, arrived at the DC within about 8 d of ripener receival, and arrived at the DC within 13 d of packing. There was little difference in the average between the two seasons, although there was a reduced range in 2011/12. Note that these "delayed" dispatches may only represent a small percentage of the volume of each lot.

[1] Commercial experience indicates that quality suffers if part ripened fruit are cold stored on arrival. Hence all consignments are fully ripened before holding.

Table 49 The average days between packing and arrival at the ripener, ripener arrival to retail distribution centre (DC) arrival, and packing to DC arrival for 'B74' mango, based on the lot number and dispatches data from The Harvest Company.

	Average days		Range	
	2010/11	2011/12	2010/11	2011/12
Packing to ripener arrival	4.7	5.1	1-14	1-18
Ripener arrival to DC arrival	8.0	8.4	0-42	0-31
Packing to DC arrival	12.8	13.6	2-44	1-35

4.9.4.2. Percentage of dispatches

Table 50 shows that 93-95% of lots arrived at the ripener within 8 d of packing for both seasons. About 97% of lots arrived at the DC within 15 d of arrival at the ripener, and 98% (2010/12) and 92% (2011/12) of lots arrived at the DC within 20 d of packing.

Table 51, Table 52 and Table 53 suggest that the time window where the greatest percentage of lots or dispatches arrived at ripener or DC was a few days later in 2011/12, compared with 2010/11. This suggests that fruit movement was a bit slower between ripener and DC arrival in 2011/12.

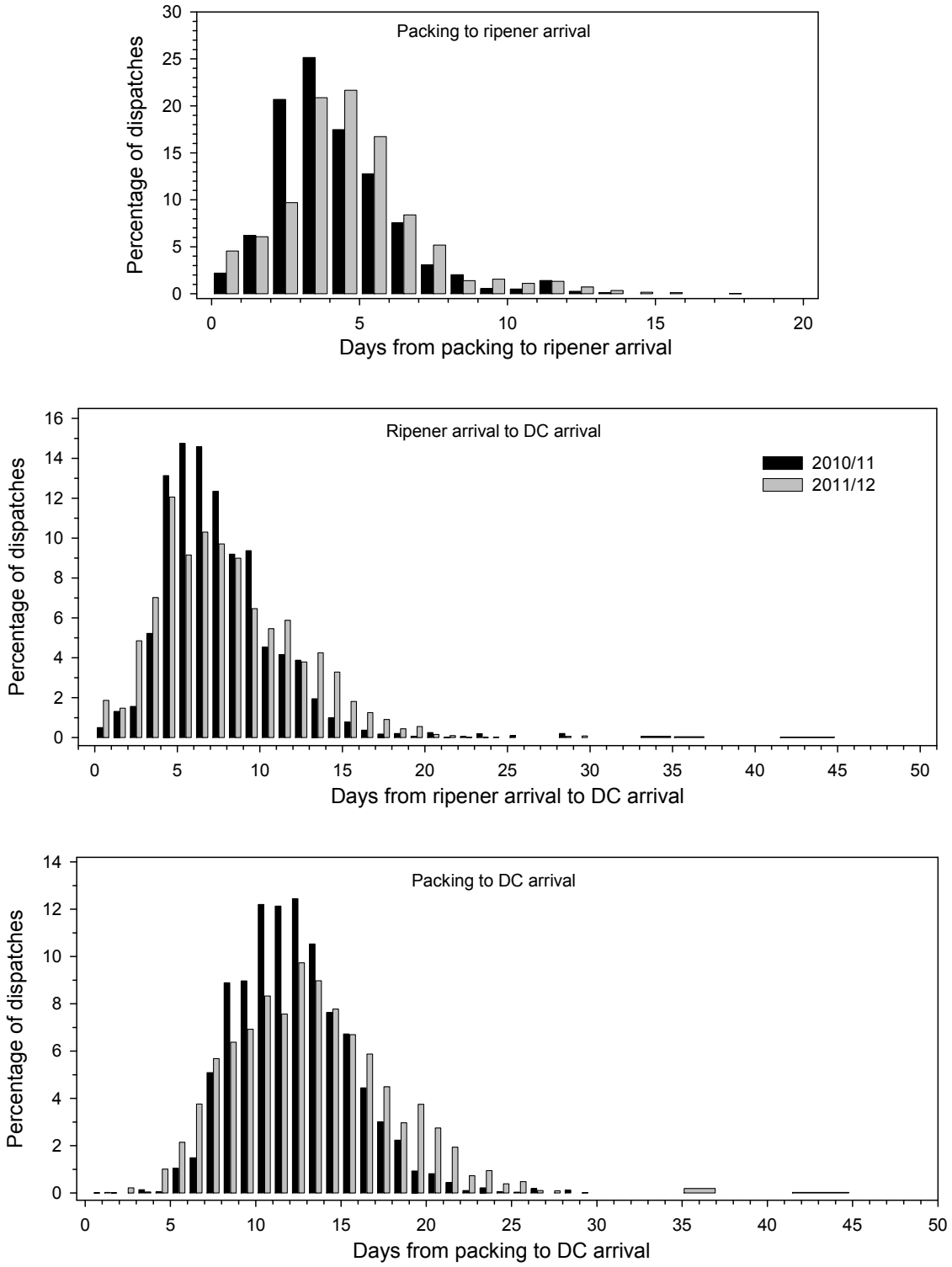


Figure 15 Detailed histograms of the percentage of dispatches that arrived at the ripener during specified days of packing (top graph) or arrived at the Coles or Woolworths distribution centre (DC) during specified days after arrival at the ripener (middle), or arrived at the DC during specified days of packing (bottom). Data are for 'B74' mango through The Harvest Company for the 2010/11 and 2011/12 seasons.

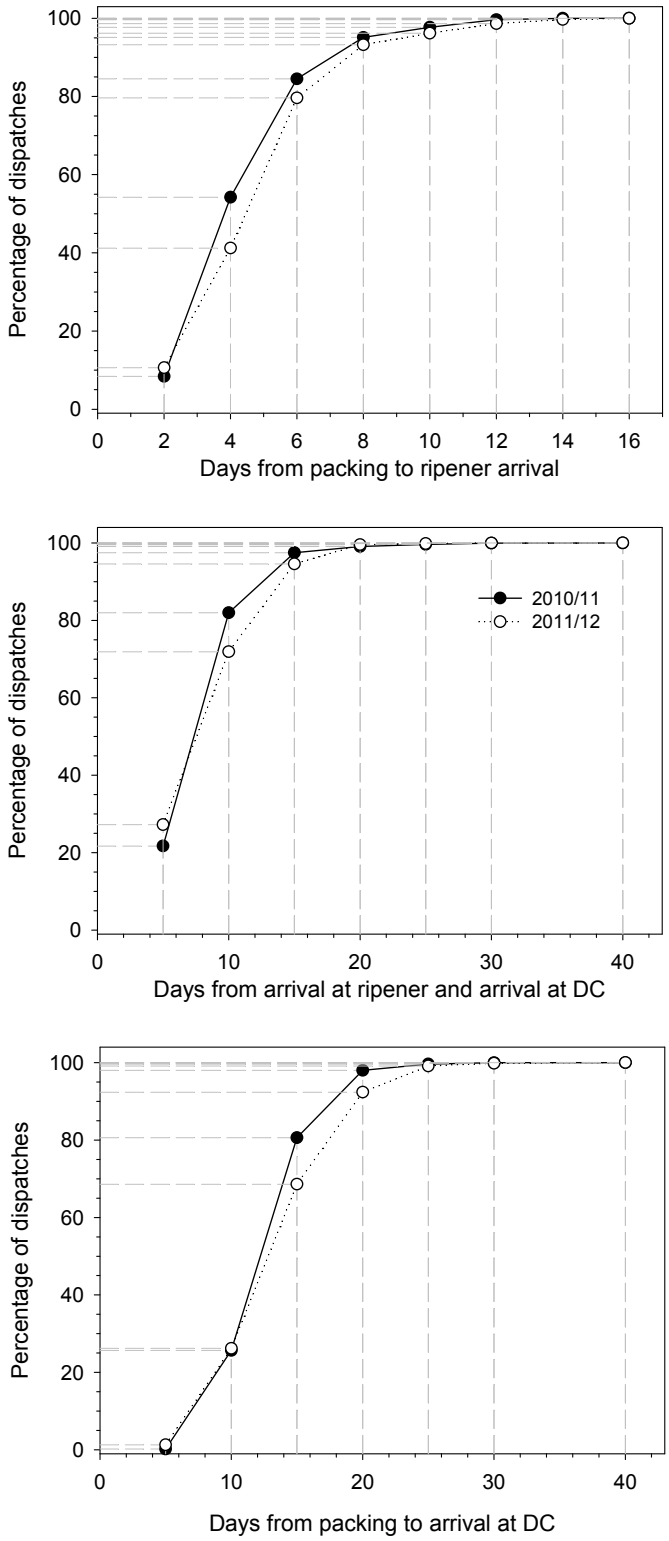


Figure 16 The total accumulated percentage of dispatches that arrived at the ripener within specified days of packing (top graph) or arrived at the Coles or Woolworths distribution centre (DC) after specified days after arrival at the ripener (middle), or arrived at the DC after specified days of packing (bottom).

Table 50 The percentage of dispatches that arrived at the ripener within specified days of packing, arrived at the retail chain distribution centre (DC) within specified days of arrival at the ripener, or arrived at the DC within specified days of packing. For example 8% of the dispatches arrived at the ripener within 2 d of packing in 2010/11.

% of dispatches within:	% of dispatches dispatched		
	Packing to arrival at ripener	Ripener arrival to DC arrival	Packing to DC arrival
2010/11 season			
2 days	8		
4 days	54		
6 days	84		
8 days	95		
10 days	98	82	25.7
12 days	100		
15 days		97	80.6
20 days		99	98.0
25 days		100	99.6
30 days		100	100
2011/12 season (to 13 Feb)			
2 days	11		
4 days	41		
6 days	80		
8 days	93		
10 days	96	72	26.2
12 days	99		
15 days		95	68.6
20 days		100	92.4
25 days		100	99.1
30 days		100	99.8

Table 51 The percentage of 'B74' mango dispatches that arrived at the ripener between specified days after packing. For example, 8.4% of lots arrived between 0 and 2 days of packing in 2010/11.

Percentage of dispatches that arrived at the ripener between "x and y" days of packing	Percentage of dispatches	
	2010/11	2011/12
0 and 2	8.4	10.6
3 and 4	45.8	30.6
5 and 6	30.3	38.4
7 and 8	10.6	13.6
9 and 10	2.6	3.0
11 and 12	1.9	2.4

Table 54 indicates that those lots taking longer to arrive at ripener originated mainly from the NT and Dimbulah in 2010/11, and mainly Dimbulah in 2011/12. Fruit taking longer from ripener arrival to DC arrival usually originated from Carnarvon and the NT and sold in Darwin and Perth in 2010/11, and fruit from the NT sold in Perth and Darwin in 2011/12.

Table 52 The percentage of 'B74' mango dispatches that arrived at the distribution centre (DC) between specified days of arrival at the ripener. For example, 21.7 % of dispatches arrived at the DC between 0 and 5 days of arrival at the ripener in 2010/11.

Percentage of dispatches that arrived at the DC within the "x and y" days of arrival at the ripener	Percentage of dispatches	
	2010/11	2011/12
0 and 5	21.7	27.3
6 and10	60.2	44.6
11 and 15	15.5	22.7
16 and 20	1.6	5.0
21 and 25	0.5	0.3
26 and 30	0.3	0.1
30 or more days	0.1	0.1

Table 53 The percentage of 'B74' mango dispatches that arrived at the DC between specified days of packing. For example, 0.2 % of dispatches arrived at the DC between 0 and 5 days of packing in 2010/11.

Percentage of dispatches that arrived at the DC within "x and y" days of packing	Percentage of dispatches	
	2010/11	2011/12
0 and 5	0.2	1.3
6 and10	25.5	24.9
11 and 15	54.9	42.4
16 and 20	17.4	23.8
21 and 25	1.6	6.8
26 and 30	0.4	0.7
30 or more days	0.0	0.2

4.9.5. Discussion and conclusions

The data recorded by THC allowed good analysis of lots and dispatch movements. The only significant data not collected was time of dispatch from the ripener.

The analysis generally supports the THC estimates of times at each stage from farm to DC arrival. For example 92-98% of all dispatches arrived at the DC within 20 d of packing. The greatest percentage of dispatches arrived at the DC between 11-15 d after packing.

Mango fruit usually require at least 1 d for cooling after packing, at least 3 d for transport from the NT, at least 6 d for ripening, 1 d for cooling before dispatch, and about 2 d for transport to the DC; a total of about 13 d of essential operations. Almost 75% of all lots were dispatched to the retailer within 15 d of packing, and 95% within 20 d of packing. This allowed 2-7 d to balance the flow between harvest and retailer demand. Therefore, the data suggests generally good practice given the distance from farm to market and the ripening times required, but reducing the % of consignments taking longer than 15 d to reach the retailer should be targeted.

Delays between packing and ripener arrival are influenced by holding time on the farm (dependant on cooling time and transport availability) and transit time.

The longer times from NT farms to the ripener were likely influenced by distance and transport availability, and most likely transport availability for Dimbulah. Longer times between ripener arrival and DC arrival were usually associated with Northern Territory fruit sold in Darwin and Perth. These longer durations may affect fruit appearance and flavour.

The use of higher transport temperatures in 2011/12 to allow in-transit ripening should have reduced the time required at the ripener by about 3-5 d. However, the data do not support this since the days between ripener arrival and DC arrival were generally more in 2011/12. Further discussion on this would be worthwhile.

Table 54 More detailed data for those batches that took longer to arrive at ripener or DC. NT = Northern Territory, NQ = North Queensland, WA = Western Australia.

Days between each stage	Farm location	Distribution centre location
Days from packing to ripener arrival		
2010/11		
9	Approx. 50% from NT, 50% from Dimbulah (NQ)	
10	Approx. 30% NT, 70% Dimbulah	
11	Mainly Mataranka (NT)	
12	Approx. 30% from NT, 60% from Dimbulah	
13-14	NT	
2011/12		
10 days	Mainly Dimbulah	
11 days	Mainly Dimbulah	
12	Acacia (NT)	
14	Mareeba (NQ)	
Days between ripener arrival and DC arrival		
2010/11		
23	Dimbulah	Gold Coast
24	Mataranka	Darwin
25	Acacia	Darwin
29	Carnarvon (WA)	Perth
32	Carnarvon	Perth
2011/12		
19	Mainly Mataranka and Katherine (NT)	MKay, Perth, Coss, Cosm
20	Mataranka, Acacia	Perth, Darwin
21	Mataranka	Perth, Coss
22	Katherine and Mataranka	Perth, Coss
23	Mataranka	Perth
29	Acacia	Darwin
30	Acacia	Darwin
31	Acacia	Darwin

5. Market access

5.1. Effect of maturity on lenticel discolouration

Roberto Marques, Minh Nguyen, Peter Hofman, Jonathan Smith and Barbara Stubbings

5.1.1. Summary

Previous studies have shown that lenticel discolouration (LD) on 'B74' mango fruit can increase with advancing harvest maturity. In addition, studies over three seasons showed that irradiation at disinfestation doses (200-400 Gy) often causes considerable LD and skin browning (SB) on 'B74' fruit. However, the relative susceptibility of fruit of different maturities to irradiation-induced lenticel discolouration has not been investigated.

Fruit were harvested from a farm in south-east Queensland four times over a five week period from selected trees. The fruit were exposed to 0 or approx. 350 Gy gamma irradiation, and then ripened. Some fruit were also dipped into a solution of "Brilliant Blue" food dye at harvest as a potential indicator of water uptake into the lenticels, which may be one of the mechanisms increasing lenticel sensitivity to discolouration. The key findings were:

- LD increased gradually and markedly with advancing harvest maturity. From the first to the fourth harvest, LD (0-5 scale) at full yellow (ripe) increased from 0.3 to 2.5 for non-irradiated fruit, and from 1.5 to 3.6 for irradiated fruit.
- At each harvest time, irradiated fruit had consistently more LD than non-irradiated fruit.
- Dipping fruit in food dye at harvest resulted in blotchy and spotty dye uptake patterns around specific lenticels, and there were significant positive correlations between the degree of blotchy and spotty dye uptake and LD.
- The incidence of dyed lenticels showing a blotchy or spotty pattern increased in later harvested fruit.

These results confirm that LD can be more severe in more mature fruit, thereby increasing the risk of commercially significant LD following irradiation. This maturity effect may be partly caused by the increased ease of water penetration into the lenticels, since previous research has consistently shown that water exposure after harvest increases LD following irradiation. The dye test may be a useful indicator of the commercial risk of LD because of significant correlations between LD and extent of dye uptake.

5.1.2. Introduction

Lenticels are modified stomata that become non-functional as the fruit expands (du Plooy *et al.*, 2006). Discolouration of lenticels is readily visualised as a darkening of the cells and tissues immediately surrounding the lenticels (Bezuidenhout *et al.*, 2005; Hofman *et al.*, 2010b). This discolouration can seriously reduce the external appearance of the fruit and downgrade its commercial value (Rymbai *et al.*, 2012). There is little research done on the impact of fruit maturity or harvest date on LD of mango fruit. In previous preliminary studies with 'B74' fruit from south east Queensland, LD severity increased in fruit harvested later in the season (Whiley *et al.*, 2006).

'B74' fruit have greater numbers of lenticels per unit area than other Australian cultivars (section 4.1) and this often results in more obvious LD on the skin after irradiation. Although delaying mango harvest can improve fruit flavour and size, the potential loss of external quality due to increased LD needs to be considered. This is particularly important if fruit are to be irradiated, and when heavy rain around harvest is predicted.

Studies over three mango seasons showed that irradiation at disinfestation doses (200-400Gy) can significantly reduce the visual quality of commercially picked and packed 'B74' fruit. This loss in quality mainly arises from LD and SB (Hofman *et al.*, 2010b). However, the effect of fruit maturity on the susceptibility to irradiation-induced LD was not investigated.

Preliminary results from the year 2010/11 showed that fruit harvested at a later maturity had fewer lenticels per unit area. However, a relatively greater number of these lenticels took up "Brilliant Blue" dye, resulting in a higher dye uptake score. Taken together, this suggested that the main cause for increased LD with advancing fruit maturity may be greater penetration of water into the lenticels of more mature fruit. However, results were inconclusive as there was little effect of harvest date on dry matter due to unusually high rainfall during the harvest period and excessive fruit drop. These observations needed to be confirmed and further experiments were required to establish the correlation between LD and dye uptake.

To test this, fruit from a commercial farm in south east Queensland were harvested four times over a five wk period from selected trees. Fruit were assessed for dry matter (DM) and flesh colour (FC) at harvest (as indicators of fruit maturity), either irradiated or not at 350Gy, ripened, and assessed for external quality (including LD and SB) at full yellow and 7 d later. Additional fruit were dipped into a solution with "Brilliant Blue" food dye before being assessed for external quality and dye uptake.

5.1.3. Materials and methods

5.1.3.1. Trial 1: Maturity and irradiation

Fruit harvest

Fruit adjacent to those used for the 'Production Factors' trial (refer to section 4.3) at a commercial farm at Childers in south east Queensland were harvested in early 2012 for this trial. Twenty four trees were selected and tagged (1-24), subdivided into four groups of six trees each (A-D, with each group corresponding to one harvest time), in a completely randomised design across three rows, as follows:

Row 1	1-B	2-C	3-D	4-A	5-C	6-A	7-C	8-D
Row 2	9-C	10-B	11-B	12-D	13-B	14-A	15-D	16-A
Row3	17-C	18-A	19-C	20-A	21-D	22-B	23-D	24-B

Fruit were harvested four times as follows:

Harvest	Targeted DM (%)	Harvest date
A. Early	13.5-14.0	31/1/12
B. Early-mid*	14.0-15.0	6/2/12
C. Mid-late	16.0-17.0	20/2/12
D. Late	>17.0	29/2/12

* = Commercial harvest on the block was done around 6/2/12.

For each harvest time, 32 fruit were picked from each of six trees (tree replication). The fruit were of average size for the tree, and were harvested from all aspects of the tree. They were picked with long stems and carefully placed on the ground. Three fruit from each tree were set aside to be used for Trial 2 (see below). The stems were removed, de-sapped on paper towel, labelled (tree number) and placed into cardboard trays with plastic inserts. They were not exposed to water during desapping.

The remaining fruit had the stem removed, held for a couple of seconds with the stem end facing down (to remove spurt sap), then dipped into a 50 L plastic container with Mango

Wash® solution (75 g powder per 30 L of water) for 4 min. The fruit were then placed into labelled (tree number) plastic crates, and transported by car to the postharvest laboratory at the DAFF Maroochy Research Facility (MRF) in Nambour. A temperature logger was inserted into a fruit in one of the crates.

Sorting and irradiation

Five fruit per plastic crate were set aside and labelled (tree number) to determine flesh % dry matter (DM) and flesh colour (FC), following the procedures given in the "'B74' Best Practice Guide" (Hofman and Whiley, 2010). Both DM and FC were also estimated using a Near Infrared Spectrometry (NIRS) handgun model 'Nirvana' (Spectronics Pty. Ltd., Australia). The remaining 24 fruit per plastic crate were dipped into fungicide solution (55 mL of Sportak.100L^{-1} of water) for 30 s. Fruit were allowed to dry before being individually labelled (tree number), divided into two lots of 12 fruit each per tree, and re-packed into cardboard labelled ("control" or "irradiated") trays with plastic inserts.

Fruit from all trees were randomised across the trays, so each tray had three fruit from all six trees (a total of 18 fruit per tray), and those fruit were also randomised within each tray. The fruit were then air-freighted (total of eight trays per harvest) to Sydney (Lucas Heights) the next day, and half of the trays were irradiated at 350 Gy within 24 h of arrival in Sydney. After irradiation, fruit were air-freighted back to MRF the same day. All fruit were then treated with 10 µl.L^{-1} of ethylene for 2 d at 20°C then ripened at 20°C.

Fruit quality assessment

Fruit were individually assessed based on the rating systems described in the "'B74' Quality Assessment Manual" (Hofman *et al.*, 2010a). On arrival at the laboratory after irradiation, the % dry matter and flesh colour were estimated using the 'Nirvana' handgun, and LD visually assessed (refer to section 4.1.3.3). When each fruit reached colour stage 6 (more than 90% of the non-red area of the skin surface turned from green to yellow), the days from harvest to full yellow (ripe) was recorded and the fruit rated for firmness and the severity of LD and SB. The fruit were again rated 7 d later.

5.1.3.2. Trial 2: Maturity and dye treatment

The same trees were used for as in Trial 1, with no exposure to water during harvesting. On arrival at the laboratory, the fruit were assessed for LD as above. Half the fruit were exposed to the dye infiltration treatment (see below), and the other half left untreated (control). The fruit were then treated with 10 ppm ethylene for 2 d at 20°C, then ripened at 20°C, and assessed for LD at full yellow and 7 d later as described above.

Dye uptake treatment and lenticel density

Dye uptake techniques and rating scales, and lenticel density assessments are described in section 4.1.3.3.

The % surface area dyed within each quadrant was recorded as:

1 = less than 20% of the surface area with dye;
2 = 21-40%;
3 = 41-80%;
4 = 81-100%.

5.1.3.3. Statistical analysis

Statistical analyses were performed by Genstat® 11 for Windows™ (VSN International Ltd., UK). Analysis of variance (ANOVA) used the 'General Analysis of Variance' model, with a factorial design of harvest time by treatment (e.g. irradiation, blotchy/spotty dye uptake, proportion of dyed lenticels, % surface area dyed) as 'treatment' structures, and tree as 'block' structure. The protected least significant difference (LSD) procedure at P=0.05 was used to test for differences between treatment means. The relationships between LD severity

and dye uptake parameters were established using correlation analysis on the means per harvest date. The significance of the correlations was determined by linear regression analysis (P = 0.05), and the strength by the correlation coefficient (r).

5.1.4. Results and discussion

5.1.5. Trial One: Maturity and irradiation

Dry matter and flesh colour at harvest

As expected, both DM and FC at harvest increased with later harvests (Table 55).

The NIRS estimates for fruit DM were generally higher than the actual values, although within a narrow range of 0.5% for the first two harvests. However, the difference between estimate and actual DM increased with the last two harvests. This suggests that as fruit maturity increases over the season the accuracy of the NIRS handgun may decrease, possibly requiring a re-calibration of the equipment a couple of weeks after harvest starts. The NIRS estimates for FC were much lower than the actual results, suggesting that the calibration model used was not reliable for FC. This has been observed in other trials.

Table 55 Estimated (near infrared spectroscopy; NIRS) and actual fruit dry matter (%) and flesh colour (1-11) at harvest of 'B74' mango grown on a commercial farm in Childers as affected by harvest date

Harvest date (2012)	Dry matter at harvest (%)		Flesh colour at harvest (1-11)	
	NIRS*	Actual	NIRS*	Actual
31/Jan	14.1 [b]	13.5 [c]	4.9 [b]	7.6 [c]
06/Feb	14.6 [b]	14.1 [b]	5.4 [b]	8.4 [c]
20/Feb	16.1 [a]	15.1 [a]	5.8 [ab]	9.9 [b]
29/Feb	16.8 [a]	15.0 [a]	6.6 [a]	10.7 [a]

*NIRS = near infrared spectroscopy.
Means (n=30) in each column with the same letters are not significantly different at P=0.05 as tested by LSD.

Lenticel discolouration

The severity of LD increased gradually and markedly with later harvest dates (up to the third harvest) and with irradiation, both 1 d after irradiation and when fruit reached the full yellow (ripe) stage (Figure 17, top and middle graphs; Plate 24). As a result, the % of acceptable fruit (those with a severity rating of 3 or less for lenticel discolouration) reduced markedly in harvests three and four compared with harvests one and two, especially with irradiated fruit (Figure 17, bottom graph). This effect of harvest date on LD confirms previous preliminary studies with non-irradiated 'B74' fruit (Whiley *et al.*, 2006). Similarly, in 'Langra' and 'Dashehari' mangoes, more rapid change in lenticels from creamy white to brown colour was associated with late-harvested fruit, thus suggesting a greater susceptibility to LD with more mature fruit (Mann and Singh, 1976). In contrast, little difference in LD severity between early and mid season fruit was reported in 'Tommy Atkins' mango that had been waxed after harvest, held at 9°C for 2 weeks before ripening at 23^{o}C (Self *et al.*, 2006), but only six replicates (individual fruit) per treatment were assessed in that study.

Generally, there was little difference in LD between the third and fourth harvests (Figure 17, top graph). At full yellow, there was also no difference in LD between the first and second harvests for irradiated fruit. At each harvest time, LD severity was consistently higher for

irradiated fruit compared to non-irradiated, both at 1 d after irradiation and at full yellow. Interestingly, LD was consistently more severe even at 1 d after irradiation, at all harvest times.

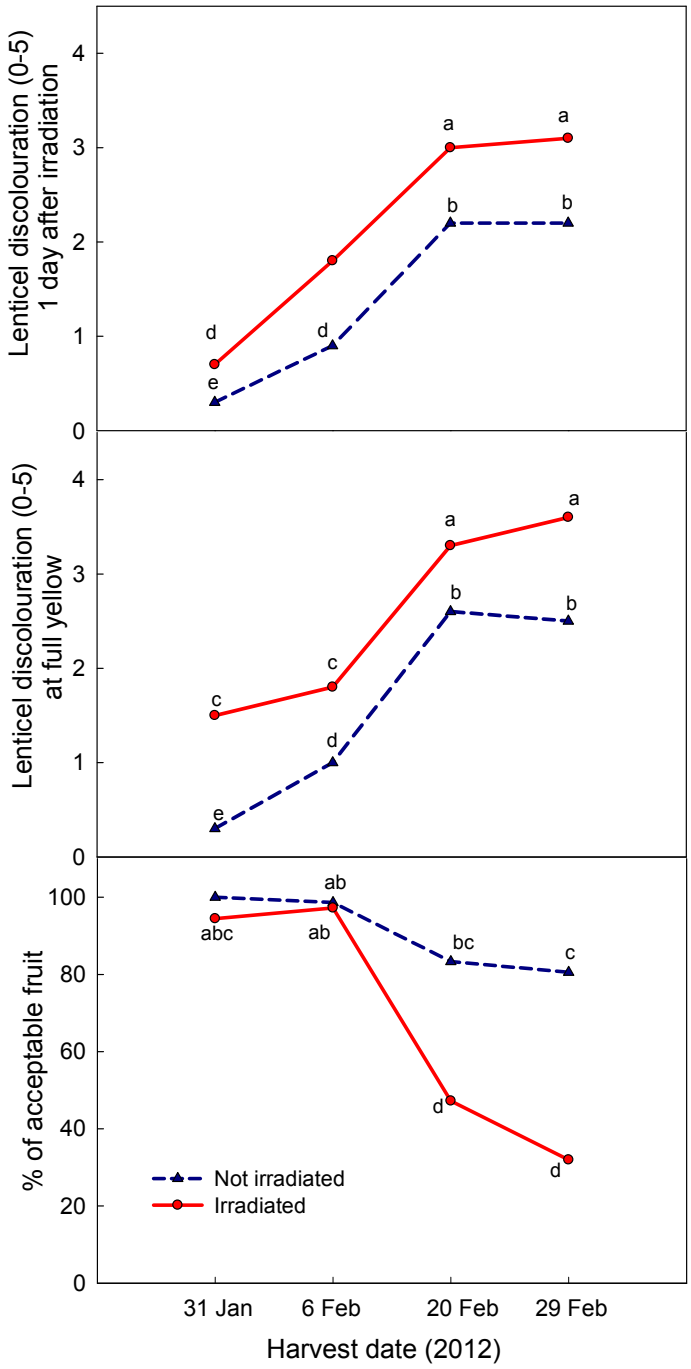


Figure 17 Severity (0-5) of lenticel discolouration 1 d after harvest (top graph) and at full yellow (middle graph) as affected by harvest date and irradiation. The percentage of acceptable fruit (those with a severity rating of 3 or less for lenticel damage) is presented in the bottom graph. Data is for 'B74' mango from a commercial farm in Childers (QLD). In each graph, data points (n=72) with the same letters are not significantly different (P=0.05) as tested by LSD.

The interaction between harvest time and irradiation was not significant for LD 7 d after full yellow (data now shown). However, it increased significantly with harvest time up to the third harvest, from 1.8 and 2.6, to 3.7 (on a 0-5 scale) for harvests one, two and three. Similarly, across all harvest times, LD 7 d after full yellow was higher in irradiated fruit (3.2) compared to non irradiation fruit (2.6).

Early-harvested 'B74' fruit after irradiation and ripening at 20°C

Late-harvested 'B74' fruit after irradiation and ripening at 20°C

Plate 24 Lenticel damage in early-harvested (photo on the left) or late-harvested (photo on the right) 'B74' mango after irradiation treatment and ripening. Fruit were harvested from a commercial farm in Childers (QLD).

Other fruit quality parameters

The severity of SB on full yellow fruit was generally low for all harvest dates (Table 56). Skin browning was more severe in irradiated fruit from the 3rd and 4th harvests compared to non-irradiated or irradiated fruit from the first two harvests. With the exception of the 1st harvest, the severity of SB always increased during shelf life (to full yellow plus 7 d), particularly in fruit that received the irradiation treatment.

Table 56 Skin browning in 'B74' mango grown on a commercial farm in Childers upon reaching the full yellow (ripe) stage and 7 d later, as affected by the interaction between harvest time and irradiation.

Harvest date (2012)	Skin browning* (0-5) at full yellow		Skin browning (0-5) 7 d after full yellow	
	Not irradiated	Irradiated	Not irradiated	Irradiated
31 Jan	0.0 [b]	0.0 [b]	0.0 [d]	0.1 [d]
06 Feb	0.1 [b]	0.1 [b]	0.9 [c]	2.4 [ab]
20 Feb	0.2 [b]	0.4 [a]	0.7 [c]	2.6 [a]
29 Feb	0.2 [b]	0.5 [a]	0.7 [c]	2.1 [b]

* Rated as severity, based on the visual assessment of the skin surface area affected (0=nil; 5=more than 25%). Means (n=72) either at full yellow or 7d after with the same letters are not significantly different (P=0.05) as tested by LSD.

The interaction between harvest date and irradiation was not significant for days to full yellow and for firmness at full yellow (data now shown). However, both harvest date and irradiation affected these parameters separately (Table 57). Fruit from the 4th harvest reached full yellow more rapidly and were firmer at that stage than fruit sampled from the first three harvests. The irradiation treatment delayed the time to reach the full yellow stage by approx. 1 d relative to non-irradiated control fruit.

Table 57 Days from harvest to full yellow and firmness at full yellow for 'B74' mango grown on a commercial farm in Childers, as affected by the interaction between harvest date and irradiation.

Effect	Days to full yellow	Firmness at full yellow*
Harvest date		
31 Jan	5.9 [a]	2.7 [ab]
6 Feb	5.6 [a]	2.9 [a]
20 Feb	5.9 [a]	2.6 [b]
29 Feb	3.4 [b]	2.1 [c]
Irradiation		
No	4.8 [b]	2.5 [b]
Yes	5.6 [a]	2.6 [a]

* Rated using hand pressure based on a 0-4 scale (0=hard; 4=soft). Means (n=72) in columns for either harvest time or irradiation with the same letters are not significantly different (P=0.05) as tested by LSD.

5.1.5.1. Trial Two: Maturity and dye treatment

The number and proportion of lenticels that took up "Brilliant Blue" dye did not consistently vary for fruit picked at different harvest maturities (Table 58). Between 50 and 67% of lenticels took up the dye irrespective of harvest maturity. The dye accumulated around lenticels as blotches (>1mm) or minor spots (<1mm) (Table 59). The proportion of lenticels displaying blotchy and spotty features at the first (early-maturity) harvest was relatively high at 11-30% and 31-50%, respectively. An increasing proportion of lenticels exhibited the blotchy and spotty dye features on fruit harvested at a more advanced maturity (Plate 25).

Table 58 The number and proportion (%) of lenticels on 'B74' mango fruit that took up "Brilliant Blue" dye immediately after harvest. Fruit were harvested at sequential stages of maturity during the season.

Harvest date	Number of dyed lenticels/cm^2	Proportion (%) of dyed lenticels/cm^2
31 Jan	20.8 [b]	61 [b]
6 Feb*	18.0 [a]	50 [a]
20 Feb	24.5 [c]	60 [b]
29 Feb	22.4 [bc]	67 [b]

Means (n=9) in columns with the same letters are not significantly different (P=0.05) as tested by LSD.
* Represents fruit harvested at a green mature commercial stage of maturity.

There were significant positive linear correlations between the severity of LD in non-irradiated fruit at each harvest date and the rating of either blotchy or spotty dye uptake (correlation coefficient, r=0.91, and 0.93 (Figure 18), respectively.

Likewise, the severity of LD in irradiated fruit at each harvest date and the rating of blotchy (but not spotty) dye uptake was significant (r=0.98).

In contrast, correlations between the severity of LD (in either non-irradiated or irradiated fruit) and either the number of dyed lenticels or the proportion (%) of dyed lenticels were not

significant. Further work and evaluation of expanded datasets would be required to confirm these relationships and the potential for dye uptake to help predict LD.

Table 59 The degree of blotchy and spotty lenticels on 'B74' fruit immediately after harvest and dye uptake treatment

Harvest date	Dye uptake score (1-6)	
	Blotchy (spots >1mm)	Spotty score (1-6)
31 Jan	3.2 [a]	4.9 [a]
6 Feb*	3.1 [a]	5.1 [ab]
20 Feb	4.3 [b]	5.4 [bc]
29 Feb	4.2 [b]	5.5 [c]

Means (n=9) in columns with the same letters are not significantly different (P=0.05) as tested by LSD.
* Coincided with commercial harvest for the block.

Early-harvested 'B74' fruit dipped in food dye solution

Late-harvested 'B74' fruit dipped in food dye solution

Plate 25 Blotchy or spotty patterns in non-irradiated 'B74' fruit dipped in food dye solution at harvest. Fruit were harvested from a commercial farm in Childers (QLD) early in the season (photo on the left) or 29 d later photo on the right). Note that late-harvested fruit had more blotchy dye patterns than early-harvested fruit.

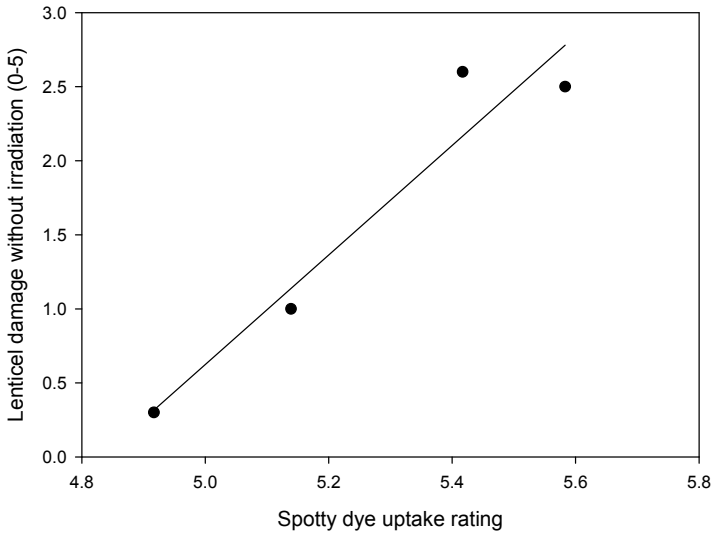


Figure 18 Relationship between lenticel discolouration severity and spotty dye uptake of lenticels in non-irradiated ‘B74’ mango fruit, at four harvests. Each harvest date is represented by a data point in the graph. Harvest dates were 31/1/12 (Early), 6/2/12 (Early-mid/commercial), 20/2/12 (Mid-late) and 29/2/12 (Late).

5.2. Cultivar responses to irradiation

Tran Anh San, Peter Hofman, Daryl Joyce, Andrew Macnish

This chapter forms part of a current PhD program (Anh). The results presented here have not yet been published. The chapter was drafted by the Ph.D. student, and reviewed by the project team.

5.2.1. Summary

Mango fruit generally require disinfestation treatments to reduce the risk of quarantine pests being introduced into importing countries. Irradiation is an effective disinfestation treatment against fruitfly, but can also cause damage to the fruit at the common irradiation doses of 400-500 Gy. To determine the response of 'B74'(trading as 'Calypso'™), 'Kensington Pride' ('KP'), 'Honey Gold' ('HG') and 'R2E2' mango to disinfestation irradiation, mature fruit were obtained from the end of the packing line from farms in the Northern Territory and north and south east Queensland over two seasons, then treated at about 500 and 1000 Gy of gamma irradiation and ripened.

Irradiation generally retarded softening in the early stages of ripening, but usually had little effect after 7-9 d. Irradiation also retarded the loss of green colour in most instances, resulting in eating soft fruit with less yellow skin colour. 'Honey Gold' was the least affected by irradiation, with 'KP' generally having the least yellow colour at eating soft. Irradiation at 500 Gy significantly increased LD, with generally little increase between 500 and 1000 Gy. 'Honey Gold' had the least LD in both years. Irradiation did not affect total soluble solids at eating soft, but 500 Gy increased titratable acidity in all cultivars except 'B74', and increased titratable acidity in 'KP' by more than 100%. Irradiation also reduced volatiles concentrations in 'KP'. There were few effects at 500 Gy, but at 1000 Gy the concentrations of all of the measured volatiles were significantly reduced compared to no irradiation.

These results indicate that irradiation at typical disinfestation doses significantly reduces the external appearance of 'B74', 'KP', 'HG' and 'R2E2' by reducing the yellow colour and increasing LD at eating soft. 'Honey Gold' was generally the least affected by irradiation. It is likely that irradiation may also affect the flavour of 'KP' by increasing titratable acidity and reducing volatiles concentrations at ripe.

5.2.2. Introduction

Mango (*Mangifera indica* L.), considered as "The King of Fruits", is one of the main fruit crops in the tropics and subtropics (Tharanathan *et al.*, 2006). The fruit is highly nutritious and a rich source of amino acids, carbohydrates, fatty acids, minerals, organic acids, proteins, vitamins and antioxidants (Vazquez-Salinas and Lakshminarayana, 1985; Kohli *et al.*, 1987; Rocha-Ribeiro *et al.*, 2007) . Mango fruit are susceptible to various insect pests of quarantine significance (e.g. fruit fly, mango seed weevil) (Boag *et al.*, 1990). Susceptible fruit require appropriate preventative measures and postharvest disinfestation treatments to minimise the risk of pest incursion into importing countries. Irradiation is an effective quarantine/phytosanitary treatment used postharvest to minimise quarantine pest presence in traded foods (Bustos *et al.*, 2004).

Disinfestation treatments, and particularly physical treatments, are often a balance between delivering adequate insect mortality without damaging the product. In relation to mango, irradiation can provide quarantine security, but it can also reduce ripe fruit skin quality by increasing discolouration of the lenticels and reducing the rate of green colour loss normally associated with mango fruit ripening (Hofman *et al.*, 2010c). The changes in colour of ripening mango fruit peel are typically associated with increased chlorophyllase and peroxidise activity (Ketsa *et al.*, 1999), resulting in loss of the green pigment allowing expression of the underlying yellow carotenoid pigments. Irradiation treatment has also been

associated with lower and higher carotene levels in mangoes as compared with non-irradiated fruit (Thomas and Beyers, 1979; El-Samahy *et al.*, 2000).

In addition to affecting pigmentation, irradiation may potentially affect the production of aroma volatiles. Lower production of terpenes in irradiated 'Chok Anan' mangoes was evident by headspace gas chromatography (GC) and mass spectra as compared with non-treated samples (Laohakunjit *et al.*). However, other studies have shown that there was no marked difference between the GC profiles of aroma volatile compounds in irradiated and non-irradiated 'Kent' and 'Alphonso' mangoes (Blakesley *et al.*, 1979; Gholap *et al.*, 1990).

Our research investigated the effects of gamma irradiation on four commercially important mango cultivars ('B74', 'KP', 'HG' and 'R2E2') in terms of their physicochemical properties. In particular, we hypothesised that irradiation would differentially induce LD, delay the loss of green skin colour, and diminish aroma volatile production among these four cultivars. In the medium term, better understanding of LD, de-greening and aroma synthesis processes should assist the formulation of postharvest practices to optimise the quality of irradiated fruit.

5.2.3. Materials and methods

5.2.3.1. Plant material

Four mango cultivars were used, namely 'KP', 'B74', 'R2E2' and 'HG'. Sixty fruit of each of the four cultivars were harvested to provide 15 fruit per cultivar for each of the three irradiation treatments. Similar procedures were used in the 2012/13 and the 2013/14 seasons.

'B74', 'HG', 'KP' and 'R2E2' mango fruit were obtained from the Northern Territory (NT), north Queensland and south east Queensland as detailed in Table 60. All fruit were grown, harvested and packed under standard commercial conditions, including the use of Mango Wash® during harvesting, and postharvest fungicide and insecticide applications during packing. Fruit from the NT and north Queensland arrived in Brisbane by airfreight within 1-2 d of harvest, while fruit from south east Queensland were driven by road in an air-conditioned vehicle, arriving within 1.5 d of harvest.

Table 60 The locations of farms from which 'B74', 'Kensington Pride', 'Honey Gold' and 'R2E2' mango fruit were obtained for the trials in 2012/13 and 2013/14.

Cultivar	Farm location		
	Northern Territory	north Queensland	south east Queensland
2012/13			
'B74'	Katherine	Dimbulah	Childers
'Kensington Pride'	Katherine	Mutchilba	Childers
'Honey Gold'	Katherine	Mutchilba	Childers
'R2E2'	Katherine	Dimbulah	Childers
2013/14			
'B74'	Katherine	Dimbulah	Goodwood
'Kensington Pride'	Katherine	Mareeba	Childers
'Honey Gold'	Katherine	Dimbulah	Electra
'R2E2'	Katherine	Dimbulah	Childers

5.2.3.2. Treatments

Following arrival at the Maroochy Research Facility (MRF) laboratory in Nambour, the fruit were randomly assigned to treatment lots and labelled with treatment number. The fruit from the different cultivars and treatments were then randomly assigned to each of six single layer trays holding about 10 kg of fruit each. Two dosimeters (Opti-chromic detectors FWT-70-40M) were placed in two corners of each tray, then the tray covered with 4 mm plywood lids combined with 15 mm low density foam lining to prevent fruit and dosimeter movement. The fruit were transported by air conditioned car to the irradiation facility (Steritech, Narangba,

Queensland) for exposure to gamma irradiation from a Cobalt 60 source. They were exposed to 0 Gy (control), or the preferred commercial dose of about 500 Gy (called 500 Gy), or twice the commercial dose of about 1000 Gy (called 1000 Gy). Table 61 indicates the actual average dose and the minimum and maximum doses recorded for each of the trials.

Table 61 The average and minimum and maximum doses recording for each experimental irradiation session at Steritech (Narangba, Queensland) during the 2012/13 and 2013/14 trials. Four cultivars from three growing regions were used (NT=Northern Territory, NQ=north Queensland and SEQ=southeast Queensland).

Farm location	Cultivar	Dose (Gy)			
		Targeted 500 Gy)		Targeted 1000 Gy	
		Average	Min-max	Average	Min-max
2012/13					
NT	'Kensington Pride'				
	'B74'	492	408-629	1015	1001-1029
	'Honey Gold'				
	'R2E2'				
NQ	'Kensington Pride'	548	525-568	1040	1001-1073
	'B74'				
	'Honey Gold'	581	502-656	1047	1033-1061
	'R2E2'	548	525-568	1040	1001-1073
SEQ	'Kensington Pride'	580	568-591	1235	1204-1266
	'B74'	541	506-653	1037	1025-1049
	'Honey Gold'	541	568-591	1037	1204-1266
	'R2E2'	580	506-653	1235	1025-1049
2013/14					
NT	'Kensington Pride'				
	'B74'	424	410-449	786	774-810
	'Honey Gold'				
	'R2E2'				
NQ	'Kensington Pride'				
	'B74'	342	320-381	833	824-846
	'Honey Gold'				
	'R2E2'				
SEQ	'Kensington Pride'				
	'B74'	520	493-577	1043	1003-1079
	'Honey Gold'				
	'R2E2'				

Immediately after irradiation the fruit were transported back to MRF, treated with 10 $\mu L.L^{-1}$ (ppm) ethylene for 2 d at 20ºC then ripened at 20ºC. Quality was assessed every 2 d, then at eating soft.

5.2.3.3. Quality assessment

External appearance

External appearance was rated based on the procedures of Hofman *et al*. (2010a) outlined in section 4.1.3.3. Fruit firmness was assessed subjectively by hand firmness every 1-2 d using the following rating scale: 0 = hard, 1 = rubbery, 2 = sprung, 3 = soft and 4 = very soft. Skin colour was rated subjectively as 1=0-10% of the non-blush skin with yellow colour, and 6= 90-100% yellow. Lenticel discolouration and skin browning (SB) on each fruit at harvest and during ripening was rated using the 0-5 rating scale.

External appearance and internal quality (see below) were also assessed at the eating soft stage (hand firmness 3 for 'B74' and 'R2E2', and rating 4 for 'KP' and 'HG').

Total soluble solids (TSS) and titratable acidity (TA)

When the fruit had reached the eating soft stage, one cheek of the fruit was removed, a vertical section of the flesh (from stem to base) chopped into small pieces then frozen at -18°C. Within two months of freezing the samples were thawed at room temperature and immediately homogenised. The Brix value (an estimate of TSS) was recorded using an Atago digital hand held "Pocket" Refractometer PAL-1 (Padda *et al.*, 2011). A 10 g sample of the homogenized pulp was used to determine the titratable acidity using 0.1 M NaOH and tritrating to pH 8.1 with an automated titrator (Padda *et al.*, 2011). TA is expressed as percent citric acid.

Chlorophyll concentration

The chlorophyll concentration was determined using the method described by Ketsa *et al.* (1999) with modification. Peel samples on the non-blush area of each fruit were taken when fruit reached eating soft and were stored at -20ºC for 2-4 weeks, then at -80ºC. Approx 3 g of the frozen mango peel was ground to a powder in liquid nitrogen, then 0.5 g of peel powder extracted with 80% cold acetone in a 50 mL falcon tube for 2 h then centrifuged at 4000 rpm for 15 min at -9ºC. This was repeated about three times until the supernatant was colourless. The combined supernatant was made up to 50 mL with cold acetone and kept in the dark until chlorophyll was measured.

Chlorophyll concentration was measured using a spectrophotometer (Beckman Coulter DU 530 UV Vis) at 663, 646 and 710 nm. Chlorophyll a (Ca), chlorophyll b (Cb) and the total chlorophyll (C) concentration, expressed as µg chlorophyll per g fresh weight was calculated as follows (Hartmut, 1983):

$Ca = (12.21A663 – 2.81A646) \times 100$

$Cb = (20.13A646 – 5.03A663) \times 100$

$C = Ca + Cb$

Aroma volatiles

The aroma volatile standards used included 2-carene, 3-carene, α-terpinene, α-terpinolene, β-cymene, D-limonene, ethyl octanoate and hexanal (Sigma-Aldrich, Australia). Internal standards were d15-ethyl octanoate, d15-hexanal, d6-α-terpinolene, d5-α-terpinene and d6-D-limonene (CDN Isotopes). These compounds have not been previously found in mango volatile analysis and thus served as suitable internal standards.

The conditions for headspace solid-phase microextraction (SPME) sampling were as follows: The frozen flesh samples prepared as described above were stored in bottles at -20ºC for 4-6 weeks until analysis. The samples were transferred to 4ºC for 12 h to thaw prior to sample preparation. Thawed samples were blended using an Ultraturrax stick blender then 2.5 g of flesh was added to a 20 mL HS-SPME vial containing 2.5 mL of saturated sodium chloride (NaCl) solution and a magnetic stir flea (5 mm x 2 mm), and the vial immediately crimp-capped (magnetic, Teflon lined rubber septum). Subsequently, 100 µL of the internal standards solution (as described above) was injected through the septum and the vial was shaken well. The 100 µL volume of a solution of standards contained approximately 30 $\mu g.L^{-1}$ of d12-hexanal, 106 $\mu g.L^{-1}$ of d5-α-terpinene, 106 $\mu g.L^{-1}$ of d6-D-limonene and 50 $\mu g.L^{-1}$ of d15-ethyl octanoate.

Headspace sampling was conducted using a MPS2XL multipurpose sampler (Gerstel). The vial and its contents were heated to 40°C with stirring at 250 rpm for 2 min followed by extraction with a Supelco divinylbenzene/carboxen/polymethylsiloxane (DVB/CAR/PDMS, 'grey', 1 cm) 50/30 µm fibre which was exposed to the headspace for 30 min. An 5890N gas chromatograph (Agilent) equipped with a Phenomen ZB-5ms column (30 m x 250 µm i.d. 0.25 µm, J&W Scientific, Folsom, CA) was used for volatiles analysis. The SPME fibre was injected into the PTV injector (GERSTEL), which was set to splitless mode at 200°C and desorbed for 8 min. The carrier gas was helium (BOC, ultra high purity), set at a linear velocity of 44 $cm.s^{-1}$ and constant flow rate of 1.5 $mL.min^{-1}$. The mean pressure for the front

inlet was 75.7 kPa and total flow was 70.5 mL.min^{-1}. The oven was held at 40°C for 2 min then increased to 80ºC at 20ºC.min^{-1}, then to 220ºC at 40ºC.min^{-1} then held constant for another 5 min. Eluting compounds were transferred to a positive ion electron impact Agilent 5975 mass spectrometer (MS) at 70 eV. The transfer line was 280ºC and the mass scan range was 35–350 mass units. The total runtime was 32 min and the data was collected using the Agilent Technologies MSD Statistical analysis ChemStation (USA) software.

5.2.3.4. Statistical analysis

Statistical analysis of the data was carried out using Genstat software (VSN international Ltd, 2012) using analysis of variance (ANOVA), and ANOVA with repeated measures for data measured over time (e.g. change in LD during ripening). Where appropriate a factorial model was used with three irradiation treatments and four cultivars as treatments and farm location as block. Single fruit replications were used with 15 fruit for external appearance assessment, and five fruit for internal quality. The difference between means was assessed using Fisher's protected LSD at $P < 0.05$.

5.2.4. Results and discussion

5.2.4.1. Irradiation doses

The small variation in the irradiation doses in 2012/13 (Table 61) were unlikely to have a significant effect on fruit responses. The 2013/14 doses were generally lower (except for southeast Queensland) than 2012/13. Hence seasonal comparisons for the NT and north Queensland farms should be carefully considered.

Also, the results can not be interpreted to indicate the performance of fruit from the three production regions since only one farm was sampled from each region. Region is used in the following text to indicate farm location, rather than whole of production region responses.

5.2.4.2. External appearance during ripening

Firmness

Mango fruit softened (higher hand firmness rating scores) with increased ripening time (Figure 19 and Figure 20). In most cases and in both years, irradiated fruit were more firm in the early stages of ripening compared with no irradiation, but there was often little irradiation effect on firmness by 7 and 9 d. The obvious exceptions were that north Queensland 'HG' mango in 2012-2013 and NT 'HG' in 2013-2014 irradiated at 1.0 kGy were significantly firmer at 11 d than fruit irradiated at 0-500 Gy. Also, non-irradiated 'R2E2' mango fruit from south east Queensland were firmer than 500 Gy-treated fruit.

Irradiation generally had a greater effect in reducing softening during early ripening in 2013-3014 compared with 2012-2013, and there was little treatment difference between 500 Gy and 1500Gy.

Similar results have been observed with other mango cultivars. 'Tommy Atkins' mangoes irradiated at 1000 and 1500 Gy were softer by 50.0% and 66.9%, respectively, compared with no irradiation (Moreno *et al.*, 2006). They suggested that fruit softening induced by irradiation may be partly caused by the changes in the cell structure such as cracks and depressions on the surface and disruption of the cells and its components. 'Tommy Atkins' mangoes exposed to 1000 Gy can also soften more during storage at 22ºC for 12 d (Sabato *et al.*, 2009a).

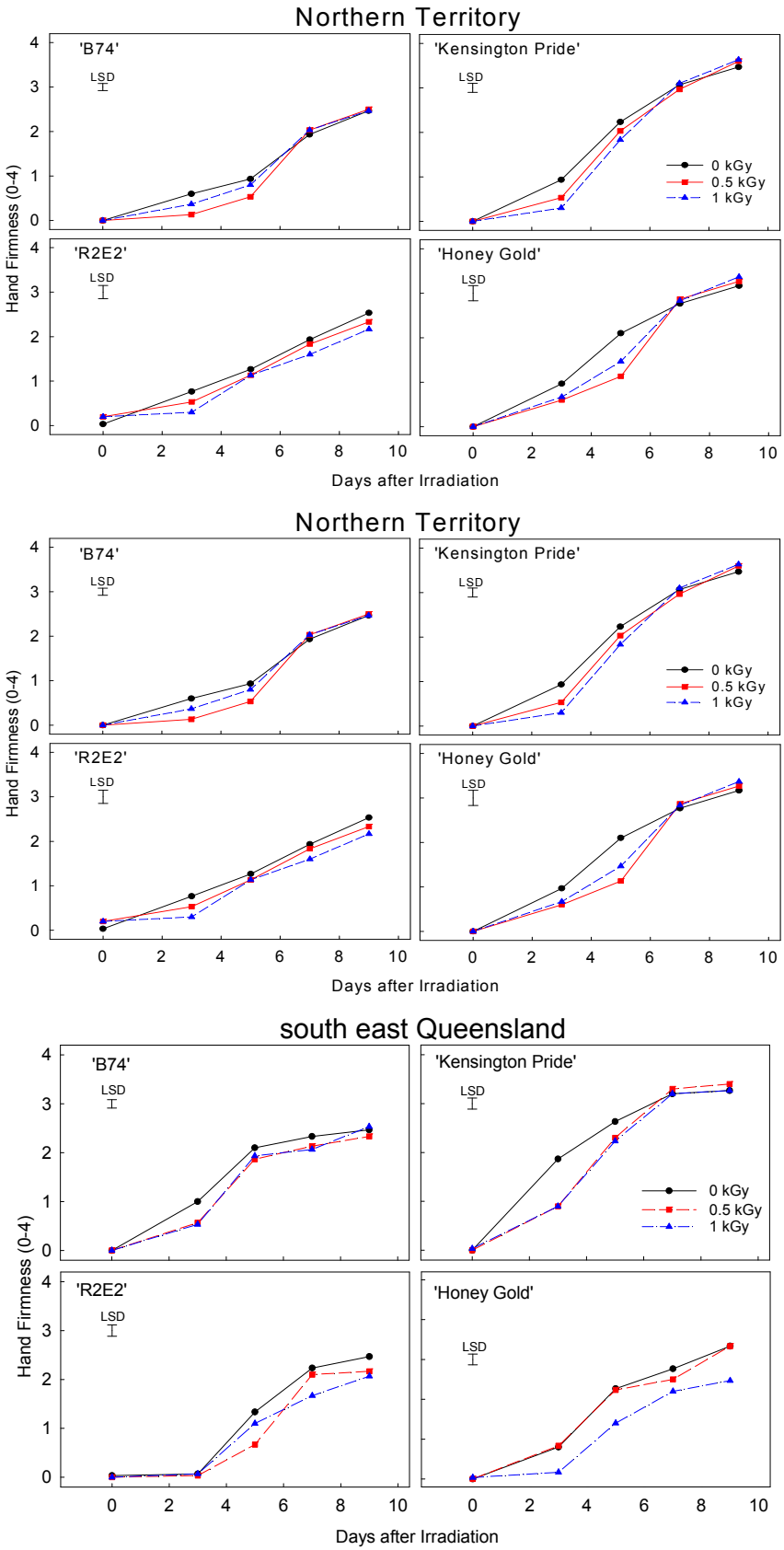


Figure 19 Effect of irradiation (0-1000 Gy) on the hand firmness of ripening 'Kensington Pride', 'B74', 'R2E2' and 'Honey Gold' mango fruit from the Northern Territory, and north and south east Queensland in 2012-2013. The LSD bar indicates LSD value at P<0.05.

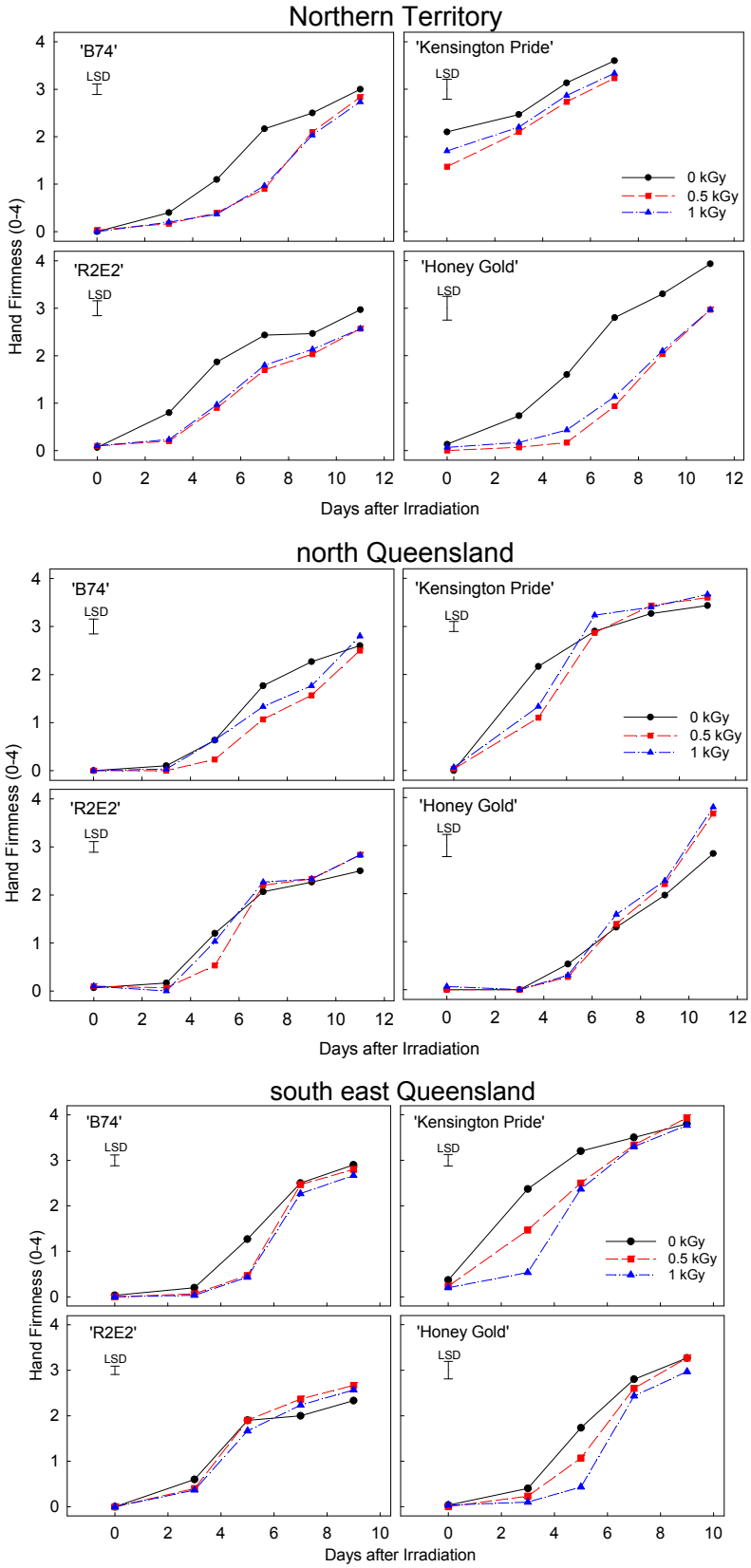


Figure 20 Effect of irradiation (0-1000 Gy) on the hand firmness during ripening of 'Kensington Pride', 'B74', 'R2E2' and 'Honey Gold' mango fruit from the Northern Territory, and north and south east Queensland in 2013-2014. The LSD bar indicates LSD value at P<0.05.

Skin colour

In all treatments, the skin changed from green to yellow (higher colour scores) as the fruit ripened (Figure 21 and Figure 22). In most cases, irradiation at either 500 or 1000 Gy significantly reduced the loss of green colour during ripening, so that irradiated fruit had less yellow colour on the skin at 9-11 d, compared with no irradiation. This effect appeared to be less in fruit from the NT in 2012-2013, but considerably greater in 'R2E2' and 'HG' fruit from this region in 2013-2014. There was generally little difference in skin colour response between 500 and 1000 Gy.

Irradiation at 750 Gy also retarded the loss of green skin colour in 'KP' (Boag *et al.*, 1990), and loss of green skin colour of 'Tommy Atkins' mango during storage was retarded by 3100 Gy (Moreno *et al.*, 2006).

Lenticel discolouration

Lenticel discolouration either did not increase significantly, or increased slightly during ripening of non-irradiated fruit (Figure 23 and Figure 24). In all cultivars LD was more severe following irradiation, and in most cases there was little difference in LD between 500 and 1000 Gy. With irradiated fruit LD usually increased significantly within 2-4 d of treatment and increased more slowly or not at all with further ripening.

'B74' fruit usually showed the greatest increase in LD with irradiation compared with the other cultivars, but this was not consistent in all seasons and locations. 'Honey Gold' generally had the least LD compared with the other cultivars.

Similar effects were noted by Johnson *et al.* (1990) in 'KP', with fruit treated with doses in excess of 600 Gy developing unacceptable LD.

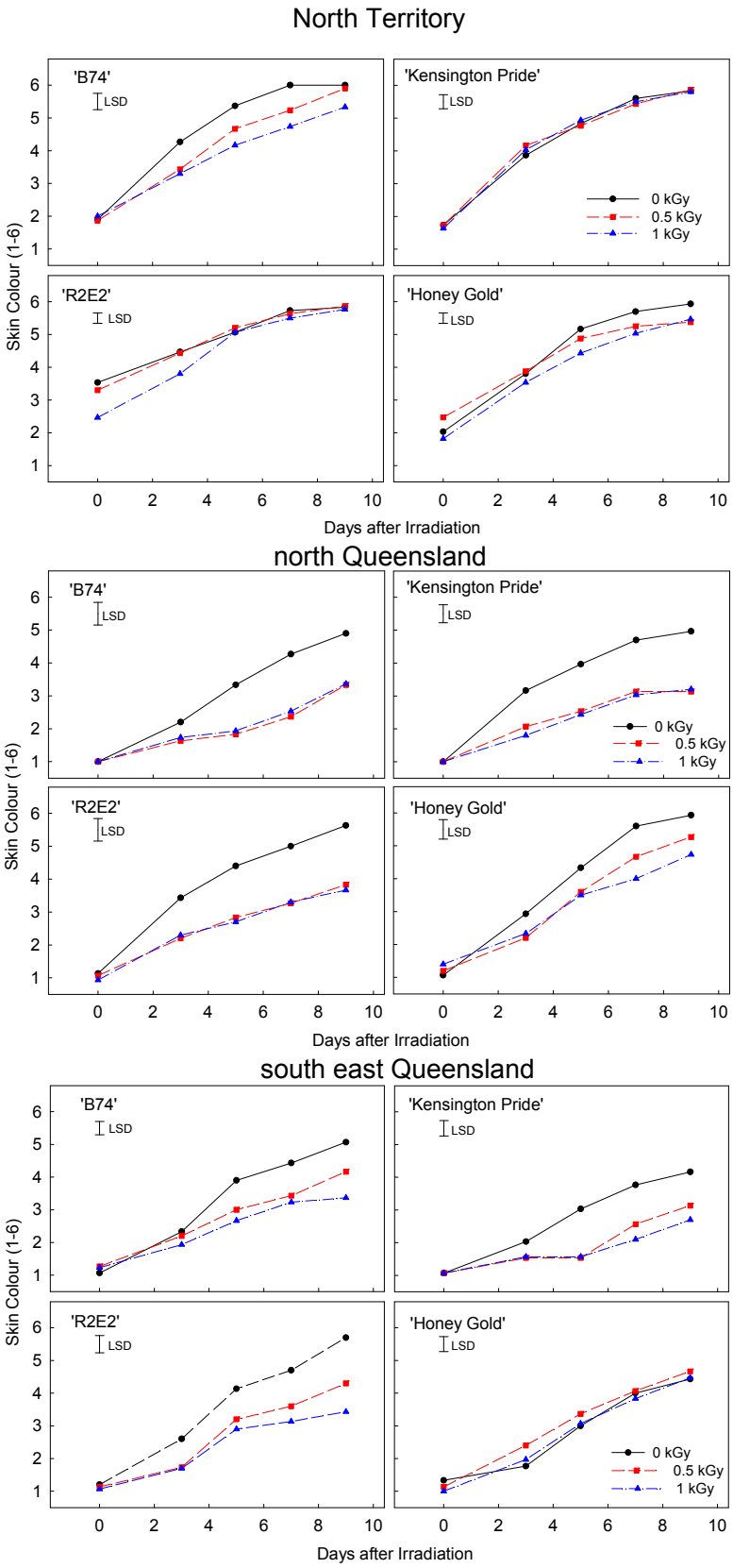


Figure 21 Effect of irradiation (0-1000 Gy) on the change of skin colour during ripening of 'Kensington Pride', 'B74', 'R2E2' and 'Honey Gold' mango fruit from the Northern Territory, and north and south east Queensland in 2012-2013. The LSD bar indicates LSD value at P<0.05.

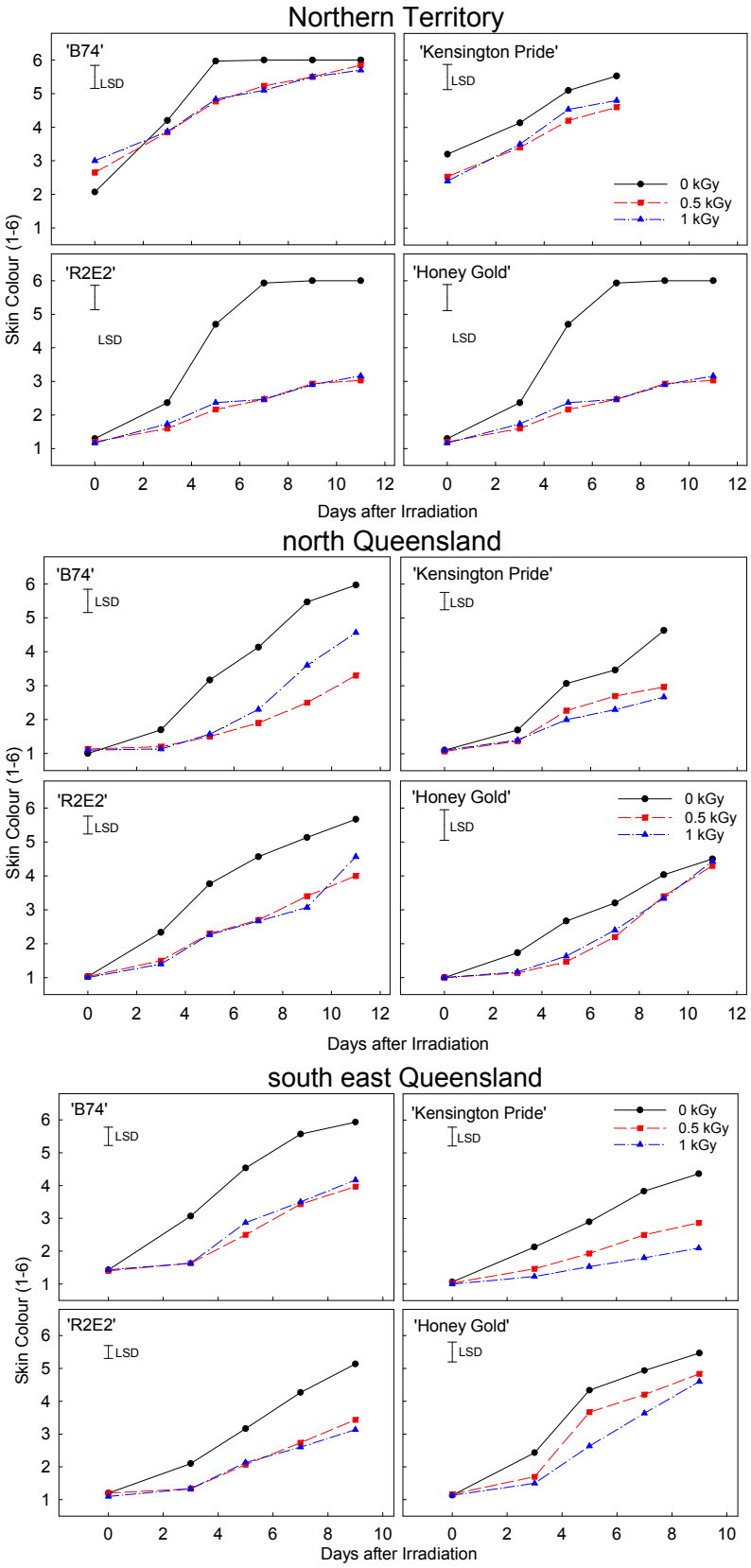


Figure 22 Effect of irradiation (0-1000 Gy) on the change of skin colour during ripening of 'Kensington Pride', 'B74', 'R2E2' and 'Honey Gold' mango fruit from the Northern Territory, and north and south east Queensland in 2013-2014. The LSD bar indicates LSD value at P<0.05.

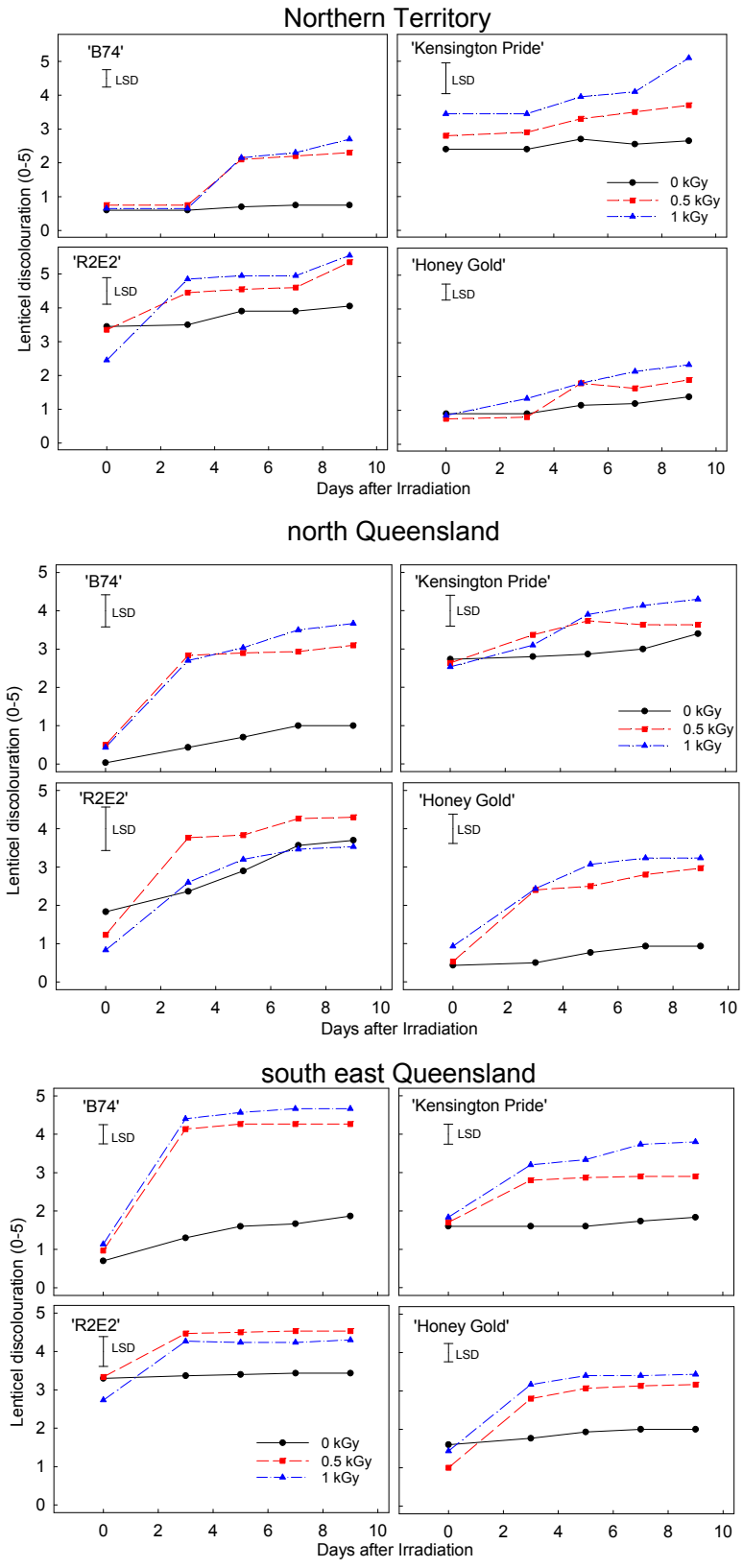


Figure 23 Effect of irradiation (0-1000 Gy) on the lenticel discolouration during ripening of 'Kensington Pride', 'B74', 'R2E2' and 'Honey Gold' mango fruit from the Northern Territory, and north and south east Queensland in 2012-2013. The LSD bar indicates LSD value at P<0.05.

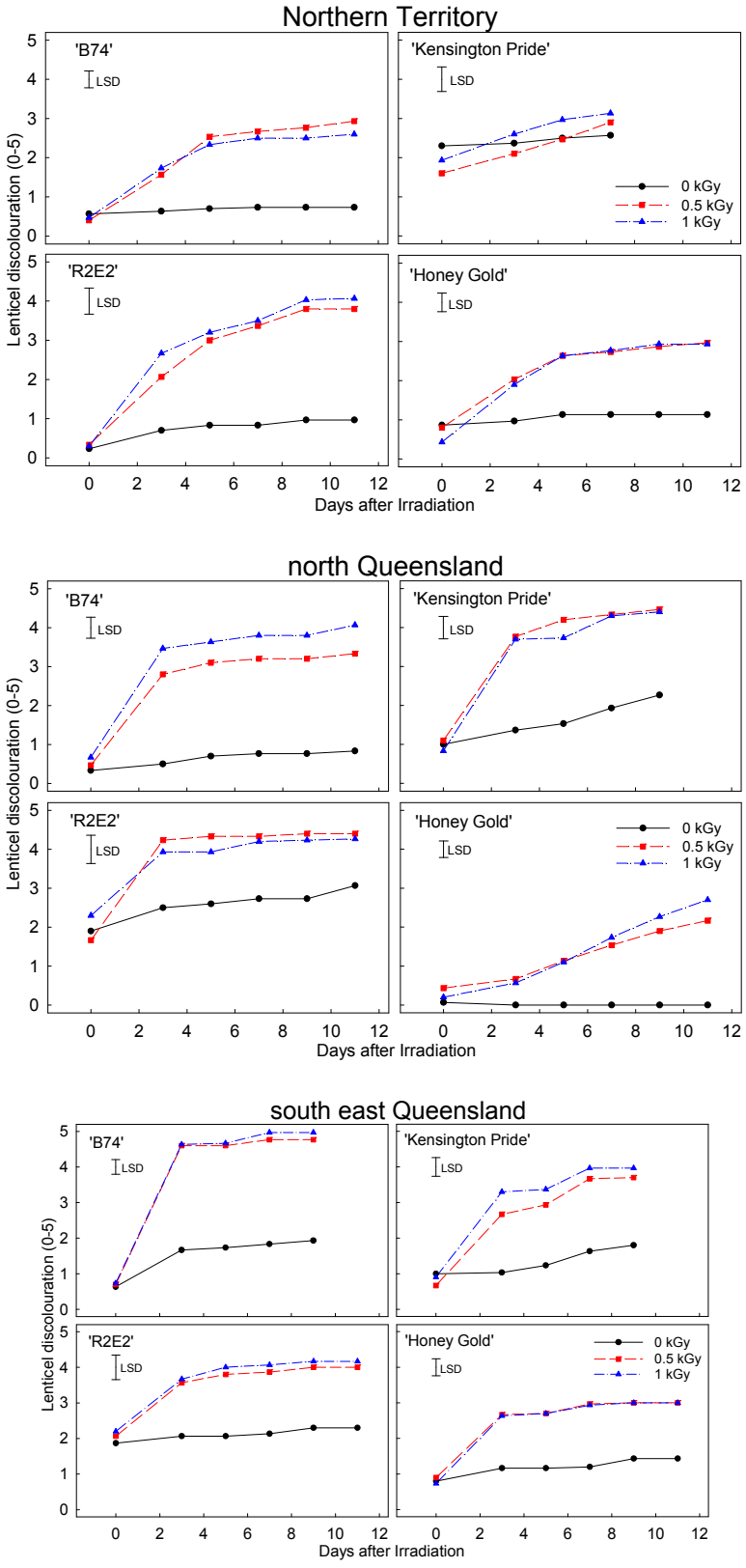


Figure 24 Effect of irradiation (0-1000 Gy) on lenticel discolouration during ripening of 'Kensington Pride', 'B74', 'R2E2' and 'Honey Gold' mango fruit from the Northern Territory, and north and south east Queensland in 2013-2014. The LSD bar indicates LSD value at P<0.05.

5.2.4.3. External appearance at eating soft (ripe)

Table 62 indicates that LD and skin colour at eating soft varied between cultivar in response to irradiation in both years (that is, a significant interaction between cultivar and irradiation), and with flesh acidity in 2012/13. There were no significant interactions between cultivar and irradiation on hand firmness in neither year, nor a significant effect of irradiation on firmness in 2013/14.

Table 62 Factorial analysis of the interaction between cultivar and irradiation on lenticel discolouration and colour of the skin, and total soluble solids (TSS) and acidity of the flesh of 'Kensington Pride', 'B74', 'Honey Gold', and 'R2E2' mango fruit at eating soft. P values <0.05 are considered statistically significant.

Treatment	P value			
	Lenticel discolouration	Skin colour	TSS	Acidity
2012/13				
Cultivar	<.001	<.001	<.001	<.001
Irradiation	<.001	<.001	0.762	<.001
Cultivar.Irradiation	<.001	0.008	0.764	<.001
2013/14				
Cultivar	<.001	<.001		
Irradiation	<.001	<.001		
Cultivar.Irradiation	<.001	0.004		

Skin colour

With no irradiation, all cultivars reached colour rating 5 or above at eating soft, except 'KP' in 2013/14 (Table 63). 'R2E2' had the most yellow at eating soft in 2012/13, and 'B74' in 2013/14. For all cultivars and seasons, 1000 Gy irradiation resulted in significantly less yellow skin colour at eating soft compared with no irradiation. 'Honey Gold' was the only cultivar where skin colour at eating soft was similar between 0 and 500 Gy irradiation. Following 500 and 1000 Gy irradiation, 'KP' had the least yellow colour in both seasons, while 'HG', and 'B74' and 'R2E2' to a lesser extent generally had the most yellow colour.

Table 63 Effect of cultivar and irradiation on the skin colour (0=1-10% of the non-blush skin area with yellow colour, to 6=90-100% yellow) of 'Kensington Pride', 'B74', 'Honey Gold', and 'R2E2'mango at eating soft 2013-2014 and year 2013-2014. Farm location was the block. Means with the same letters are not significantly different (P=0.05) as tested by LSD.

	Irradiation dose (Gy)		
	0	500	1000
2012/13			
'B74'	5.3 ^{gh}	4.5 ^{cd}	4.0 ^{ab}
'Honey Gold'	5.4 ^{hi}	5.1 ^{fgh}	4.9 ^{ef}
'Kensington Pride'	5.0 ^{efg}	4.0 ^{ab}	3.8 ^{ab}
'R2E2'	5.7 ^{i}	4.7 ^{de}	4.3 ^{bc}
lsd	0.4		
2013/14			
'B74'	6.0 ^{efg}	4.4 ^{cd}	4.8 ^{c}
'Honey Gold'	5.4 ^{d}	4.5 ^{cd}	4.5 ^{cd}
'Kensington Pride'	4.8 ^{c}	3.5 ^{ab}	3.2 ^{ab}
'R2E2'	5.8 ^{de}	3.7 ^{b}	3.8 ^{bc}
lsd	0.5		

Skin chlorophyll concentration

Without irradiation, 'KP' had higher chlorophyll concentrations in the non-blushed skin at eating soft compared with 'B74', 'HG' and 'R2E2' in both years (Table 64). Irradiation at 500 Gy significantly increased chlorophyll concentrations in all instances except 'HG' in 2012/13. This confirms the visual data that 'HG' is more resistant to irradiation effects on skin colour. Increasing irradiation from 500 to 1000 Gy further increased chlorophyll concentrations in 'B74' in both years and 'KP' in 2013/14.

The inhibition of de-greening was presumably caused by an irradiation induced suppression of the synthesis or activity of enzyme systems involved in the regulation of chlorophyll breakdown.

Table 64 Effect of cultivar and irradiation on the chlorophyll concentration (µg/g) of 'Kensington Pride', 'B74', 'Honey Gold', and 'R2E2'mango at eating soft 2013-2014 and year 2013-2014. Means with the same letters are not significantly different (P=0.05) as tested by LSD.

	Irradiation dose (Gy)		
	0	500	1000
2012/13			
'B74'	4.2 ^{a}	33.3 ^{c}	67.0 ^{d}
'Honey Gold'	18.0 ^{ab}	32.5 ^{bc}	30.2 ^{bc}
'Kensington Pride'	29.6 ^{bc}	69.2 ^{d}	71.5 ^{d}
'R2E2'	14.2 ^{a}	36.8 ^{c}	39.4 ^{c}
LSD	14.9		
2013/14			
'B74'	34.1 ^{ab}	47.2 ^{c}	64.1 ^{d}
'Honey Gold'	31.2 ^{a}	33.7 ^{ab}	42.0 ^{bc}
'Kensington Pride'	48.6 ^{c}	93.4 ^{e}	106.2 ^{f}
'R2E2'	39.1 ^{abc}	72.9 ^{d}	64.5 ^{d}
LSD	10.1		

Lenticel discolouration

With all cultivars and in both seasons, irradiation significantly increased LD at eating soft (Table 65). There was little significant increase between 500 and 1000 Gy, except with 'B74' and 'KP' in 2012/13. With no irradiation, 'KP' and 'R2E2' had the highest LD severity in both seasons. 'Honey Gold' had the least LD following irradiation at either dose in both years, with 'R2E2' having the most LD following 500 Gy.

These results suggest that the 'HG' lenticels are least affected by irradiation, compared with no irradiation, with arguably 'R2E2' being the most sensitive.

5.2.4.4. Flesh TSS

There was no significant effect of irradiation on TSS at eating soft (Table 62). Averaging across irradiation treatments and locations in 2012/13, 'B74' had the lowest, and 'HG' the highest TSS at eating soft (Table 66).

The TSS of 'Keitt' and 'Tommy Adkins' fruit were also affected little by 1000 Gy treatment (Lacroix *et al.*, 1990; Sabato *et al.*, 2009b), but was reduced by 3100 Gy (Moreno *et al.*, 2006).

5.2.4.5. Titratable flesh acidity

With no irradiation, titratable acidity was highest in 'HG' at eating soft (Table 67) which, combined with its higher TSS would contribute towards its stronger flavour. Irradiation generally increased acidity, except with 'B74' where there was no irradiation effect. The

increase in acidity with irradiation was relatively small for 'HG' and 'R2E2', but more than doubled in 'KP'. Similar responses were noted with 'Zebda' mango (El-Samahy *et al.*, 2000).

Table 65 Effect of cultivar and irradiation on lenticel discolouration (0-5) of 'Kensington Pride', 'B74', 'Honey Gold', and 'R2E2'mango at eating soft in 2013-2014 and 2013-2014. Farm location was the block. Means with the same letters are not significantly different (P=0.05) as tested by LSD.

	Irradiation dose (Gy)		
	0	500	1000
2012/13			
'B74'	1.2 [a]	3.2 [de]	3.7 [f]
'Honey Gold'	1.5 [a]	2.7 [bc]	3.0 [cd]
'Kensington Pride'	2.6 [b]	3.4 [ef]	4.4 [g]
'R2E2'	3.7 [f]	4.7 [g]	4.5 [g]
LSD	0.4		
2013/14			
''B74''	1.2 [a]	3.7 [d]	3.9 [de]
'Honey Gold'	0.9 [a]	2.7 [c]	2.9 [c]
'Kensington Pride'	2.2 [b]	3.7 [d]	3.8 [de]
'R2E2'	2.1 [b]	4.1 [e]	4.2 [e]
LSD	0.4		

Table 66 Total soluble solids content (measured as ºBrix) of mangoes exposed to different irradiation treatments in 2012/13. Means with the same letters are not significantly different (P=0.05) as tested by LSD.

Cultivar	Brix
'B74'	12.5 [a]
'Honey Gold'	16.4 [d]
'Kensington Pride'	15.1 [c]
'R2E2'	14.3 [b]
LSD	0.5

Both flesh acidity and green skin colour decreases during mango fruit ripening (Palafox-Carlos *et al.*, 2012). The lower skin colour rating (more green colour) and higher acidity in 'KP' fruit at eating soft suggests that these changes are slowed more by irradiation compared with the other three cultivars, resulting in more acid, green fruit at eating soft.

Table 67 Titrable acidity of mangoes in function of different irradiation treatments in 2012-2013. Means with the same letters are not significantly different (P=0.05) as tested by LSD.

Cultivar	Irradiation dose (Gy)		
	0	500	1000
'B74'	0.16 [a]	0.23 [ab]	0.23 [ab]
'Honey Gold'	0.46 [c]	0.60 [de]	0.52 [cd]
'Kensington Pride'	0.25 [b]	0.63 [e]	0.58 [de]
'R2E2'	0.15 [a]	0.28 [b]	0.26 [b]
LSD	0.08		

5.2.4.6. Aroma volatiles

The aroma volatiles in the flesh of ripe 'B74' and 'KP' mangoes from south east Queensland were quantified. 'Kensington Pride' fruit exposed 1000 Gy had significantly lower concentrations of the major volatiles compared with no irradiation, expect hexanal (Table 68).

5.2.5. Conclusions

Irradiation of the main Australian mango cultivars can affect commercial value mainly through effects on LD and delayed loss of green colour.

In general, firmness changes are not significantly affected by irradiation, although there can be slightly slower firmness loss initially. The loss of green skin colour during ripening is retarded, even at 500 Gy, resulting in less yellow skin colour at eating soft. This response was strongest in 'KP' and least in 'HG' and 'B74'. This is not surprising given the commercial experience of these cultivars developing yellow skin colour more consistently after harvest. All cultivars increased LD with irradiation but 'HG' was the least affected. The increase in titratable acidity of 'KP' with irradiation, coupled with reduction in volatiles with higher doses, may indicate an irradiation effect on flavour as well. The higher TSS of 'HG' would help to counteract any irradiation effects on flavour with this cultivar.

Therefore, 'HG' was the most tolerant of the four cultivars to irradiation.

Table 68 Effect of irradiation treatment on aroma volatile concentrations of ‘Kensington Pride’ mangoes in 2013/14. For each cultivar and volatile, means with the same letters are not significantly different (P=0.05) as tested by LSD.

	Compound (µg/L)							
Dose (Gy)	Hexanal	2-Carene	3-Carene	α-terpinolene	β-cymene	D-Limonene	α -terpinene	ethyl octanoate
‘Kensington Pride’								
0	20.9 [b]	43.1 [a]	425.8 [a]	23,399 [a]	15.5 [a]	303.2 [a]	49.3 [a]	1.0 [a]
500	80.7 [a]	39.1 [a]	361.7 [a]	17,533 [a]	11.6 [a]	262 [a]	42.7 [a]	0.6 [b]
1000	106.1 [a]	12.4 [b]	93.1 [b]	4,577 [b]	7.1 [b]	125.7 [b]	12.7 [b]	0.6 [b]

5.3. Preventing browning - antioxidants

Guoqin Li (PhD candidate), Peter Hofman, Daryl Joyce, Andrew Macnish

This chapter forms part of a current PhD program (Guoqin Li). The results presented here have not yet been published. The chapter was drafted by the Ph.D. student and reviewed by the project team.

5.3.1. Summary

Lenticel discolouration (LD) reduces appearance and value of 'B74' mango. Irradiation used as a quarantine treatment increases LD. The discolouration is likely caused by a browning reaction requiring oxygen. The discolouration of lenticels is likely an oxidation reaction similar to browning of cut surfaces of apple and avocado. Antioxidants can reduce these oxidation reactions in plants. The potential for several concentrations of ascorbic and citric acid, and calcium ascorbate to reduce LD in 'B74' was tested over two seasons. The trials showed little potential for antioxidants to reduce LD following irradiation. In most cases they increased LD and skin browning (SB).

5.3.2. Introduction

Lenticels on mango fruit generally originate from stomata on the young fruit which play a role in respiration and transpiration during early fruit growth (Rymbai *et al.*, 2012). However, they can develop a brown/dark discolouration around the lenticel after harvest, resulting in lenticel discolouration (LD). It can be a significant postharvest disorder in 'B74' mango fruit which influences fruit appearance and can result in economic loss for growers. Brown discolouration in plant tissue, such as is obvious following a bruising event or cutting of plant tissue, is a common enzymatic plant response. It typically involves the oxidation of phenols and other plant constituents to dark pigments in the presence of oxygen.

Antioxidants are a common treatment to reduce these browning reactions. Surface treatments involving dipping fruit pieces into aqueous solutions containing anti-microbial agents, antioxidants and calcium salts are widely practiced to improve quality of fresh-cut fruit (Oms-Oliu *et al.*, 2010). Ascorbic acid, citric acid, and sulfur-containing amino acids have been used as substitues for sulfite to prevent enzymatic browning (Pizzocaro *et al.*, 1993) Several chemical browning inhibitors at different concentrations and combinations have been studied for this purpose (Laurila *et al.*, 1998). Among them, citric acid (CA) and ascorbic acid (AA) at 0.5% to 2% have been commonly used.

Antioxidants have also been used to reduce the expression of skin disorders. For example, dipping apple fruit in ascorbic acid (0.1 or 0.3 mM for 10 min) before storage can decrease the area affected by skin spots (Grimm *et al.*, 2012). A sequential dip treatment including sodium hypochlorite, potassium metabisulfite, and hydrochloric acid containing ascorbic acid (2%) has been used prior to gamma irradiation (Kumar *et al.*, 2012). Also, 2% ascorbic acid has been used to prevent browning of potato, fresh-cut artichokes (Amodio *et al.*, 2011) apples (Son *et al.*, 2001) and pears (Gorny *et al.*, 2002). 0.5% citric acid has been used to reduce browning of lettuce (Altunkaya and Gokmen, 2009), 5% and 10% citric acid reduced pericarp browning of longan fruit (Whangchai *et al.*, 2006), 0.5%-1% citric acid has also been used to control artichoke heads browning (Amodio *et al.*, 2011), and 5 $g.L^{-1}$ citric acid used for fresh-cut mango flesh colour (Chiumarelli *et al.*, 2011)). The presence of Ca^{2+} increased the cohesion of cells walls (Demarty *et al.*, 1984) and 4% calcium chloride (w/v) increased cell thickness 'Kensinton Pride' ('KP'), 'Irwin','Sensation' and 'Palmer' mango fruit flesh, especially outside flesh (de Assis *et al.*, 2009). Calcium chloride (1% w/v) delayed fruit ripening and maintained structural integrity of cell walls of strawberry (Lara *et al.*, 2004).

Discolouration around lenticels is likely to be an oxidation reaction involving enzymes, phenols and oxygen. It is suggested that treatments such as irradiation damages cell

membranes which allows phenols to mix with enzymes (e.g. polyphenol oxidase; PPO) and oxygen resulting in an oxidation reaction and brown pigmentation. The following experiments aimed to evaluate the effects of several antioxidants (citric acid and ascorbic acid) at 50-500 mM and calcium ascorbate at 10-100 mM on lenticel discolouration following irradiation. Other parameters such as skin colour, firmness, skin browning, titratable acidity, total soluble solids and weight loss were also monitored.

5.3.3. Materials and methods

5.3.3.1. Fruit

Green mature mango (*Mangifera indica.* cv. 'B74') fruit grown under standard commercial conditions were collected from the end of the packing line from a farm in the Childers area of south east Queensland during January 2012 and 2013. The fruit in single layer trays were driven to the Maroochy Research Facility (MRF) Nambour for treatment within 2 d of harvest. The fruit from the trays were randomly allocated to treatments.

5.3.3.2. Experiment 1. Preliminary trail; 2012

Five fruit per treatment were dipped in the following solutions for 10 min; distilled water (control), 100mM and 500mM ascorbic acid (BDH, Product code-100704Y, Australia), and 100mM and 500mM citric acid (BDH, Australia). The fruit were dried at room temperature (about 30°C) for about 2 h. They were then assessed for quality (see below) then randomly allocated to single layer, 10 kg trays and transported to Steritech (about 1 h drive). Half the fruit were irradiated as described in section4.4.3.1. The average dose received was 537 Gy (min-max; 492-563 Gy). All fruit were then driven to the Ecoscience Precinct (ESP) Brisbane (1 h drive from Steritech) and ripened at 20ºC and 80-90% RH.

5.3.3.3. Experiment 2. Anti-oxidant concentrations and calcium chloride; 2013

Fifteen fruit per treatment were dipped in the following solutions for 10 min; distilled water (control), 100mM calcium chloride (BDH, Product code-10303, BDH Australia Pty ltd, Australia), 100mM ascorbic acid (BDH, Product code-100704Y, Australia), and 10mM, 50mM and 100mM calcium ascorbate (Melrose, Product code-9312628120352, Melrose Laboratories Pty ltd). The fruit were dried at room temperature (about 30°C) for about 2 h, assessed for quality, then not irradiated or irradiated as described above. The mean dose was 523 Gy (min-max; 502-562 Gy). All fruit were then driven to the ESP and ripened as above.

5.3.3.4. Quality assessment

External fruit quality was assessed as described in section 4.1.3.3, and total soluble solids (°Brix) and titratable acidity assessed as described in section 5.2.3.3.

The fruit were considered ripe at a hand firmness of 3.

5.3.3.5. Experimental design and data analysis

A completely randomised design was used for both experiments. Quality parameters over time were analysed as factorials by repeated measures ANOVA analysis with GenStat (Version 14). Quality parameters at ripe were analysed as factorials by general ANOVA. The significance between treatment means was tested with the unprotected Fisher's test at the 5% level.

5.3.4. Results and Discussion

5.3.4.1. Experiment 1. Preliminary trail; 2012

Lenticel discolouration

Lenticel discolouration was significantly higher in irradiated fruit than non-irradiated fruit by 1 d after treatment, but changed little thereafter (Figure 25). This suggests that the irradiation effects on lenticels can be rapid, so that most of the sensitive lenticels express discolouration quickly after treatment with little further expression as the fruit ripens.

Antioxidants had no effect on lenticel discolouration (data not shown).

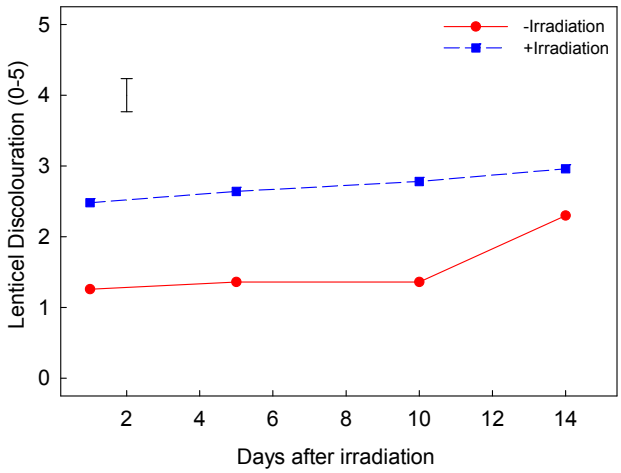


Figure 25 Effects of irradiation on lenticel discolouration of 'B74' mango fruit. Bar indicates LSD at P=0.05.

Skin Browning

With no irradiation, 100 mM ascorbic acid resulted in a rapid increase in skin browning from 1 to 5 d (Figure 26). However, the same response was not observed with 500 mM ascorbic acid, suggesting that this high concentration may suppress the skin browning that is caused by lower concentrations. However, it was not possible to determine whether 100 mM ascorbic acid could reduce skin browning caused by irradiation because of little significant irradiation effect on skin browning.

Skin Colour

Irradiation significantly reduced development of yellow skin colour during ripening (Figure 27), as observed in previous experiments. However, the antioxidants had no significant effect on skin colour change during ripening (data not presented).

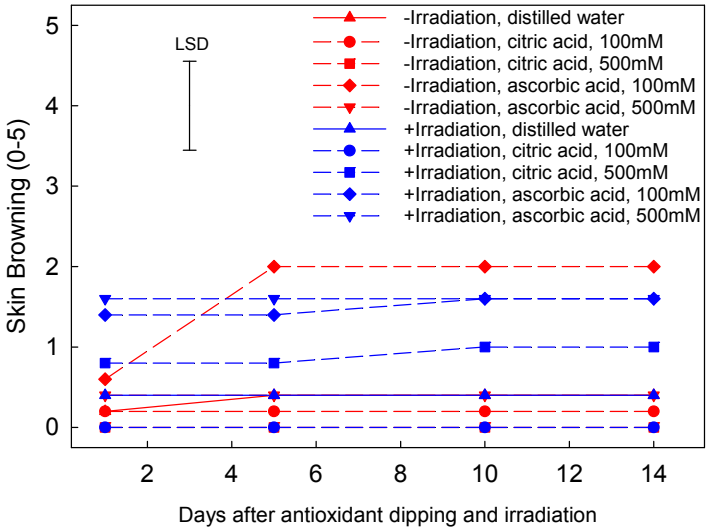


Figure 26 Effects of irradiation and several concentrations of antioxidants on skin browning of 'B74' mango fruit (2011/12). Bar indicates LSD at P=0.05.

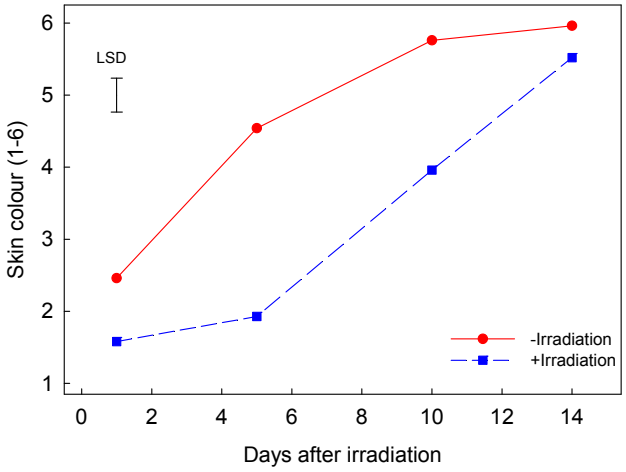


Figure 27 Effects of irradiation on yellow skin colour of 'B74' mango fruit (2011/12). Bar indicates LSD at P=0.05.

Firmness

Irradiation delayed softening during the early stages of ripening, but at 14 d they were softer than non-irradiated fruit (Figure 24). However the effects were small. There were no antioxidant effects on firmness.

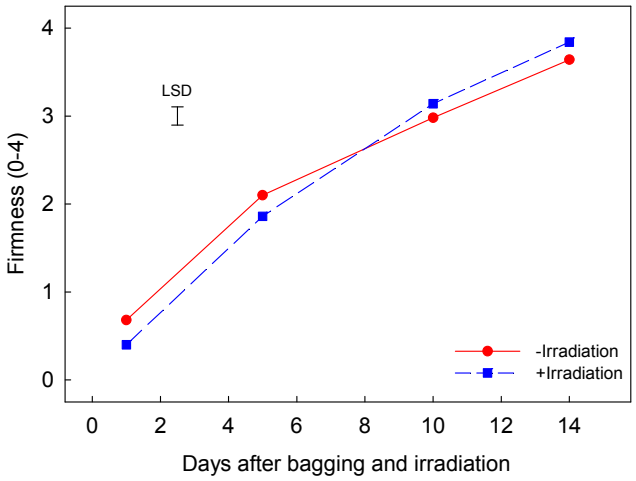


Figure 28 Effects of irradiation on fruit firmness of of 'B74' mango fruit (2011/12). Bar indicates LSD at P=0.05.

Quality at eating soft

Irradiation increased LD at eating soft (Table 69). However, antioxidants did not reduce LD. A significant interaction between irradiation and antioxidants was found on skin colour. Citric acid reduced skin colour significantly compared to distilled water.

With no irradiation, 100mM ascorbic acid increased skin browning compared with distilled water, while and 500mM had not effect. This suggests that the higher concentrations may overcome the negative effects of the 100 mM treatment. However, with irradiation, both concentrations increased skin browning compared with water. Citric acid had not effect on skin browning.

These results indicate that these antioxidants may have little potential to reduce LD caused by irradiation, and may actually reduce skin appearance.

5.3.4.2. Experiment 2. Anti-oxidant concentrations and calcium chloride; 2013

Lenticel discolouration

Significant interactions occurred between irradiation and antioxidant type, but not concentration. Irradiation significantly increased lenticel discolouration by 4 d after irradiation (Figure 29A). With no irradiation treatment, ascorbic acid, calcium ascorbate and calcium chloride increased LD at 12 d compared to distilled water. With irradiation, none of the antioxidants reduced lenticel discolouration compared to distilled water, while calcium chloride and ascorbic acid increased lenticel discolouration significantly compared to distilled water. Antioxidants at all the tested concentrations resulted in more LD at 12 d compared with distilled water (Figure 29B).

Table 69. Lenticel discolouration (0-5), skin yellow colour (1-6) and skin browning (0-5) of 'B74' mango fruit with/without irradiation and with/without different concentrations of antioxidant at eating soft (2011-12). Means in the same column and main treatment or treatment interaction with the same letter are not significantly different at P=0.05.

Treatment	Lenticel Discolouration	Skin Colour	Skin Browning
Irradiation			
-Irradiation	1.3 [b]		
+Irradiation	2.8 [a]		
Irradiation x Antioxidants			
-Irradiation, +distilled water		6.0 [c]	
-Irradiation, +ascorbic acid		6.0 [c]	
-Irradiation, +citric acid		5.4 [c]	
+Irradiation,+distilled water		4.5 [b]	
+Irradiation, +ascorbic acid		3.9 [ab]	
+Irradiation, +citric acid		3.5 [a]	
Irradiation x (antioxidant.concentration)			
-Irradiation,+distilled water			0.4 [a]
-Irradiation,+ascorbic acid, 100mM			2.0 [b]
-Irradiation,+ascorbic acid, 500mM			0.4 [a]
-Irradiation,+citric acid, 100mM			0.2 [a]
-Irradiation,+citric acid, 500mM			0 [a]
+Irradiation,+distilled water			0.4 [a]
+Irradiation,+ascorbic acid, 100mM			1.6 [b]
+Irradiation,+ascorbic acid, 500mM			1.6 [b]
+Irradiation,+citric acid, 100mM			0 [a]
+Irradiation,+citric acid, 500mM			1.0 [ab]

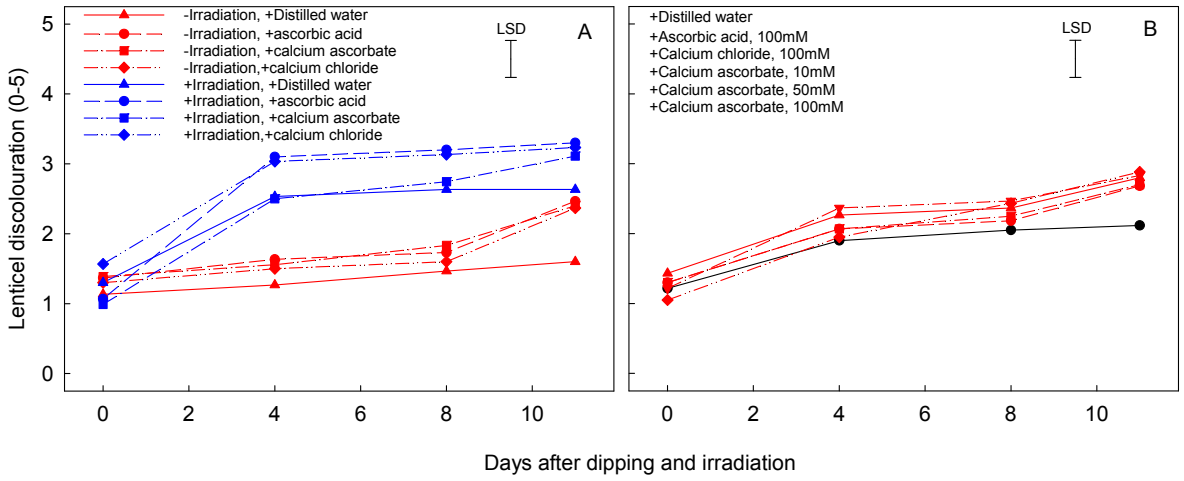


Figure 29 Effects of irradiation and types and concentrations of antioxidant and calcium chloride on lenticel discolouration of 'B74' mango fruit (2012/13). (A): Irradiation and types of antioxidants. (B): the main effects of concentrations. Bars indicate LSDs at P=0.05.

Skin Browning

Significant interactions occurred between irradiation and antioxidant treatments (Figure 30A). Irradiation generally resulted in more skin browning compared to no irradiation. Irrespective of irradiation treatment, calcium ascorbate and especially ascorbic acid, resulted in considerably more skin browning during ripening compared with distilled water and calcium

chloride. There was no evidence that the antioxidants reduced skin browning following irradiation

In relation to the main effects, calcium chloride had little effect on skin browning (Figure 30B). All other treatments increased skin browning compared with water only, and particularly ascorbic acid.

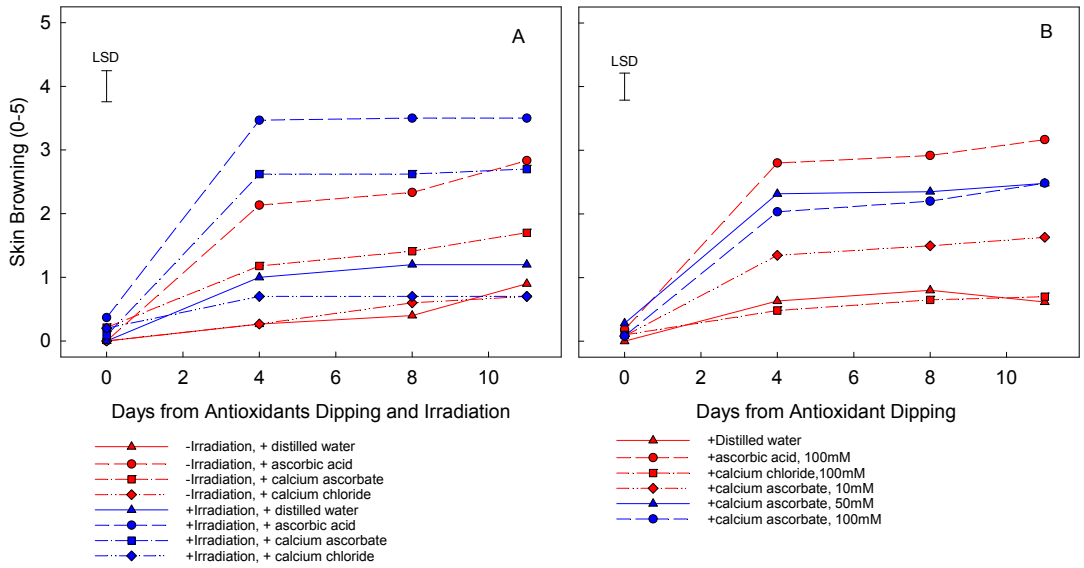


Figure 30 Effects of irradiation and types and antioxidants concentrations and calcium chloride on skin browning of 'B74' mango fruit (2012/13). (A): Irradiation and types of antioxidants. (B): The main effects of concentrations. Bars indicate LSDs at P=0.05.

Quality at eating soft

Irradiation increased both LD and SB severity at eating soft (Table 70). The antioxidant treatments and calcium chloride increased rather than decreased LD. Also, all concentrations increased skin browning, with ascorbic acid causing the most SB.

Table 70. Lenticel discolouration and skin browning of 'B74' mango fruit with/without irradiation and with/without different concentrations of antioxidant at eating soft (2012-13). Means in the same column and main treatment or treatment interaction with the same letter are not significantly different at P=0.05

Treatment	Lenticel discolouration	Skin browning
Irradiation		
-Irradiation	2.3 [b]	1.5 [b]
+Irradiation	3.1 [a]	2.2 [a]
Antioxidants		
+distilled water	2.1 [a]	
+calcium chloride	2.8 [b]	
+ascorbic acid	2.9 [b]	
+calcium ascorbate	2.8 [b]	
Antioxidant concentration		
+distilled water		0.6 [a]
+calcium chloride, 100mM		0.7 [a]
+ascorbic acid, 100mM		3.2 [d]
+calcium ascorbate, 10mM		1.6 [b]
+calcium ascorbate, 50mM		2.5 [c]
+calcium ascorbate, 100mM		2.5 [c]

5.3.5. Conclusions

Antioxidants can reduce oxidation reactions in plants. The discolouration of lenticels is likely an oxidation reaction similar to browning of cut surfaces. However, the current trials showed little potential for antioxidants to reduce LD. In most cases they increased LD and SB.

5.4. Preventing browning - postharvest coatings

Guoqin Li (PhD candidate), Peter Hofman, Daryl Joyce, Andrew Macnish

This chapter forms part of a current PhD program (Guoqin Li). The results presented here have not yet been published. The chapter was drafted by the Ph.D. student and reviewed by the project team.

5.4.1. Summary

Lenticel discolouration (LD) reduces appearance and value of 'B74' mango. Irradiation used as a quarantine treatment increases LD. The discolouration is likely caused by a browning reaction requiring oxygen. Surface coatings can also extend storage and shelf life of climacteric fruit like mango by restricting movement across the fruit surface, thereby reducing oxygen concentrations in the fruit tissue. The current trials were aimed at reducing oxygen concentrations around the lenticels to reduce the browning reaction associated with LD. 'B74' mango fruit were coated with several dilutions of Natural Shine® designed for use on mango. However, the waxing treatments had either nil effect on LD, or reduced LD but retarded skin colour development or softening. Therefore, it is unlikely that fruit coatings can reduce the LD effects of irradiation on 'B74' mango.

5.4.2. Introduction

Lenticel discolouration (LD) is a significant postharvest disorder in 'B74' mango fruit which influences fruit appearance and can result in economic loss to growers. Lenticels play a role in respiration and transpiration during the early development of mango fruit (Rymbai *et al.*, 2012). However, they can develop a dark discolouration around the lenticel after harvest.

Brown discolouration in plant tissue, for example the discolouration associated with flesh bruising, is a common oxygen-requiring reaction in plant tissues. Treatments to reduce the concentration of oxygen around the lenticel and the adjacent cells during and after irradiation may help reduce the development of the brown pigments that often appear to be associated with LD.

Surface coatings are applied to fruits to maintain quality and extend storage and shelf-life. Edible coatings are used mainly to retard moisture loss, reduce decay (McGuire and Hallman, 1995), and improve appearance and flavour (Hagenmaier and Baker, 1993). They can create a modified atmosphere (MA) by reducing gas exchange across the skin, resulting in lower oxygen and higher carbon dioxide concentrations inside the fruit (Amarante *et al.*, 2001). Edible coatings such as chitosan, shellac wax and carnauba wax have been used on fresh-cut pears (Xiao *et al.*, 2011), avocado (Bower and Palpi, 2006) and (Baldwin *et al.*, 1999; Dang *et al.*, 2008). Carnauba wax is a natural edible coating material obtained from the underside of the leaves of the Brazilian palm tree. The beneficial role of carnauba wax is well known for enhancing shelf life and maintaining postharvest quality of several fruits such as mango (Dang *et al.*, 2008) and avocado (Feygenberg *et al.*, 2005). Hence it is possible that carnauba wax applied to 'B74' fruit will lower oxygen concentrations and prevent the enzymatic browning around lenticels. For example a 20% and 40% carnauba-based wax emulsion reduced friction discolouration on pear (Amarante and Banks, 2001).

This report describes experiments aimed at testing whether carnauba wax applied at different concentrations and number of coatings to 'B74' mango can restrict oxygen concentrations sufficiently to retard the discolouration reaction around lenticels, but without negatively affecting other ripening characteristics and ripe fruit quality.

5.4.3. Materials and methods

5.4.3.1. Fruit

Green mature mango (*Mangifera indica)* 'B74' fruit grown under standard commercial conditions were collected from the end of the packing lines following commercial harvesting with Mango Wash® (Bally *et al.*, 1997), packing line washing, brushing, fungicide and insecticide treatment from the Childers area (south east Queensland) in January 2012 and 2013. The fruit in single layer trays were transported to the Maroochy Research Facility, (MRF) Nambour for treatment within 2 d of harvest. The fruit from the trays were randomly allocated to treatments.

5.4.3.2. Experiment 1. Wax concentrations; 2012

The fruit (average dry matter 13.6%) were randomly allocated to treatments the day after arrival at the laboratory. Fruit was dipped in 10%, 20%, 40% and 80% v:v carnauba wax (Sunshine Carnauba, Pace International):distilled water for 10 seconds, then dried on racks outside in the shade (approx. 30ºC) for about 2 h. Distilled water was used as the control. After quality assessment, fruit were completely randomized and placed in the 10 kg single layer trays, then transported to Steritech Pty Ltd and irradiated as described in section 5.2.3.2. The average dose received was 537 Gy (min-max; 492-563 Gy). The fruit were transported to the Ecoscience Precinct (ESP) Brisbane and ripened at 20^{o}C, and 80-90% RH. Ten individual fruit applications were used, with five wax treatments without and with irradiation.

5.4.3.3. Experiment 2. Multiple wax coatings; 2013

The fruit (average dry matter=13.8%) were randomly allocated to treatments the day after harvest. The fruit were dipped in 75% v:v carnauba wax as above. Other fruit were dipped another two times to provide three coatings. Distilled water was used as the control. After quality assessment, fruit were completely randomized across 10 kg single layer trays and irradiated as above at an average dose of 557 Gy (min-max; 524-587 Gy). The fruit were transported to the MRF and ripened at 20^{o}C and 75-90% RH. Fifteen individual fruit applications were used per treatment, with three wax treatments (including control), and without and with irradiation.

5.4.3.4. Quality Assessment

Fruit firmness (0-4), LD (0-5), skin colour (as the percentage of yellow colour on the non-blushed area; 1-6) and skin browning (0-5), were assessed as described in section 4.1.3.3. Brix (as an indicator of total soluble solids) and titratable acidity of the fruit at eating soft (firmness rating 3) were assessed as described in section 5.2.3.3.

Fruit were considered eating soft at firmness 3.

5.4.3.5. Experimental design and data analysis

A completely randomised design was used. Quality parameters over time were analysed as factorials by repeated measures ANOVA analysis with GenStat (Version 14). Total soluble solids and titratable acidity at eating soft were analysed by General Analysis of Variance. The significance between treatment means was tested with the unprotected Fisher's test at the 5% level.

5.4.4. Results and discussion

5.4.4.1. Experiment 1. Wax concentrations; 2012

Lenticel discolouration

The interaction of wax treatment and irradiation over time was not significant, suggesting that the waxing effect was similar without and with irradiation. By 10 d, the fruit treated with 80% wax had less LD compared with the no wax fruit (Figure 31). Also, irradiation increased LD compared with no irradiation.

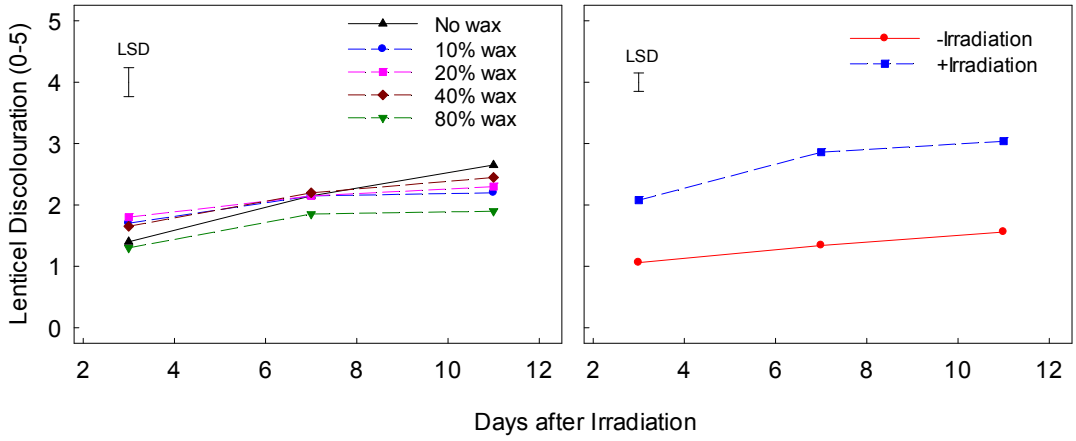


Figure 31 Effects of different concentrations (0, 10%, 20%, 40% and 80%) of Sunshine Carnauba wax on 'B74' mango fruit lenticel discolouration with/without irradiation (2011/12). (A): effect of wax concentrations and (B): effect of irradiation. Bars represent LSDs at P=0.05.

Skin colour

There was also no significant interaction between irradiation and wax over time on skin colour. However, increasing wax concentrations resulted in less yellow colour on the skin, even up to 11 d after treatment (Figure 32A). Even 10% and 20% wax delayed the loss of yellow colour compared to no wax. Irradiation also resulted in less yellow colour on the skin during ripening (Figure 32B).

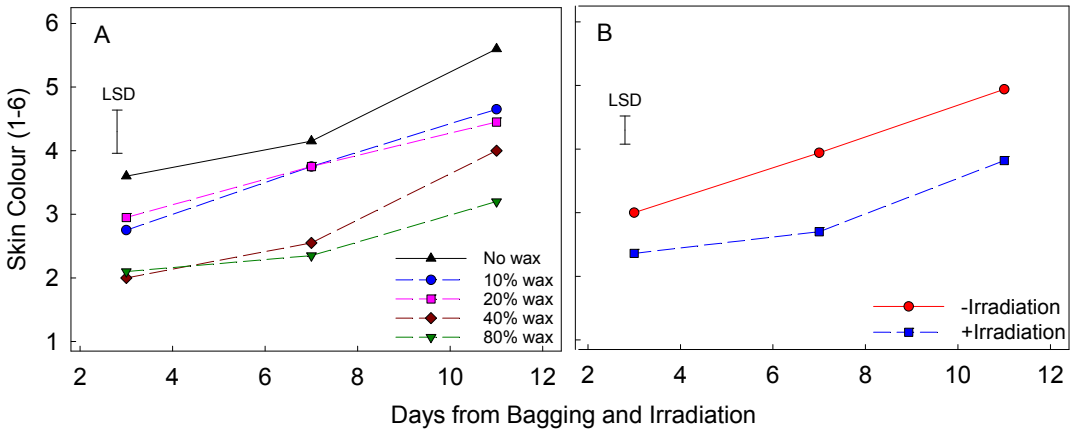


Figure 32 Effects of different concentrations (0, 10%, 20%, 40% and 80%) of wax on 'B74' mango fruit skin colour with/without irradiation (2011/12). (A): effect of wax concentrations on skin colour; (B): effect of irradiation on skin colour. Bars represent LSDs at P=0.05.

Firmness

The 80% wax treatment also significantly reduced firmness at 11 d compared with no wax (Figure 33), suggesting that the higher concentrations delayed ripening.

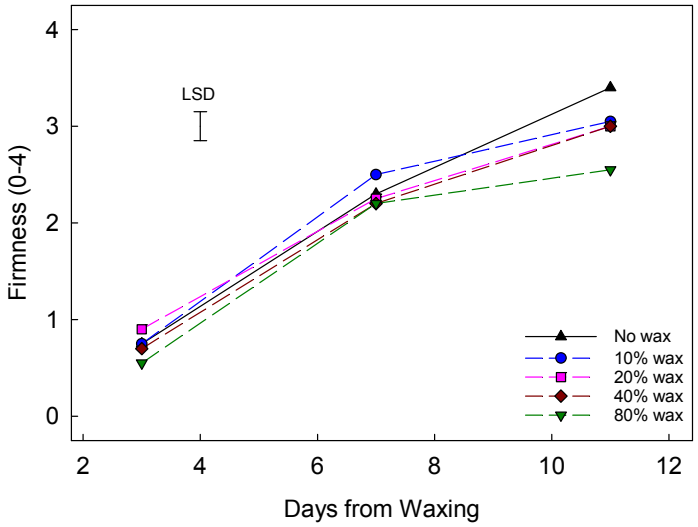


Figure 33 Effects of different concentrations (0, 10%, 20%, 40% and 80%) of wax on 'B74' mango fruit firmness (2011/12). Data were collected at 3, 7 and 11 days after waxing and irradiation. The bar represents LSD at P=0.05.

Skin browning

The wax treatments did not affect SB, but irradiation increased SB severity from 0.24 to 0.82.

Quality at eating soft

Irradiation increased LD and SB and reduced yellow skin colour at eating soft (Table 71). Waxing treatment had no effect on LD at eating soft. 40-80% wax reduced SB but all wax treatments reduced the yellow colour in the ripe fruit, and especially with 40-80% wax.

Thus, there was no benefit of the wax treatment in reducing LD of irradiated fruit, and it significantly retarded the development of yellow skin colour during ripening.

Table 71 Lenticel discolouration (0-5), skin colour (1-6) and skin browning (0-5) of 'B74' mango fruit with/without irradiation and with several wax concentrations at the eating soft stage (2011-12). Data were collected on the day fruit reached eating soft (firmness=3). Letters followed by data stand for least significant difference (0.05).

Treatment	Lenticel discolouration	Skin browning	Skin colour
Irradiation			
-Irradiation	1.6 ^{b}	0.08 ^{b}	5 ^{a}
+Irradiation	3.1 ^{a}	0.6 ^{a}	3.9 ^{b}
Wax			
-Wax		0.6 ^{b}	5.6 ^{c}
+10% wax		0.3 ^{ab}	4.7 ^{b}
+20% wax		0.6 ^{b}	4.5 ^{b}
+40% wax		0.2 ^{a}	4 ^{ab}
+80% wax		0.1 ^{a}	3.5 ^{a}

5.4.4.2. Experiment 2. Multiple wax coatings; 2013

Lenticel discolouration

Irradiation of non-waxed and 1 waxed fruit increased LD compared with no irradiation (Figure 34). In the non-irradiated treatments, 1 wax reduced LD compared with not waxed, but this was not the case with 3 wax. With irradiation, the 3 wax treatment reduced LD to non-irradiation levels. Therefore, this treatment showed potential to reduce irradiation damage, assuming no negative effects on fruit quality.

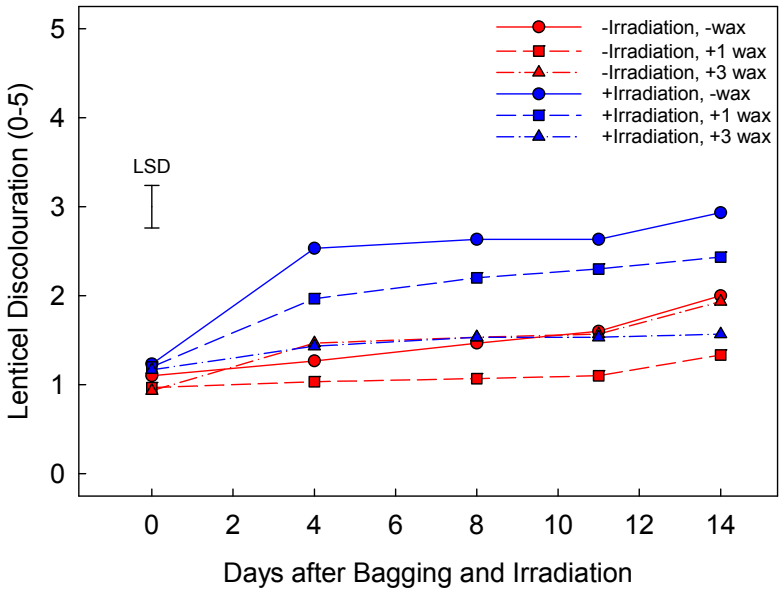


Figure 34 Effect of one (+1 wax) or three (+3 wax) coatings with 75% Sunshine Carnauba, with and without irradiation on 'B74' mango fruit lenticel discolouration during ripening (2012/13). The bar represents LSD at P=0.05.

Skin Colour

With no irradiation, one and three coatings reduced the % yellow on the ripening skin, and especially with three coatings (Figure 35). Irradiation reduced the % yellow with no wax, had no effect with one coating, but also reduced yellow colour with three coatings. Hence, while three coatings reduced LD, it also significantly reduced the development of yellow skin colour during ripening.

Firmness

Irradiation did not affect firmness during ripening. However one and three coatings reduced the loss of firmness during ripening (Figure 36) so the fruit would take longer to reach eating soft.

Skin Browning

Irradiation increased SB compared to non-irradiation (Figure 37). Wax treatments had no effect on reducing SB of the non-irradiated treatments. With irradiation, three coatings resulted in less SB at 4 d but more at 14 d compared with no wax and one coating.

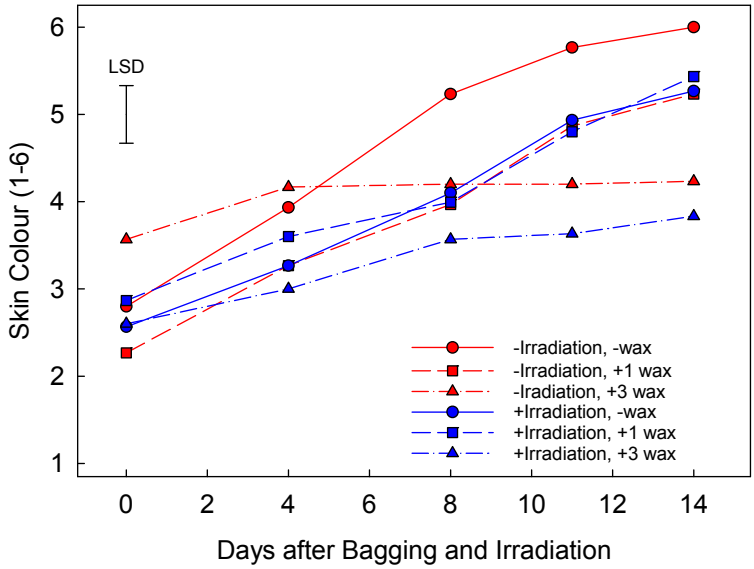


Figure 35 Effect of one (+1 wax) or three (+3 wax) coatings with 75% Sunshine Carnauba, and irradiation on 'B74' mango fruit skin colour (2012/13). Data were collected at 0, 4, 8, 11 and 14 days from waxing and irradiation. The bar represents LSD at P=0.05.

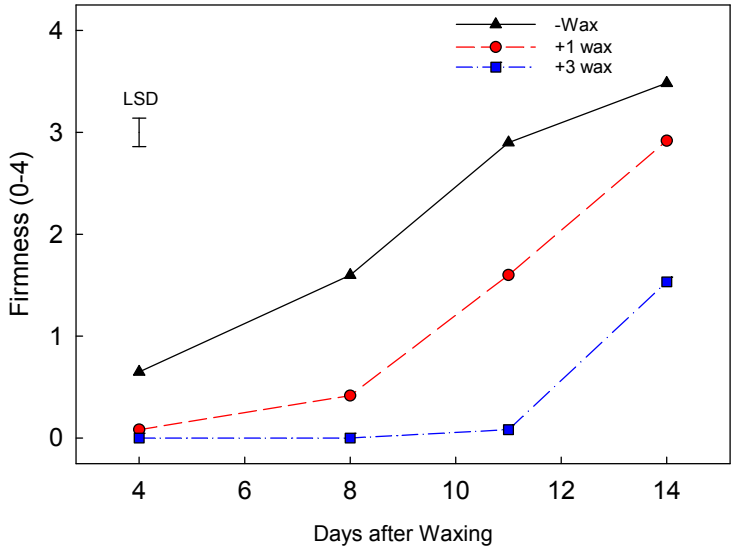


Figure 36 Effect of one (+1 wax) or three (+3 wax) coatings with 75% Sunshine Carnauba on 'B74' mango fruit firmness (2012/13). The bar represents LSD at P=0.05.

Weight Loss

No waxing resulted in significantly higher % weight loss during ripening compared with waxing (Figure 38), as expected. Irradiation generally resulted in more weight loss, with three coatings resulting in greater % weight loss at 14 d compared with no waxing and one coating. This was unexpected but may represent significant physiological damage to fruit because of restricted gas movement into and out of the fruit.

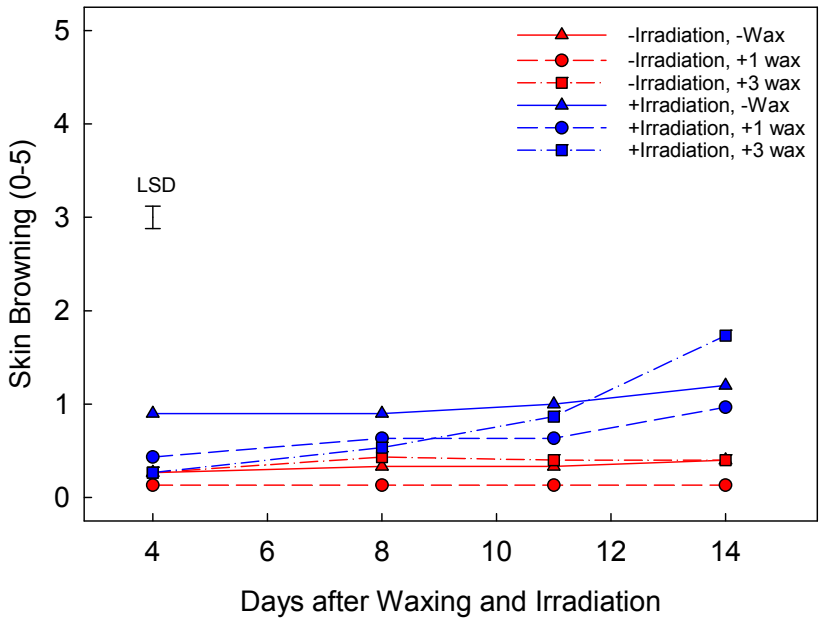


Figure 37 Effect of one (+1 wax) or three (+3 wax) coatings with 75% Sunshine Carnauba and irradiation on 'B74' mango fruit skin browning (2012/13). Data were collected at 4, 8, 11 and 14 days from waxing and irradiation. The bar represents LSD at P=0.05.

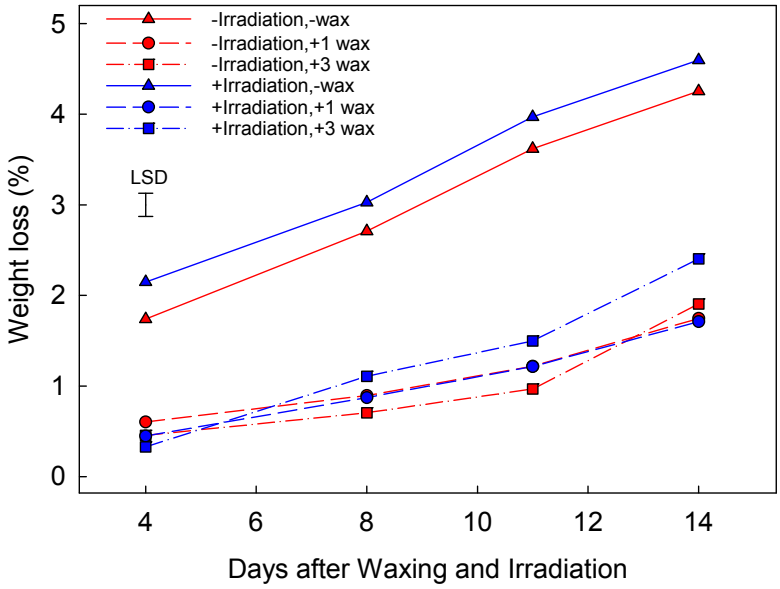


Figure 38 Effect of one (+1 wax) or three (+3 wax) coatings with 75% Sunshine Carnauba and irradiation on 'B74' mango fruit weight loss (2012/13). The bar represents LSD at P=0.05.

Quality at eating soft

Irradiation increased LD on the ripe fruit but waxing had no effect (Table 72). Irradiation increased SB, but three coatings of wax reduced LD. However, three coatings also reduced

the yellow skin colour at ripe, and several of the irradiated and three coatings fruit did not attain firmness 3 (eating soft) after 20 d.

Table 72 Lenticel discolouration (0-5), skin browning (0-5) and yellow skin colour (1-6) on 'B74' mango fruit with/without irradiation and with/without nil, one or three Sunshine Carnauba wax coatings at the eating soft stage (2012-13). Means in the same column for each main treatment or interaction with the same letters are not significantly different at P=0.05.

Treatment	Lenticel discolouration	Skin browning	Skin colour
Irradiation			
-Irradiation	1.6 [a]	0.4 [a]	
+Irradiation	2.5 [b]	1.3 [b]	
Wax			
- wax		0.8 [ab]	
+ 1 wax		0.6 [a]	
+ 3 wax		1.2 [b]	
Irradiation x wax			
-Irradiation, -wax			6 [d]
-Irradiation, + 1 wax			5.2 [bc]
-Irradiation, + 3 wax			4.7 [b]
+Irradiation, -wax			4.9 [bc]
+Irradiation, + 1 wax			5.4 [cd]
+Irradiation, + 3 wax			4.1 [a]

5.4.5. Conclusions

Surface coatings can add value to fruit by improving the gloss and reducing weight loss. They can also extend storage and shelf life of climacteric fruit like mango by restricting gas movement across the fruit surface and delaying ripening. However, too much restriction can result in unfavourable gas concentrations in the fruit, disrupting the ripening processes and reducing quality.

The current trials were aimed at reducing oxygen concentrations around the lenticels to reduce the browning reaction associated with LD. However, the waxing treatments had either nil effect on LD, or reduced LD but retarded skin colour development or softening. In some cases the fruit did not reach the full ripe stage. Therefore, it is unlikely that fruit coatings can reduce the LD effects of irradiation on 'B74' mango.

5.5. Preventing browning - postharvest bagging

Guoqin Li (PhD candidate), Peter Hofman, Daryl Joyce, Andrew Macnish

This chapter forms part of a current PhD program (Guoqin Li). The results presented here have not yet been published. The chapter was drafted by the Ph.D. student and reviewed by the project team.

5.5.1. Summary

Lenticel discolouration (LD) on the skin of mango fruit reduces appearance and value. The 'B74' cultivar is relatively susceptible to LD, and particularly after irradiation. The discolouration is most likely a browning reaction requiring oxygen from the atmosphere. Treatments that reduce oxygen concentrations around the fruit during and after irradiation may reduce LD on the ripe fruit. To test this, 'B74' mango fruit were placed in plastic or paper bags, and in some cases the bags flushed with nitrogen before irradiation. Some treatments included saturated water crystals to increase relative humidity. The fruit were then irradiated at typical disinfestation doses (about 500 Gy), then ripening and fruit quality assessed. In most cases bagging had little beneficial effect on ripe fruit LD, but in the positive cases, other quality parameters such as skin colour and skin browning (SB) were affected. This effect was generally worse with irradiation. Using macro-perforated plastic bags or paper bags had little effect. Treating ripening fruit reduced LD and improved yellow skin colour on the ripe fruit, but the 'B74' fruit need to be at least 70% colour for this treatment to be effective. This limits the distribution life of the treated fruit, but still may allow airfreight and 4-7 day seafreight as long as there are efficient chains in the importing country to ensure rapid distribution to the consumer.

5.5.2. Introduction

Mango fruit are grown in over 90 countries and is one of the popular tropical fruit because of its good flavour (Sivakumar *et al.*, 2011). Lenticel discolouration (LD; also known as lenticel damage), involves browning in the cells surrounding the lenticels. It is a common disorder that can reduce the appearance of mango fruit, and its consumer appeal (Bally *et al.*, 1997). Of the mango cultivars commonly grown in Australia, 'B74' is one of the more susceptible cultivars to LD (section 5.2). Lenticels play an important role in facilitating gas exchange and water loss, especially during early fruit growth (Rymbai *et al.*, 2012). Some studies suggested that disruption of the cells surrounding lenticels (Bezuidenhout *et al.*, 2005) caused by terpinolene from the nearby resin ducts (Lalel and singh, 2003) contributes to LD, however, LD was also suggested to be a self-defence mechanism without any disruption to the structure of the cells (du Plooy *et al.*, 2006). Hence the mechanisms causing LD are unclear.

Approx 8.6 million tonnes of mango fruit are lost each year because of the short shelf life, high susceptibility to chilling injury and postharvest disorders (Kader, 2005). Irradiation has been used commercially as a safe and effective technology for microorganism and insect (disinfestation) treatment (El-Samahy *et al.*, 2000). However, irradiation of mango for quarantine disinfestation can significantly increase LD (Hofman *et al.*, 2010c). Few studies have been published on the mechanisms and control of LD in mango fruit after irradiation.

Brown discolouration in plant tissue, for example the discolouration associated with flesh bruising, is a common reaction. It is often associated with oxidation of phenols and other plant constituents, which requires the presence of oxygen. We hypothesise that treatments to reduce oxygen concentrations around the lenticel and adjacent cells during and after irradiation may help reduce the development of the brown pigments that often appear to be associated with LD. In addition, treatments such as bagging may alter the water relations

around the lenticel and modulate damage to the adjacent cells. This chapter reports on the potential for several bagging treatments to reduce LD after irradiation. The treatments included flushing the bags with nitrogen to quickly reduce oxygen concentrations around the fruit, and treatments to moderate relative humidity within the bag. Lenticel discolouration, as well as other quality parameters such as skin colour, skin browning (SB), firmness, weight loss, titratable acidity and total soluble solids were recorded following treatment.

5.5.3. Materials and Methods

5.5.3.1. Plant material

Green mature mango (*Mangifera indica* cv. 'B74') fruit grown under standard commercial conditions were collected from the end of the packing lines following commercial harvesting using the desapping detergent Mango Wash® (Bally *et al.*, 1997), packing line washing, brushing, fungicide and insecticide treatment. The fruit were obtained from farms in the Katherine area (Northern Territory) in November 2013, and from the Childers area (south east Queensland) in January 2012, 2013 and 2014. The fruit in single layer trays from Katherine area were air-freighted to Brisbane the day after packing, then driven to either the Maroochy Research Facility (MRF), Nambour, or the Ecoscience Precinct (ESP), Brisbane for treatment within 2-3 d of harvest. The fruit for each trial were removed from the transport trays and randomly allocated to treatments.

5.5.3.2. Experiment 1. Bags and nitrogen; 2011/12

'B74' fruit were obtained from the Childers area (south east Queensland) in January 2012. The fruit in single layer trays were car-transported to MRF within 3 h. Within 2 d of harvest, fruit were allocated to treatments comprising without and with irradiation, without and with bags for 1 d or 2 d, and without or with nitrogen flushing of the bags before sealing (Table 73).

Table 73. Postharvest bagging treatments with 'B74' mango including bagging with/without nitrogen and with/without irradiation (2011/12).

Treatment code	Treatment details
No bags,-Irr	No bagging, no irradiation
Bags 1 d, -N, -Irr	Bagging for 1 d, no nitrogen flushing, no irradiation
Bags 1 d, +N,-Irr	Bagging for 1 d, nitrogen flushed, no irradiation
Bags 2 d, -N,-Irr	Bagging for 2 d, no nitrogen flushing, no irradiation
Bags 2 d, +N,-Irr	Bagging for 2 d, nitrogen flushed, no irradiation
No bags,-Irr	No bagging, irradiation
Bags 1 d, -N, -Irr	Bagging for 1 d, no nitrogen flushing, irradiation
Bags 1 d, +N,-Irr	Bagging for 1 d, nitrogen flushed, irradiation
Bags 2 d, -N,-Irr	Bagging for 2 d, no nitrogen flushing, irradiation
Bags 2 d, +N,-Irr	Bagging for 2 d, nitrogen flushed, irradiation

For the bagging treatment each fruit was placed in a GLAD® sandwich bag (24x24cm, polyethylene plastic). For the "bags without nitrogen" treatment, the bags were sealed with a heat sealer without any adjustment to the atmosphere in the bag. With "bags with nitrogen", the fruit was placed in the bag, the bag partly heat sealed, the atmosphere flushed with industrial nitrogen (BOC) for about 1 min using a tube inserted into the non-sealed section of the bag, then the remainder of the bag heat sealed. Fruit were then placed into 10 kg single layer trays, completely randomized across trays and treatments, then transported to Steritech Pty Ltd (a commercial irradiation facility near Caboolture) in 1 h. Two dosimeters (Opti-chromic detectors FWT-70-40M) were placed in opposite corners of each tray before irradiation (section 5.2.3.2). Typical doses were approx. 500 Gy. All fruit were then driven to

ESP early the next morning and ripened at 20°C and 80-90% RH. The fruit were removed from the bags after either 1 or 2 d.

Ten single fruit replications were used for each treatment.

5.5.3.3. Experiment 2. Bag types; 2012/13

'B74' mango fruit were obtained from the Childers area (south east Queensland) in January 2013. The fruit in single layer trays were car-transported to MRF within 3 h. Within2 d of harvest fruit were allocated to several bagging treatments as described in Table 74 and Table 75.

Table 74 Postharvest bagging treatments with 'B74' mango including bagging with/without nitrogen and with/without irradiation (2012/13).

Treatment code	Treatment details
No bags	No bagging
Macro-perforated	Macro-perforated LifeSpan bags, 200x200 mm, with 18 holes in each bag of approx 2 mm diam. Gas permeability was 27,000 $mL.bag^{-1}.day^{-1}.atm^{-1}$ O_2 and 21,600 $mL.bag^{-1}.day^{-1}.atm^{-1}$ CO_2 (LifeSpan)
Macro-perforated, water crystals	As above, but with water-saturated crystals
Polyethylene bags	Polyethylene LifeSpan bags, 200x200 mm, no perforations; LifeSpan-L335 bags with 24,000 $mL.bag^{-1}.day^{-1}.atm^{-1}$ O_2 and 19,000 $mL.bag^{-1}.day^{-1}.atm^{-1}CO_2$ (LifeSpan)
Polyethylene bags, nitrogen	As above, but with the bags flushed with nitrogen
Paper	Brown paper bags (80 GSM; Labtek)

The treatments were applied without and with nitrogen flushing of the polyethylene bags, without and with wet water crystals inside the macro-perforated bags, and without and with irradiation (Table 75).

Bagging with polyethylene bags, including nitrogen flushing, was done as described in Experiment 1. For the "wet water crystal" treatment, water absorbing crystals (Rain Saver water storing crystals, Hortex Australia, Epping) were placed in the porous plastic/paper material used to retain the water absorbing material in adult diapers Woolworths Homebrand, Australia). About 20 g of the crystals were placed in the porous bags (approx. 40x70mm), the bags heat sealed, immersed in water for 3 h to fully saturate the crystals, then placed in the macro-perforated bags and the bags heat sealed. Other fruit were placed in the paper bags and the bag opening folded over and stapled with about three staples along the edge.

Table 75 Bagging treatments applied to 'B74' mango to investigate effects on lenticel discolouration (2012/13).

Treatment code	Treatment details			
	Bag	Nitrogen flushing	Wet crystals	Irradiation
No Bag,-Irr	None	-	-	-
Macro,-Crystals,-Irr	Macro-perforated	-	-	-
Macro,Crystals,-Irr	Macro-perforated	-	+	-
Poly,-N,-Irr	Polyethylene	-	-	-
Poly,+N,-Irr	Polyethylene	+	-	-
Paper,-Irr	Paper	-	-	-
No Bag,+Irr	None	-	-	+
Macro,-Crystals,+Irr	Macro-perforated	-	-	+
Macro,Crystals,+Irr	Macro-perforated	-	+	+
Poly,-N,+Irr	Polyethylene	-	-	+
Poly,+N,+Irr	Polyethylene	+	-	+
Paper,+Irr	Paper	-	-	+

Fruit were randomly placed in 10 kg single layer trays, and irradiated as described in Experiment 1, and received an average dose of 575 Gy (min.-max. of 527-613 Gy). All fruit were then driven back to MRF the following morning and ripened at 20ºC and 75-90% relative humidity (RH). The fruit were removed from the bags after 8 d. Fifteen single fruit replications were used per treatment.

5.5.3.4. Experiment 3. Bagging and ripeness SEQ; 2013/14

Fruit were obtained from the Childers area (south east Queensland) in January 2014. The fruit in single layer trays were car-transported to MRF within 4 h of collection. After 1.5 d at 16^{o}C and 0.5 d of 20ºC, the fruit were car-transported to the ESP in 2 h.

Bagging and irradiation was applied at the following ripeness stages:

- Firmness 0 (average skin colour stage of 2 and 2 d after harvest)
- Firmness 1-2 (average skin colour stage of 3 and about 5 d after harvest at 20ºC), and
- Firmness 3 (average skin colour stage of 6 and 10 d after harvest at 20ºC)

The fruit, (except firmness 0) were held at 20ºC until the required firmness stage. The bagging and nitrogen flushing treatments (Table 76) were applied as described in Experiment 1.The bagging treatment used a polyethylene bag (L335, LifeSpan), with permeability characteristics of 24,000 mL.bag-1.day-1.atm-1 O_2 and 19,000 mL.bag-1.day-1. atm-1 CO_2. All fruit were placed randomly into 10 kg single layer trays then transported to Steritech Pty Ltd and treated as described in Experiment 1. The dose characteristics for the three firmness stages are described in Table 77. All fruit were transported back to ESP the following morning and ripened at 20ºC, 80-90% RH of ripening. Ten individual fruit replications were used per treatment.

Table 76 Bagging treatments applied to 'B74' mango to investigate effects on lenticel discolouration (2012/13).

Treatment code	Treatment details			
	Fruit firmness	Bagged	Nitrogen flush	Irradiation
Firm 0,-Bag,-Irr	0	-	-	-
Firm 0,+Bag,-N,-Irr	0	+	-	-
Firm 0,+Bag,+N,-Irr	0	+	+	-
Firm 0,-Bag,+Irr	0	-	-	+
Firm 0,+Bag,-N,+Irr	0	+	-	+
Firm 0,+Bag,-N,+Irr	0	+	+	+
Firm 1-2,-Bag,-Irr	1-2	-	-	-
Firm 1-2,+Bag,-N,-Irr	1-2	+	-	-
Firm 1-2,+Bag,+N,-Irr	1-2	+	+	-
Firm 1-2,-Bag,+Irr	1-2	-	-	+
Firm 1-2,+Bag,-N,+Irr	1-2	+	-	+
Firm 1-2,+Bag,-N,+Irr	1-2	+	+	+
Firm 3,-Bag,-Irr	3	-	-	-
Firm 3,+Bag,-N,-Irr	3	+	-	-
Firm 3,+Bag,+N,-Irr	3	+	+	-
Firm 3,-Bag,+Irr	3	-	-	+
Firm 3,+Bag,-N,+Irr	3	+	-	+
Firm 3,+Bag,-N,+Irr	3	+	+	+

Table 77 The mean and the minimum and maximum doses received by 'B74' mango for the postharvest fruit bagging trials in 2013/14.

Firmness when irradiated	Irradiation dose (Gy)		
	Average	Minimum	Maximum
Experiment 3			
0	520	493	577
1-2	669	624	716
3	530	495	564
Experiment 4			
0	485	465	505
1	498	477	519
2-3	490	469	510

5.5.3.5. Experiment 4 Ripeness NT; 2013/14

'B74' fruit (dry matter=14.8%±0.7%) were obtained from the Katherine area (Northern Territory) in November 2013. The fruit in single layer trays were air-freighted to Brisbane airport the following day. Within 3 d of harvest, the fruit from the trays were randomly allocated to several firmness treatments as follows:

- Firmness 0 (skin colour stage of 2 and 2 d after harvest)
- Firmness 1 (average skin colour stage of 3 and about 5 d after harvest at 20ºC), and
- Firmness 2-3 (average skin colour stage of 5 and 8 d after harvest at 20ºC)

The fruit, (except firmness 0) were held at 20ºC until the required firmness stage. Fruit from the different firmness stages were either not irradiated, or irradiated using gamma irradiation from a cobalt-60 source at the Gamma Technology Research Irradiator (GATRI) at the Australian Nuclear Science and Technology Organisation (ANSTO) facility at Lucas Heights, Sydney The fruit were air-freighted from Brisbane to Sydney, then irradiated the following day and immediately airfreighted back to Brisbane. Before irradiation, Fricke dosimeters were placed throughout the array at the expected minimum and maximum dose zones to measure the dose received. The average dose, and the minimum and maximum doses received at each firmness stage are presented in Table 77.

After irradiation the fruit were ripened at 20ºC and 80-90% RH. Ten individual fruit replications were used per treatment.

5.5.3.6. Quality assessment

Lenticel discolouration, skin colour, and firmness were assessed as described in the "'B74' Quality Assessment Manual" (Hofman *et al.*, 2010a). Lenticel discolouration was assessed as: 0= no lenticel spotting, 1= dense, pronounced spots on less 10% of skin, not star-shaped or cracked; 2= dense, pronounced spots on more than 10% and less than 25% of skin, not star-shaped or cracked lenticel damage; 3= dense, pronounced spots on more than 25% and less than 50% of skin, not star-shaped or cracked; 4= dense, pronounced spots on more than 50% of skin; 5= lenticels cracked and open. Skin colour assessment was based on the percentage of the non-blush area with yellow skin colour using the following scale: 1=0-10% yellow, 2=10%-30% yellow, 3=30%-50% yellow, 4=50%-70% yellow, 5=70%-90% yellow, 6=90%-100% yellow. Firmness was assessed by hand using the following scale: 0=hard (no 'give' in the fruit), 1=rubbery (slight 'give' in the fruit with strong thumb pressure), 2= sprung (flesh deforms by 2-3mm with moderate thumb pressure), 3=firm soft (whole fruit deforms with moderate hand pressure) and 4=soft (whole fruit deforms with slight hand pressure).

5.5.3.7. Weight loss

Fruit weight was measured before bagging and irradiation treatment (Experiment 1, 2 and 3) or after bagging and irradiation (Experiment 4) and at different times during ripening. The percent weight loss was expressed as percentage of the initial value (Hu *et al.*, 2013).

5.5.3.8. Total soluble solids and titratable acidity

Both cheeks of each fruit was collected, the skin removed, then the flesh diced and stored at -20ºC for total soluble solids and titrable acidity analysis within two months. On the day of analysis, the samples were removed from the freezer, flawed, and a sample of juice collected by squeezing through cheesecloth. The total soluble solids was estimated using a pocket refractometer PAL-1 (ATAGO, Japan), and presented as Brix. The titratable acidity content was determined on a 5 gm subsample by tritration with 0.1 M NaOH to PH=8.1 using a Titrino-719 S (Metrohm USA).

5.5.3.9. Oxygen and carbon dioxide concentrations

The O_2 and CO_2 concentrations in the relevant bag treatments were analysed using an OxyBaby gas analyzer (HTK, Hamburg, Germany) by inserting the gas analyser needle into the bags for about 15 s (Aday *et al.*, 2011). The analyser was calibrated with 0% O_2 (pure nitrogen), 100% CO_2 and outdoor air with 20.9% O_2 and 0.04% CO_2. Ten single fruit bag replicates were measured and the results expressed as on a v/v percentage basis.

5.5.3.10. Enzyme activity and total phenols

Polyphenol oxidase (PPO) and peroxidise (POD) activity were assayed according to Cao *et al*. (2010) with modification. Frozen mango peel was grounded in liquid nitrogen to a fine powder with a tissue lyaser (Qiagen, San Francisco), then 0.1 g weighed into pre-cooled 0.5M, pH 6.5 phosphate buffer containing 5% (w/v) polyvinyl pyrrolidone. The homogenate was centrifuged at 14,000 x g for 20 min at 4ºC and the supernatant used to assay for enzyme activities. For PPO, a 1.2 mL solution containing 0.6 mL 0.05M pH 6.5 phosphate buffer, 0.3mL crude enzyme extract and 0.3mL 0.3M catechol (Sigma-Aldrich) was placed in a spectrophotometer and the absorbance at 420 nm recorded. One unit of activity was defined as the increase in A_{420} of 0.01 min^{-1} and expressed as units.mg^{-1} protein. For POD, a 1.21mL reaction mixture containing 0.6ml 0.05M pH 6.5 phosphate buffer, 0.3mL crude enzyme extract, 0.3ml 0.3% (v/v) guaiacol (Sigma-Aldrich) and 0.01mL 0.4% H_2O_2 (Lab-tek) and absorbance recorded. One unit of activity was defined as an increase in A_{470} of 0.01 min^{-1} expressed as units mg^{-1} protein.

To measure total phenols, 0.1 g of the powder was added to 1ml 1% HCl-ethanol for 2 h at 4ºC and centrifuged in 4ºC for 20 min at 14,000 x g. The supernatant were diluted with milli-Q water 20-fold and thoroughly mixed. A 1 mL reaction mixture of 0.05mL diluted supernatant, 0.1 mL Folin-Ciocalteu reagent and 0.85 mL sodium carbonate was made. The absorbance at 765 nm was measured and used to calculate the total phenols content using gallic acid as a standard, expressed as mg of garlic acid equivalent.gm^{-1} fresh wt.

5.5.3.11. Statistical analysis

Completely randomised designs were used in all four experiments, which 10 single fruit replicates in Experiment 1, 3 and 4 and 15 single fruit replicates in Experiment 2. Quality parameters over time were analysed by repeated measurements ANOVA analysis with GenStat (Version 14). Total soluble solids, titratable acidity at eating soft, weight loss and O2 and CO2 concentrations were analysed by factorial analysis. Quality parameters were analysed by General Analysis of Variance without factors. The significance between treatment means was tested with the unprotected Fisher's test at the 5% confidence level.

5.5.4. Results and discussion

5.5.4.1. Experiment 1. Bags and nitrogen; 2011/12

Lenticel discolouration

There were no significant interactions between bagging and irradiation, however irradiation and bagging both affected LD (Figure 39). Irradiation increased LD as soon as 3 d after irradiation. Bagging did not affect LD up to 6 d after treatment, but increased LD by 11 d.

A significant interaction between irradiation and duration of bagging was noted (Figure 40). Irradiation resulted in higher LD at all assessment times compared with no irradiation. In addition, bagging for 2 d reduced LD at 3 d after bagging/irradiation, but this effect was not observed at 7 d and later.

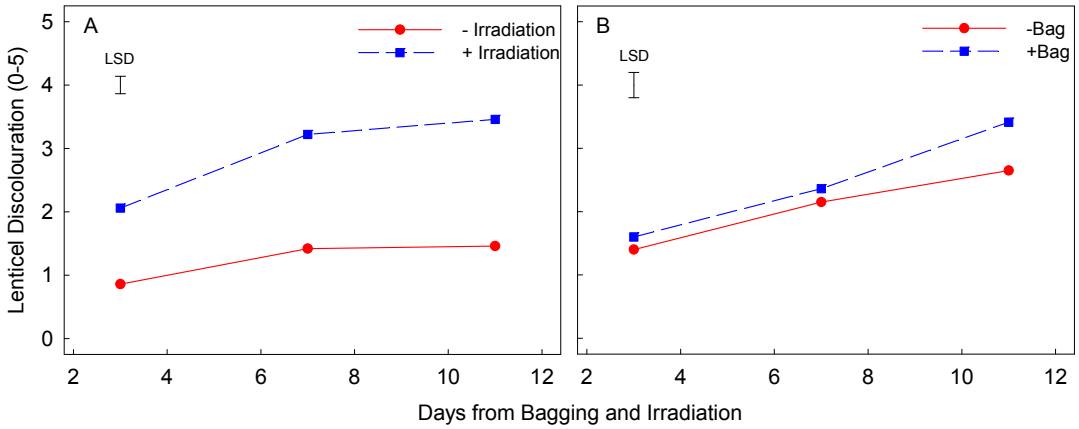


Figure 39 Effects of bagging (GLAD® sandwich bag) for 1 d and 2 d, and with and without nitrogen flushing and with and without irradiation on lenticel discolouration on 'B74' mango fruit (2011/12). (A) effect of irradiation on lenticel discolouration and (B) effect of bagging on lenticel discolouration. Data were collected on the day 3, 7 and 11 days after bagging and irradiation. Bars indicates LSD at P=0.05.

Skin Colour

There was no significant interaction between irradiation, bagging and duration of bagging.

A significant interaction was found between irradiation and bagging. In the absence of irradiation, bagging reduced the percentage of skin with yellow colour (lower skin rating score) at 3 d, but there was no effect at 7 and 11 d (Figure 41A). Irradiation reduced the increase in yellow colour irrespective of bagging, with bagging plus irradiation having the greatest reduction on yellow colour development.

A significant interaction was noted within the bagging treatments. At 3 d, bagging for 24 h with no nitrogen or irradiation resulted in more yellow colour than bagging with nitrogen for 48 h (Figure 41B). At 7 d, irradiation resulted in less yellow colour in all treatments. By 11 d the 48 h bag treatments with irradiation had developed very little yellow colour (about 10% yellow).

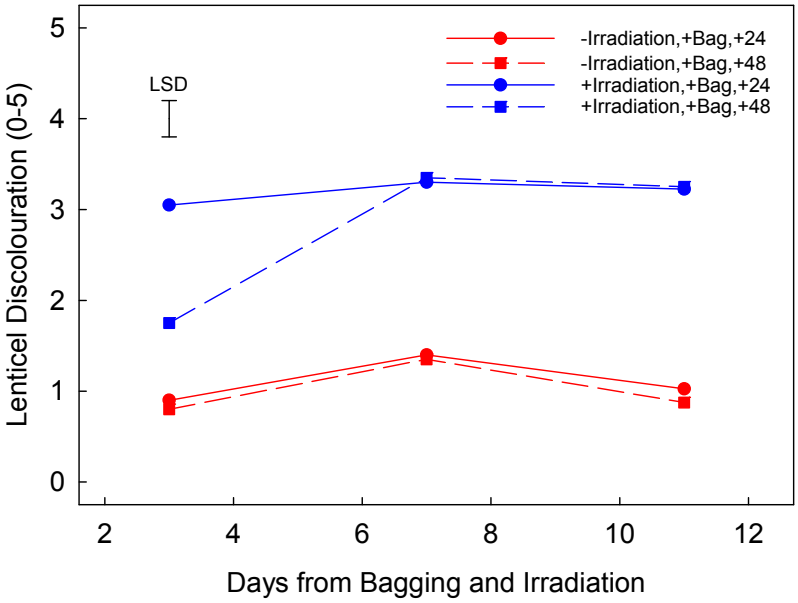


Figure 40 Effects of bagging (GLAD® sandwich bag) for 1 d and 2 d with and without irradiation on lenticel discolouration on 'B74' mango fruit (2011/12). Data were collected 3, 7 and 11 d after bagging and irradiation. Bar indicates LSD at P=0.05.

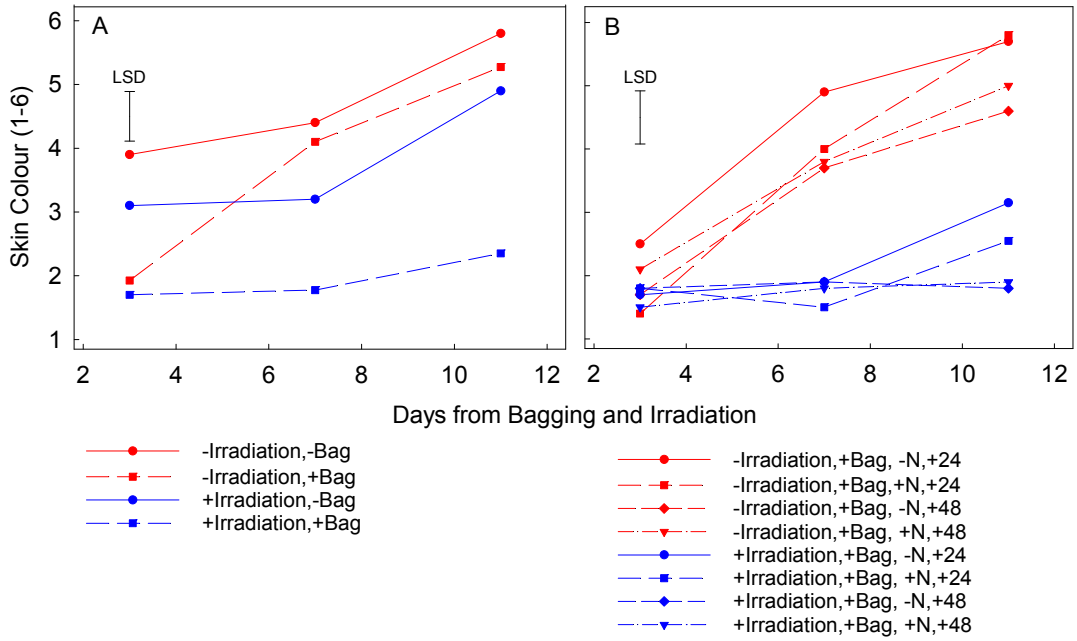


Figure 41 Effects of bagging (GLAD® sandwich bag) for 1 and 2 d and with/without nitrogen flushing and with/without irradiation on skin colour of 'B74' mango fruit (2011/12). n=10. (A) effect of irradiation and duration of bagging and (B) effect of bagging duration and nitrogen flushing within bagging treatments. Data were obtained at 3, 7 and 11 d from bagging and irradiation. Bars indicate LSDs at P=0.05.

Firmness

The only significant treatment effects were an interaction between bagging and assessment date, and between the nitrogen treatments. Bagged fruit were slightly firmer at 7 d compared to no bagging, but the effect was very small and transient (Figure 42A). Nitrogen increased softening (higher firmness score) but only very slightly (Figure 42B).

Skin browning

Bagging and irradiation affected SB. At 3 d, bagging reduced SB in irradiated fruit to levels similar to no irradiation (Table 78). However, this effect was not observed at 11 d. Nitrogen flushing had no effect on SB at 3 d, but actually increased SB in the irradiated fruit at 11 d. Hence bagging and N had no beneficial effect on SB.

Gas concentrations

1 d bagging marginally increased the O_2 concentration in the bag compared with 2 d bagging (Table 79). Irradiation consistently increased the CO_2 concentration, especially with 2 d bagging.

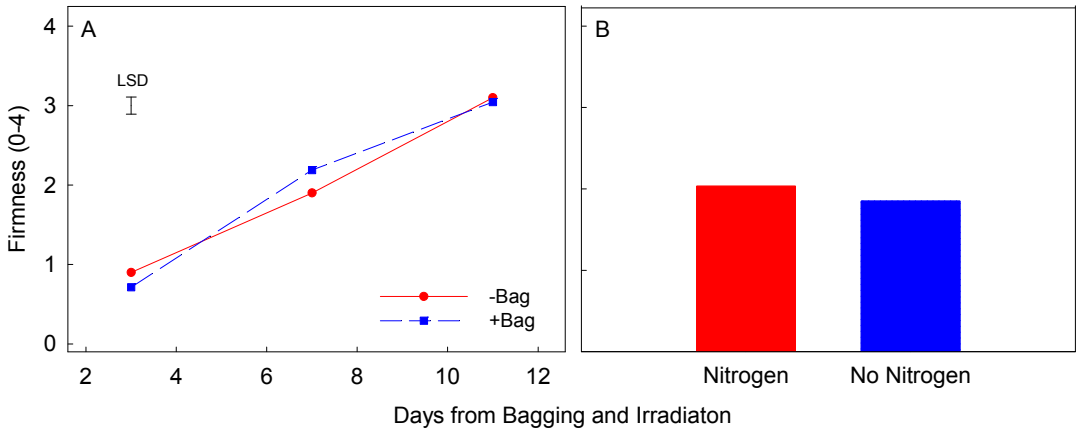


Figure 42 Effects of bagging (GLAD® sandwich bag) with/without nitrogen flushing, with/without irradiation on firmness of 'B74' mango fruit (2011/12). n=10. (A): Effect of bagging on firmness during ripening; Data were collected at 3, 7 and 11 days from bagging and irradiation and expressed as means. B): Effect of nitrogen and bagging treatments. Bar in graph A represents LSD (P=0.05).

Quality at eating soft

Bagging reduced LD in the absence of irradiation, but not with irradiation (Table 80). There was no effect of bagging duration or nitrogen flushing on LD. Bagging had no effect on yellow skin colour rating in the absence of irradiation. Irradiation reduced yellow skin colour at eating soft compared with no irradiation, and this effect was stronger with bagging. Longer bagging also reduced yellow skin colour at eating soft. Skin browning was highest with bagging+irradiation, with 2 d bagging, and with nitrogen flushing and irradiation.

Table 78 Effects of bagging (GLAD® sandwich bag) with/without nitrogen flushing with and without irradiation on skin browning of 'B74' mango fruit (2011/12). Means with different letters in the same column are significantly different at P< 0.05

Treatment	Skin browning (0-5)	
	Day 3	Day 11
Bagging x Irradiation		
-Bagging,-irradiation	0.4 [a]	0.4 [a]
+Bagging,-irradiation	0.5 [a]	0.5 [a]
-Bagging, +irradiation	1.3 [b]	1.3 [b]
+Bagging,+irradiation	0.6 [a]	1.5 [b]
Nitrogen x irradiation		
+Bagging,-Nitrogen,- Irradiation	0.1 [a]	0.1 [a]
+Bagging,+Nitrogen,- Irradiation	0.1 [a]	0.1 [a]
+Bagging,-Nitrogen,+ Irradiation	0.6 [a]	0.9 [b]
+Bagging,+Nitrogen,+ Irradiation	0.7 [a]	2.2 [c]

Table 79 Effect of duration of bagging, nitrogen flushing and irradiation on oxygen (O_2) and carbon dioxide (CO_2) concentrations in the polyethylene bag with 'B74' mango fruit. The results are for the significant main effects and the significant interactions. Data were collected just before removing the bags. Means in the same row and for the same main or interaction effects are not significantly different at P=0.05.

Treatment	Concentration (%)	
	O_2	CO_2
Bagging duration		
1 d	4.96 [a]	
2 d	4.12 [b]	
Nitrogen with bagging x irradiation		
-Nitrogen,- Irradiation	5.13 [b]	16.86 [a]
+Nitrogen,- Irradiation	3.79 [a]	15.21 [a]
-Nitrogen,+ Irradiation	4.37 [ab]	32.37 [c]
+Nitrogen,+ Irradiation	4.87 [b]	23.71 [b]
Bag duration x irradiation		
1 d,- Irradiation		14.79 [a]
2 d,- Irradiation		17.28 [a]
1 d,+ Irradiation		24.18 [b]
2 d,+ Irradiation		31.9 [c]

5.5.4.2. Experiment 2. Bag types; 2012/13

Lenticel discolouration

Significant interactions occurred between irradiation and bag type. All irradiation treatments resulted in more LD at 10 d compared to no irradiation (Figure 43A). With irradiaton, the macro-perforated and paper bags had no effect on LD, however the polyethylene bag reduced LD at 8 d, and to a lesser degree at 10 d, compared to the other irradiation treatments.

Nitrogen flushing with polyethylene bags had no effect on LD. However, the wet water crystal treatment resulted in increased LD at 8-10 d compared to no water crystals (Figure 43B).

Table 80 Lenticel discolouration (0-5), skin colour (1-6), and skin browning (0-5) of 'B74' mango fruit with and without irradiation, and with and without bagging for different durations (1 or 2 d) and with and without nitrogen flushing at the eating soft stage (firmness=3) (2011-12). Means with the same letter in the same row and for the same main or interaction effects are not significantly different at P<0.05.

Treatment	Lenticel discolouration (0-4)	Skin colour (1-6)	Skin browning (0-5)
Bag x Irradiation			
-Bag, -Irradiation	1.9 ^{b}	6 ^{c}	0
+Bag, -Irradiation	1.4 ^{a}	5.3 ^{bc}	0.1 ^{a}
-Bag, +Irradiation	3.4 ^{c}	4.9 ^{b}	0.8 ^{b}
+Bag, +Irradiation	3.5 ^{c}	2.5 ^{a}	2.2 ^{c}
Bagging duration x Irradiation			
1 d, -Irradiation			0
2 d, -Irradiation			0.1 ^{a}
1 d, +Irradiation			0.8 ^{b}
2 d, +Irradiation			2.2 ^{c}
Nitrogen x irradiation			
-Nitrogen,- Irradiation			0.1 ^{a}
+Nitrogen,- Irradiation			0.1 ^{a}
-Nitrogen, + Irradiation			0.9 ^{b}
+Nitrogen,+ Irradiation			2.2 ^{c}
Bagging duration			
1 d		4.4 ^{a}	
2 d		3.5 ^{b}	

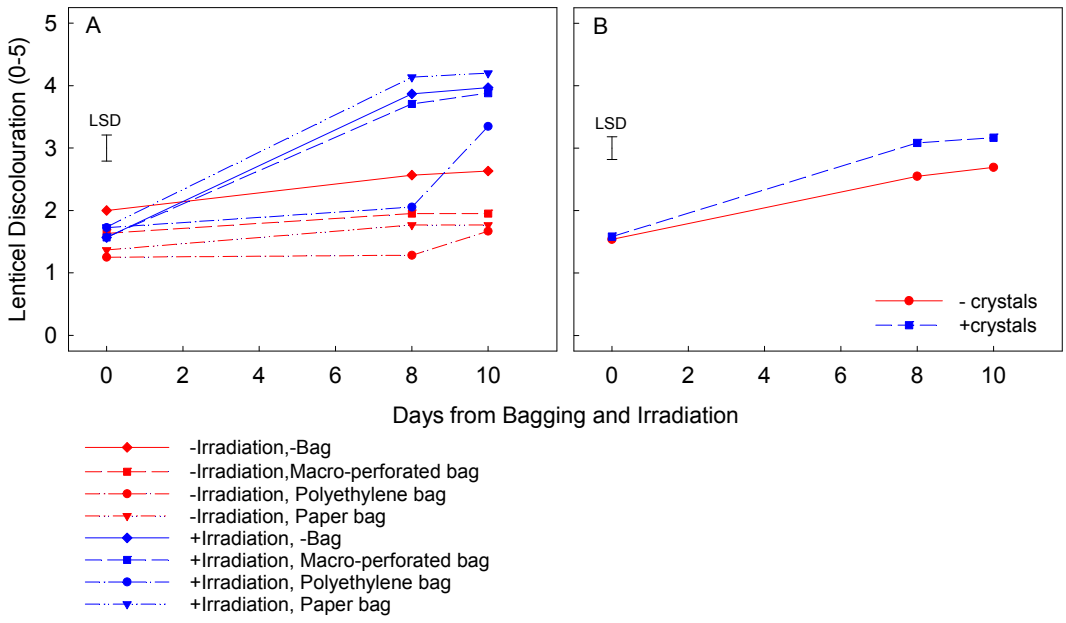


Figure 43 Effect of irradiation, bag type and presence of water crystals on lenticel discolouration of 'B74' mango fruit during ripening (2012/13). (A): effect of irradiation and bag type and (B): effect of wet water crystal. Data were collected at 0, 8 and 10 d from bagging and irradiation. The bars represent LSDs at P=0.05.

Skin Colour

All irradiation treatments resulted in less yellow colour on the skin at 8 and 10 d, compared with no irradiation or bagging, except for polyethylene bags (Figure 44A). In non-irradiated fruit, the macro-perforated, and especially the polyethylene bags, resulted in less yellow colour at 8-10 d compared with no bagging or paper bags. A similar bagging effect was noted with irradiation, but the effect was not as marked.

Significant interactions between irradiation and the water crystal treatment were also noted (Figure 44B). With no irradiation, fruit in bags with the water crystals had significantly more yellow skin colour at 8 d compared with no crystals, although there was no treatment effect at 10 d. There was little crystal effect with irradiation at any assessment time.

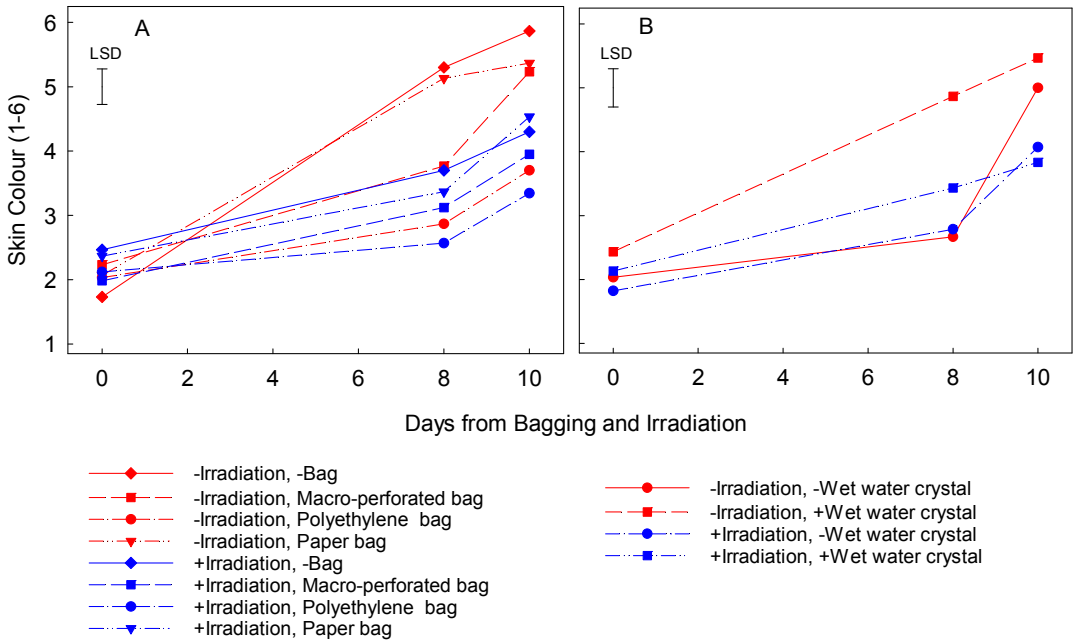


Figure 44 Effect of irradiation, bags type and presence of water crystals on skin colour of 'B74' mango fruit during ripening (2012/13). (A): effect of irradiation and bag type, and (B): effect of irradiation and wet water crystals inside the macro-perforated bag on skin colour. Data were collected at 0, 8 and 10 d from bagging and irradiation. Bars indicate LSDs at P=0.05.

Firmness

Irradiation resulted in firmer fruit (lower firmness rating) with micro-perforated and polyethylene bags at 8 d only (Table 81). There was no irradiation effect on firmness at 10 d but the polyethylene bag resulted in firmer fruit compared with all other treatments.

Skin browning

Irradiation increased SB at 8 and 10 d (Table 82). Polyethylene bags reduced SB at both 8 and 10 d compared with no bagging, while the other bags had no effect. Nitrogen flushing and wet water crystals did not affect SB.

Table 81 Effect of irradiation and bagging on firmness (0-4) of 'B74' mango fruit during ripening (2012/13). Data were collected 8 and 10 days bagging and irradiation and are expressed as means. Means in the same column with the same letter are not significantly different at P=0.05.

Bag treatment	Day 8		Day 10	
	Not irradiated	Irradiated	Not irradiated	Irradiated
-bag	2.4 de	2.8 e	3.2 b	3.2 b
+macro-perforated bag	2.5 de	1.8 bc	3.2 b	3 b
+polyethylene bag	1.5 b	0.8 a	1.9 a	1.5 a
+paper bag	2.6 de	2.2 cd	3.3 b	3.3 b

Table 82 Effect of bag type and irradiation on skin browning (0-5) of 'B74' mango fruit at 8 and 10 days during ripening (2012/13). Means with the same letter in the same column are not significantly different at P=0.05.

Treatment	Day 8	Day 10
Irradiation		
No	0.3 a	0.3 a
Yes	0.6 b	0.7 b
Bagging		
-Bag	0.5 ab	0.5 ab
+Macro-perforated bag	0.6 b	0.7 b
+Polyethylene bag	0.2 a	0.3 a
+Paper bag	0.4 ab	0.5 ab

Weight loss

Irradiation of non-bag fruit had no effect on percent weight loss (Table 82). All bagging treatments reduced percent weight loss compared to no bags, irrespective of irradiation treatment. Macro-perforated bags resulted in the lowest percent weight loss that both 8 and 10 d.

Table 83 Effect of irradiation and bag treatment on the percent weight loss of 'B74' mango fruit during ripening (2012/13). Data were collected at 8 and 10 days after bagging. Means in the same column with the same letter are not significantly different at P=0.05.

Treatment	Weight loss (%)			
	Day 8		Day 10	
	No irradiation	Irradiation	No irradiation	Irradiation
No bagging	3.6 f	3.6 f	4.2 f	4.2 f
Macro-perforated bags	1.2 a	1.1 a	1.8 a	1.8 a
Polyethylene bags	1.8 b	2.2 c	2.4 b	2.8 c
Paper bags	2.6 d	2.9 e	3.2 d	3.6 e

Quality at eating soft

With no irradiation, all bagging treatments resulted in less LD at eating soft compared with no bagging (Table 84). Irradiation increased LD at ripe in all treatments and there was no effect of bagging ion LD of the irradiated fruit. Nitrogen did not affect LD, but the presence of wet water crystals increased LD at ripe.

Irradiation reduced yellow colour on the skin at eating soft (Table 84), as did both plastic bag treatments. In fact, some of these fruit did not reach the soft ripe stage. Irradiation increased SB but there were no significant effects of the other treatments on SB.

Irradiation of non-bag and macro-perforated bag fruit had no effect on titratable acidity at eating soft (Table 85). However, the polyethylene bag treatment resulted in significantly higher titratable acidity in the non-irradiated fruit compared with the irradiated fruit. Also, in the non-irradiated fruit, polyethylene bags resulted in higher titratable acidity compared with the non-bag and the other bag treatments. This suggests that polyethylene bagging retarded the loss of acidity during ripening, but irradiation enhanced acids breakdown so that there was little effect of bagging with irradiation. There were no significant treatment effects on total soluble solids (data not presented).

Table 84 Lenticel discolouration (0-5), skin colour (1-6) and skin browning (0-5) of 'B74' mango fruit at eating soft treated with and without irradiation, or with and without bagging or with/without wet water crystals (2012/13). For each quality criterion, means with the same letter in the same main or interaction effects are not significantly different at P=0.05.

Treatment	Lenticel discolouration	Skin colour	Skin browning
Bag type x irradiation			
-Bag, -Irradiation	2.6 [b]		
Macro-perforated bag, -Irradiation	2 [a]		
Polyethylene bag, -Irradiation	2.1 [a]		
Paper bag, -Irradiation	1.8 [a]		
-Bag, +Irradiation	4 [c]		
Macro-perforated bag, +Irradiation	3.9 [c]		
Polyethylene bag, +Irradiation	3.7 [c]		
Paper bag, +Irradiation	4.1 [c]		
Water crystal in macro-perforated bag			
-wet water crystal	2.7 [a]		
+wet water crystal	3.2 [b]		
Irradiation			
-Irradiation		5.3 [a]	0.3 [b]
+Irradiation		4.1 [b]	0.9 [a]
Bag type			
-Bag		5.1 [c]	
Macro-perforated bag		4.6 [ab]	
Polyethylene bag		4.5 [a]	
Paper bag		5.0 [bc]	

Table 85 Effect of irradiation and bagging on titratable acidity of the flesh of 'B74' mango fruit at eating soft (2012/13). Means with the same letter are not significantly different at P=0.05.

Treatment	Titratable acidity (%)	
	No irradiation	Irradiation
-Bag	0.11 [ab]	0.15 [bc]
+Macro-perforated bag,-Wet water crystal	0.14 [abc]	0.16 [c]
+Macro-perforated bag,+Wet water crystal	0.13 [abc]	0.14 [abc]
+Polyethylene bag, -N_2	0.37 [e]	0.11 [abc]
+Polyethylene bag, +N_2	0.30 [d]	0.12 [abc]
+Paper bag	0.11 [a]	0.16 [c]

5.5.4.3. Experiment 3. Bagging and ripeness SEQ; 2013/14

Lenticel discolouration

Irradiation increased LD in non-bagged fruit at 8 d (Figure 45) irrespective of when the fruit were bagged and irradiated. However irradiating 8 d after harvest would allow the fruit to reach the eating soft stage more quickly, when LD would likely be less. Bagging reduced LD at bag removal (8 d bagging) irrespective of irradiation. In the irradiated fruit LD increased rapidly after bag removal but LD was still less after 4 d compared with no bag.

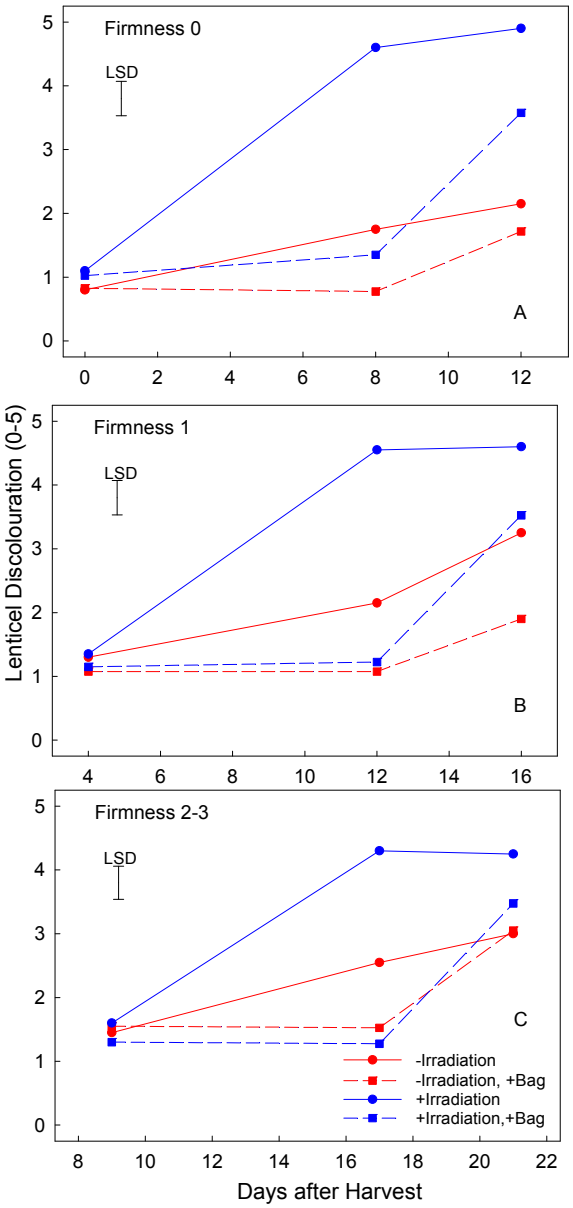


Figure 45 Effect of irradiation, bagging and nitrogen flushing on lenticel discolouration of 'B74' mango fruit during ripening (2013/14). (A): effect of irradiation and bagging on lenticel discolouration of 'B74' mango fruit of firmness 0. (B): effect of irradiation and bagging on skin colour of 'B74' mango fruit of firmness 1 at 4, 12 and 16 days and expressed as mean. (C): effect of irradiation and bagging on skin colour of 'B74' mango fruit of firmness 2-3. Data were collected at 9, 17 and 21 days and expressed as mean. Bars represent LSDs at P=0.05.

Skin Colour

Irradiation and bagging resulted in less yellow colour on the non-blush area of the skin when fruit were irradiated at firmness zero and one (Figure 46). For fruit irradiated at firmness 2-3, there was little effect of irradiation on skin colour, but bagging often reduced yellow skin colour 8 d after treatment (no irradiation).

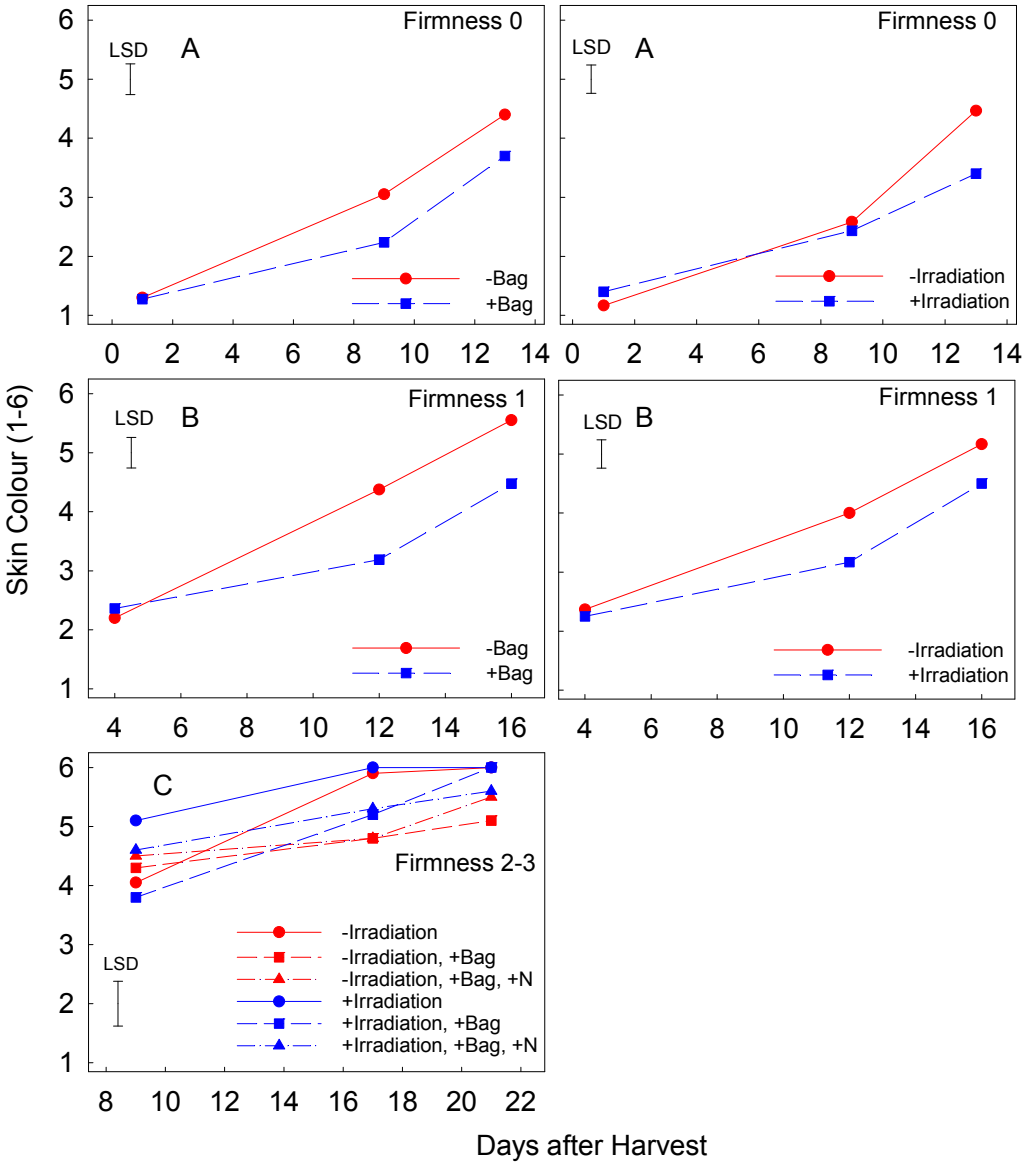


Figure 46 Effect of irradiation, bagging and nitrogen flushing on skin colour of 'B74' mango fruit during ripening (2013/14). (A): effect of irradiation and bagging treatment at firmness 0 and (B) and firmness 1. (C): effect of irradiation, bagging and nitrogen flushing treatment at firmness 2-3. Bars indicate LSDs at P=0.05.

Firmness

When fruit were irradiated at firmness 0, bag fruit softened significantly slower than non-bag fruit (Figure 47). There was no effect of irradiation on these fruit. With firmness 1, bagging resulted in firmer fruit that 8 and 12 d in the absence of irradiation. However, bagging had little effect on firmness loss with irradiation.

A significant interaction of irradiation, bagging and nitrogen flush was found with firmness 2-3. At 12 d, the bagged, non-irradiated fruit were firmer than most of the irradiated treatments. There was no obvious effect of nitrogen flushing.

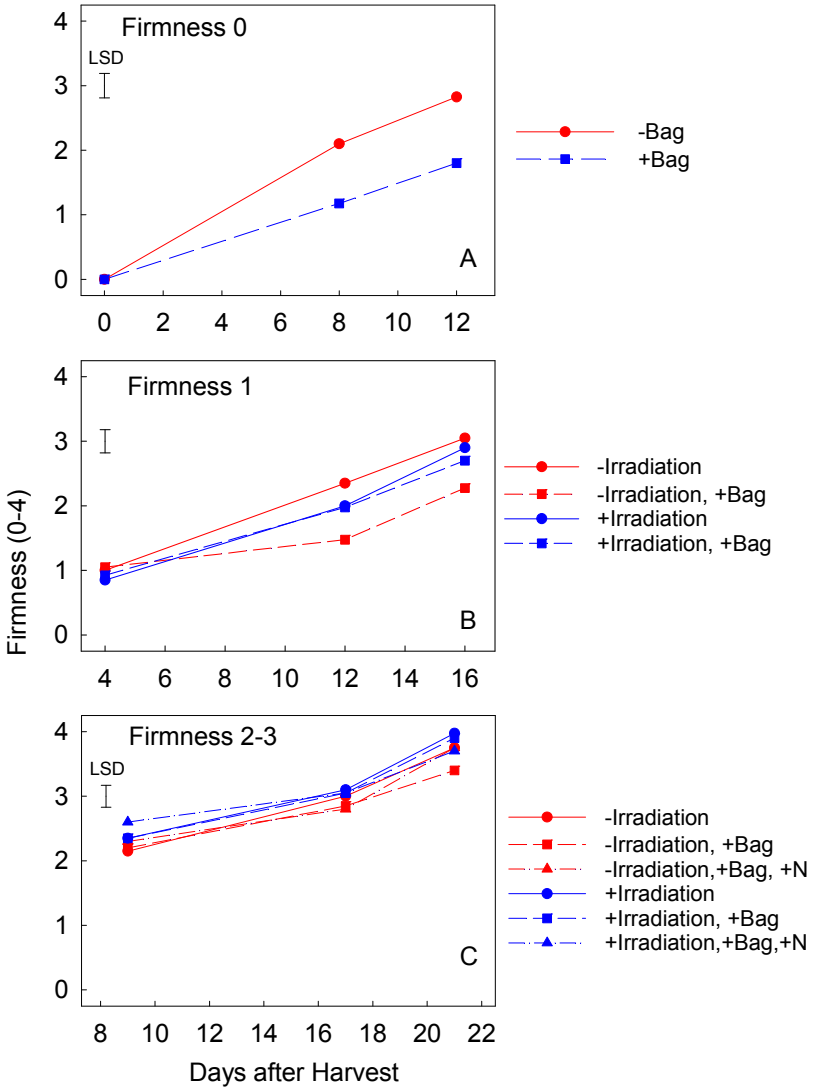


Figure 47 Effect of irradiation, bagging and nitrogen flushing on firmness of 'B74' mango fruit during ripening (2013/14). (A): effect of bag treatment at firmness 0. (B): effect of irradiation and bagging at firmness 1. (C): effect of irradiation, bagging and nitrogen flushing treatment at firmness 2-3. Bars indicate LSDs at P=0.05.

Gas concentrations

Oxygen concentrations at 8 d after bagging was higher in bags with irradiated fruit (11.54%) compared with non-irradiated fruit (6.67%). There was no effect of nitrogen flushing on O2 concentrations.

Carbon dioxide concentrations were higher in the bags of non-irradiated fruit (41.7%) compared with irradiated fruit (23.9%).

Quality at eating soft

Irradiation at firmness 0 (hard green fruit) resulted in very high LD at eating soft (Table 86). However, irradiation of fruit at firmness 2-3 resulted in similar LD to non-irradiated fruit at eating soft. Similar effects were noted with skin colour; irradiation reduced the yellow colour at ripe but irradiating at firmness 2-3 resulted in no reduction.

Bagging of irradiated fruit also reduced LD at eating soft, but it also reduced the yellow skin colour at eating soft (Table 86).

Table 86 Lenticel discolouration (0-5) and yellow skin colour (1-6) on 'B74' mango at the eating soft stage, either with/without irradiation or with/without bagging. The fruit were bagged and/or irradiated at either firmness stage 0, 1 or 2-3. For each quality criterion, means with the same letter in the same main or interaction effects are not significantly different at P=0.05

	Lenticel discolouration	Skin colour
Irradiation x firmness		
firmness 0, -Irradiation	1.9 [ab]	5.5 [c]
firmness 1, -Irradiation	2.1 [b]	5.6 [c]
firmness 2-3, -Irradiation	1.7 [a]	5.2 [c]
firmness 0, +Irradiation	4.0 [c]	3.6 [a]
firmness 1, +Irradiation	3.8 [c]	4.5 [b]
firmness 2-3, +Irradiation	2.0 [ab]	5.5 [c]
Irradiation x Bag		
-bag, -Irradiation	2.1 [a]	
+bag, -Irradiation	1.8 [a]	
-bag, +Irradiation	4.3 [c]	
+bag, +Irradiation	2.8 [b]	
Bag		
-bag		5.4 [a]
+bag		4.5 [b]

5.5.4.4. Experiment 4. Ripeness NT and SEQ; 2013/14

Lenticel discolouration

Lenticel discolouration increased significantly over time with irradiation, but irradiating at firmness 2-3 reduced the increase in LD with further ripening (Figure 48A and B).

Skin Colour

Irradiation of northern Territory fruit had no effect on skin colour during ripening. With south east Queensland fruit. irradiation at firmness 0 and 1 fruit resulted in retarded expression of yellow skin colour from 2- d after irradiation, compared with no irradiation (Figure 49). This effect was not noted when firmness 2-3 fruit were irradiated because of more yellow colour on the skin when irradiated.

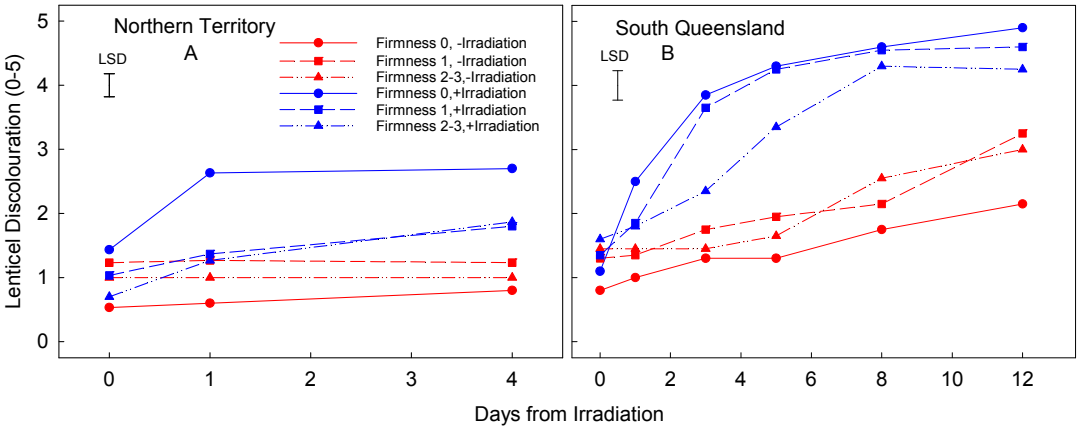


Figure 48 Effect of firmness at irradiation on lenticel discolouration of 'B74' mango fruit during ripening (2013/14). (A): fruit from the Northern Territory and (B) south east Queensland. Bars indicate LSDs at P=0.05.

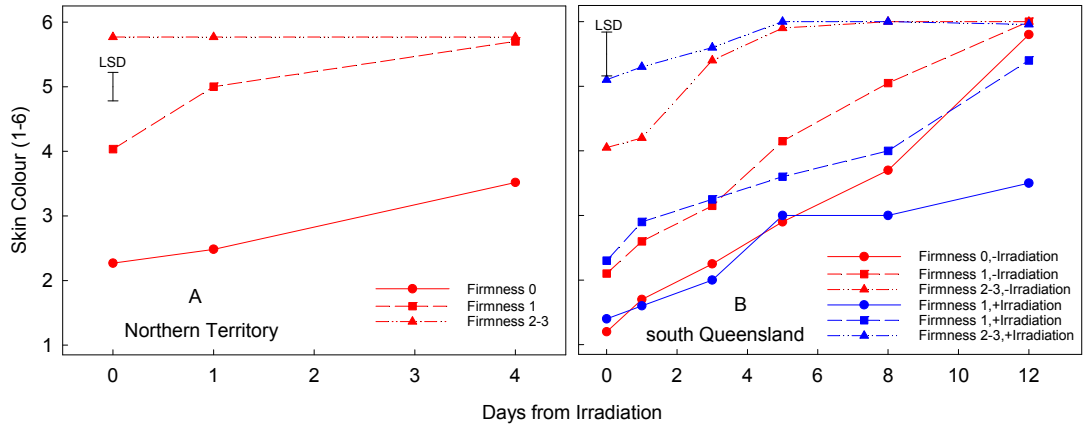


Figure 49 Effect of firmness at irradiation, and irradiation on skin colour of 'B74' mango fruit during ripening (2013/14). (A): fruit from the Northern Territory and (B): south east Queensland. Fruit were irradiated at either firmness 0 (hard green), 1 or 2-3. Bars indicate LSDs at P=0.05.

Firmness

There were no significant irradiation effects on firmness during ripening (data not presented).

Quality at eating soft

Irradiation increased LD, and reduced the yellow skin colour on the ripe fruit from both regions (Table 87). However, irradiating fruit at the 2-3 firmness stage significantly reduced LD compared with irradiating less firm fruit, although the LD at ripe was still greater than the non-irradiated controls. Lenticel discolouration was consistently higher in fruit from south east Queensland, and the results suggest that, even with irradiating firmness 2-3 fruit, the LD severity would be commercially unacceptable.

Table 87 Effect of fruit firmness when irradiated, and irradiation treatment on lenticel discolouration (0-5) and yellow skin colour (1-6) of 'B74' mango fruit at the eating soft stage. The fruit were obtained from commercial farms from the Katherine (Northern Territory) and Childers (south east Queensland) areas. Means in the same column with the same letter are not significantly different at P=0.05.

Treatment	Northern Territory		South east Queensland	
	Lenticel discolouration	Skin colour	Lenticel discolouration	Skin colour
Firmness 0, -Irradiation	0.9 [a]	5.5 [c]	2.2 [a]	5.8 [c]
Firmness 1, -Irradiation	1.2 [a]	5.6 [c]	2.2 [a]	5.9 [c]
Firmness 2-3, -Irradiation	1 [a]	5.2 [c]	1.9 [a]	6 [c]
Firmness 0, +Irradiation	3.2 [c]	3.6 [a]	4.9 [c]	3.5 [a]
Firmness 1, +Irradiation	2.2 [b]	4.5 [b]	4.5 [c]	5.2 [b]
Firmness 2-3, +Irradiation	1.9 [b]	5.5 [c]	3.4 [b]	6 [c]

5.5.4.5. Total Phenols and enzyme activity

Preliminary statistical analysis of samples from experiment 3 indicated that there were no significant treatment effects on total phenols concentration, nor PPO or POD activity. Average PPO activity in the skin was 32.4 units.mg^{-1} protein at 8 d and 42.2 at 12 d. The POD activity was 32.4 units.mg^{-1} protein at 8 d and 49.8 at 12 d.

5.5.5. Conclusions

The results of these trials confirmed the consistent impact of irradiation on LD and skin colour at ripe, as has been observed with other mango cultivars. The discolouration is likely an oxidation reaction requiring oxygen, similar to the browning observed with cut plant surfaces such as apple and avocado. In theory, reducing oxygen concentrations around the lenticel could help reduce the oxidation reaction causing the browning. Humidity around the lenticels may also contribute to LD by causing excessive water loss from the thinner layer of cells around the lenticel opening. The treatments tested aimed to provide a range of gas and humidity effects around the fruit during and after irradiation to identify possible remediation treatments.

In most of the trials polyethylene bagging resulted in delays in skin colour development and increased SB, with little or small positive effects on LD. However the overall effects on ripe fruit quality were negative. With irradiation, LD was generally worse with polyethylene bags, and with nitrogen gassing, presumably because of the added stress of irradiation to the fruit.

Macro-perforated bags can reduce the impact on oxygen and carbon dioxide concentrations around the fruit while still maintaining fairly high relative humidity. However these had relatively little benefit either. Interestingly, adding water saturated crystals increased LD slightly, perhaps indicating that very high relative humidities may not be the solution.

Delaying irradiation until the fruit were close to ripe was the most effective treatment for reducing the negative effects of irradiation on ripe fruit quality. This approach allows the yellow skin colour to develop, and presumably the cells around the lenticel to become less sensitive so that ripe fruit quality is not affected. The 'B74' fruit need to be at least firmness 2 (70% yellow colour) to have the desired effect. Obviously this presents other challenges because of reduced time for distribution to consumers, and especially for export. However, efficient supply chains may still allow airfreight to suitable export markets. A 4-7 d seafreight duration may also be possible because of reasonable storage capacity of ripened 'B74' (Whiley *et al.*, 2006), but very efficient distribution systems will be required in the importing country to minimise time from arrival to consumer.

5.6. Improving yellow skin colour after irradiation

Roberto Marques, Peter Hofman, Jonathan Smith, Barbara Stubbings

5.6.1. Summary

Irradiation can successfully disinfest mango fruit of fruit fly and mango seed weevil and thereby satisfy entry requirements of quarantine authorities in export markets. However, irradiation can also reduce the visual quality of the fruit and delay the loss of green skin colour during ripening. Previous research suggested that treatment with ethylene and/or raising the ripening temperature (e.g. to 24°C) could accelerate the typical change in skin colour more rapidly than flesh softening. Exposure to even higher ripening temperatures may elicit more profound effects, plus also increase flavour especially in combination with a long ethylene exposure. To test this, 'B74' mango fruit from south east Qld were irradiated and, along with non-irradiated controls, treated with 10 $\mu L.L^{-1}$ ethylene for 0-6 d at either 20, 24, or 28°C. Fruit were then assessed for external quality (at 4 d after placing into the ripening temperatures and at full yellow) and internal quality (flesh brix, acidity and flavour) at full yellow.

The results suggest that ripening at 28°C with ethylene may result in irradiated fruit losing green colour more rapidly relative to loss of firmness, but this effect is likely to be small, and was not reflected in the data at full yellow. In addition, ripening at 28°C usually resulted in a more glossy, greasy feel to the ripe fruit (similar to 'Honey Gold' mango) which may cause customer/consumer concern. Four days ethylene and lower ripening temperatures resulted in slightly higher Brix and acidity in the ripe fruit, but this did not translate into a detectable flavour improvement.

5.6.2. Introduction

Australian mango fruit are hosts to several economically significant insect pests such as fruit fly and mango seed weevil. Treatment of fruit with gamma irradiation at doses of 200-400 Gy can successfully disinfest fruit of these pests and thereby satisfy entry requirements of quarantine authorities in the importing countries. However, irradiation can also reduce the visual quality of mango fruit, including the loss of green skin colour during ripening (Boag *et al.*, 1990; McLauchlan *et al.*, 1990). While treatment with ethylene and/or raising ripening temperatures generally accelerates mango ripening, there is some evidence that these treatments may differentially affect the loss of green skin colour relative to flesh softening. For example, our previous research (Whiley *et al.*, 2006) showed that ripening 'B74' mangoes at 15-24°C in the presence of 10 $\mu L.L^{-1}$ ethylene for 0-5 days had little effect on the relative changes of flesh firmness and skin colour. However, there were indications that ripening the fruit at 24°C for 1.5 days with ethylene could accelerate the typical change in skin colour more than flesh softening. Exposure to higher ripening temperatures (e.g. 28°C) may elicit more profound effects, plus also affect flavour and volatile production (Lalel and Singh, 2004) especially in combination with a long ethylene exposure.

To test this, the response of irradiated and non-irradiated 'B74' mango fruit from south east Qld to approx. 10 $\mu L.L^{-1}$ ethylene for 0-6 days at either 20, 24, or 28°C was tested. Fruit were than assessed for external quality (at 4 days after placing into ripening temperatures and at full yellow), as well as internal quality (including flesh Brix, acidity and flavour) at full yellow.

5.6.3. Materials and methods

5.6.3.1. Fruit and treatments

Twenty cardboard P-84 trays (7.5 Kg fruit per tray) of commercially picked and packed 'B74' mango fruit count 18 (total of 360 fruit) were collected at commercial maturity (14% dry

matter) on 6/2/2012 from a farm near Childers (QLD). The fruit were transported to the Maroochy Research Facility (MRF) at Nambour within 4 h and cooled to 13°C overnight. The fruit were removed from 13°C to 20ºC for several hours to allow them to warm with minimal condensation. Half of the fruit were then transported by air conditioned car to Steritech Pty Ltd (a commercial irradiation facility near Brisbane about 60 min from MRF) and treated as secsribed in section 4.4.3.1. Dosimeters readings ranged from 346 to 753 Gy. The dose recorded per tray is displayed in Figure 50. The control treatment received no irradiation.

Top of rack	
Tray 1 482 Gy	Tray 2 753 Gy
Tray 3 346 Gy	Tray 4 496 Gy
Bottom of rack	
Tray 5 595 Gy	Empty
Tray 6 451 Gy	Tray 7 442 Gy

Figure 50 Plan of irradiation racks showing tray numbers and dosimeter readings

Immediately after irradiation, the fruit were transported back to MRF and the following treatments (3 temperature x 4 ethylene times x 2 irradiation doses = total of 24 treatments) were imposed (15 fruit per treatment):

- Ripening temperatures: 20, 24, 28°C
- Ethylene exposure (targeted at 10 $\mu L.L^{-1}$) duration: 0, 2, 4, 6 days

5.6.3.2. Ethylene treatment chambers

The ethylene treatment occurred in a chamber in each of three coldrooms. Each chamber was a 0.9 x 0.9 x .1.2 m aluminium frame with high density polyethylene or aluminium sides (Plate1). Air samples from each of these chambers was in turn circulated through an ICA518 ethylene sensor (International Controlled Atmosphere Ltd Kent, UK) with analogue output, and additional ethylene from a RipeGas cylinder (4% ethylene in CO_2) injected into the relevant chamber through a computer-controlled solenoid system. Carbon dioxide concentrations were measured via a PP Systems infrared gas analyser (measuring range 0-10% CO_2) connected before the ethylene sensor. Relative humidity in the chambers was not controlled but was measured using Vaisala HMP50 humidity probes. Air circulation was achieved with two 15 cm muffle fans inside each chamber, placed over a “chimney” in the centre of the rows of trays. An ethylene concentration of 10 $\mu L.L^{-1}$ was targeted, and average ethylene concentrations over the 6 d of 10.3, 10.3 and 11.9 $\mu L.L^{-1}$ achieved for the 20, 24 and 28ºC treatments, respectively.

Plate 26 Ethylene treatment chamber used in the trial.

5.6.3.3. Fruit quality assessments

Individual fruit were visually assessed for skin colour and the severity of lenticel discolouration (LD) and skin browning (SB), and for fruit firmness using hand pressure (section 4.1.3.3). Assessments were based on the rating systems in the "'B74' Quality Assessment Manual" (Hofman *et al.*, 2010a) at the following times:

- On arrival at laboratory after irradiation: for LD;
- Four days after placing into ripening temperatures: for skin colour and fruit firmness
- At the full yellow (ripe) stage: for fruit firmness, and the severity of LD and skin browning.

When each fruit reached full yellow, both cheeks were removed and the flesh diced and mixed, then frozen. Within two months, a portion of each cheek was thawed and measured for Brix (using an Atago bench refractometer) and titratable acidity (using a Metrohm Titrino autotitrater, with the results expressed as % citric acid).

The remaining sample per fruit was pooled with another four fruit that ripened on the same day. This was repeated with the next five, then the next five fruit to give three composite replications of five fruit each. Flavour was then assessed using a tasting panel of about 12 staff at MRF, based on a hedonic rating scale (1=dislike extremely and 9=like extremely); a rating of 5.5 was considered to indicate an acceptable eating quality. Half of the treatments (the ones at 0 and 4 d ethylene exposure) were selected (total of 12 treatments) to avoid an excessive number of samples given to the tasters. Two tastings per day were conducted over 3 d, with six samples per tasting.

5.6.3.4. Statistical analysis

Statistical analyses were performed by Genstat® 11 for Windows™ (VSN International Ltd., UK). Analysis of variance used the 'General Analysis of Variance' model, with a factorial design (ripening temperature by ethylene exposure time by irradiation) as 'treatments' structure, no 'block' structure, and 15 single fruit replications per treatment. Flavour was analysed with three replications (5 fruit per replication based on days to full yellow). The protected least significant difference (LSD) procedure at P = 0.05 was used to test for differences between treatment means.

5.6.4. Results and discussion

5.6.4.1. Skin colour and firmness four days after placing into ripening temperatures

At removal from the ripening chambers after 4 d, both non-irradiated and irradiated fruit ripened at 24 or 28°C with ethylene for 2 or 4 d had generally lost more green colour and were softer than fruit ripened at 20°C or without ethylene (Table 88). Within each batch of fruit, non-irradiated and irradiated, there was little difference in both skin colour and firmness between 2 and 4 d ethylene exposure regardless of the ripening temperature. Non-irradiated fruit had generally lost more green colour than irradiated fruit when ripened at either 20 or 24°C, but there were little difference at 28°C. Non-irradiated fruit were softer than irradiated fruit when ripened without ethylene at 20 or 24°C, or when ripened with ethylene at 20°C.

In more general terms, irradiation with no ethylene retarded loss of green colour at 4 d irrespective of ripening temperature. Irradiation had a similar effect on firmness with 20 and 24°C, but this effect was negated at 28°C, possibly because ripening had progressed more at this higher temperature, allowing the irradiation effects on firmness to be overcome.

Ethylene treatment for 2-4 d did not enhance the loss of green colour on the radiating fruit compared to no irradiation, irrespective of ripening temperature (except at 28°C and 2 d). In addition, ethylene and 24-28°C resulted in similar firmness after 4 d irrespective of radiation, which suggests that these treatments resulted in less green colour on fruit of similar firmness, thereby enhancing the negative effects of radiation on skin colour loss. The only exception was 28°C with 2 d ethylene, where similar skin colour and firmness were observed irrespective of irradiation treatment.

Table 88 Skin colour (1-6) and firmness (0-4) of 'B74' mango fruit four days after the start of the ripening treatments as affected by ripening temperature, ethylene exposure and irradiation. The 6 d ethylene treatment was not included.

Ripening temperature	Days with ethylene					
	0		2		4	
	Not-irrad.	Irradiated	Not-irrad.	Irradiated	Not-irrad.	Irradiated
Skin colour (1-6)						
20°C	4.5 ^{cde}	3.5 ^{g}	5.0 ^{bc}	3.7 ^{fg}	5.0 ^{bc}	3.7 ^{fg}
24°C	4.6 ^{cde}	2.5 ^{h}	5.9 ^{a}	4.4 ^{cde}	5.7 ^{a}	4.3 ^{def}
28°C	5.0 ^{bcd}	4.2 ^{efg}	4.8 ^{cde}	4.6 ^{cde}	5.6 ^{ab}	5.0 ^{bcd}
Firmness (0-4)						
20°C	2.4 ^{cd}	1.7 ^{e}	2.3 ^{d}	1.8 ^{e}	2.3 ^{d}	1.9 ^{e}
24°C	2.3 ^{cd}	1.7 ^{e}	2.5 ^{bcd}	2.5 ^{bcd}	2.6 ^{abcd}	2.5 ^{abcd}
28°C	2.6 ^{abcd}	2.5 ^{bcd}	2.8 ^{ab}	2.9 ^{a}	2.6 ^{abc}	2.8 ^{ab}

Means (n=15) for either skin colour or firmness with the same letters are not significantly different (P=0.05) as tested by LSD.

5.6.4.2. External quality at full yellow colour

For days to full yellow, there were no significant interactions between ripening temperature, days with ethylene and irradiation (data not shown), but the interactions between ripening temperature and days with ethylene (Table 89) and between ripening temperature and irradiation (Table 90) were significant.

Generally, fruit ripened at 24-28°C with 4-6 d ethylene reached the full yellow stage about 1.5 to 3 days quicker than fruit ripened at 20°C with 0-2 days of ethylene (Table 89). The general trend suggests an additive effect of both factors (temperature and ethylene) on ripening time, but results were not always consistent.

Table 89 Ripening time (days to full yellow) of 'B74' mango fruit as affected by ripening temperature and ethylene exposure.

Ripening temperature	Days with ethylene			
	0	2	4	6
20°C	9.6 [ab]	9.4 [b]	9.1 [bc]	10.7 [a]
24°C	8.9 [bc]	7.3 [de]	8.1 [cd]	7.4 [de]
28°C	8.7 [bc]	9.5 [b]	8.0 [cd]	6.6 [e]

Means (n=15) with the same letters are not significantly different (P=0.05) as tested by LSD

Both non-irradiated and irradiated fruit reached full yellow more quickly at higher ripening temperatures (Table 90), suggesting that higher ripening temperatures or ethylene reverse the irradiation effects on days to full colour.

Table 90 Ripening time (days to full yellow) of 'B74' mango fruit as affected by ripening temperature and irradiation at about 550 Gy.

Temperature	Not irradiated	Irradiated
20°C	8.8 [b]	10.6 [a]
24°C	6.8 [d]	9.0 [b]
28°C	7.9 [c]	8.5 [bc]

Means (n=60) with the same letters are not significantly different (P=0.05) as tested by LSD

There was no main effect of temperature on firmness at full yellow (Table 91), suggesting that higher temperatures accelerate skin colour and softening changes at approximately similar rates. However, more than 2 d ethylene treatment resulted in firmer fruit at full colour, while irradiation had the opposite effect.

Table 91 Firmness (0-4) of Calypso mango fruit at the full yellow (ripe) stage as affected by ripening temperature, ethylene exposure and irradiation.

Treatment	firmness (0-4)
Ethylene duration (days)	
0	3.7 [a]
4	3.7 [a]
2	3.7 [a]
6	3.4 [b]
Irradiation	
No	3.3 [b]
Yes	3.7 [a]

Means (n=15) with the same letters are not significantly different (P=0.05) as tested by LSD

As expected, non-irradiated fruit had less lenticel damage and skin browning (average ratings of 2.3 and 1.0, respectively on a 0-5 scale) than non-irradiated fruit (ratings of 3.9 and 2.8 respectively) at full yellow (data now shown). There was little effect of ripening temperature or ethylene treatment on lenticel damage and skin browning (data not shown).

Interestingly, fruit ripened 24°C, and especially at 28°C developed a more glossy, greasy feel when ripe (not rated) (Plate 27). This appearance and feel was similar to typical, ripe 'Honey Gold' mango, but is considerably different to commercially ripened fruit, and may result in customer/consumer concern.

		
Ripened at 20°C	Ripened at 24°C	Ripened at 28°C
Plate 27 Appearance of non-irradiated 'B74' fruit after six days ethylene treatment and ripening at 20°C, 24°C and 28°C. Fruit ripened at 24°C and 28°C developed a more glossy appearance, and a more greasy feel, than fruit ripened at 20°C.		

5.6.4.3. Internal quality at full yellow colour

The factorial analysis indicated no interaction between ripening temperature, ethylene exposure and irradiation; hence the results were averaged across these treatment effects when justified by the analysis.

Neither irradiation nor ripening temperature had a significant effect on Brix in the flesh at full yellow (data not presented). Ethylene had a statistically significant but small effect by increasing Brix from 12.5 (no ethylene) to 12.9° with 4 d treatment (Table 92). Irradiation also had no significant effect on fresh acidity at full yellow. However, increasing ripening temperatures reduced acidity and exposure to 4 d ethylene increased acidity compared to no ethylene.

During ripening, the fruit chlorophyll (green colour) concentration in the skin decreases to expose the background yellow colour, the total soluble solids (mainly sugars, and measured by ºBrix) increases, and acidity decreases. However the relative rates of these changes can be affected by postharvest treatments and ripening conditions, resulting in differing fruit quality when ripe (Johnson and Hofman, 2009). The present results suggest that ethylene enhances Brix production more than the loss of green skin colour, but enhances loss of green colour more than loss of acidity during ripening. Likewise, higher ripening temperatures enhanced the rate of acidity loss more than the rate of green colour loss, resulting in fruit with less acidity at full yellow.

Based on the above, fruit treated with ethylene and ripened at 20°C would be expected to have improved flavour compared with no ethylene and higher ripening temperatures, however neither ripening temperatures nor ethylene affected flavour at full yellow. The only significant treatment effect on flavour was irradiation resulting in a significant but slight reduction in flavour at full yellow (Table 92).

'B74' mango fruit generally lose all fruit skin colour (reached at full yellow stage) about 2-3 d before fruit reach eating soft. Irradiation for disinfestation consistently retards the loss of green colour, so that the fruit remained in a less attractive green/yellow stage for longer and generally lose all green colour just before eating soft. This trial investigated whether higher ripening temperatures and longer ethylene durations could enhance the loss of green colour more quickly than softening so that the fruit reach full yellow well before eating soft stage. The results obtained provided no evidence that these treatments have potential to achieve this. Ethylene and higher temperatures increased the rate of green colour loss four days after the start of treatment, but generally had no similar effects on firmness. Firmness data at full yellow, suggested no consistent or commercially significant treatment effects.

Table 92 Brix (º), acidity (%) and flavour (1-9) of 'B74' mango fruit at the full yellow (ripe) stage as affected by ripening temperature, ethylene exposure and irradiation. The statistical analysis indicated no interaction between ethylene duration, ripening temperature and irradiation so the main treatment effects are presented.

Treatment	Brix (º)	Acidity (%)	Flavour (1-9)
Ethylene duration			
0 d	12.5 [b]	0.16 [b]	
4 d	12.9 [a]	0.21 [a]	
Ripening temperature (ºC)			
20		0.24 [a]	
24		0.17 [b]	
28		0.14 [c]	
Irradiation			
No			6.0 [a]
Yes			5.7 [b]

Means for either brix, acidity (n=15) or flavour (n=3) with the same letters are not significantly different (P=0.05) as tested by LSD

It was also hypothesised that higher ripening temperatures may reduce volatiles production based on evidence with 'Kensington Pride' (Lalel *et al.*, 2004). There was some evidence of temperature and ethylene effects on Brix and acidity in this study, but this did not translate into significant flavour effects.

As expected, higher ripening temperatures and ethylene treatment resulted in more rapid loss of green colour when removed from ethylene and a reduction in days between harvest and full yellow skin colour.

In summary, the results suggest that ripening at 28°C with ethylene may result in irradiated fruit losing green colour more rapidly relative to loss of firmness, but this effect is likely to be small, and was not reflected in the data at full yellow. In addition, ripening at 28°C usually resulted in a more glossy, greasy feel to the ripe fruit (similar to 'Honey Gold' mango) which may cause customer/consumer concern. Four days ethylene and lower ripening temperatures resulted in slightly higher Brix and acidity in the ripe fruit, but this did not translate into a detectable flavour improvement.

5.7. Irradiating ripening fruit at several doses

Roberto Marques, Peter Hofman, Jonathan Smith

5.7.1. Summary

Disinfestation treatments are required for marketing in several export markets such as New Zealand and the USA. Irradiation is a potentially useful disinfestation treatment against fruit fly and seed weevil in mango and can be used in these markets. Previous studies showed that delaying irradiation treatment until 'B74' fruit have partially ripened results in less lenticel discolouration (LD) than fruit irradiated at green mature. This strategy may be a commercially viable option for minimising LD where fruit can be sea-freighted short distances to selected markets, or air-freighted.

This trial aimed at confirming if fruit sensitivity to irradiation-induced LD and effects on green colour loss during ripening decreases when treating ripening fruit, and at characterising the fruit responses to irradiation doses required for different markets. 'B74' fruit were sampled from three commercial farms in the Northern Territory and ripened without irradiation, or with irradiation at 150, 300, 500, or 800 Gy after the fruit had been held for 3 or 8 d at 20ºC. To ensure dose accuracy, fruit were irradiated at the facilities of the Australian Nuclear Science and Technology Organisation in Sydney.

Regardless of the dose, irradiating partially ripened fruit (8 d after harvest; DAH) reduced LD by 36-47% compared to irradiating mature green fruit (at 3 DAH). As a result, the % of acceptable fruit (those with a severity rating of less than 3 on a 0-5 scale) irradiated 8 DAH was close to 100% and not different from non-irradiated fruit, but reduced to 50-60% in fruit irradiated 3 DAH at doses of 300-800 Gy. Irradiating partially ripened fruit also minimised any retardation in the loss of green colour during fruit ripening. The results suggest that fruit sensitivity to irradiation-induced LD decreases as fruit ripen. They also suggest that delaying irradiation treatment until fruit have partially ripened may be a commercially viable option to minimise loss of external quality due to LD, as long as fruit can be marketed quickly enough to compensate for the reduction in fruit shelf life.

5.7.2. Introduction

Disinfestation treatments for fruit fly and seed weevil are required for marketing mangoes in some export markets such as New Zealand and the USA. With increasing restrictions on chemical disinfestation treatments due to the risk of residues, irradiation is a potentially useful disinfestation alternative for mangoes, and the preferred method in markets like New Zealand and the United States (Johnson and Hofman, 2009; Bustos-Griffin *et al.*, 2012).

In previous trials (Hofman *et al.*, 2010b) irradiation significantly reduced the visual quality of commercially picked and packed 'B74' fruit, mainly due to lenticel discolouration (LD) and skin browning (SB). Similar responses have been reported in 'Kensington Pride' mango (Johnson *et al.*, 1990; McLauchlan *et al.*, 1990). Earlier studies done in one season also showed that delaying irradiation treatment until 'B74' fruit have partially ripened can result in fruit with less severe LD than fruit irradiated at green mature (Hofman *et al.*, 2010b). This strategy may be a commercially viable option for minimising LD where fruit can be quickly freighted to some markets. As the irradiation treatment in that study was done in one season only, and at a commercial irradiation facility with some variation in dose, it was warranted to confirm if fruit sensitivity to irradiation-induced LD decreases as fruit ripens, and to characterise the fruit responses to more accurate irradiation doses required for different markets.

5.7.3. Materials and methods

5.7.3.1. Fruit sampling and handling

Commercially picked and packed 'B74' fruit were sampled on 19/11/2013 (1 d after harvest) from each of three commercial farms in the Northern Territory (NT), in the Darwin, Katherine and Mataranka areas (10 P-84 trays count 18 per farm, total of 30 trays). One day after collection, a batch of 12 trays (four trays per farm) were air-freighted from Darwin to Sydney for irradiation treatment the next morning (3 d after harvest; DAH) at four doses (one tray per farm per dose), as detailed in the following section. The target ripeness was a colour stage 1-2 or about 10% yellow. Fruit were airfreighted from Sydney to the Sunshine Coast (SC) immediately after irradiation treatment then transported by car to the postharvest laboratory at the Maroochy Research Facility (MRF), Nambour. The remaining 18 trays of fruit were air-freighted from Darwin to Brisbane, then transported by car to MRF.

Upon arrival at MRF, one tray per farm were set aside and flesh % dry matter determined on 12 fruit per farm using the % dry matter maturity test as described in the ''B74' Best Practice Guide'' (Hofman and Whiley, 2010). Three trays (one tray per farm) were kept at MRF as controls. All fruit were assessed for external quality (as described below) and ripened at 20ºC. In the first 2 d of ripening, fruit were exposed to 10 $\mu L.L^{-1}$ of ethylene.

Three days after ethylene treatment (8 DAH), a second batch of 12 trays (four trays per farm) were air-freighted to Sydney for irradiation treatment (in similar conditions as the first batch). The target was skin colour stage 3-4 or about 50% yellow. However, the fruit reached this stage more quickly than anticipated and there was no capacity at the irradiation facility to provide an earlier irradiation date. Hence, the fruit were at stage 4-5 (about 70% yellow) when irradiated. Fruit were air freighted from Sydney to the SC immediately after irradiation treatment. The next day, all fruit were again rated for external quality as described below.

5.7.3.2. Irradiation treatment

To ensure dose accuracy, fruit were irradiated at the Gamma Technology Research Irradiator (GATRI) at the Australian Nuclear Science and Technology Organisation (ANSTO) facility at Lucas Heights, Sydney. Gamma radiation from a cobalt-60 source was used at target doses of 150, 300, 500 and 800 Gy. Fricke dosimeters were placed throughout the array at the expected minimum and maximum dose zones as detailed in section 4.3.3.4.

5.7.3.3. Fruit quality assessment

Fruit were individually assessed for skin colour, fruit firmness and the severity of LD (section 4.1.3.3) based on the rating systems in the "'B74' Quality Assessment Manual" (Hofman *et al.*, 2010a). Assessment times were:

- 4 DAH: 1 d after 1st irradiation treatment
- 9 DAH: 1 d after 2nd irradiation treatment
- 11 DAH: when non-irradiated fruit reached the full yellow (ripe) stage
- 15 DAH: when most fruit reached the full yellow (ripe) stage.

Fruit firmness was assessed at 4, 11, and 15 DAH. Skin browning was assessed at 15 DAH.

The days between harvest and full yellow (days to full yellow) were determined when at least 80% of the fruit in each tray (15 fruit) reached the full yellow (ripe) stage (rating of 5.5-6.0, or 90-100% yellow on the fruit skin surface).

The proportion of acceptable fruit within each treatment and farm was calculated as the % of fruit with a LD severity rating lower than 3.0 in relation to the total number of fruit per treatment and farm.

5.7.3.4. Statistical analysis

Statistical analyses were performed by Genstat® 16 for Windows™ (VSN International Ltd., UK). A completely randomised design was used with nine treatments and three trays (replicates; one tray per farm) each of 18 fruit per treatment (54 fruit per treatment; total of 486 fruit). The 'General Analysis of Variance' model was used to analyse the data, with each treatment as the 'treatment' factor and farm by tray as the block factor. The least significant difference (LSD) procedure at P = 0.05 was used to test for differences between treatment means.

5.7.4. Results and discussion

5.7.4.1. Irradiation dosimetry results

The doses absorbed by the fruit complied with required specifications in both irradiation dates (Table 93). The dose rate was approx. 7.7 Gy.min^{-1}.

Table 93 Irradiation doses received by 'B74' mango fruit from three farms in the Northern Territory treated at 3 and 8 d after harvest.

Irradiation date	Target dose (Gy)	Minimum dose (Gy)	Maximum dose (Gy)	Average dose (Gy)
21/11/13 (3 d after harvest)	150	143 ± 4	155 ± 4	149 ± 3
	300	282 ± 7	307 ± 8	295 ± 6
	500	465 ± 8	505 ± 10	485 ± 7
	800	747 ± 11	813 ± 13	780 ± 9
26/11/13 (8 d after harvest)	150	141 ± 4	153 ± 4	147 ± 3
	300	281 ± 7	306 ± 8	294 ± 6
	500	477 ± 9	519 ± 10	498 ± 7
	800	758 ± 11	825 ± 13	792 ± 9

5.7.4.2. Lenticel discolouration

During ripening

The severity of LD from 4 DAH onwards was significantly affected by the time between harvest and irradiation (i.e. the stage of ripeness of the fruit when irradiated) and the irradiation dose (Figure 51; Plate 28). Generally, delaying irradiation by 5 d until fruit was partially ripened (about 50% yellow skin colour) reduced LD regardless of the irradiation dose. In fruit irradiated 8 DAH, there was little difference in LD severity between doses, as well as little difference between the lower doses (150-300 Gy) and non-irradiated fruit.

In fruit irradiated at 3 DAH, LD was significantly higher within 1 d of radiation. It was less severe at 150 Gy compared with the other doses, with little difference between 300, 500 and 800 Gy. However, even at the lowest dose of 150 Gy, LD was more severe than non-irradiated fruit, especially at 15 DAH. A separate analysis of the irradiation treatments (non-irradiated fruit excluded) showed that the interaction between time of irradiation and irradiation dose for LD was not significant at any assessment time (data not shown). Overall, the effect of the time of irradiation after harvest appeared stronger than that of the irradiation dose.

At full yellow (ripe)

When each treatment and replication reached the full yellow stage, fruit irradiated 3 DAH, even at 150 Gy, had more severe LD than non-irradiated fruit (Figure 51). Lenticel discolouration increased to 300 Gy, with little increase with higher doses.

In contrast, fruit irradiated 8 DAH with 150-300 Gy had similar LD to non-irradiated fruit (Figure 51), and 500-800 Gy was required to significantly increase LD above non-irradiated fruit. At all doses, fruit irradiated 8 DAH had considerably less LD than those irradiated at 3 DAH.

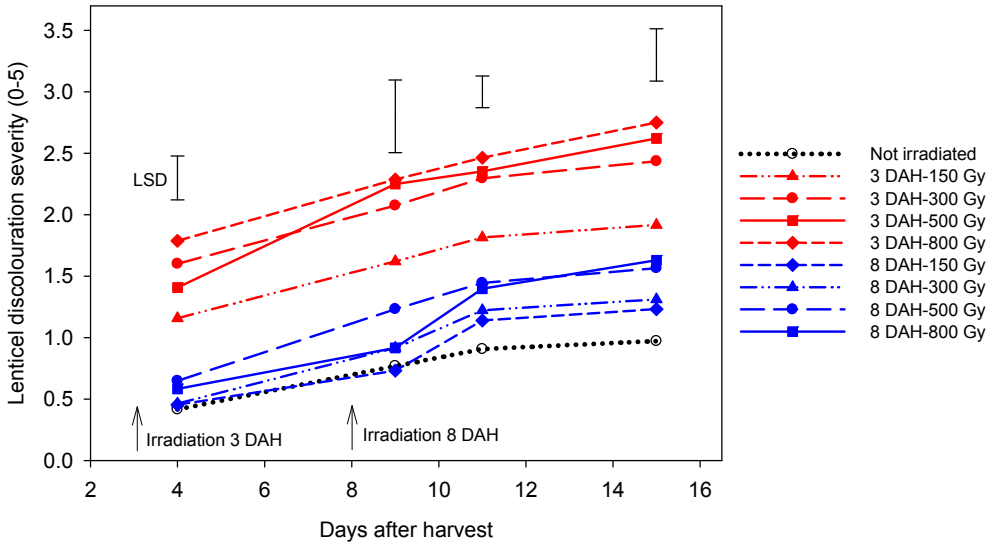


Figure 51.Lenticel discolouration severity in 'B74' mango fruit during ripening at 20°C as affected by the time between harvest and irradiation, and the irradiation dose. Fruit were not irradiated or irradiated either 3 or 8 days after harvest (DAH) at 150, 300, 500, or 800 Gy. Ratings are based on visual assessment of the skin surface area affected (0=nil, 5=more than 50%). LSD bars indicate the least significant difference at P=0.05 for each assessment time. Arrows indicate irradiation treatment times. Each data point on the graph is the mean of 54 fruit from three commercial farms (18 fruit per farm) in the Northern Territory.

These results confirm previous work with fruit from south-east Queensland, in which longer delays between harvest and irradiation resulted in less severe LD after irradiation and during fruit ripening (Hofman *et al.*, 2010b). This would suggest that fruit sensitivity to irradiation-induced LD decreases as fruit ripen. The reasons for that are not clear. 'Tommy Atkins' mango is reportedly more tolerant to other skin disorders such as chilling injury (CI) as they ripen, presumably because CI changes may be inhibited in the riper fruit (Mohammed and Brecht, 2002). Similar processes could be involved in the resistance of 'B74' mango to irradiation damage of lenticels, but that would require further investigation.

Similar LD responses to irradiation has been observed with 'Kensington Pride', where fruit treated with 300-600 Gy had more severe LD than those irradiated at 75 Gy (McLauchlan *et al.*, 1990). However, the impact of irradiation dose in the current study was generally not as strong as observed in previous work with 'B74' mango (Hofman *et al.*, 2010b), although the irradiation doses across treatments in the previous trials were not as targeted as in the current work.

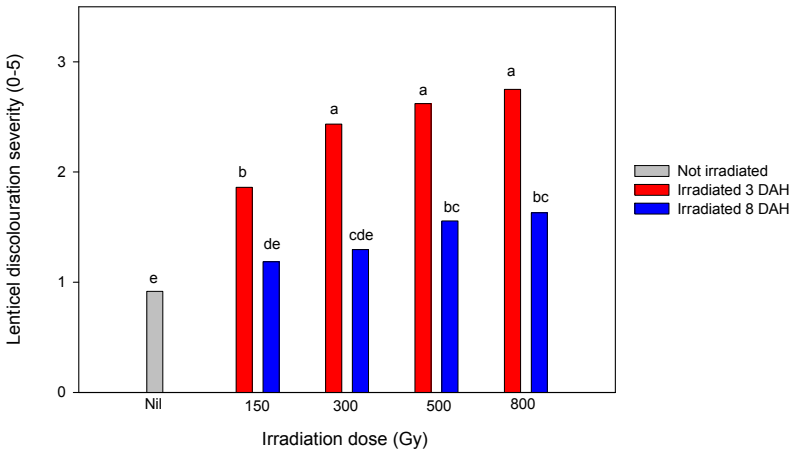


Figure 52. At full yellow: Lenticel discolouration severity in 'B74' mango as affected by the time between harvest and irradiation treatment and the irradiation dose. Fruit were not irradiated or irradiated either 3 or 8 days after harvest (DAH) at 150, 300, 500, or 800 Gy. Ratings are based on visual assessment of the skin surface area affected (0=nil, 5=more than 50%). Bars with the same letter are not significantly different at P=0.05 as tested by LSD. Each bar is the mean of 54 fruit from three commercial farms (18 fruit per farm) in the Northern Territory.

5.7.4.3. Fruit acceptability

The proportion of acceptable fruit (those with a LD severity rating lower than 3.0 in relation to the total number of fruit per treatment) at the full yellow (ripe) stage declined to about 45-60% in fruit irradiated 3 DAH at 300-800 Gy (Figure 53). In contrast, there was no significant reduction in the % acceptability in fruit not irradiated, or irradiated 8 DAH (at any dose), or fruit irradiated 3 DAH at 150 Gy. This again confirms the commercial potential of irradiating partly ripe fruit to minimise the irradiation effect on LD.

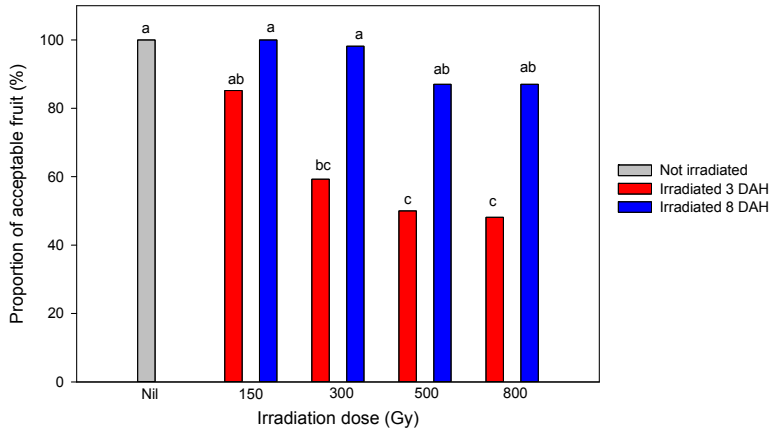


Figure 53 At full yellow: The proportion (%) of acceptable 'B74' mango fruit (those with a severity rating lower than 3 for lenticel discolouration in relation to the total number of 54 fruit per treatment) as affected by the time between harvest and irradiation treatment and the irradiation dose. Fruit were not irradiated or irradiated either 3 or 8 days after harvest (DAH) at 150, 300, 500, or 800 Gy. Ratings are based on visual assessment of the skin surface area affected (0=nil, 5=more than 50%). Bars with the same letter are not significantly different at P=0.05 as tested by LSD.

5.7.4.4. Ripening time, firmness and skin colour development

Both the days from harvest to full yellow and fruit firmness at full yellow were significantly affected by the time between harvest and irradiation (Table 94). 'B74' fruit irradiated 3 DAH took about 3 d longer to reach the full yellow (ripe) stage and were softer at full yellow compared to non-irradiated fruit. In contrast, there was no difference in days to full yellow or firmness between fruit irradiated 8 DAH and non-irradiated fruit. There was not effect of irradiation dose on either days to full yellow or firmness at full yellow.

Within each irradiation dose, fruit irradiated 3 DAH were softer at full yellow than those irradiated 8 DAH (Table 94), most likely because the 3 DAH fruit required more days to reach the full yellow stage.

Table 94 The days from harvest to full yellow and fruit firmness (0-4) of 'B74' mango fruit at full yellow as affected by the time between harvest and irradiation treatment and the irradiation dose. Fruit were not irradiated or irradiated either 3 or 8 days after harvest (DAH) at 150, 300, 500, or 800 Gy, and then ripened at 20°C.

Irradiation dose (Gy)	Days to full yellow			Fruit firmness at full yellow (0-4)		
	No irradiation	Irradiation at 3 DAH	Irradiation at 8 DAH	No irradiation	Irradiation at 3 DAH	Irradiation at 8 DAH
0	12.3 [b]			2.5 [d]		
150		13.7 [ab]	12.3 [b]		2.9 [ab]	2.4 [d]
300		15.0 [a]	12.3 [b]		3.1 [a]	2.6 [cd]
500		15.3 [a]	12.3 [b]		3.0 [ab]	2.6 [cd]
800		15.7 [a]	13.7 [ab]		2.9 [abc]	2.7 [bcd]

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD.
Skin colour ratings are based on visual assessment of the background skin colour (non-red area; 1=0-10% yellow; 6=90-100% yellow).
Fruit firmness determined using hand pressure and a 0-4 scale (0=Hard, 4=soft).

The differences between treatments for fruit firmness during ripening were not significant when assessed at 4, 11 and 15 DAH (data not shown). The mean firmness (on a 0-4 scale) across all treatments was 1.1, 2.5, and 2.9 for assessments at 4, 11, and 15 DAH respectively. It is likely that fruit irradiated 3 DAH were softer at full yellow due to the longer time to reach that stage compared to non-irradiation, as observed in previous work with 'B74' fruit (Hofman *et al.*, 2010b). In contrast, little effect on fruit firmness was reported in irradiated 'Kensington Pride' mango (Boag *et al.*, 1990).

The time between harvest and irradiation also affected skin colour during fruit ripening at 20°C. The loss of green skin colour in fruit irradiated 3 DAH was delayed at 9 DAH (at doses of 300-800 Gy) and 11 DAH (at doses of 500-800 Gy) compared to non-irradiated fruit and fruit irradiated 8 DAH (Figure 54). This effect was obvious at 150 Gy, and greater at higher doses.

However, there was no irradiation effect on % yellow colour when fruit were irradiated at 8 DAH (Figure 54), likely because most fruit had reached a colour stage of at least 5.0 (70% yellow) by the time the fruit were irradiated. Ongoing loss of green colour to the full yellow stage was not affected by the irradiation treatment.

These findings also confirm previous studies with 'B74' (Hofman *et al.*, 2010b) and with 'Kensington Pride' (Boag *et al.*, 1990); (McLauchlan *et al.*, 1990), that irradiation slows down the loss of green skin colour during ripening.

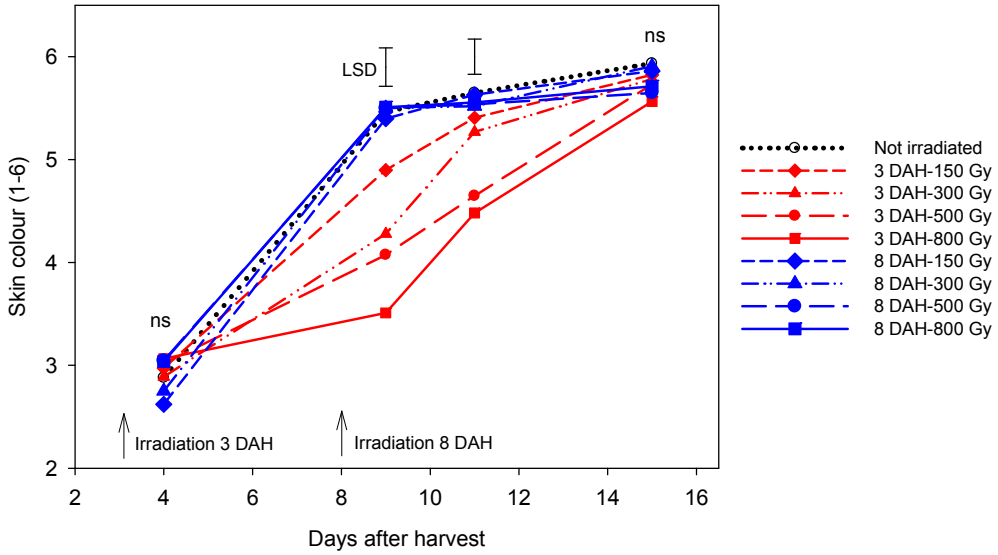


Figure 54. Skin colour development in 'B74' mango fruit during ripening at 20°C as affected by the time between harvest and irradiation, and the irradiation dose. Fruit were not irradiated or irradiated either 3 or 8 days after harvest (DAH) at 150, 300, 500, or 800 Gy. Ratings are based on visual assessment of the background skin colour (non-red area; 1=0-10% yellow; 6=90-100% yellow) done at 4, 9, 11, and 15 DAH. LSD bars indicate the least significant difference at P=0.05 for each assessment time. ns=not significant. Arrows indicate irradiation treatment times. Each data point in the graph is the mean of 54 fruit from three commercial farms (18 fruit per farm) in the Northern Territory.

5.7.4.5. Production location effects

There was significant variation in fruit % dry matter at harvest, days to full yellow, firmness, LD severity and the proportion of acceptable fruit at full yellow across the three farms (Table 95). Generally, fruit from the Darwin farm had lower DM at harvest, took longer to reach the full yellow stage, and had more LD at full yellow than the other two locations. In contrast, fruit from the Mataranka farm had the lowest LD severity and the highest % of acceptable fruit. Farm effects on irradiation-induced LD have also been observed in previous studies with 'B74' mango (Hofman *et al.*, 2010b).

Overall, regardless of the dose up to 800 Gy, irradiating partially ripened (average 70% yellow) 'B74' mango fruit resulted in ripe fruit with significantly less LD than irradiating mature green fruit. Irradiating partially ripened fruit also eliminated any negative effects on the development of yellow skin colour during ripening. These results suggest that fruit sensitivity to irradiation-induced LD decreases as fruit ripen, and waiting until fruit have partially ripened before irradiation may be a suitable option to reduce loss of external quality. However, the shelf life of the fruit will also be reduced, so this strategy may be a commercially viable option only where fruit can be quickly freighted to the consumer.

Table 95 Fruit dry matter (%), days to full yellow, firmness (0-4) and lenticel discolouration severity (0-5) in 'B74' mango fruit, and the proportion (%) of acceptable fruit, as affected by production location. Fruit were not irradiated or irradiated either 3 or 8 days after harvest at 150, 300, 500, or 800 Gy.

Location	Dry matter at harvest (%)	Days to full yellow	At full yellow		
			Firmness (0-4)	Lenticel discolouration severity (0-5)	Proportion of acceptable fruit (%)
Darwin	14.8 [c]	15.1 [a]	2.8 [b]	2.3 [a]	66 [b]
Katherine	18.6 [b]	12.6 [b]	3.0 [a]	1.8 [b]	78 [ab]
Mataranka	16.6 [a]	13.2 [b]	2.4 [c]	1.3 [c]	94 [a]

Means (n=162) in columns with the same letters are not significantly different at P=0.05 as tested by LSD.
Skin colour ratings are based on visual assessment of the background skin colour (non-red area; 1=0-10% yellow; 6=90-100% yellow).
Fruit firmness determined using hand pressure and a 0-4 scale (0=Hard, 4=soft).
Lenticel discolouration ratings are based on visual assessment of the skin surface area affected (0=nil, 5=more than 50%).
The proportion of acceptable fruit was calculated as the % of fruit with a severity rating lower than 3.0 for LD in relation to the total number of fruit per treatment.
Values for each location are the means of nine treatments (with one tray of 18 fruit per treatment).

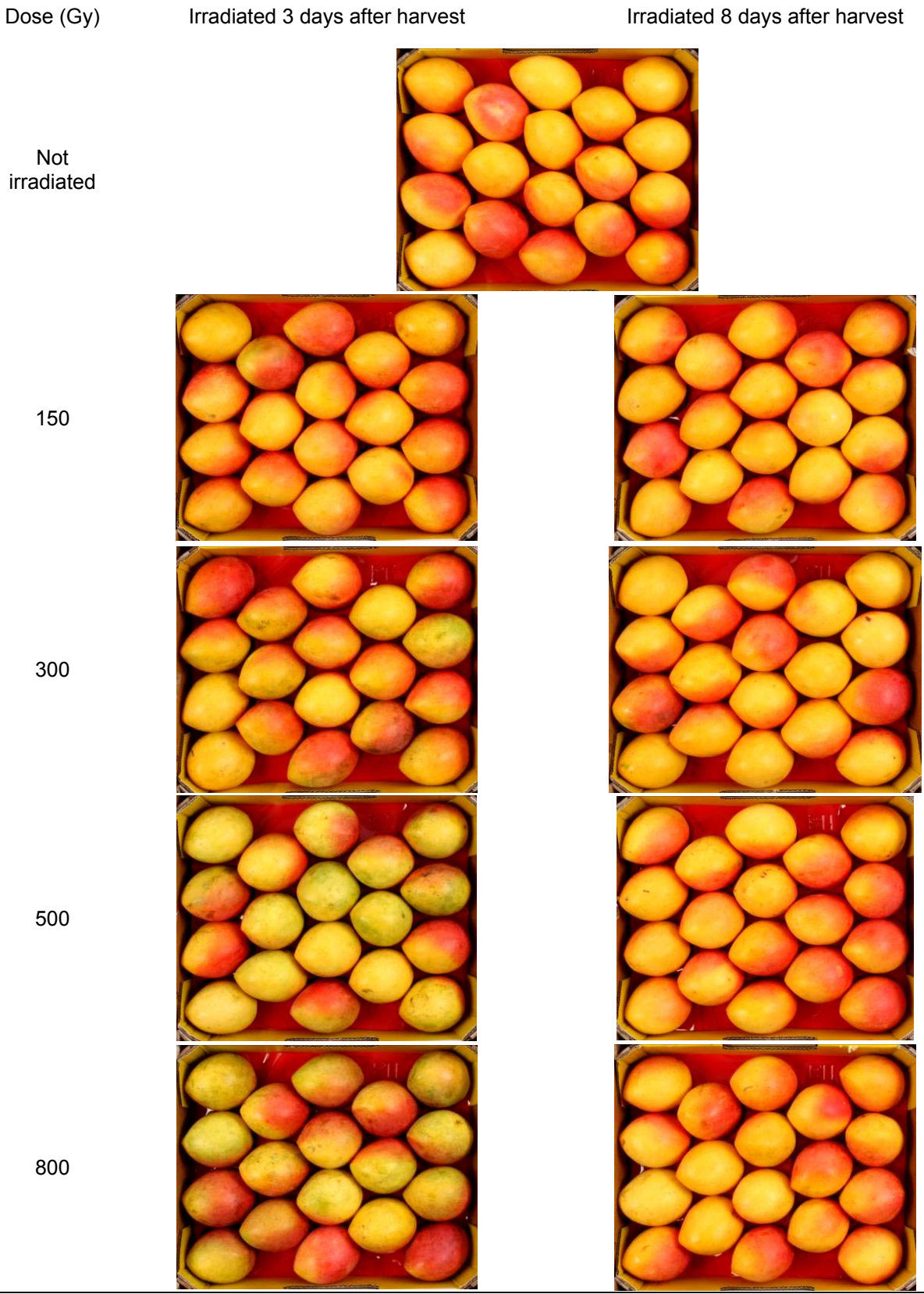


Plate 28 External quality of 'B74' mango fruit at 15 days after harvest (DAH) as affected by the time between harvest-irradiation and the irradiation dose. Fruit were not irradiated or irradiated either 3 or 8 DAH at 150, 300, 500, or 800 Gy.

6. Increasing fruit size - Screen

Tony Whiley

6.1.1. Summary

In the 2008 and 2009 cropping seasons the mean fruit size of 'B74' mango grown at Mataranka and Katherine in the Northern Territory (NT) was below the national average for this cultivar. The purpose of this research was to evaluate at a semi-commercial level the effect of Screen Duo® on fruit size at maturity of 'B74' mangoes grown at Katherine and Mataranka. Effective removal of the product at the packhouse was also assessed. Experiments were carried out at Katherine and Mataranka during the 2010 and 2011 cropping seasons comparing fruit treated with Screen Duo® with untreated controls.

In 2010 data from two of the experimental sites showed a reduction in crop yield with larger fruit sizes on those trees treated with Screen Duo®. The 2010 season had significant pre-harvest rain resulting in adequate fruit size across the whole farm. Thus, the size increase moved a significant percentage of the fruit into grades that returned lower prices hence together with lower yield the value of the crop decreased. This shift into lower priced sizes is unlikely to occur in normal seasons because of smaller average fruit size, so that the yield reduction would possibly be compensated by increased returns from high-priced fruit sizes.

The spray residue was effectively removed from the fruit during packing in 2010, largely because the water dump at the start of the pack line moistened the residue and allowed easier removal during brushing. Therefore the treatment did not significantly reduce visual appearance of the packed product because of residues, nor did it affect red skin blush.

The water dump was removed from the start of the pack line in 2011 to increase packhouse operation efficiency. However this resulted in the Screen Duo® not being removed from the fruit during packing so the fruit were non-saleable. To avoid further loss the fruit were not placed over the packing line and no data were collected.

The conclusion of this research is that Screen Duo® can increase fruit size and returns, but it's commercial benefits are restricted to seasons where fruit growth is retarded by "stressful" growing conditions, and to packing lines that include a water dump or similar to remove the spray residue.

6.1.2. Introduction

In the 2008 and 2009 cropping seasons the mean fruit size of 'B74' mango grown at Mataranka and Katherine in the Northern Territory (NT) was below the national average for this variety. This led to some difficulties in marketing the crop at acceptable returns. The smaller fruit size was likely due to reduced cell division period in these fruit because of a short time between fruit-set and the start of very high daily maximum temperatures. Once temperatures exceed 35°C photo-inhibition occurs and mango trees fix less atmospheric carbon essential for fruit growth (Schaffer *et al.*, 2009). Research with other crops showed that Screen Duo® can reduce leaf temperatures by as much as 10°C, thereby maintaining photosynthetic activity for longer daily periods.

This research was conducted to evaluate the effect of Screen Duo® on fruit size at maturity of 'B74' mangoes grown at Katherine and Mataranka at a semi-commercial level. Experiments were carried out at Katherine and Mataranka during the 2010 and 2011 cropping seasons comparing fruit treated with Screen Duo® with untreated controls.

6.1.3. Materials and methods

6.1.3.1. Treatments

The Screen Duo® treatment was applied to plots which consisted of a full block of trees on the K1, K2 and M1 'B74' mango farms at Katherine and Mataranka, NT. The plots consisted of 50% of two blocks with the other 50% being untreated controls. This represented 1280 Screen Duo®-treated and 1280 control trees at K1, 1800 Screen Duo®-treated and 1800 control trees at K2 and 1044 Screen Duo®-treated and 1044 control trees at M1.

The following treatments were applied:

1. Control – standard management practice with no Screen Duo® applied.
2. Screen Duo® applied immediately after the termination of flowering (fruitlets no larger than pea size). For this treatment Screen Duo® was applied on a regular basis through to harvest. The first application was a 2.5% formulation followed at 7 d intervals with a 1.25% formulation through to stone-hardening (six, weekly applications). Following this period subsequent applications were at 14-21 day intervals (depending on rainfall) using a 1.25 % application. Screen Duo® was applied with Du-Wett®.

6.1.3.2. Data collected

The following data was collected at a fruit collection depot on each farm and at the pack-house:

- Block biological yield (the total weight of fruit harvested off each block).
- Block pack out percentages (commercial yield).
- Assessment of effective removal of the product at the packhouse.
- Fruit quality assessments – blush colour (intensity) was assessed to determine whether that there had been a reduction by treatment.

6.1.4. Results and discussion

6.1.4.1. 2010 crop

At Mataranka (M1) the data indicated an increase in fruit size from the Screen Duo® treatment Figure 55. There were fewer kgs of bulks and sizes 22 and 20 from the Screen Duo® but an increased volume in the larger sizes of 18, 16 and 12. The inconsistency in the data was size 14 where there was a greater volume of fruit from untreated trees (Fig. 5). The unexpected result from this trial was the 7% reduction in yield from the Screen Duo™ which was consistent across both treated blocks. This effect has not been reported by the manufacturer of Screen Duo® or in related literature. The mean tree yield across the experiment was 37.2 and 42.3 kg/tree for Screen Duo® and untreated trees, returning $133.8 and $148/tree respectively. Due to unprecedented pre-harvest rain in 2010 fruit size across the Mataranka farm was not an issue as it had been in previous years. The increased fruit size achieved by the Screen Duo® treatment actually reduced fruit value as it shifted a greater volume of fruit into grades where returns were lower. The reverse may occur in a more "normal" season where significant rainfall events are not expected during fruit growth hence the evaluation of this product was repeated in the 2011 season.

At the Katherine K1 farm the two blocks treated were 11 and 16. Unfortunately fruit harvested from block 16 were not correctly labelled when sent to the packing facility and consequently no data is available from this site. Block 11 was harvested separating fruit from the Screen Duo® and control treatments.

Block 11 was amongst the last to be harvested which was at a time when much of the fruit left had become too mature hence much of the product was dumped (Figure 56). This event

distorted the results from this experiment and their reliability is in doubt hence no interpretation has been made.

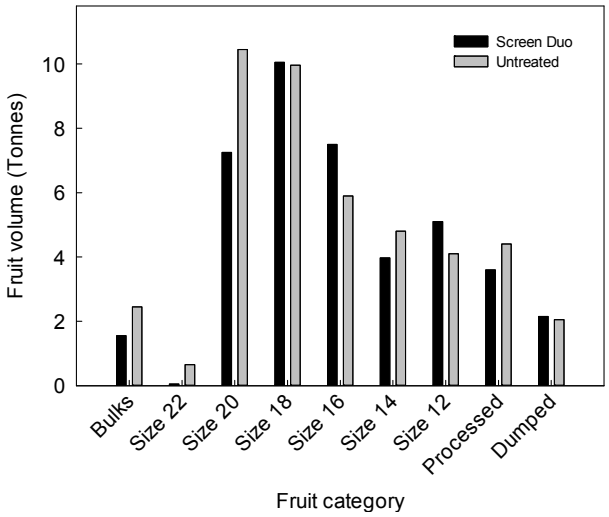


Figure 55 Effect of Screen Duo® on fruit size of 'B74'™ grown at Mataranka in 2010. Data are the combined results from blocks 20 and 41.

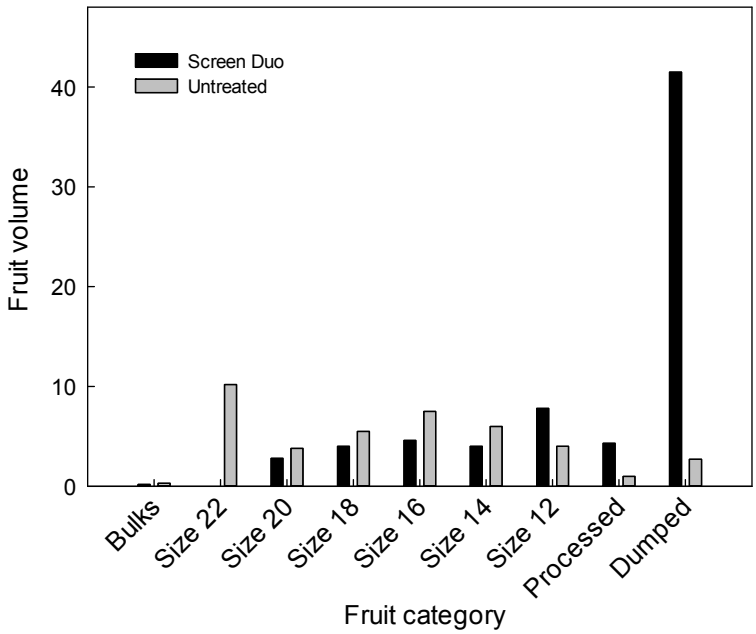


Figure 56 Effect of Screen Duo® on fruit size of 'B74'™ grown at K1 in 2010. Data are from Block 11.

At the Katherine K2 site blocks 32 and 48 were treated with Screen Duo®. An error during harvest occurred where treated and untreated fruit from block 48 were not separated and no data was available from this part of the experiment. Results from block 32 clearly demonstrated that Screen Duo® improved fruit size as there were no fruit packed in the 23 and 22 sizes but more fruit than from the control trees packed in the larger sizes of 16-12 (Figure 57). Similarly to the Mataranka site there was a reduction in crop load on the Screen Duo® treated trees (27.0 kg/tree) when compared to the untreated trees (30.5 kg/tree). As a

greater percentage of the crop from Screen Duo® treated trees fell into the lower-priced larger sizes and crop load was overall lighter there was a significant reduction in returns per tree when compared to untreated trees: $83.92/tree to $104.26/tree, respectively.

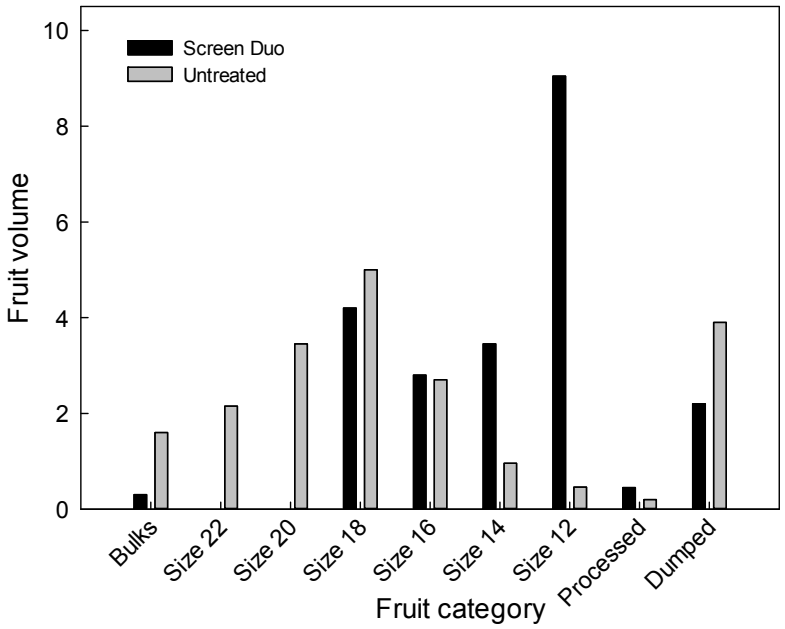


Figure 57 The effect of Screen Duo® on 'B74'™ grown at K2 in 2010. Data are from block 32.

At harvest, treated fruit had a film of white Screen Duo® covering the skin which was mostly easy to remove when on the packing line which had a water dump followed by eight roller brushes. For the most part the intensity of the blush was similar to untreated fruit with the exception being a few fruit on the outside of trees and closer to the spray rig. These fruit finished up with a thicker coating of Screen Duo® that created a speckled appearance in the blushed area.

6.1.4.2. 2011 Crop

The 2011 'B74' mango crop was the most productive to date across the farms. Screen Duo® was applied to each of the designated blocks on each of the three farms and a clearly visible coating of the product was achieved both on fruit and trees. However, at harvest, the packing line was unable to remove the coating of Screen Duo® from the fruit. Consequently, due to the significant increase in the volume of fruit that had to be harvested and packed in a three week period Oolloo Farms decided not to harvest the Screen Duo® experiments so no data was available for the 2011 fruiting season.

The difficulty in removing Screen Duo® from the fruit appeared to be related to the removal of the water dump from the packing line in 2011. The effect of immersing fruit in water prior to the brushing was to soften the product allowing it to be removed easily without damaging the skin. Passed over the brushes dry, the Screen Duo® firmly adhered to the skin of the fruit, rendering the product unsaleable.

6.1.5. Conclusions

Results from the Screen Duo® 2010 evaluations indicated there was consistent evidence that the product reduced total crop load and increased fruit size. There may be use for the latter during a normal season when the crop is grown entirely on irrigation and there is likely to be

a higher percentage of smaller fruit. However, the difficulty of removing the product from the skin of the fruit in the absence of a water dump remains a commercial problem which is likely to stop any future use of Screen Duo® on ‘B74’ mango if the company wishes to run a dry packing line.

7. Increasing the harvest window

7.1. Manipulating flowering time

Tony Whiley

7.1.1. Summary

The harvest window for each mango cultivar in each farm is about three weeks. Spreading the harvest window will reduce the peak infrastructure and labour required, and reduce the market peak and risk of over-supply. The harvest window can be potentially extended by manipulating the flowering time in certain blocks on the same farm, and by understanding how long fruit can remain on the tree without significant loss.

Delaying flowering

In subtropical climates mango trees are known to re-flower if the inflorescences are removed prior to fruit set as the prolonged cool temperatures induct new floral buds from axillary positions on the shoots (Crane *et al.*, 2009). This experiment was carried out to test if this procedure would result in re-flowering with later cropping in the more tropical climates of the Northern Territory.

The experiment was carried out on 'B74' farms at Katherine and Mataranka, since the latter has lower night minimum temperatures. Two hundred paired trees were selected for the study on each farm with 100 de-flowered and the other 100 allowed to flower normally as controls. Inflorescences were snapped at their base by hand with all flowers removed from these trees. Data was collected on re-flowering and tree yield (kg of fruit/tree).

The results showed that trees re-flowered at both sites within eight weeks after removing blossoms, but in general the flowering was patchy with only about one third of the canopy responding. The smaller fruit observed on re-flowered inflorescences generally did not make it through to maturity, most likely due to abortion from the very high ambient temperatures. The yield decreased by about 80%, hence there is no commercial potential for this treatment.

Earlier flowering

Pre-flowering Ethephon (Ethrel®) sprays have been successfully used to promote early flowering (leading to earlier fruit maturity) in 'B74' growing at Darwin in a humid, tropical climate. This experiment was set up to evaluate the effects of pre-bloom foliar sprays of Ethephon growing in a semi-arid, tropical climate at Katherine and Mataranka which are located inland and south of Darwin.

Effective spray concentrations used in Darwin had no effect at both field sites. It is possible that higher concentrations are required. The Australian mango industry had commissioned research to support registration of Ethephon on mango, but a suitable supplier could not be found at that time so the product was not available for commercial use. There was no justification to repeat this work using higher Ethephon concentrations unless access was granted for use through label registration.

7.1.2. Introduction

The production of mangoes in the tropics and subtropics of Australia provides different challenges across the divergent environments where the crop is grown. With the commercialisation of 'B74' a deliberate strategy was developed to plant trees in locations with sequential maturity extending from the earliest (Darwin) to the latest (Bundaberg) harvest times. The ability to compete for market share with a new cultivar lies in the

availability for a consistent, high quality product delivered to retail shelves each and every week for the period the commodity is in season. This is dependent on each location where the crop is grown to reliably produce quality fruit over successive years. Since the harvest period for mangoes is relatively short (15-20 d), agronomic practices that can either advance or delay flowering are beneficial in spreading the harvest operation at any one locality. Ethylene is a powerful growth regulator in plants, having many different effects on growth and fruit development. Ethephon (Ethrel) breaks down to ethylene in the plant cell and promotes flowering in mangoes (Barba, 1974; Bondad, 1976).Flower pruning and foliar Ethephon sprays were carried out on farms at Katherine and Mataranka to evaluate if maturity could be delayed or advanced, respectively.

In relation to delayed flowering, in subtropical climates mango trees are known to re-flower if the inflorescences are removed prior to fruit set as the prolonged cool temperatures induct new floral buds from axillary positions on the shoots. This experiment was carried out to test if this procedure would result in re-flowering with later cropping in the more tropical climates of the Northern Territory where delayed maturity would provide greater management options through the harvest period.

Pre-flowering *Ethephon* (Ethrel®) sprays have been successfully used to promote early flowering (leading to earlier fruit maturity) in 'B74' growing at Darwin in a humid, tropical climate. This experiment was set up to evaluate the effects of pre-bloom foliar sprays of Ethephon growing in a semi-arid, tropical climate at Katherine and Mataranka which are located inland and south of Darwin.

7.1.3. Materials and methods

7.1.3.1. Flower pruning to delay flowering

This experiment was carried out on 'B74' farms at both Katherine and Mataranka since the latter has lower night minimum temperatures. Two hundred paired trees were selected for the study on each farm with 100 de-flowered and the other 100 allowed to flower normally as controls. Inflorescences were snapped at their base by hand with all flowers removed from these trees. Data was collected on re-flowering and tree yield (kg of fruit/tree).

Mataranka

The full flower extension date used to predict fruit maturity for the Mataranka Farms was the 13/07/2010. The experimental trees were flower-pruned from the 8-14th July at full inflorescence extension and prior to any fruit being set. Re-flowering data were collected on the 14/10/2010. Trees were harvested on the 6th (controls) and 11th (de-flowered) November 2010.

Katherine

The full flower extension date used to predict fruit maturity for the K1 farm was the 14/07/2010. The experimental trees were flower-pruned from the 12-13th July prior to any fruit being set. Re-flowering data were collected on the 15th September 2010. Trees were harvested on the 4th (controls) and 10th (de-flowered) of November 2010.

7.1.3.2. Pre-bloom Ethephon foliar applications

At both sites, fruiting five-year-old 'B74' trees growing in an orchard situation were selected for the experiment. The experimental design was three treatments replicated 10 times in a 3 x 10 randomised block design. The treatments applied to trees were:

1. Control – untreated
2. Ethephon as a pre-bloom foliar spray at 0.08% a.i. with surfactant.
3. Ethephon as a pre-bloom foliar spray at 0.1% a.i. with surfactant.

Trees were sprayed to run-off shortly after the post wet-season flush had matured but prior to any visual sign of flower bud development. Trees were monitored for a change in leaf colour (previous treatments at Darwin caused a yellowing of leaves with many in the under canopy being shed within 10-14 d of treatment).

Flowering dates of trees were also recorded (floral bud break and peak flowering) and maturity checked with a near infra red spectroscopy (NIRS) gun at harvest.

7.1.4. Results and discussion

7.1.4.1. Delaying flowering

Mataranka

As indicated in Table 96, 67% of the flower-pruned trees re-flowered between pruning and 14/9/2010. However across the 67% of trees that re-flowered only 38.6% of the canopy area produced new flowers. At the time of data collection 48% of the re-flowering trees had set fruit while 19% of trees were flowering without any current fruit set. Re-flowering resulted in two different fruit set events with the largest fruit on trees approximately 40 mm in diameter and the next 10-15 mm in diameter. A third set was possible from current flowering. About 41% of trees had some fruit of about 40 mm diameter that were likely to reach maturity, however crop loads were small. It was doubtful that the 10-15 mm diameter fruit or those subsequently set would make commercial size or be retained on trees.

The mean yield from the control trees was 35.1 kg/tree, and 6.4 kg/tree from the de-flowered trees.

Table 96 Re-flowering and fruit set data for trees flower-pruned at Mataranka on the 8-14th July 2010. Data are mean values from 100 trees on the 14th September 2010.

% of trees re-flowering	% of canopy re-flowering	% of re-flowering trees with fruit set
67	39	48

Katherine

Re-flowering data in Table 97 shows that 95% of the flower-pruned trees re-flowered between pruning and the 15th September 2010. However across the 95% only 32% (one third) of the canopy area produced new flowers. At the time of data collection 93% of the re-flowering trees had set fruit while 2% of trees were flowering without any current fruit set. For the most part fruit diameter was less than 5 mm and it was doubtful that they will make commercial size or be retained on trees.

The mean yield from the control trees was 9.2 kg/tree, and 1.3 kg/tree from the de-flowered trees.

Table 97 Re-flowering and fruit set data for trees flower-pruned at K1 on the 12-13th July 2010. Data are mean values from 100 trees on the 15th September 2010

% of trees re-flowering	% of canopy re-flowering	% of re-flowering trees with fruit set
95	32	93

The results show that trees re-flowered at both sites within an eight week period after removing blossoms, however in general the flowering was patchy with approximately one

third of the canopy responding. The smaller fruit observed on re-flowered inflorescences on the 14-15th September for the most part did not make it through to maturity. Embryos usually abort with prolonged exposure to 33°C or higher. Maximum temperatures reach 36°C in Katherine and Mataranka during this time, so it is likely that that fruitlet loss was due to heat stress. The yield figures from Mataranka and Katherine indicate that de-flowering delays maturity by about 5 d, but the loss in production (≥ 80%) is commercially unacceptable. No further testing is justified.

7.1.4.2. Pre-bloom Ethephon foliar applications

Unlike the Darwin experiments, there was no change in leaf colour or drop in the three weeks following spraying, indicating that the treatments had not worked at these two sites. Similarly, there were no differences in flowering dates or fruit maturity between the three treatments.

The Australian mango industry has commissioned research with Ethephon on 'Kensington Pride' trees in the Darwin region to induce early flowering and maturity and the technology at this location has been shown to be effective. However, in 2011 they have been unable to get a supplier of Ethephon to register its use on mangoes, hence the product still remained inaccessible for use by mango growers. There was no intention to repeat this work using higher Ethephon concentrations unless access was granted for use through label registration.

7.2. How long can the fruit hang on the tree?

Peter Hofman, Roberto Marques, Jonathan Smith, Barbara Stubbings

7.2.1. Summary

Understanding how long fruit can remain (hang) on the tree without significant commercial loss is important to help increase the harvest window of 'B74' mango, which can reduce peak equipment requirements and pressure on labour, as well as improving fruit size and flavour. However, excessive delays can affect returns by increasing fruit drop, fruit damage on the tree and reduce flavour because of over-maturity. Factors that may increase hanging potential are a more even and single flowering peak resulting in very even maturity and high crop load. Previous results suggested that fruit drop may be a key determinant of the end of the harvest window of 'B74'. Further work was required to confirm those results and to investigate the impact of seasonal effects. Trials were established on five representative commercial farms in the in the Northern Territory (NT), north Queensland (QLD) and south-east QLD over three seasons. Growing conditions and tree/fruit characteristics in these locations were monitored, focusing on the development of fruit maturity using estimated dry matter (% DM) and flesh colour (FC) on the tree over time using a near infrared spectroscopy (NIRS) handgun.

Results showed large variation in the development of fruit % DM and FC across the five sites and over the three seasons. The average weekly increase in % DM was generally higher in the NT locations (0.7-0.8%) than in the QLD ones (0.5-0.6%). The stage of fruit maturity in which more than 10% of the fruit dropped off the trees varied considerably across locations. The final % DM before fruit dropped off the trees was generally higher in the NT sites (17.1-18.4%) than in the QLD ones (15.9-17.6%). The average tree harvest window (defined as the time in which each tree reached the minimum acceptable maturity of about 14% DM and the time in which the tree retained at least 90% of all its fruit) also varied considerably across locations. Generally, the harvest window was wider in trees from the hotter production areas in the NT (4.0-6.1 wk), reducing to the cooler areas in north QLD (2.7-4.2 wk), and being narrowest in trees from the coolest production area in southeast QLD (2.2-3.1 wk). The % of terminals flowering at full bloom was generally more variable in trees from QLD farms (especially Mareeba and Childers, ranging from 34-97%) compared with those from the NT farms (ranging from 69-92%). This suggests a greater spread of flowering and possible greater range of fruit maturity at harvest in QLD farms, which may be a contributing factor to their narrower harvest window compared to the NT ones.

The results highlight the challenges of a relatively narrow harvest window for 'B74' mango, especially if grown in cooler regions. Effective orchard maturity mapping, and reliable maturity estimates, are essential to allow an effective harvest schedule for each farm and season. This will allow fruit to be harvested at an adequate fruit maturity stage to ensure good eating quality, whilst minimising fruit loss.

7.2.2. Introduction

Increasing the harvest window can have considerable commercial benefit by reducing peak equipment requirements and pressure on labour. Delaying the start of harvest can also improve fruit size and flavour. However, excessive delays can affect returns by increasing fruit drop, fruit damage on the tree and during harvesting and marketing (because of increased skin sensitivity), fruit internal disorders, and possible loss of flavour because of over-maturity. Understanding how long fruit can remain (hang) on the tree without significant commercial loss is important to help increase the harvest window of 'B74' mango.

Factors which may increase hanging potential are a more even and single flowering peak (resulting in very even maturity, so that the more mature fruit will not drop while the others are still maturing) and high crop load (less leaves to feed the fruit). Preliminary results over one season suggested that fruit drop may be a key determinant of the end of the harvest window of 'B74' fruit (Hofman *et al.*, 2010b). Further work was required to confirm these results and to investigate the impact of seasonal effects. Therefore, the development of fruit maturity on the tree over time and fruit drop was investigated over three seasons (2010-11, 2011-12 and 2012-13).

Trials were established on five representative commercial farms in the main production areas in Australia: two farms in the Northern Territory (NT; at Darwin and Katherine), two farms in north Queensland (NQld; at Dimbulah and Mareeba) and one farm in south-east QLD (SEQld; at Childers). Growing conditions and tree characteristics in these locations were monitored. Several weeks before expected maturity, about 20 fruit per tree were tagged and numbered, and the percentage dry matter (% DM) and flesh colour (based on a 1-11 colour chart from the "'B74' Picking Guide") of each fruit was estimated over time using a near infrared spectroscopy (NIRS) handgun. Readings were taken every one to two weeks from about 14% DM until about 50% of the fruit had dropped off the tree. The percentage of fruit that dropped off the tree was also determined weekly.

7.2.3. Materials and methods

7.2.3.1. Sites and production characteristics

Refer to section 4.3.3.1 for details on the trial sites, and recording of climactic conditions, flowering assessments, vegetative growth assessments and tree characteristics at harvest.

Fruit maturity assessments

A few weeks before fruit reached minimum percentage dry matter (% DM, 14%), 20 fruit in each of the marked trees were tagged with a coloured ribbon (about 20 cm long) and numbered with a permanent marker to allow individual fruit assessment over time. The area of the fruit to be scanned (in the middle of the blush cheek) was marked with four dashes (on the top, bottom, left, right; Plate 13). Fruit representing all aspects of the tree were selected, proportionally including fruit from sun-exposed positions (about 30-50% blush on the fruit), and fruit further inside the canopy. Fruit that were too small, sunburnt, heat stressed, or misshapen were excluded.

The 'Nirvana' NIRS handgun (Integrated Spectronics Pty. Ltd., Sydney) was used (Plate 13) to non-destructively estimate fruit % DM and flesh colour (FC; based on the 1-11 scale of the "'B74' Picking Guide"). The handgun was calibrated and used in the field as described in the ''B74' Best Practice Guide" (Hofman and Whiley, 2010).

Assessments started initially on three trees randomly chosen along the row. When maturity reached approx. 14% DM, tagged fruit on all 15 trees were assessed until about 50% of the fruit dropped from the tree. Whenever possible, assessments were done weekly or up to two weeks intervals.

The NIR guns were also used commercially by farmers to predict maturity across the farms, which sometimes created challenges in relation to availability and suitable calibration. As a result, the assessments of FC in fruit from Mareeba and Childers in some years were not done.

Fruit drop

At 14% DM, the total number of fruit on each tree was counted. From that stage on, the number of fruit that had dropped from the tree was recorded weekly. The % of fruit retention on the tree was then calculated over time for each location as the cumulative number of fruit that dropped from the tree at each interval in relation to the total number of fruit in the tree. At

each assessment, all fallen fruit were removed to avoid any confusion with fruit that fell during the next week.

The harvest window for each tree was individually calculated as the time between 14% DM and the stage in which more than about 10% of fruit dropped from each of the selected trees. The means of the 15 trees was then averaged to provide the harvest window for each location.

Plate 29 'Nirvana' NIRS handgun in use on a 'B74' mango tree (left) and close up of the unit (right)

7.2.3.2. Statistical analysis

Statistical analyses were performed by Genstat® 11 for Windows™ (VSN International Ltd., UK). Analysis of variance (ANOVA) used the 'General Analysis of Variance' model, with trees as single replicates, farm as 'treatments' structure and no 'block' structure. The protected least significant difference (LSD) procedure at P=0.05 was used to test for differences between treatment means. The relationships between tree and fruit characteristics were established using correlation analysis on the means for each tree. The significance of the correlations was determined by linear regression analysis (P=0.05), and the strength by the correlation coefficient (r).

7.2.4. Results and discussion

7.2.4.1. Climatic conditions during fruit growth

Field temperature (ranging from 24.1 to 30.7°C) and relative humidity (ranging from 56 to 88%) generally varied moderately across the three seasons and across the five locations during 28 and 56 d before harvest (Table 98). In contrast, rainfall varied markedly across years and locations at different periods (from 7 to 56 d) before harvest (Table 98). In general, temperatures were higher and rainfall was lower in the NT sites compared to Qld ones, while relative humidity was higher in the NQld sites compared to Katherine and Childers.

It is likely that the above climatic variation was a contributing factor to the large variation between seasons and locations observed in this trial in fruit maturity development as detailed below.

7.2.4.2. Fruit maturity development on the tree

There was considerable variation among seasons and locations in fruit maturity development, as indicated by flesh % DM (Figure 58) and FC (Figure 59), based on the weeks before and after commercial harvest for each location, and NIRS assessments.

For each location, the relationship between % DM, FC and fruit retention on the trees over time is shown in Figure 60, Figure 61 and Figure 62. The stage of fruit maturity in which more than 10% of the fruit dropped from the trees (as indicated by the dotted vertical line on the graphs) varied considerably across locations. Generally, trees in the NT farms dropped more than 10% of fruit at a higher %DM (around 17.5%) than trees at the Qld farms (15-16.5%).

Table 98 Total rainfall (mm), average air temperature (°C) and relative humidity (%) at the five 'B74' mango trial sites in 2010/11, 2011-12 and 2012-13 up to 56 days before harvest

Location	Harvest date	Total rainfall (mm)				Mean Temp. (°C)		Mean RH (%)	
		Days before harvest							
		7	14	28	56	28	56	28	56
Darwin (NT)									
2010-11	19/10/10	20	104	151	175	28.5	27.2	77	70
2011-12	12/10/11	49	49	49	49	27.7	26.1	74	68
2012-13	22/10/12	0	42	42	42	27.8	26.7	73	71
Mean		23	65	81	89	28.0	26.7	75	70
Katherine (NT)									
2010-11	29/10/10	5	31	78	82	29.5	30.0	58	52
2011-12	22/11/11	86	142	176	181	29.6	30.1	70	62
2012-13	14/11/12	0	33	35	65	30.7	28.0	61	56
Mean		30	69	96	109	29.9	29.4	63	57
Dimbulah (NQld)									
2010-11	7/12/10	32	32	183	228	25.3	24.9	88	80
2011-12	12/12/11	3	3	3	14	26.1	25.1	71	72
2012-13	10/12/12	3	3	27	27	25.6	24.6	60	61
Mean		13	13	71	90	25.7	24.9	73	71
Mareeba (NQld)									
2010-11	14/12/10	65	79	281	357	25.1	24.4	88	85
2011-12	13/12/11	54	93	100	145	25.2	24.1	67	65
2012-13	11/12/12	0	0	36	36	24.5	23.6	77	77
Mean		40	57	139	179	24.9	24.0	77	76
Childers (SEQld)									
2010-11	8/2/11	1	5	59	135	25.8	25.3	69	73
2011-12	6/2/12	3	227	312	510	24.6	24.1	67	65
2012-13	4/2/13	0	469	471	476	26.0	25.6	67	63
Mean		1	234	281	374	25.5	25.0	68	67

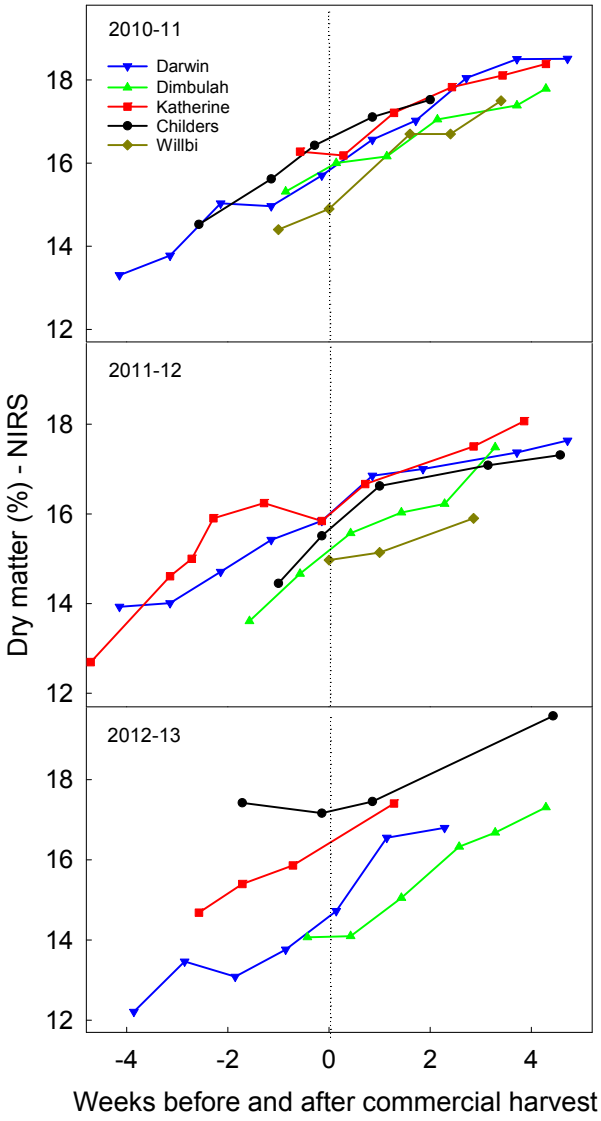


Figure 58 Changes in estimated flesh dry matter (%) in 'B74' mango trees grown in five commercial locations during the 2010-11, 2011-12 and 2012-13 seasons. Each point in the graphs is the mean of 20 selected fruit from each of 15 selected trees (total of 300 fruit) per location per season. Fruit were assessed non-destructively on the tree with a NIRS handgun for approx. 2-4 weeks before and after harvest. The dotted vertical line indicates the start of commercial harvesting for all locations.

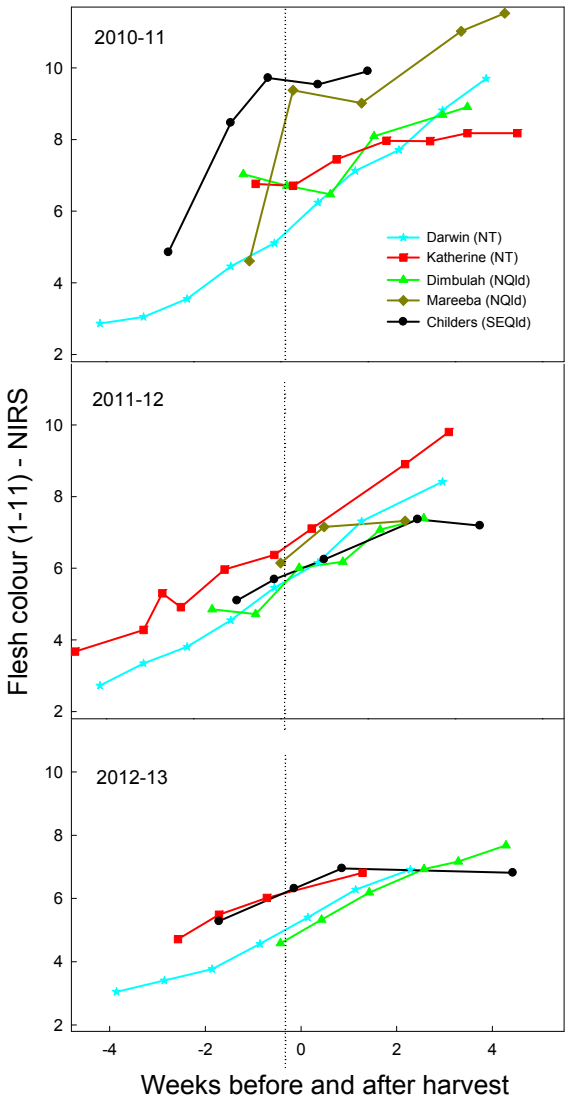


Figure 59 Changes in estimated flesh colour (1-11) in 'B74' mango trees in five locations during the 2010-11, 2011-12 and 2012-13 seasons. Each point in the graphs is the mean of 20 selected fruit from each of 15 selected trees (total of 300 fruit) per location per season. Fruit were assessed non-destructively on the tree with a NIRS handgun approx. 2-4 weeks before and after harvest. The dotted vertical line indicates the start of commercial harvesting for all locations.

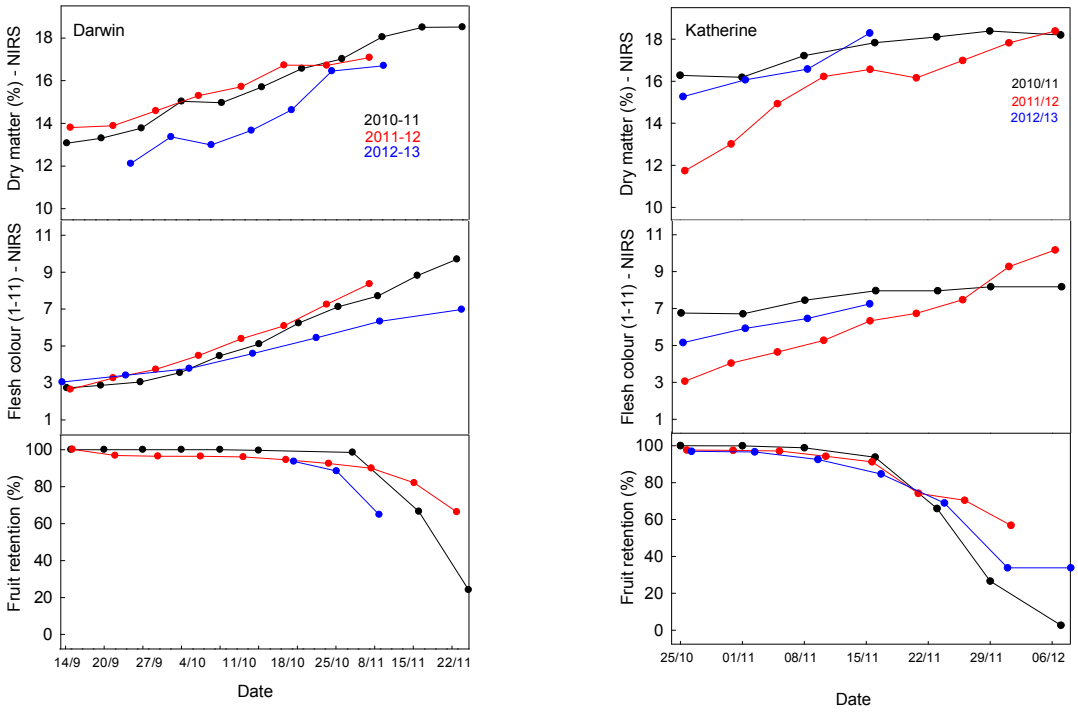


Figure 60 Changes in fruit dry matter (%), flesh colour (1-11) and the percentage of fruit retention on 'B74' mango trees in two locations in Northern Territory during the 2010-11, 2011-12 and 2012-13 seasons. For dry matter and flesh colour, each point in the graphs is the mean of 20 fruit from each of 15 selected trees (total of 300 fruit) per location. Fruit were assessed non-destructively on the tree with NIRS. For fruit retention, data is based on all fruit from each of the 15 selected trees

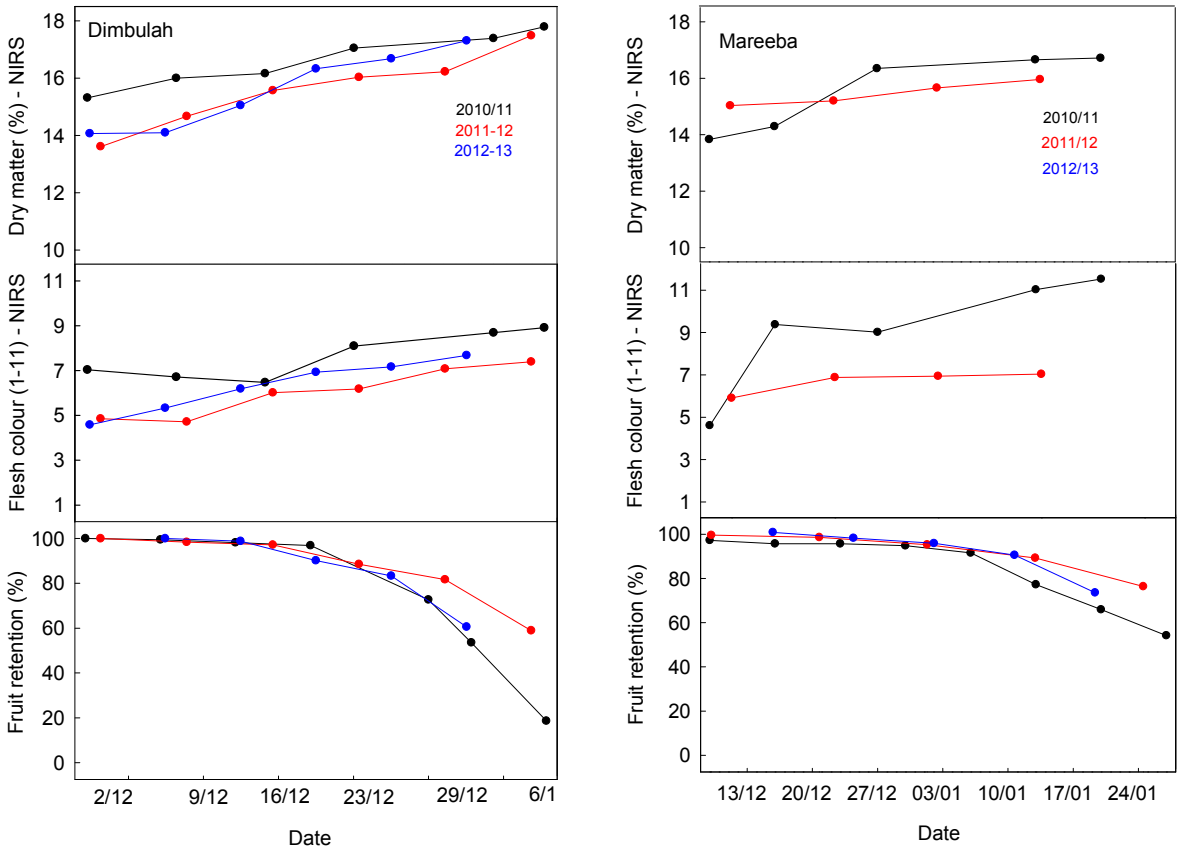


Figure 61 Changes in fruit dry matter (%), flesh colour (1-11) and the percentage of fruit retention on 'B74' mango trees in two locations in north Queensland during the 2010-11, 2011-12 and 2012-13 seasons. For dry matter and flesh colour, each point in the graphs is the mean of 20 fruit from each of 15 selected trees (total of 300 fruit) per location. Fruit were assessed non-destructively on the tree with NIRS. For fruit retention, data is based on all fruit from each of the 15 selected trees.

7.2.4.3. Tree and fruit characteristics across sites

The percentage of terminals flowering and the % of terminals flushing (at harvest) varied significantly across locations (Table 99). Except for Katherine in 2011-12 (which was an atypical situation with very low crop load in that block), trees from the NT farms generally had a higher % of terminals flowering than those in the NQld farms (especially Mareeba and Childers).

Likewise, the final stage of fruit maturity before the fruit dropped from the tree (as indicated by % DM assessed on the tree with the NIRS handgun), varied significantly across locations in the first two seasons (Table 99). Generally, fruit from the NT farms dropped off the trees at a higher %DM (17.1-18.4%) than fruit from the trees in the Qld farms (15.9-17.6%), confirming the previous results with fruit retention Figure 60), which were based on all the fruit on the trees rather than only 20 fruit selected fruit per tree.

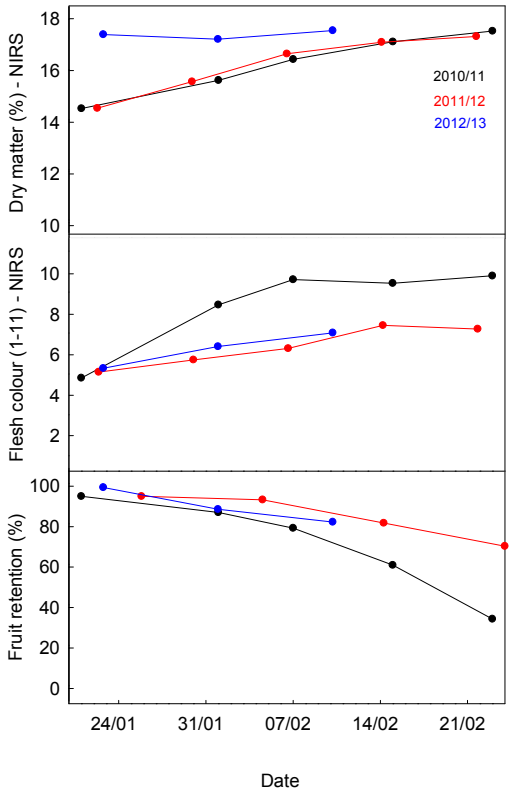


Figure 62 Changes in fruit dry matter (%), flesh colour (1-11) and the percentage of fruit retention on 'B74' mango trees in south east Queensland (Childers) during the 2010-11, 2011-12 and 2012-13 seasons. For dry matter and flesh colour, each point in the graphs is the mean of 20 fruit from each of 15 selected trees (total of 300 fruit) per location. Fruit were assessed non-destructively on the tree with NIRS. For fruit retention, data is based on all fruit from each of the 15 selected trees.

7.2.4.4. Harvest window

The average tree harvest window (the time in which each tree reached the minimum acceptable maturity of about 14% DM as assessed on the tree by the NIRS handgun, and the time in which the tree retained at least 90% of all its fruit, i.e. no more than 10% of fruit dropped off the trees) also varied considerably across locations and seasons (Figure 63). Regardless of the season, the harvest window was generally wider (4.0-6.1 weeks) in trees from the hotter production areas in the NT, reducing to the cooler areas in NQld (2.7-4.2 weeks) and being narrowest in trees from the coolest production area in SEQld (2.2-3.1 weeks). It is possible that the differences in climatic conditions between the regions were a major factor involved in these differences in harvest window. Higher field temperatures may allow the fruit DM to accumulate more rapidly compared with cooler climates, so that the fruit attains the minimum % DM more quickly relative to when fruit start dropping from the tree. The results also suggest that fruit from hotter climates attain a higher % DM before starting to drop. This longer harvest window in the hotter climates would allow a harvest start at higher % DM, thus improving flavour of the ripe fruit while still having an adequate harvest window.

Overall, the results above highlight the challenges of a relatively narrow harvest window for commercially grown 'B74' mango, especially in cooler regions. Effective orchard maturity mapping and reliable maturity estimates are essential for developing an effective harvest schedule for each farm and season. That will allow fruit to be harvested at an adequate fruit maturity stage to ensure good eating quality, whilst minimising fruit loss.

Table 99 The effect of production location on terminals flowering at about full bloom (%), terminals vegetatively flushing at harvest (%), the latest recorded fruit % dry matter before fruit dropped off the tree (assessed with NIRS handgun), from 2010-11 to 2012-13.

Season	Darwin (NT)	Katherine (NT)	Dimbulah (NQld)	Mareeba (NQld)	Childers (SEQld)
% of terminals on the tree at full flowering					
2010-11	78 ab	93 a	53 c	28 d	74 b
2011-12	94 a	16 c	94 a	40 b	92 a
2012-13	82 a	90 a	89 a	15 c	37 b
% of terminals vegetatively flushing at harvest					
2010-11	0 c	0 c	30 a	14 b	31 a
2011-12	9 b	42 a	6 b	3 b	7 b
2012-13	28 a	7 c	7 c	0 c	17 b
% DM before fruit drop					
2010-11	18.4 a	18.0 a	17.3 b	16.7 b	17.2 b
2011-12	17.6 a	17.8 a	17.5 a	15.9 b	17.3 a
2012-13	17.1	17.4	17.3	17.3	17.6

Tree data are the means of 10 trees per location. Fruit data are the means of 20 selected fruit and individually assessed in each of 20 marked trees (total of 200 fruit) per location.
Means in each row with the same letter are not significantly different (at P=0.05) as measured by LSD. Absence of letters in a row indicates that differences are not significantly different

Correlation analysis with the tree data shown above, fruit maturity characteristics and harvest window from all farms across the three seasons showed no strong correlations between these parameters (data not shown).

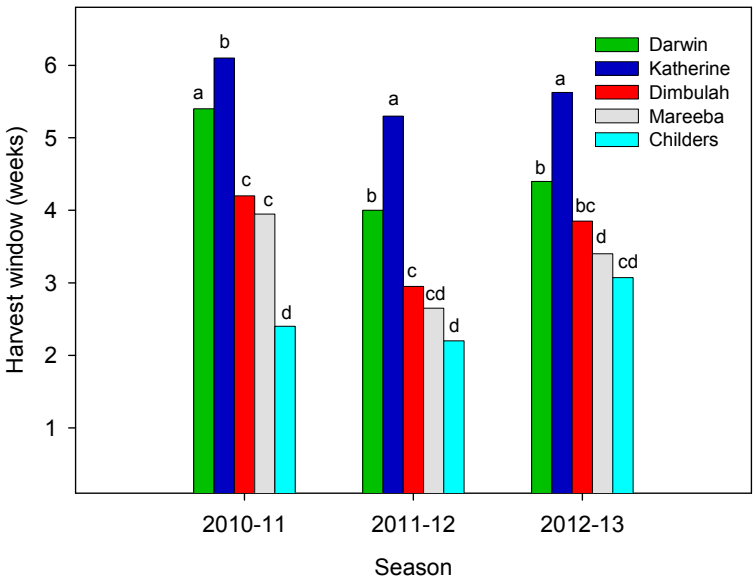


Figure 63 Harvest window (weeks), the time in which each tree reached the minimum acceptable maturity of about 14% DM and the time in which the tree retained at least 90% of all its fruit (e.g. no more than 10% of the fruit dropped off from the tree) for each farm during 2010-13). Bars with the same letter in each year are not significantly different (at P=0.05) as measured by LSD.

8. Fruit movement in the paddock

8.1. Assessment of improvements to in-field movement of fruit

Henrik Christiansen and Kieren Brown

Prior to the 2010 mango season, 'B74' fruit were removed from the field using field bins, bin runners and tractors. Two different sizes were available – 4 and 5 bins per unit. Once the trees are in full production, harvest requirements per farm will be approximately 300 tonnes per day. This requires approximately 25-30 harvest aids per farm and 8-10 bin runners and tractors per farm. It is unlikely that this tractor requirement could be sourced economically and, if they could, the number of vehicle movements in the orchard would pose a safety hazard. In addition, it is unlikely that the required number of qualified tractor drivers would be available.

A number of options have been identified to solve the problem:

1. Construct a higher capacity tractor-drawn bin runner to reduce bin runner and tractor requirements.
2. Reduce the travel time for tractors and bin runners by establishing in-field bin staging facilities and transfer full bins in-field to a flat deck truck for transport to the fruit store.
3. Evaluate more efficient methods of transferring bins from harvest aids to flat deck trucks and eliminate the need for bin runners and for bins to touch the ground during picking.
4. Establish mobile fruit stores in field and despatch to packhouse directly from the field.

The following progress was made:

An engineering business prepared to design and build a high capacity (10-12 bins) bin runner could not be identified. However, a six bin capacity bin runner was tested. While this unit is more expensive than a five bin unit, the construction cost per bin space remains constant at around $2,500. The increase in capacity corresponds to a decrease of 17% in the number of trips back and forth from the fruit store.

In field concrete pads were constructed at Katherine and Mataranka. Bin runners deposited full bins on concrete pads and a forklift then loaded the bins onto a flat deck truck for transporting to the fruit store. The reverse applies for the delivery of empty bins to the field. This system was more than double the cost compared with bin runners to the fruit store, for a typical harvest season on one farm (Table 100). Observations included:

- Nil negative quality effects were experienced on fruit transported by the flat deck trucks.
- Greater volumes of fruit were moved infield but with a 60-70% reduction in bin runner movements to the fruit store.
- There was reduced congestion at the fruit store.
- There was an 55% increase in payload per movement.
- Less wear and tear on tractors and bin runners.
- The time taken to deliver bins to fruit stores doubled and often exceeded our best practice target of 30 minutes from harvest aid to fruit store.

Thus, while there were some advantages this option was not considered commercially feasible.

A high volume harvest aid was trialled during the 2012/13 season. In addition to high volume, the field bins can be placed directly from the harvest aid onto a flat deck truck without the bins touching the ground (causes contamination, potential fruit damage from sand and grit and food safety issues), and with no forklift requirements.

This was tested in Katherine. In terms of bin yield, the harvest aid only achieved similar output to the current model of harvest aids. It certainly did not achieve the brief of yielding the same output per day as three current models of harvest aids. It also had a higher support labour contingent which made it twice as expensive to run in terms of cost per kg of fruit harvested. The seamless full bin transfer onto a flat deck truck was not achieved because the flat deck frame was not built to specification. One success however was the practice of filling four bins on the move, and on the actual machine platform, meaning the bins never touched the ground. The learnings from this trial could be transferred to the design of an improved high-volume prototype.

No progress was made with the establishment of mobile fruit stores. One of the major problems to solve is IT-related because of poor in field communications, due to remoteness making it difficult to collect the required data for traceability (harvest aid ID, bin numbers, bin runners). Also this process meant double handling in terms of equipment movements and infrastructure required, and also an increase in labour to service these stores

In conclusion, the proposed methodology of this component of the project could not be followed through because an engineering business prepared to design and construct a prototype could not be found. As a result, several other alternatives to improve in-field fruit movement were tested. To date, none of these were successful, apart from comparing a six bin trailer unit with the standard four or five bin unit.

Table 100 Cost comparisons of using bin runners direct to the fruit store on farm, compared with using in field concrete pads and flat bed trucks to transfer the fruit to the holding area

Bin runner method, not using flat deck truck

Fuel	
$/L	$1.67
L used per day	70
No of bin runners and tractors per day	3
No of harvest days	35
Total fuel costs	**$12,275**
Labour (drivers)	
Tractor drivers	3
$/hr	$22.70
No of hrs	10
No of harvest days	35
	$23,835
Total cost; bin runner	**$36,110**

Flat deck trucks from loading pad in the orchard

Fuel	**Trucks**	**Forklifts**	**For bin runners to concrete pad**
$/L	$1.67	$1.67	$1.67
L used per day	50	35	50
No flat deck trucks per day	2	2	3
No of harvest days	35	35	35
	$5,845	**$4,092**	**$8,768**
Total fuel costs			**$18,704**
Labour	**Truck**	**Forklifts**	**Bin runners**
Drivers	2	2	3
$/hr	$21.20	$22.70	$22.70
No of hrs	10	10	10
No of harvest days	35	35	35
	$14,840	**$15,890**	**$23,835**
Total labour			**$54,565**
Total fuel + labour	**$20,685**	**$19,982**	**$32,603**
			$73,269
Truck hire	**9 t truck**	**15 t truck**	
$/day	$201	$240	
days	35	28	
	$7,035	**$6,720**	
Total			**$13,755**
Total cost; flat deck			**$87,024**

9. In-transit ripening

9.1. Temperature, ethylene and carbon dioxide control

Peter Hofman, Rod Jordan, Daryl Joyce, Binh Ho, Bhesh Bhandari, Roberto Marques

9.1.1. Summary

Current recommendations for Australian mangoes requiring more than 2-3 d transit time from farm to ripener is to cool on farm to 12-13°C within 24 h of harvest, and transport at this temperature. However there are compelling commercial benefits to initiating ripening in transit. Risks are associated with controlling the three key variables that influence mango fruit ripening behaviour; temperature, ethylene and carbon dioxide (CO_2), which can cause loss of value if not controlled within acceptable limits. To evaluate the potential for in transit ripening, the performance of relatively new road/rail containers was evaluated. The results indicated that the containers can reduce 'Calypso' mango fruit temperatures for average 21ºC to the set temperature of 13ºC, and maintain the temperature of fruit of average pulp temperature of about 21ºC in a container set at 18ºC. However, pulp temperature control was variable across loads, partly because of poor loading (a gap between the front of the container and the first row of pallets). Hence, loading containers with warm fruit, and inappropriate loading can allow fruit temperatures to increase, and increase the temperature gradient across the container and the risk of chilling of fruit on the top layers of the front pallets.

CO_2 concentrations after the 3-4 d journey from the NT to Adelaide often reach 8-12%. Laboratory trials confirmed that 'B74' mango fruit ripening is not affected when held in more than 10% CO_2 for the first 6 d of ripening. However above 3% CO_2 is a safety issue because of the risk of asphyxiation. Live trials indicated that CO_2 concentrations can be held below about 3% in rail containers set at 18ºC by using 200 kg of hydrated lime in the container. Also preliminary trials suggest that an ethylene-releasing powder and ethylene released from permeable bags can maintain ethylene concentrations at 5-40 $\mu L.L^{-1}$ (ppm). However these systems require further refinement.

Based on the above, in transit ripening of 'B74' mango is achievable, however the risks need to be well controlled by using containers with good refrigeration and air circulation, the pallets cooled to within 2ºC of the set temperature, the container loaded properly and more testing of the co and ethylene release systems to reduce variability.

9.1.2. Introduction

Current recommendations for Australian mangoes requiring more than 2-3 d transit time from farm to ripener is to cool on farm to 12-13°C within 24 h of harvest, and transport at this temperature (Ledger and Barker, 2009; Ledger *et al.*, 2012). These recommendations are based on the assumptions that transport containers do not have sufficient refrigeration capacity to cool fruit, or to maintain the temperature of ripening fruit within acceptable limits.

However, there are several valid reasons to re-consider these recommendations:

- Farms/packhouses sometimes have insufficient cooling capacity during peak harvest periods, so that mangoes are often loaded at higher than recommended temperatures.
- The newer road/rail containers/trailers likely have higher refrigeration capacity and airflow, thus allowing limited cooling and better temperature control during transit.

- Ripening in transit has several significant cost advantages. These include reducing on-farm precooling and in-market ripening room floor space, reducing the time from harvest to market allowing access to higher prices at the start of the season, and reducing energy requirements by not cooling the fruit as much on-farm, running the trucks at higher temperatures, and not requiring warmup of fruit in market before ripening.

However there are several challenges:

- Ripening fruit produce considerable heat from respiration. Inadequate cooling capacity or airflow in the transport container will allow fruit temperatures to increase, further increasing heat production, and resulting in "runaway" temperatures.
- Ripening fruit also produce carbon dioxide (CO_2) during respiration. High CO_2 concentrations can result in green ripe 'Kensington Pride' mango fruit with more disease (Nguyen, 2003). Also concentrations above about 4% will cause asphyxiation.
- The fruit should be treated with ethylene at the start of ripening. Ethylene treatment on-farm before dispatch is less practical because of the need for increased cool room space, and transporting these ripening fruit will increase the risk of excess heat and CO_2 generation as respiration increases rapidly the fruit ripens. Therefore, fruit should be transported soon after harvest and ethylene treated in transit. Delaying the ethylene treatment until arrival will reduce the benefits of ethylene treatment.

Several systems have been developed for ethylene release in cartons or containers based on very small release canisters (Sharrock *et al.*, 2010 ; Sharrock and Henzell, 2010) and from ethylene encapsulated (trapped in a sugar matrix) (Ho *et al.*, 2013) which may have application to transport containers. In addition, hydrated lime with at least 85-90% available lime index can effectively absorb CO_2 (Bartsch, 2004), and is used in controlled atmosphere rooms to minimise CO_2 accumulation.

To evaluate the potential for consistent in transit ripening we tested the following over three seasons:

- The performance of refrigerated road/rail containers. We focussed only on relatively new (less than 2-3 y old) 45 ft containers because of their potential to perform better and more consistently. They would also have more consistent leakage rates because of better door seals etc, which is important for predictable ethylene application.
- Several approaches to maintain CO_2 below about 3%. We also tested the sensitivity of 'B74' to high CO_2 concentrations to provide a target maximum CO_2 concentration (section 9.2).
- Several approaches for ethylene treatment at 10-50 $\mu L.L^{-1}$ (ppm) for at least 2 d. Previous research with 'Kensington Pride' indicated negligible difference in ripening responses between 10-50 $\mu L.L^{-1}$ ethylene, but at least 2 d exposure was required to reduce variability in fruit ripening.

The trials were conducted on 'B74' mango fruit harvested from Darwin or Katherine, and mostly rail-freighted to Adelaide within 3-5 d.

9.1.3. Materials and methods

9.1.3.1. Fruit and treatments

The 'B74' mango fruit were picked, packed, cooled and transported under typical commercial conditions. The only "intervention" was specifying particular pulp temperatures container set temperatures at loading, and inserting ethylene and CO_2 modulating treatments, and logging

equipment to test container performance. In most cases, the temperature loggers were inserted during palletising.

The results presented here are selected from the trials over the three seasons to best illustrate the key findings from this research. The treatments applied to the loads are summarised in Table 101.

Table 101 Rail container refrigeration set temperatures and average fruit pulp temperatures at loading, of selected commercial 'B74' consignments from the NT to Adelaide from 2011-2013. The comments relate to temperature management in consignments where the temperature data are not presented in the report. The refrigeration units on loads 6-7 were set to vent to provide fresh air exchange. Some of the containers had hydrated lime in 5 kg bags on top of pallets. Other consignments had either ethylene releasing powder or semipermeable bags of different thickness filled with ethylene.

Load no	Temperature (ºC)		Comments	Container venting	Lime	Ethylene
	Container set	Pulp at loading				
1	13	16		No	No	No
2	13	21		No	No	No
3	13	21.5	Gap between front and first pallet	No	No	No
4	18	21		No	No	No
5	16	25		No	No	No
6	18	15		Yes	40bags of x5kg each	No
7	18	16		Yes	No	No
8	18	14	Good	No	No	Powder
9			Good	No	No	Powder
10	18	14	Good	No	No	20 bags 35 µm
11	18	14	Good	No	40x5kg	20 bags 35 µm
12			Poor. Increased to 24ºC by journey end	No	20x5	6 bags 50 µm
13	18	13	Good	No	No	Powder
14	18	13	Good	No	No	Powder
15	18	13	Poor. Increased to 24ºC by journey end	No	No	9 bags 50 µm
16	18	13	Good	No	18x5 kg	9 bags 50 µm

9.1.3.2. Container and transport details

- 45 foot rail/road containers, mostly less than about 3-4 y old; loaded with 18-20 pallets, and about 70 cm between the top of the pallet and the container roof. 18 pallets were loaded to reduce the weight over the rear axle of the prime mover. In most of these cases the first row of pallets were placed about 1 m from the front of the container.
- Most containers were hard-wired a StarTrak system or similar, which transmits container and GPS details via satellite phone.
- Most containers were fitted with a Carrier Vector 1850 MT°, or Thermo King SB310 refrigeration units.
- The containers had no inbuilt fresh air exchange capability. Some of the Thermo King units had fresh air exchange capability through a slider that opened two vents on the refrigeration unit (Plate 30). This provided 0.71 m3/min of fresh air (personal communication, Northern Territory Freight Services).
- The floor was either non-ribbed so return air was through the pallet slats, or ribbed.

- Pallet configuration: When 18 pallets were loaded, in most cases the first row of pallets were placed about 1 m from the front of the container (Plate 31), leaving a gap allowing cold air to short circuit directly from the delivery to the return air vent. The last row of pallets was secured with spreader bars and ply sheets (Plate 32), however their arrangement varied based on truck driver preferences. The ply sheets were either placed at an angle so that the bottom of the pallets were exposed, and in other cases the ply sheets were placed vertically which prevented the cold air returning to the regeneration unit via the cavity in the bottom of the pallet. In other cases thick foam battens were placed hard against the last pallet providing a tight seal against the roof, walls and floor of the container. This prevented the delivery air from flowing down the back of the container through the pallets to the return air inlet, and likely forced more air to flow over the pallets. Load 2 had only the crossed ply sheets. Load 3 had the same foam battens as Load 1, but these were placed about 50 cm away from the back of the last pallet allowing free air movement vertically down the back of the last pallet.
- The loaded containers were transported to the Darwin or Katherine railhead by road then loaded onto the train on the same day or the next day.

Thermo King refrigeration unit fitted with the fresh air exchange ventilation lever on the top left.

Fresh air exchange lever in the closed (left) and open (right) positions.

Plate 30 Container venting system.

Plate 31 The gap between the front of the container and the first row of pallets. The spreader bar and grate maintains the 1m gap between the front of the container and the first row of pallets.

Plate 32 Examples of securing the last row of pallets. Foam battens are placed hard against the last row of pallets (left), the ply sheets placed at an angle to allow flow of air under the pallets (centre), and battens placed against spreader bars leaving about 50 cm gap between the back of the pallet and the battens (right).

9.1.3.3. Atmosphere control and monitoring

Two approaches were used for ethylene treatment:

- An ethylene powder consisted of pure ethylene "trapped" in a sugar (alpha-cyclodextrin) matrix developed by the University of Queensland. The ethylene was released from the powder by adding 200 ml water just before container closing, or using a mixture of powder and salt crystals which slowly absorb water from the humid atmosphere in the rail container to provide a slower release. The earlier trials used one container of each, while the later trials used only the salt formulation (two containers per consignment). One container holding either of these formulation was placed on the top of a pallet near the front and another near the rear of the rail container. Both containers had lids with large holes to prevent water or powder loss (Plate 33).
- A semi-permeable membrane was constructed from 35 µm thick PVC plastic tube (30 cm diameter and 1.1 m long). The bag was heat-sealed at both ends with a 20 cm "flap" which was stuck to the top of the pallet. The bag was punctured to allow filling with Ripegas (4.5% ethylene in CO_2), then sealed with adhesive tape. The bags were filled within 4 h of loading the container, and the bags were attached to the top of the pallet just before loading. Between 6-20 bags were used per container (Plate 35).

Carbon dioxide was manipulated using two approaches:

- Venting the container using the vent option on the Thermo King refrigeration unit (see above).
- Absorbing the CO_2 using hydrated lime (Adelaide Brighton, 85-95% available lime index; typically above 90%). About 5 kg was placed in standard paper bags of sufficient strength, each bag was placed into an empty tray, and the trays pallets throughout the container (Plate 35). 90-200 kg (18-40 x 5 kg bags) per container were tested.

Plate 33 Plastic container with the ethylene powder and deliquescent salt before loading into the container

Plate 34 Temperature logger and ethylene logger inserted in the tray on the fifth layer from the top of the pallet

Plate 35 Plastic bags with Ripegas, and hydrated lime in brown paper bags, on the top of selected pallets within the load.

Shipment conditions were recorded as follows:

- Temperature: Recorded using a Hobo model U12-14 data logger fitted with a T type thermocouple (Plate 34). The probes were placed into fruit on the 4th row from the top of each pallet, and inside pallets in rows 2 (2nd from front), 4 or 5 (middle) and 8 or 9 (2nd row from doors). Air temperatures near the probed fruit were also measured but not reported. In the first trials, temperature probes were also placed in fruit in the same pallets but directly facing the outside of the container to assess temperature variation. In some cases indicative pulp temperatures in most pallets at loading and on arrival in Adelaide were obtained by placing a digital temperature probe in 1-2 fruit in layers 3-5 from the top of most pallets.
- Delivery and return air data was obtained through the StarTrak system. Delivery air was also logged with a temperature logger on top of the pallet closest to the refrigeration unit.

- CO_2: Recorded using a Vaisala logger. The sensor was placed in an empty tray on the top of a pallet near the back of the container, or in later trials in an empty tray in the fourth row from the top in the middle of the container. In some cases CO_2 was measured on arrival by inserting a tube between the door seal of the container and withdrawing air samples using a Kitagawa kit with CO_2 -measuring tubes (1-20% measuring range).
- Ethylene: Recorded using a logger from CO_2 meter (measuring range of 0-200 $\mu L.L^{-1}$ ethylene) or loggers from MSR electronics (Nuremberg, Germany; measuring range of 0-100 $\mu L.L^{-1}$ ethylene; Plate 34).

A clear "do not open" notice was placed on the back door of the container. The containers were set at 18ºC and dispatched on 10th November by rail and arrived in Adelaide on 13th - 15th November.

9.1.3.4. Fruit quality assessment

About 15 trays from the same batch of fruit were pre-cooled. Temperature loggers were placed in each tray in the fourth row from the top in each of 15 pallets. The test pallets were loaded at the second, middle and second last rows from the refrigeration unit in each container. Upon arrival in Adelaide, three trays per container were removed and airfreighted to Brisbane. Fruit were ripened at 18°C (no ethylene) and assessed at full yellow colour for external appearance, Brix, and acidity.

9.1.4. Results

9.1.4.1. Temperature control

Load 1: 13°C set, 16°C pulp

The average pulp temperature across most pallets at container loading was 15.7°C (range of 11.8-22.5°C).

In the two pallets that were above 13°C at container loading, pulp temperatures decreased to the container set temperature within 6-12 h of loading (Figure 64), indicating the capacity of the container to reduce pulp temperatures to the set temperature.

Pulp temperatures were 3-5ºC lower in the front and middle row pallets compared with the second last row. This pattern was common in most monitored consignments, but the temperature gradient was not always as large.

There were differences in fruit temperature between those near the middle and those close to the wall of the container, however the temperature gradient was generally larger from the front to the back of the container.

StarTrak data indicated that return air temperature quickly settled around the set point of 13°C (Figure 65). The delivery air temperatures fluctuated between 5-18°C, presumably because of regular defrost cycles. Hobo logger data of delivery air indicated temperatures fluctuated between 8-13°C, with the average temperature gradually increasing with time as the fruit temperatures approached the set temperature.

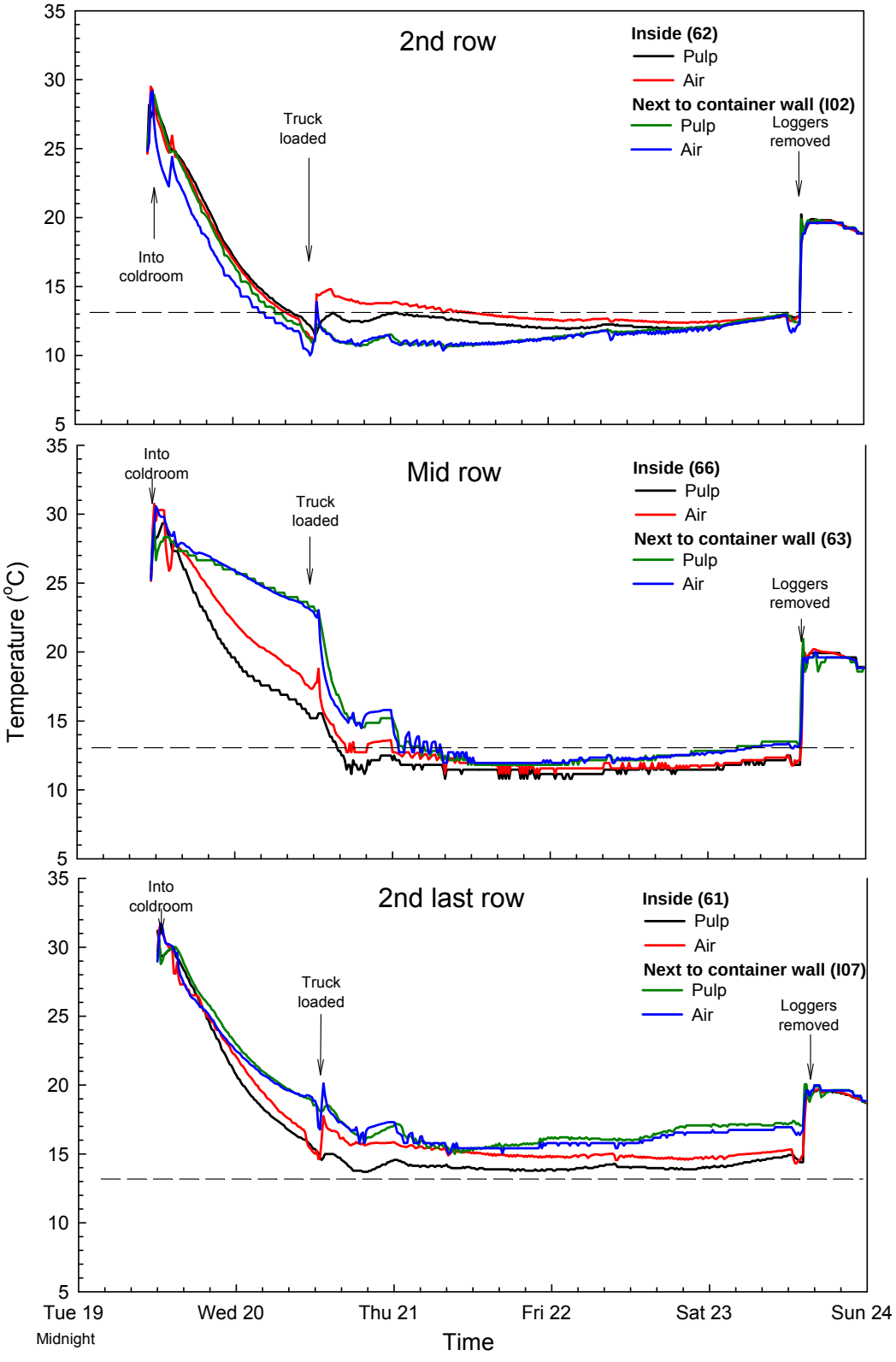


Figure 64 Load 1: 13°C container set temperature and 16°C average pulp temperature on loading. Pulp and air temperatures in a pallet second row from the front, middle and second row from the back of a 45ft refrigerated container. Loggers were placed in fruit near the face of the pallet adjacent to the other pallet in the same row (inside), and in fruit close to the container wall (next to container wall). The dashed line represents container set temperature.

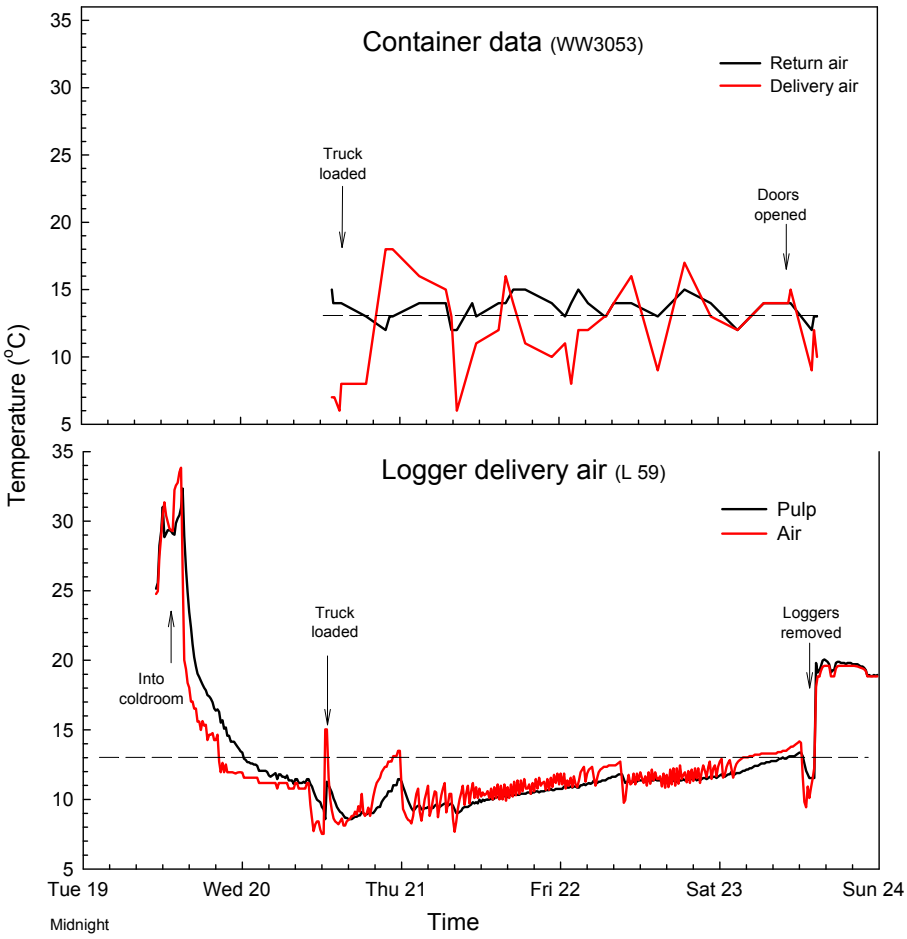


Figure 65 Load 1: 13°C container set temperature and 16°C average pulp temperature on loading. Delivery and return air temperature from the StarTrak logger attached to the container (top), and delivery temperature logged by a temperature logger on the top of the pallet nearest the refrigeration unit (bottom).

Load 2: 13°C set, 21°C pulp

The container was able to reduce temperatures of warmer fruit (Figure 66).

- Pulp temperatures generally decreased from 20°C to the 13°C container set temperature within 6-8 h of door closing. Pulp temperatures decreased more rapidly in pallets near the front of the container than those near the back. Pulp temperatures near the front declined to 3-5°C below setpoint within about 12 h, while pulp temperatures toward the back were 2-4° above the set temperature. Pulp temperatures near the front and middle approached set temperature after about two d but remained 2-4°C above set temperature near the doors. There was a 5ºC temperature differential between the front and the back.
- Fruit near the wall of the container were generally cooler than those adjacent to the next pallet in the same row.

Return air reached 13°C within about 16 h of door closing (Figure 67). Delivery air temperatures recorded by StarTrak ranged from 3-15°C. Delivery air temperatures recorded by the Hobo logger decreased to about 7°C within six h of door closing then gradually

increased to about 11°C by end of journey. Delivery air temperatures were generally lower than with Load 1, most likely because of the higher average pulp temperatures on loading.

These results indicate that the container was able to reduce pulp temperatures. However the temperature gradient from front to rear of the container was significantly large, and the delivery air temperature dropped to potentially damaging temperatures in order to reduce the delivery air temperature to the set temperature. These low delivery air temperatures could be sufficient to cause significant chilling damage to fruit on the top layers of the first few rows of pallets.

Therefore, to avoid the risk of chilling damage and significant temperature gradients which will affect ripening across the load, fruit should be loaded in the container at pulp temperatures within about 3°C of the set temperature.

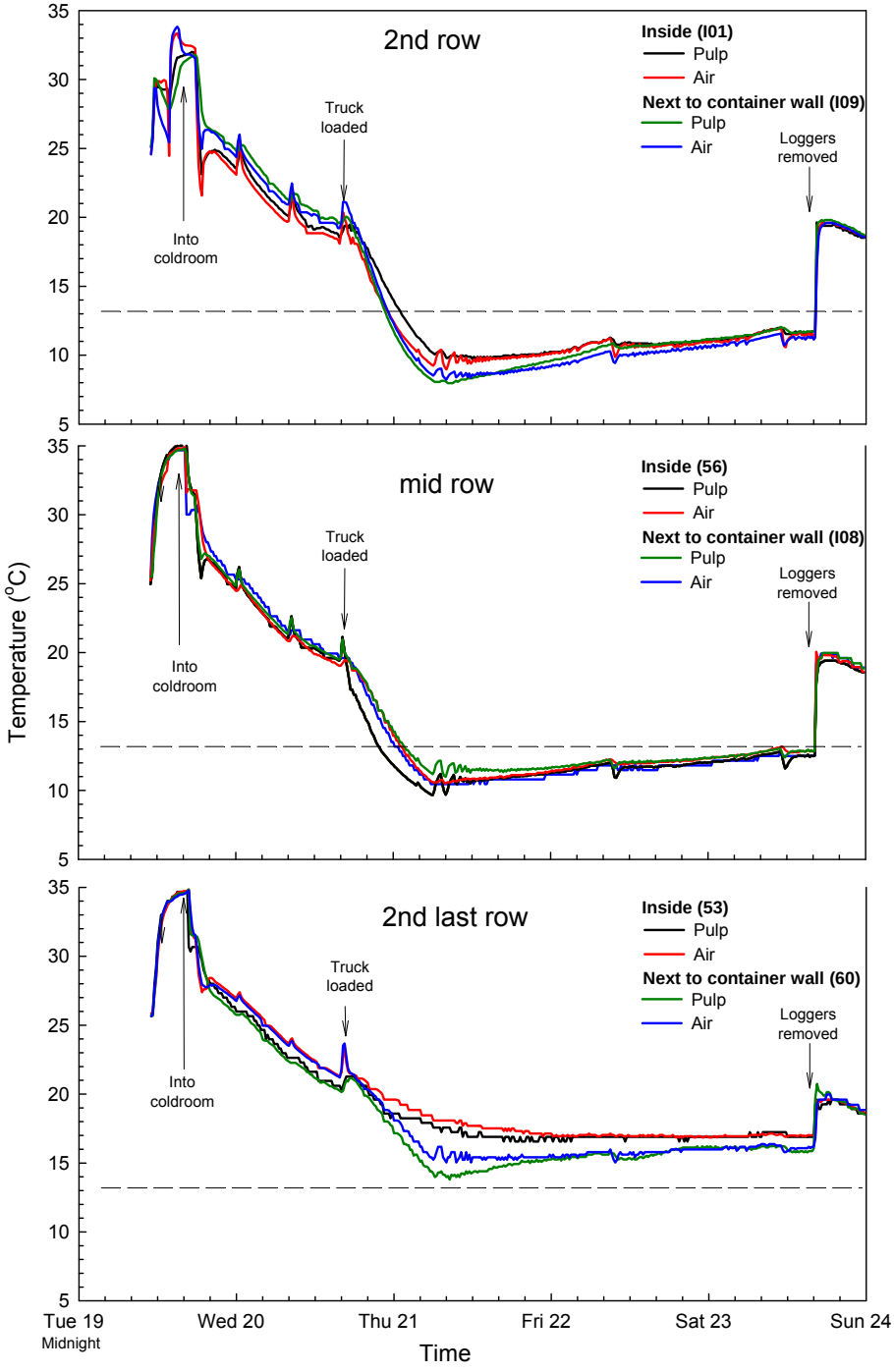


Figure 66 Load 2: 13°C container set temperature and 21°C average pulp temperature on loading. Pulp and air temperatures in a pallet second row from the front, middle and second row from the back of a 45ft refrigerated container. Loggers were placed in fruit near the face of the pallet adjacent to the other pallet in the same row (inside), and in fruit close to the container wall (next to container wall). The dashed line represents container set temperature.

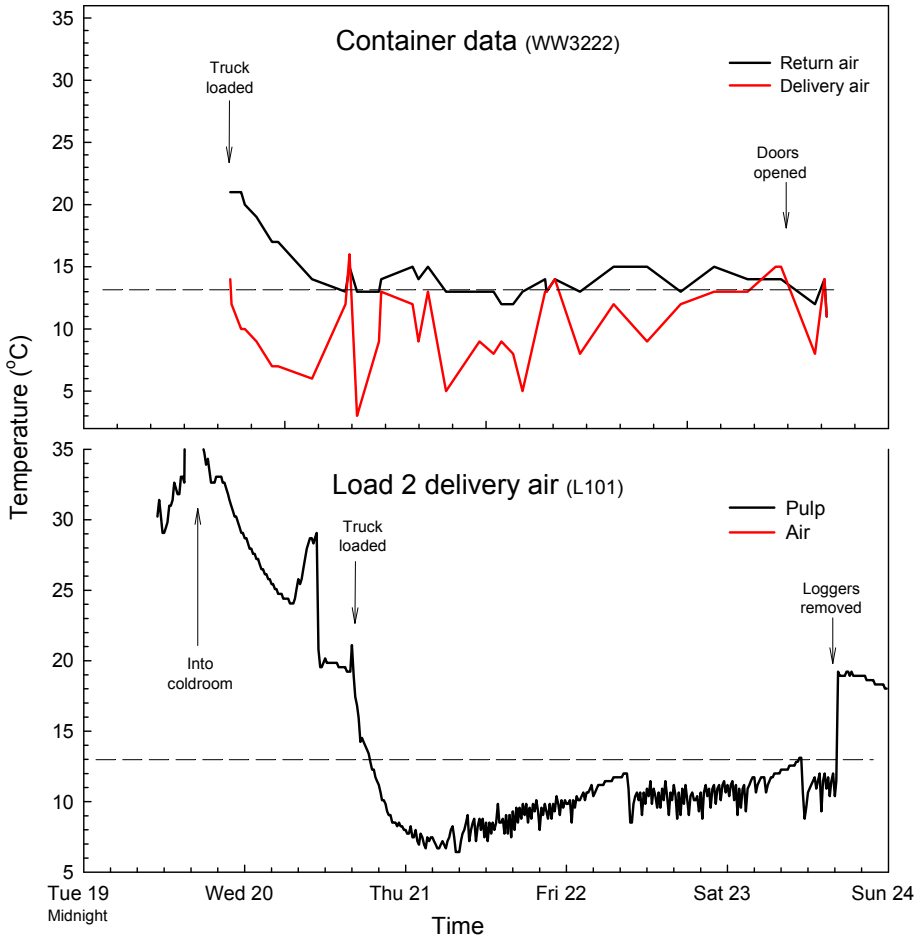


Figure 67 Load 2: 13°C container set temperature and 21°C average pulp temperature on loading. Delivery and return air temperature from the StarTrak logger attached to the container (top), and delivery temperature logged by a temperature logger on the top of the pallet nearest the refrigeration unit (bottom).

Load 3: 13°C set, 21.5°C pulp - gap at front of container

The container was loaded with 18 pallets, and with a 1 m gap between the refrigeration unit at the front of the container and the first row of pallets. The container reduced the average pulp temperature from 21 to 14ºC over the 3.5 d journey (Figure 68). However, fruit near the front of the container were up to 10°C cooler than those near the back, and those closer to the walls of the container were 5-9ºC cooler than those in the middle of the row, especially in rows near the back of the container.

The exact causes for the differing container performance between loads two and three are unclear, but it is likely that loading the container with a gap between the refrigeration unit and the first row was a significant factor. Air flow takes the path of least resistance between the delivery and return vents, so the gap would allow easy flow from the delivery to the return vent and significantly reduce cold air movement toward the back of the container.

Load 4: 18°C set, 21°C pulp - ripening fruit

Similar patterns were observed as with previous loads (Figure 69). These included:

The container again successfully reduced pulp temperatures to close to set temperature. Temperatures were maintained close to the set point in the front and middle of the container during most of the journey. However temperatures toward the rear increased during the last day, presumably because of the heat generated by respiration, and reduced cooling capacity near the back of the container. As a result pulp temperatures were very close to the set temperature toward the front and middle of the container, but 1-4°C above set temperature toward the back.

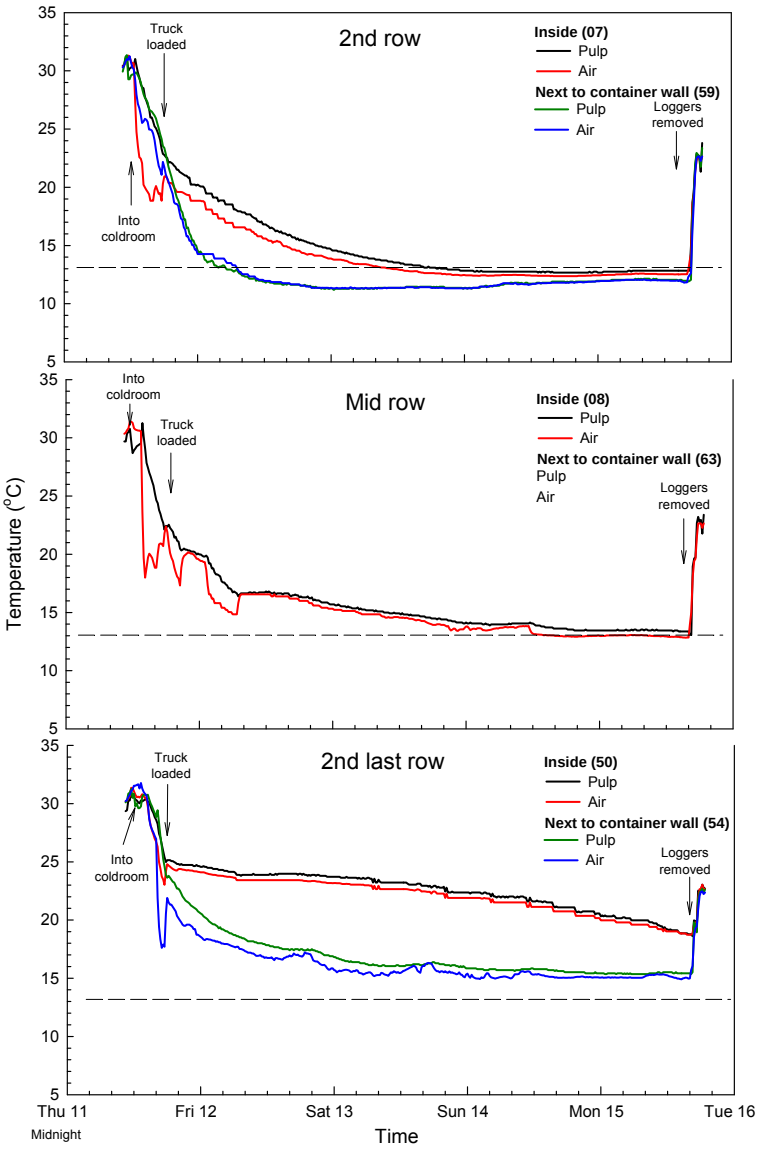


Figure 68 Load 3; 13°C container set temperature and 21°C average pulp temperature on loading. Pulp and air temperatures in a pallet second row from the front, middle and second row from the back of a 45ft refrigerated container. Loggers were placed in fruit near the face of the pallet adjacent to the other pallet in the same row (inside), and in fruit close to the container wall (next to container wall). The dashed line represents container set temperature. The container was loaded with a gap between the front of the container and the first row of pallets.

Decreasing the set temperature to 13°C toward the end of the journey rapidly reduced pulp temperatures, again illustrating the capacity of these containers to remove heat from the fruit.

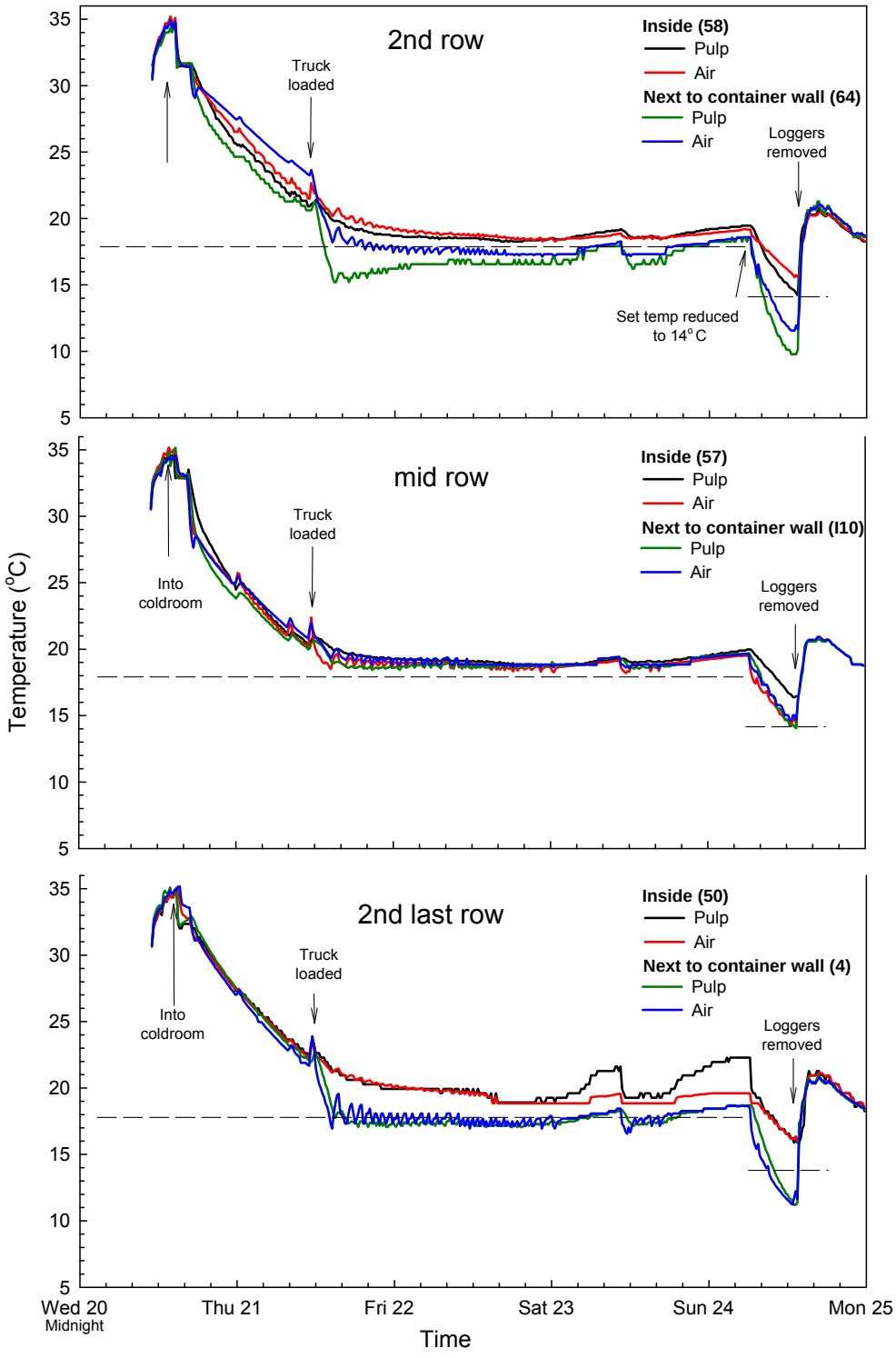


Figure 69 Load 4: 18°C container set temperature and 21°C average pulp temperature on loading. Pulp and air temperatures in a pallet second row from the front, middle and second row from the back of a 45ft refrigerated container. Loggers were placed in fruit near the face of the pallet adjacent to the other pallet in the same row (inside), and in fruit close to the container wall (next to container wall). The dashed line represents container set temperature. The container was set at 18°C, then reduced to 13°C 10 h before door opening.

Load 5: 16°C set, 25°C pulp

The fruit were loaded with higher pulp temperature because of forced air cooling else malfunction on farm. Previous loads indicated the capacity of containers to reduce pulp temperatures of warmer fruit, but the results here indicate either a container refrigeration malfunction, or the set temperature was too high (Figure 70).

9.1.4.2. Carbon dioxide

Some measurement of CO_2 concentrations on arrival were tempted using Kitagawa tubes. However, container doors were usually opened at the railhead to retrieve consignment paperwork. Only load five doors had not been opened, and 15.5% CO2 was recorded. This was likely partly due to the high arrival temperatures (approximately 25°C).

In the absence of venting or hydrated lime, CO_2 concentrations increased consistently over 2.5 d (Figure 71). The decreasing concentration thereafter was largely a result of fruit pulp temperatures decreasing from about 18 to 14°C (Figure 65). This illustrates the significant effect of fruit temperature of CO_2 production, but also the difficulty of running commercial trials on CO_2 management because of variable production rates and container performance.

The venting option on the refrigeration unit in load 7 maintained CO_2 concentrations below about 2%. The combination of venting and 200 kg of lime (load 6) maintained CO_2 below about 1%, although slightly lower recorded pulp temperatures may have contributed to lower CO_2 production in load 6.

Similar results were obtained with 200 kg lime in loads 10 and 11 (Figure 72).

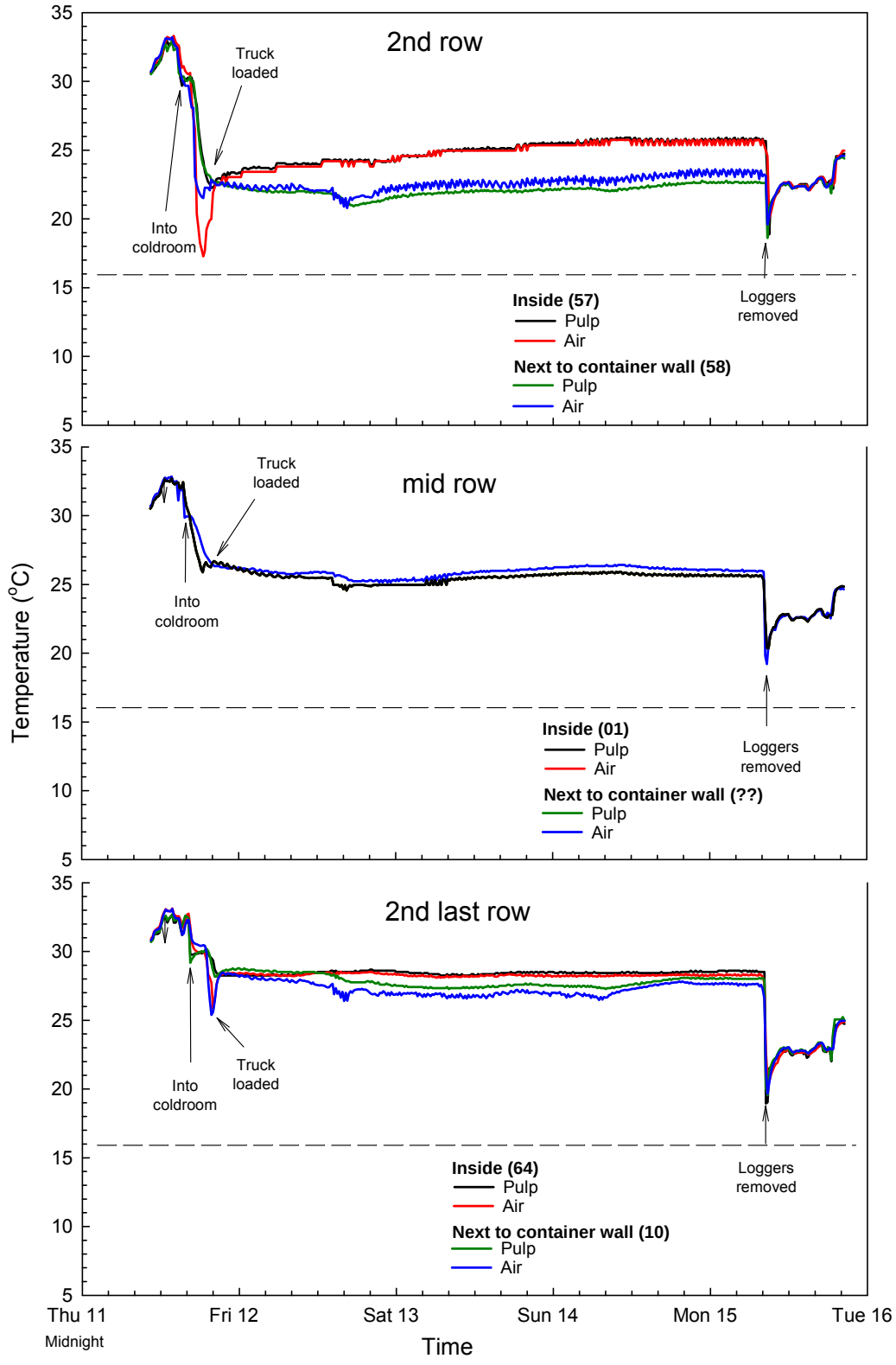


Figure 70 Load 5: 16°C container set temperature and 25°C average pulp temperature on loading. Pulp and air temperatures in a pallet second row from the front, middle and second row from the back of a 45ft refrigerated container. Loggers were placed in fruit near the face of the pallet adjacent to the other pallet in the same row (inside), and in fruit close to the container wall (next to container wall). The dashed line represents container set temperature.

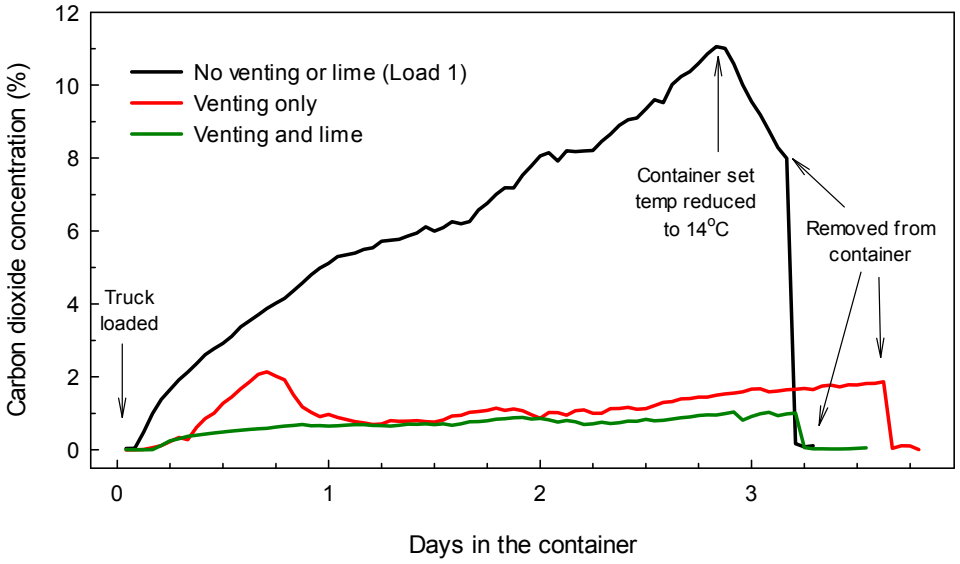


Figure 71 Loads 4, 6 and 7. Effect of container venting and hydrated lime on carbon dioxide concentrations in 45 foot refrigerated containers during rail transport of 'B74' mango from Darwin or Katherine to Adelaide. Load 1: container set at 18°C then reduced to 13°C about 10 h before door opening. Venting only and venting with lime: container set at 18ºC and pulp temperature about 18ºC. The fresh air exchange valve was open on the refrigeration units for venting, and 180 kg of hydrated lime in 36 paper bags placed on top of the 18 pallets for the lime treatment.

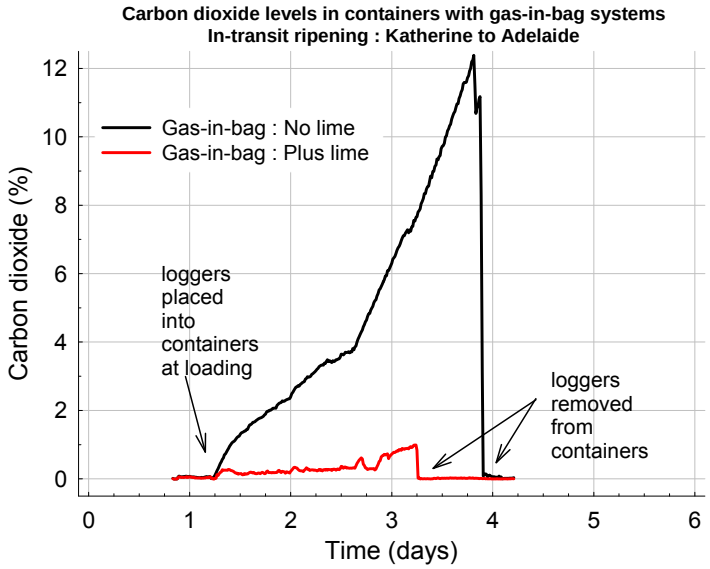


Figure 72. Loads 10 and 11 First consignments, bagged ethylene, treatment 4 (no lime) and treatment 5 (with 200 kg lime). Changes in carbon dioxide concentrations inside the rail container.

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Load 15 CO_2 concentrations increased to almost 20% in the middle of the load over 3 d (Figure 73). Fruit temperatures increased to about 24ºC in treatment 4 (data not shown) which would increase respiration rate and CO_2 production by the fruit. Previous trials have indicated that 'B74' fruit can tolerate CO_2 concentrations of more than 10% during 6 d of ripening, but the effects of almost 20% is not known. These high concentrations represent a significant safety issue because concentrations of above about 4% can be fatal.

Carbon dioxide concentrations in Load 16 were maintained below 3% over the 2 d (Figure 73). The hydrated lime would have absorbed some of the CO_2 produced, but the lower fruit temperatures compared with treatment Load 14 and therefore the lower CO_2 production, helped maintain low CO_2 concentrations. There were also two less pallets in this treatment compared with Load 15.

These results suggest that 100 kg of lime per 20 pallets may be sufficient to maintain CO_2 concentrations below the 3-4% assuming fruit temperatures are held at or below 18°C. However, further replication of this treatment is required to address variability in fruit respiration rate and container performance.

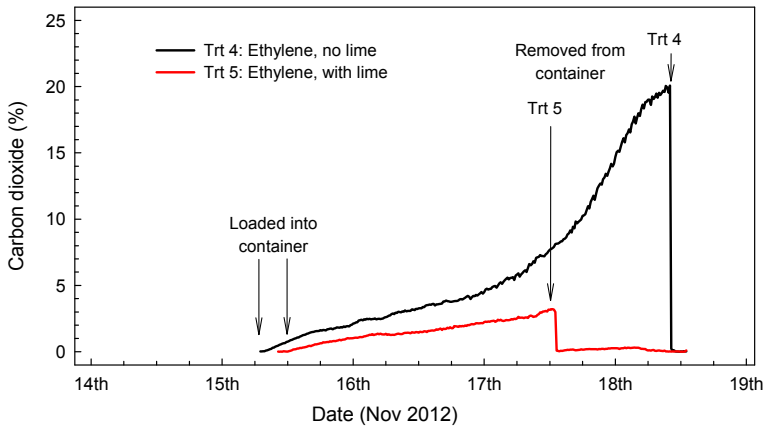

Figure 73 Loads 15 and 16 Carbon dioxide concentrations in rail containers containing 'B74' mango fruit and ethylene-releasing bags. Treatment 4 had no lime in the container and fruit pulp temperatures reached about 24ºC at arrival. Treatment 5 had 90 kg of lime (10 kg on each of 9 pallets), and recorded pulp temperatures did not exceed 18ºC.

9.1.4.3. Ethylene

In the first y of testing, the measured ethylene concentrations in the containers were variable and this suggests inconsistency in performance of the new ethylene loggers. Despite this, with the ethylene powder release system, ethylene concentrations recorded at the top of the pallet in the front of the container increased slowly over 1.5 d to about 35 ppm then decreased to about 20 ppm over the next 3 d (Figure 74). Ethylene concentrations recorded by a logger within the load were lower and may reflect relatively poor distribution of ethylene. Ethylene gas release from the 35 µm plastic bags resulted in concentrations exceeding the measuring range of the loggers but then decreasing to about 50 ppm after 3 d.

These results suggest that the ethylene powder concept has significant commercial potential, while 50-100 µm plastic bags should be tested to reduce ethylene release rate and the risk of bag puncturing during filling and installation.

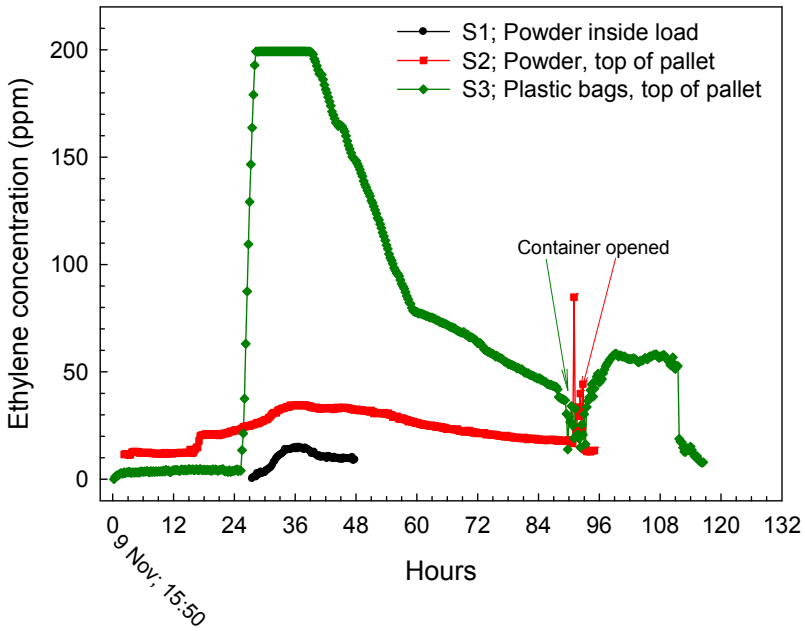

Figure 74. Loads 8-10. Ethylene concentrations that accumulated within mango containers during the first rail consignments. Ethylene was released directly from an encapsulated powder or from Ripegas trapped within semi-permeable PVC bags.

Further tests using six ethylene bags of 50 µm thickness showed increasing ethylene concentrations to about 40 ppm within about 1.5 d and maintained the concentration above 20 ppm for about 4 d (Figure 75).

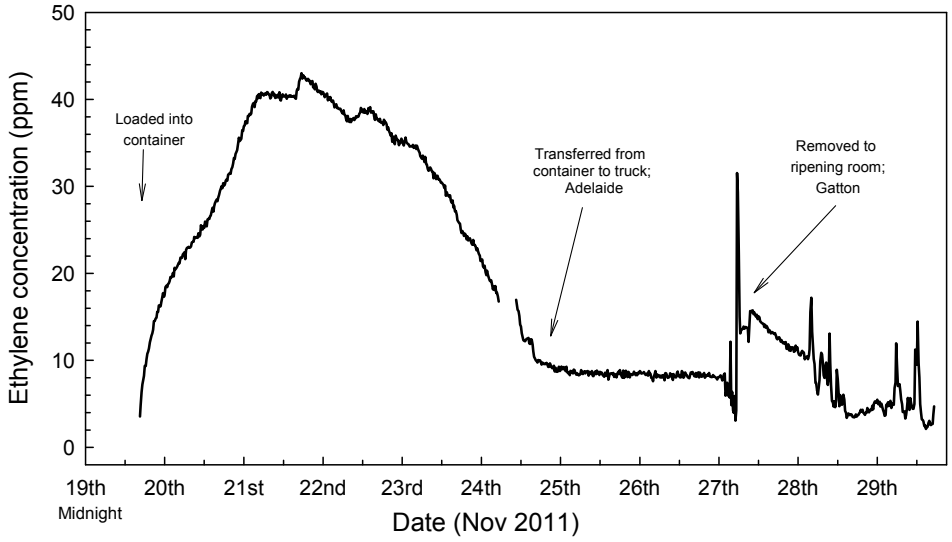

Figure 75. Load 12. Ethylene concentrations in the container containing PVC bags with Ripegas, and with lime.

Ethylene concentrations should be maintained at about 10 µL.L^{-1} for consistent ripening. However, concentrations between 5 and about 50 µL.L^{-1} will still produce satisfactory ripening responses.

2012/13. The ethylene powder increased ethylene concentrations in the middle of the load to about 5 and 10 µL.L^{-1} within about 18 h of loading (Figure 74). Treatment 2 concentrations dropped to about 2 µL.L^{-1} at removal about 2 d later, while concentrations in the treatment 3 container were maintained at about 10 µL.L^{-1}. The difference between the containers could be because of leaky door seals or joints in the treatment 2 container resulting in leakage of ethylene during transit. It could also reflect less efficient air movement in the container because the ethylene loggers were placed in the 4th row from the top of the pallet in the middle of the container.

The 10 ethylene bags in the treatment 4 container rapidly increased concentrations to over 10 µL.L-1 within 12 h, then to about 40 µL.L-1 after 3 d. This is well within the desirable range.

The Kitagawa test suggested ethylene concentrations of about 5 µL.L-1 for treatment 3 and between 5-50 µL.L-1 for treatment 4. Kitagawa measurements are less accurate, but confirm the ethylene logger results.

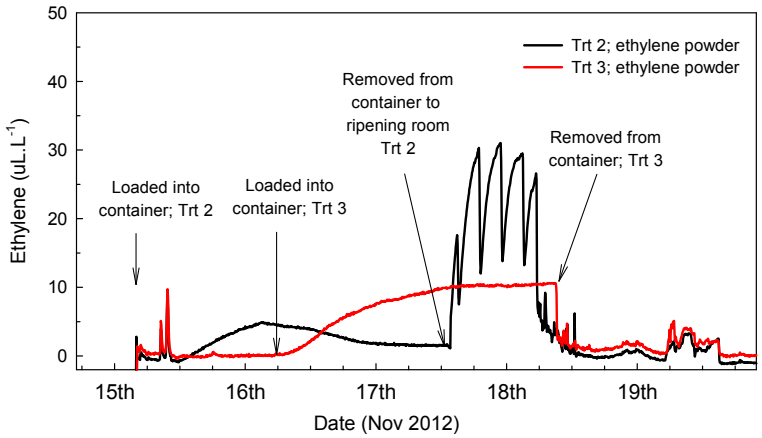

Figure 76 Loads 13 and 14. Ethylene concentrations measured in rail containers with 'B74' mango during transport from Katherine to Adelaide. Ethylene was released directly from an encapsulated ethylene powder.

9.1.4.4. Fruit quality

The fruit treated with ethylene reached full yellow colour more rapidly than the control fruit as expected, but there was no difference in fruit response to the ethylene powder and bag treatments (Table 102). The fruit treated with encapsulated ethylene had lower Brix and higher acidity at full yellow compared with untreated fruit. Among the ethylene treatments, there was little difference in Brix, while fruit treated with ethylene bags + lime had lower acidity than the other treatments. Those differences could have some impact on flavour and should be further investigated. Firmness and lenticel spotting severity at full yellow were not affected by any of the treatments.

These results illustrate that the desired effects of triggering ripening in transit were achieved, although in one other instance there was no significant ethylene effect (data not shown).

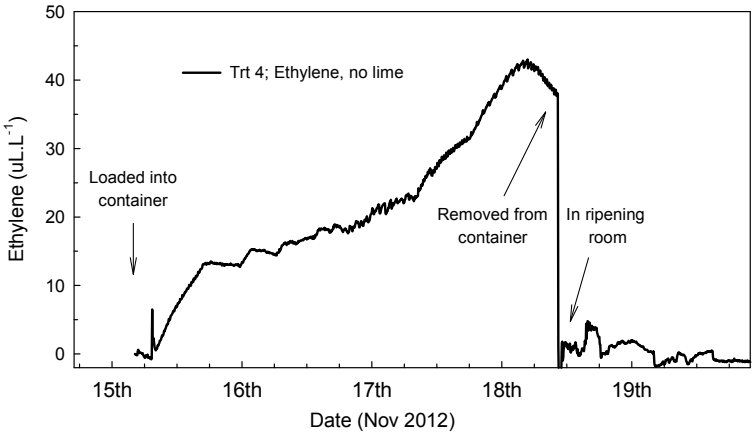

Figure 77 Load 15. Ethylene concentrations measured in rail containers with 'B74' mango during transport from Katherine to Adelaide. Ethylene was released from 10 PVC plastic bags (50 µm thickness) taped to the top of each of 10 pallets in the container.

Table 102 Effect of in-transit ethylene and hydrated lime treatment on 'B74' fruit ripening and quality. Fruit firmness, lenticel spotting severity, brix and acidity were rated when fruit reached full yellow colour.

Treatment	Days to full yellow	Firmness (0-4)	Lenticel spot. (0-5)	Brix (°)	Acidity (%)
No ethylene	6.8 [a]	2.3	0.6	14.1 [a]	0.17 [b]
Ethylene bag	3.1 [b]	2.4	0.7	13.6 [ab]	0.26 [a]
Ethylene bag + lime	2.0 [b]	2.3	1.0	13.4 [abc]	0.17 [b]
Ethylene powder Container1	3.4 [b]	2.8	0.8	12.6 [bc]	0.23 [ab]
Ethylene powder Container 2	2.4 [b]	2.6	0.6	12.5 [c]	0.29 [a]

Means in columns with the same letters are not significantly different (P=0.05) as tested by LSD. The absence of letters indicates no significant differences.

9.1.5. Conclusions and recommendations

- Rail containers can hold fruit temperatures around the set temperature of about 18ºC as long as the container is functioning properly and the pallets are loaded properly. Pallet loading configuration in the container can affect fruit temperatures. For example, a container with a 1 m gap between the front of the container and the first pallet cooled the fruit more slowly and had a greater temperature difference between the front and rear fruit, compared with a similar container with no gap between the front of the container and the first pallets. The front gap likely allowed cold air short-circuiting and less cold air going to the back of the container.
- Delivery air temperatures were as low as 6°C during the early stages of one load when 21°C fruit were placed into a 13°C set container. This is more likely if the pallets are not loaded correctly and can result in chilling of the top fruit in the front pallets.
- Both ethylene release systems showed strong potential to provide 5-40 µL.L-1 (parts per million; ppm) ethylene for at least 2 d. Concentrations can be increased by adding more powder or more bags.
- Leakage rate from, and air flow in the container will affect ethylene concentrations and consistency across the load.

- Opening the refrigeration unit fresh air exchange valve maintained CO_2 concentrations below 2%, and adding 10 kg of hydrated lime per pallet in a vented container maintained concentrations below 1%. Further trials indicated that 5 kg per container may also be effective but further testing is required.
- Higher fruit pulp temperatures have a large effect on accumulated CO_2 concentrations in the container. One of the containers had almost 20% CO_2 on arrival, which poses a significant workplace health and safety (WH&S) risk.
- The transport treatments (ethylene and CO_2 removal) did not affect external fruit quality or ºBrix, acidity, or flavour.

9.2. High CO_2 concentrations during ripening

Peter Hofman, Roberto Marques

9.2.1. Summary

Mango fruit produce carbon dioxide (CO_2) during ripening which can accumulate to unacceptable concentrations with inadequate venting. This trial investigated the sensitivity of 'B74' mango to 0-12% CO_2 during ripening at 20ºC to provide guidelines on preferred and tolerated CO_2 concentrations. 'B74' mango fruit were held in 0-12% CO_2 for 3-6 d at 20°C, then assessed at removal and again at eating soft after further ripening at 20°C. The results indicated that 'B74' fruit are surprisingly tolerant to high CO_2 concentrations, with only transient effects of delayed softening and green colour loss after 3-6 d, but no significant differences on days to eating soft. There was also little difference in flavour at eating soft, and no detectable off-flavours. Based on these results, preferred maximum CO_2 concentrations during in transit ripening is primarily a workplace health and safety rather than fruit quality issue, and should not exceed 3%.

9.2.2. Introduction

Most climacteric fruit have optimum ranges for temperature, ethylene and carbon dioxide (CO_2) conditions, and deviations from these optima can delay ripening changes or reduce quality of the ripened product. Mango fruit produce CO_2 during ripening, which can accumulate with inadequate venting of the ripening chamber and affect ripening. For example, CO_2 concentrations can reach more the 10% in 40ft rail containers with 'B74' mango at the end of the 3-4 d journey from the Northern Territory to Adelaide. Mitigation measures are possible but it is important to understand fruit CO_2 tolerances in order to determine the commercial risk and the maximum CO_2 concentration that mitigation measures need to target.

Research with 'Kensington Pride' indicated that fruit exposed to more that 3% CO_2 for longer than 3 d at 20ºC can have more disease, and more green skin colour at ripe (Nguyen, 2003), compared with fruit ripened in ambient CO_2 concentrations. It is unknown whether similar effects occur with 'B74'. This trial ripened 'B74' mango in chambers controlled to maintain 2-12% CO_2 and 20ºC; additional fruit were ripened at 20°C under ambient CO_2 conditions. The fruit were held under these conditions for 3-6 d, and the quality assessed at removal and at eating soft.

9.2.3. Materials and methods

9.2.3.1. Fruit

'B74' mango were commercially grown and harvested from one farm in Childers on 31st Jan 2012 half way through commercial harvest. The fruit were from two different production blocks on the one farm (replication 1 on sandy soil and replication 2 on red soil), and from another farm (red soil), to provide three replications. Ten premium grade trays (18-20 fruit per tray) from each block were obtained from the end of the packing line the following day (picked one day and packed the next), representing one tray per treatment per replication.

The fruit were transported to the laboratory at the Maroochy Research Facility within 5 h of packing, placed at 12ºC for 24 h, then at 18ºC overnight to allow the fruit to warm before treatment the following morning. Additional sound, reject grade fruit were used to provide a total of 24 trays per treatment chamber.

9.2.3.2. Treatments

The following treatments were applied at a pulp temperature of 20ºC:

- Control: fruit ripened at 20°C, no ethylene
- 2% CO_2: CO_2 concentration increased to 2% over about 6 h then held
- 5% CO_2: CO_2 concentration increased to 5% over about 2 d
- 9.5% CO_2: CO_2 concentration increased to 9.5% over about 2 d
- 12% CO_2: CO_2 concentration increasing to 9.5% over about 2 d, then controlled between 10-15% (average of 12%).

One tray per treatment replication was removed at 3 and 6 d, then ripened without ethylene at 20ºC.

The treatment system was designed to mimic conditions inside a rail container. Each fruit chamber was an 0.9 x 0.9 x .1.2 m aluminium frame with high density polyethylene or aluminium sides. Sufficient fruit were placed in the chamber to fill about 80% of the capacity, similar to that of a full rail container. The datum fruit were placed in the 3rd of four tray layers in the chamber, with the treatment trays randomly placed within this layer. The containers were well sealed to allow accumulation of CO_2 and fruit volatiles released during ripening. Additional CO_2 was injected from pressurised cylinders when concentrations fell below target based on regular automated measurements through a PP Systems infrared gas analyser (measuring range 0-10% CO_2). For the12 % CO_2 treatment, when measured concentration fell below 9.5% excess CO_2 was injected into the chamber to provide about 15% CO_2. Continual leakage and injection resulted in an average of about 12% CO_2 over the treatment period. Relative humidity in the chambers was not controlled but was measured using Vaisala HMP50 humidity probes. Air circulation was achieved with two 15 cm muffle fans inside each chamber, placed over a “chimney” in the centre of the rows of trays.

9.2.3.3. Quality assessment

Fruit quality was assessed after 3 and 6 d under CO_2, then again at eating soft represented by a hand firmness of three. Skin colour was assessed as described in section 4.1.3.3. The average skin colour was quantified by averaging the readings from the non-blush area on two opposite sides of the fruit using a Minolta colourmeter (model number and specifications), and using L value, chroma and hue angle (Hº). Fruit firmness was also quantified by averaging the readings from the using the Aweta Acoustic Firmness tester (Aweta, Nootdorp, the Netherlands) on two opposite sides of the fruit.

The fruit were assessed by hand to determine the days to eating soft. When at least 80% of the fruit in each tray had reached eating soft, each fruit in the replication was assessed for skin colour (visual and with the Minolta colourmeter), skin browning, lenticel damage and diseases on the 0-5 scale (Hofman *et al.*, 2010a). No indication of unique CO_2 damage was observed.

At eating soft, the most firm and most soft fruit (usually one of each) were removed. One cheek from each of the remaining fruit was cut, the flesh removed, diced, and pooled. A portion was used for sensory analysis (flavour), which was assessed over three days using a tasting panel of 7 staff at MRF based on a hedonic rating scale (1=dislike extremely and 9=like extremely); a rating of 5.5 was considered to indicate an acceptable eating quality. One composite sample per treatment per rep (3 tastings per treatment) were used.

The remainder of the pooled samples were frozen at -20°C. Within two months, the samples were thawed and juice samples taken to determine ºBrix (using an Atago bench refractometer) and titratable acidity (using a Metrohm Titrino autotitrater, with the results expressed as % citric acid).

9.2.3.4. Statistical analysis

Statistical analyses were performed by Genstat® 11 for Windows™ (VSN International Ltd., UK). Analysis of variance used the 'General Analysis of Variance' model, with a factorial design (CO_2 concentration by exposure time) as 'treatments' structure, and farm/tray/fruit (or farm/tray/taster for flavour) as 'block' structure. Trays were considered as the experimental units, with three replications (farms) per treatment. The chamber treatment was not replicated but conditions were well maintained and monitored. The protected least significant difference (LSD) procedure at P = 0.05 was used to test for differences between treatment means.

9.2.4. Results and discussion

9.2.4.1. Chamber conditions

Figure 78 illustrates the CO_2 conditions maintained in the chambers during the trial. Pulp temperatures were maintained between 19.9-20.1ºC in the chambers, and relative humidity was between 85-90% (data not shown).

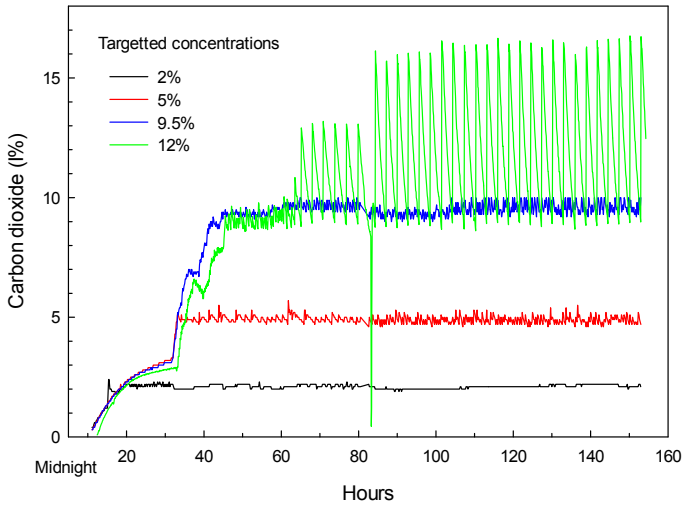

Figure 78 Carbon dioxide concentrations maintained in the treatment chambers containing 'B74' mango fruit. Target concentrations were 2, 5, 9.5 and average 12%. The sharp drop at about 85 h was to remove fruit after 3 d.

9.2.4.2. Fruit quality

At removal

Carbon dioxide treatment retarded the loss of green colour compared with control, with greater treatment effects after 6 d exposure (Figure 79). This was confirmed by the higher Hº of the skin after 6 d (Table 103). Similar treatment effects were observed for firmness measured both by hand (Figure 79) and Aweta (Table 103), suggesting that CO_2 had similar effects on these two ripening parameters.

Non-replicated tasting of samples on removal after 6 d did not suggest any unusual off-flavours.

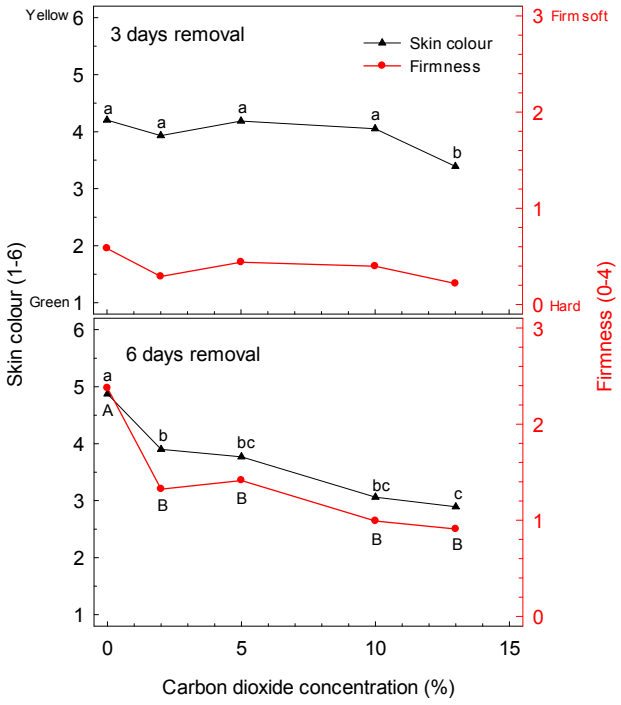

Figure 79 Skin colour (1-6) and firmness (0-4) of 'B74' mango held at 20°C in 0-12% carbon dioxide for 3-6 d. The fruit were assessed within 4 h of removal. Means with different letters for the same parameter are significantly different at LSD (P=0.05).

Table 103 Average skin colour based on hue angle (H°; lower values are more yellow than green) on two opposite sides of the non-blush areas of the skin, and firmness based on Aweta acoustic firmness (approx. 50= firm; 15-20 = eating soft) of 'B74' mango held at 20°C in 0-12% carbon dioxide for 3-6 d. The fruit were assessed within 4 h of removal.

CO_2 (%)	Skin colour (H^o)	Firmness (Aweta)
3 days removal		
0	106.9	35.6 b
2	100.1	47.3 a
5	107.9	44.0 a
9.5	103.0	45.1 a
12	105.8	46.1 a
6 days removal		
0	93.4 c	24.7 c
2	95.8 bc	33.0 ab
5	97.0 abc	31.4 b
9.5	100.9 a	36.9 a
12	100.3 ab	35.3 ab

Means with different letters for the same parameter are significantly different at LSD=0.05.

At eating soft

There were no significant treatment effects on days to eating soft, or skin colour, firmness, lenticel damage or ºBrix at eating soft. Holding the fruit in 2-12% CO_2 (irrespective of duration) reduced skin browning slightly and increased acidity and flavour of the ripe fruit

compared with ripening at ambient CO_2 concentrations. It is likely the increased acidity contributed to the improved flavour, but this effect is probably transient because of loss of acidity that can occur with further holding.

Table 104 The effect of holding 'B74' mango fruit in 2-15% CO_2 (irrespective of three or six days duration) on skin browning (0-5), titratable acidity (%) and flavour (1=like extremely: 9=like extremely) at eating soft.

Holding in carbon dioxide	Skin browning	Flesh acidity	Flavour
No	1.3 [a]	0.1 [a]	5.4 [b]
Yes	0.7 [b]	0.2 [b]	6.0 [a]

Means with different letters for the same parameter are significantly different at LSD=0.05.

These results indicate that 'B74' mango is surprisingly tolerant of high CO_2, even when held for 6 d.

10. Technology transfer

10.1. Pre- and post season meetings and training

Regular planning meetings were held before each season where potential improvements to practices were discussed. Post season meetings discussed the commercial impacts of the R&D of the past season, and how future practices can be improved.

Several training workshops were provided before the start of the seasons on 2011, 12 and 13. The workshops were aimed at improving understanding of mango fruit physiology and ripening practices. The subject areas included mango physiology, what can reduce saleability, ripening practices and ripeness indicators, ripening systems, and information tools. Key staff from One Harvest, and their agents/ripeners in the major capital cities, were usually present.

Regular farm visits by One Harvest personnel and project staff provided updates to growers, especially just before and during the season. Regular and rapid feedback on ripener receival and post ripening assessments for quality and maturity helped identify and rectify practices reducing quality.

10.2. Publications and conference presentations

Books and book chapters

Ledger, S., Barker, L., Hofman, P., Campbell, J., Jones, V., Holmes, R., Campbell, T. and Weinert, M. (2012) *Mango ripening manual*. Department of Agriculture, Fisheries and Forestry, Brisbane, Australia.

Hofman, P., Holmes, R. and Barker, L. (2013) *B74 mango quality assessment manual; 2nd edn.* Department of Agriculture, Fisheries and Forestry (Queensland), Nambour.

Popular articles

Hofman, P.J. and Jordan, R.A. 2011. Transporting mangoes by road freight, temperature and carbon dioxide management. Mango Matters May 2011:14-19.

Hofman, P.J., Macnish, A.J., Ho, B., Marques, J., Bhandari, B. and Joyce, D. (2013) Ripening mangoes during transport. *Mango Matters* 13, 28-33.

Marques, R., Nguyen, M., Hofman, P. and Joyce, D. (2013) Do late-harvested mangoes develop more lenticel damage after harvest? *Mango Matters* 13, 29-31.

Conference presentations

Subedi, P, Walsh, K. and Hofman, P. 2010. Determination of optimum maturity stages of mangoes using spectral-optical signatures. IX International Mango Symposium, Sanya, Hainan, China, 8-12 April, 2010.

Hofman, P. and Jordan, R. 2011. Road/rail freight of mango-temperatures and carbon dioxide management. 8th Australian Mango Conference, Darwin, Australia, 18-21 May 2011.

Hofman, P., Marques, R. and Joyce, D. 2011. Lenticels-what are they and what causes lenticel spotting? 8th Australian Mango Conference, Darwin Australia, 18-21 May, 2011.

Joyce, D., Hofman, P., Marques, R., and Nguyen, M. 2011. Topical Harvest and Postharvest Issues for Fresh Fruit and Vegetables: A case study of lenticel discolouration on Calypso™ mango fruit. The 9th International Conference of Food Science and Technology, 27-29 May, 2011, Hangzhou, China (Invited keynote address & abstract; repeated by request at Zhejiang Gongshang University and at Zhejiang Academy of Agricultural Sciences).

Joyce, D.C., Hofman, P., Marques, R., Nguyen, T.M., Gupta, M.L. 2011. Lenticel Damage on 'Calypso' Mango. AuSHS and the Australasian Postharvest Conference, Lorne, Australia, 18-22 September, 2011.
Hofman, P.J., Marques, J.R., Macnish, A.J., and Joyce, D.C. 2012. Interaction between production characteristics and postharvest performance and practices for fresh fruit. 7th International Postharvest Symposium, Kuala Lumpur, Malaysia, 25-29 June, 2012.
Ho, B.T., Bhandari, B.R., Hofman, P., and Joyce, D.C. 2012. Ethylene gas from an ethylene-α-cyclodextrin inclusion complex powder ripens 'Calypso' mango fruit. 7th International Postharvest Symposium, Kuala Lumpur, Malaysia, 25-29 June, 2012.
Hofman, P.J., Ledger, S., Macnish, A., Marques, J., Ho, B., Bhandari, B. and Joyce, D. (2013) Critical success factors in transporting mango fruit. 9th Australian Mango Conference. Cairns, Australia, 14-17 May 2013.
Hofman, P., Joyce, D., Macnish, A., Marques, J., Nguyen, M., Li, G. and Gupta, M. (2013) Lenticel damage - why do we get damage and can we minimise it? 9th Australian Mango Conference. Cairns, Australia, 14-17 May 2013.

10.3. Quality assessment and best practice manual

The 'B74' quality assessment manual was revised and the 2nd edition published and distributed to ripeners and retailers. The best practice manual was also revised based on the results of the current project. The manual will be distributed to the 'B74' growers and product handlers.

11. Recommendations

The following are recommendations to further develop the 'B74' supply chain to best service domestic and export markets. While the recommendations have specific benefits for 'B74', some of the experiences and principles developed during this work will have wider benefit to the whole mango industry and other horticulture industries, and these benefits would be made publicly available for wider uptake.

Lenticel discolouration.

Identifying practical solutions to reducing LD has been difficult. The present results confirm strong genetic influences suggesting that high lenticel density is a key factor in LD sensitivity after harvest. However it is possible that the smaller lenticel aperture diameter of 'B74' makes it more resistant to the field-derived lenticel damage that can result in larger, cracking lenticels. Cultivar selection programs could use lenticel density and structure characteristics as a guide to identifying LD resistant cultivars.

Past commercial, present project and overseas experience suggests the following approaches will reduce the risk of LD:

- Fruit from younger trees that have a condensed flowering time and have good graft compatibility
- Orchards grown in the hotter regions, with less rain near harvest
- Minimum exposure to water after harvest
- Smaller fruit and more mature (not over mature) fruit
- Bagging fruit on the tree for high value markets
- Possibly applying wax sprays to the frit several days before harvest.

Minimising exposure to water during and after harvest provides significant commercial challenges because of the key role of desapping solutions during harvest. However, careful hand harvested direct into small trays in the field will provide a very attractive gift pack that would likely be profitable for high value markets. Radical re-thinking of the whole harvest to

packing process may find other cost-effective solutions for “no water” systems for specific markets.

More research is warranted on the commercial feasibility of surface coating sprays, but on an experimental basis to start with.

Irradiation

The only practical solution identified to reduce irradiation effects on ‘B74’, and likely ‘Kensington Pride’ and ‘R2E2’, is to irradiate the fruit at colour stage 4-5 (2-4 d from ripe). This almost eliminates the increased LD and retarded loss of green colour associated with irradiation. It provides challenges with the supply chain but may have application to airfreight and short time seafreight to export markets. Commercial testing is warranted.

The practices to reduce LD will also assist in reducing LD after irradiation, but will have little impact on the slowing of green colour loss.

More accurate irradiation application technologies such as electron beam systems may allow doses closer to the preferred minimum of 400 Gy while still ensuring all fruit receive the minimum required dose. Evaluation of these new systems would be warranted.

Harvest window

Research on Ethephon rates to induce earlier flowering in the hot tropics could be undertaken when commercial supplies are guaranteed

Delivering fruit directly from the farm to the retailer.

Project results indicated strong commercial potential for in transit ripening to significantly reduce on-farm and at market infrastructure and energy costs, while managing the risks associated with poor ripening conditions during transit. Further commercial development of temperature, carbon dioxide, and ethylene monitoring and control strategies are warranted, combined with understanding fruit ripening performance across region/maturity/season in order to predict ripening performance. This technology could have significant benefits to other climacteric fruit chains such as banana.

Flavour

Flavour is still a limitation with ‘B74’, particularly when grown in cooler regions because of the smaller harvest window and reduced potential to harvest the fruit later without the risk of fruit drop. Most of the maturity and flavour work has been based on sugars (Brix) and acidity. Aroma compounds are also significant contributors to flavour, but were not included in these studies because previous research indicated that fruit maturity at harvest is a major determinant of flavour through sugars concentration (Brix). There is little understanding on the relative contributions to flavour from aroma compounds, sugars and acidity, and whether flavour can be improved by manipulating aroma compounds in a mango cultivar.

Consumer preferences

There is little detailed understanding of consumer preferences/tolerance to fruit appearance. For example, what are their tolerances to LD and at what severity is value reduced (the price they will pay)? Also, will they have more tolerance to LD if the fruit is well blushed? There are no clear standards for maximum severity of skin defect to guide R&D programs.

Supply chain interaction

Better interaction with all members of the ‘B74’ chain would improve cohesion and operation. An annual ‘B74’ chain meeting with growers, transporters, ripeners and retailers would improve relationships, identify opportunities for improvements, and develop effective strategies that provide benefit of all members of the chain.

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13. Appendix 1

Lenticel discolouration (LD) in 'B74' mangoes

Non-Irradiated Fruit all years combined (LDFYNI = LD at full yellow of non-irradiated fruit)

Parameters codes:

Code	Parameter	Unit
TY	Tree yield	(kg)
YE	Yield efficiency	(Kg/m2)
CA	Canopy area	(m2)
CD	Canopy density	(1-3)
TD	Trunk differ.	(cm)
F2	Flowering -2 wk	(%)
FFF	Flowering FF	(%)
FS	Flower spread	(0-7)
FIFF	Flushing FF	(%)
FVFF	Flushing vigour FF	(0-5)
FH	Flushing harv.	(%)
FVH	Flushing vigour harv.	(0-5)
DM	Dry matter	(%)
FC	Flesh colour	(1-11)
AFW	Aver. fruit weight	(g)
FB	Fruit blush	(1-6)
	LD after irrad. (NI)	(0-5)
	LD after irrad. (I)	(0-5)
DFYNI	Days to FY (NI)	
DFYI	Days to FY (I)	
FirFYNI	Firmness FY(NI)	(0-4)
FirmFYI	Firmness FY (I)	(0-4)
LDFYNI	LD at FY (NI)	(0-5)
LDFYI	LD at FY (I)	(0-5)

Model selection

```
 Response variate: LDFYNI
  Number of units: 143
     Forced terms: Constant
        Forced df: 1
       Free terms: AFW + CA + CD + DFYNI + DM + F2 + FB + FC +
                   FFF + FH + FS + FVFF + FVH + FirFYNI + FlFF +
                   TD + TY + YE
```

Stepwise (forward) analysis of variance

Regression analysis

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ FC	1	9.5054	9.5054	42.46	<.001
+ FS	1	5.6911	5.6911	25.42	<.001
+ FVH	1	2.0350	2.0350	9.09	0.003
+ DM	1	1.0754	1.0754	4.80	0.030
+ AFW	1	1.7382	1.7382	7.77	0.006
- FC	-1	-0.0232	0.0232	0.10	0.748
+ CA	1	1.1805	1.1805	5.27	0.023
+ FB	1	1.1750	1.1750	5.25	0.024
+ YE	1	0.5432	0.5432	2.43	0.122
+ TY	1	0.7514	0.7514	3.36	0.069
Residual	134	29.9965	0.2239		
Total	142	53.6686	0.3779		

Final model: Constant + FS + FVH + DM + AFW + CA + FB + YE + TY

```
 676  "Multiple Linear Regression"
 677  MODEL LDFYNI
 678  TERMS [FACT=9] FS,FVH,DM,AFW,CA,FB,YE,TY
 679  FIT [PRINT=model,summary,correlations,estimates; CONSTANT=estimate;
FPROB=yes; TPROB=yes;\
 680   FACT=9] FS,FVH,DM,AFW,CA,FB,YE,TY
```

Regression analysis

Response variate: LDFYNI
Fitted terms: Constant, FS, FVH, DM, AFW, CA, FB, YE, TY

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	8	22.90	2.8627	13.13	<.001
Residual	154	33.58	0.2180		
Total	162	56.48	0.3486		

Percentage variance accounted for 37.5
Standard error of observations is estimated to be 0.467.

Message: the following units have large standardized residuals.

Unit	Response	Residual
123	2.433	2.97
126	2.406	2.93
137	2.312	2.74

Message: the following units have high leverage.

Unit	Response	Leverage
62	1.832	0.258
72	1.677	0.220
96	0.469	0.226
97	0.800	0.193
104	0.406	0.171
151	0.417	0.153

Estimates of parameters

Parameter	estimate	s.e.	t(154)	t pr.
Constant	0.183	0.748	0.24	0.807
FS	0.2287	0.0488	4.69	<.001
FVH	0.1031	0.0458	2.25	0.026
DM	0.0772	0.0253	3.05	0.003
AFW	0.002230	0.000871	2.56	0.011
CA	-0.2335	0.0658	-3.55	<.001
FB	-0.2768	0.0842	-3.29	0.001
YE	-0.02481	0.00998	-2.49	0.014
TY	0.00621	0.00377	1.65	0.101

Correlations between parameter estimates

Parameter	ref	correlations							
Constant	1	1.000							
FS	2	-0.281	1.000						
FVH	3	0.080	0.126	1.000					
DM	4	-0.409	-0.240	-0.099	1.000				
AFW	5	-0.596	0.295	-0.241	-0.184	1.000			
CA	6	-0.594	0.358	-0.068	-0.145	0.426	1.000		
FB	7	-0.593	0.140	0.061	0.002	0.117	0.367	1.000	
YE	8	-0.637	0.096	-0.176	0.173	0.356	0.807	0.260	1.000
TY	9	0.376	-0.327	0.128	0.106	-0.254	-0.815	-0.272	-0.765
		1	2	3	4	5	6	7	8
TY	9	1.000							
		9							

Model selection

```
Response variate: LDFYNI
 Number of units: 211
    Forced terms: Constant
       Forced df: 1
      Free terms: AFW + CA + CD + DM + FB + FFF + FH + FlFF +
                  TD + TY + YE
```

Stepwise (forward) analysis of variance

Regression analysis

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ CA	1	6.8326	6.8326	24.73	<.001
+ FB	1	8.9889	8.9889	32.53	<.001
+ DM	1	2.2860	2.2860	8.27	0.004
+ FFF	1	1.2890	1.2890	4.66	0.032
+ YE	1	2.9104	2.9104	10.53	0.001
+ FH	1	2.3323	2.3323	8.44	0.004
+ TD	1	1.7503	1.7503	6.33	0.013
+ AFW	1	0.8331	0.8331	3.01	0.084
Residual	202	55.8182	0.2763		
Total	210	83.0408	0.3954		

Final model: Constant + CA + FB + DM + FFF + YE + FH + TD + AFW

```
 685  "Multiple Linear Regression"
 686  MODEL LDFYNI
 687  TERMS [FACT=9] CA,FB,DM,FFF,YE,FH,TD,AFW
 688  FIT [PRINT=model,summary,correlations,estimates; CONSTANT=estimate;
FPROB=yes; TPROB=yes;\
 689   FACT=9] CA,FB,DM,FFF,YE,FH,TD,AFW
```

Regression analysis

Response variate: LDFYNI
Fitted terms: Constant, CA, FB, DM, FFF, YE, FH, TD, AFW

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	8	27.22	3.4028	12.31	<.001
Residual	202	55.82	0.2763		
Total	210	83.04	0.3954		

Percentage variance accounted for 30.1
Standard error of observations is estimated to be 0.526.

Message: the following units have large standardized residuals.

Unit	Response	Residual
137	2.312	3.24
220	2.031	3.68

Message: the residuals do not appear to be random; for example, fitted values in the range 1.061 to 1.115 are consistently larger than observed values and fitted values in the range 1.277 to 1.332 are consistently smaller than observed values.

Message: the following units have high leverage.

Unit	Response	Leverage
62	1.832	0.201
66	1.563	0.129
72	1.677	0.199
96	0.469	0.242
97	0.800	0.153

Estimates of parameters

Parameter	estimate	s.e.	t(202)	t pr.
Constant	1.933	0.671	2.88	0.004
CA	-0.2470	0.0401	-6.16	<.001
FB	-0.5880	0.0858	-6.85	<.001
DM	0.0386	0.0231	1.67	0.096
FFF	0.01186	0.00231	5.13	<.001
YE	-0.02396	0.00748	-3.20	0.002
FH	0.01129	0.00376	3.00	0.003
TD	0.02494	0.00917	2.72	0.007
AFW	0.001503	0.000866	1.74	0.084

Correlations between parameter estimates

Parameter	ref	correlations					
Constant	1	1.000					
CA	2	-0.496	1.000				
FB	3	-0.434	0.415	1.000			
DM	4	-0.439	-0.198	-0.140	1.000		
FFF	5	-0.011	-0.224	-0.330	-0.100	1.000	
YE	6	-0.529	0.564	0.307	0.235	-0.555	1.000

FH	7	-0.031	0.079	0.087	-0.087	0.314	-0.084	1.000	
TD	8	-0.144	-0.183	-0.302	0.170	0.438	-0.247	0.205	1.000
AFW	9	-0.651	0.331	-0.001	-0.029	0.106	0.244	-0.199	0.128
		1	2	3	4	5	6	7	8

AFW	9	1.000
		9

Principal components analysis

Latent roots

1	2
4.983	3.206

Percentage variation

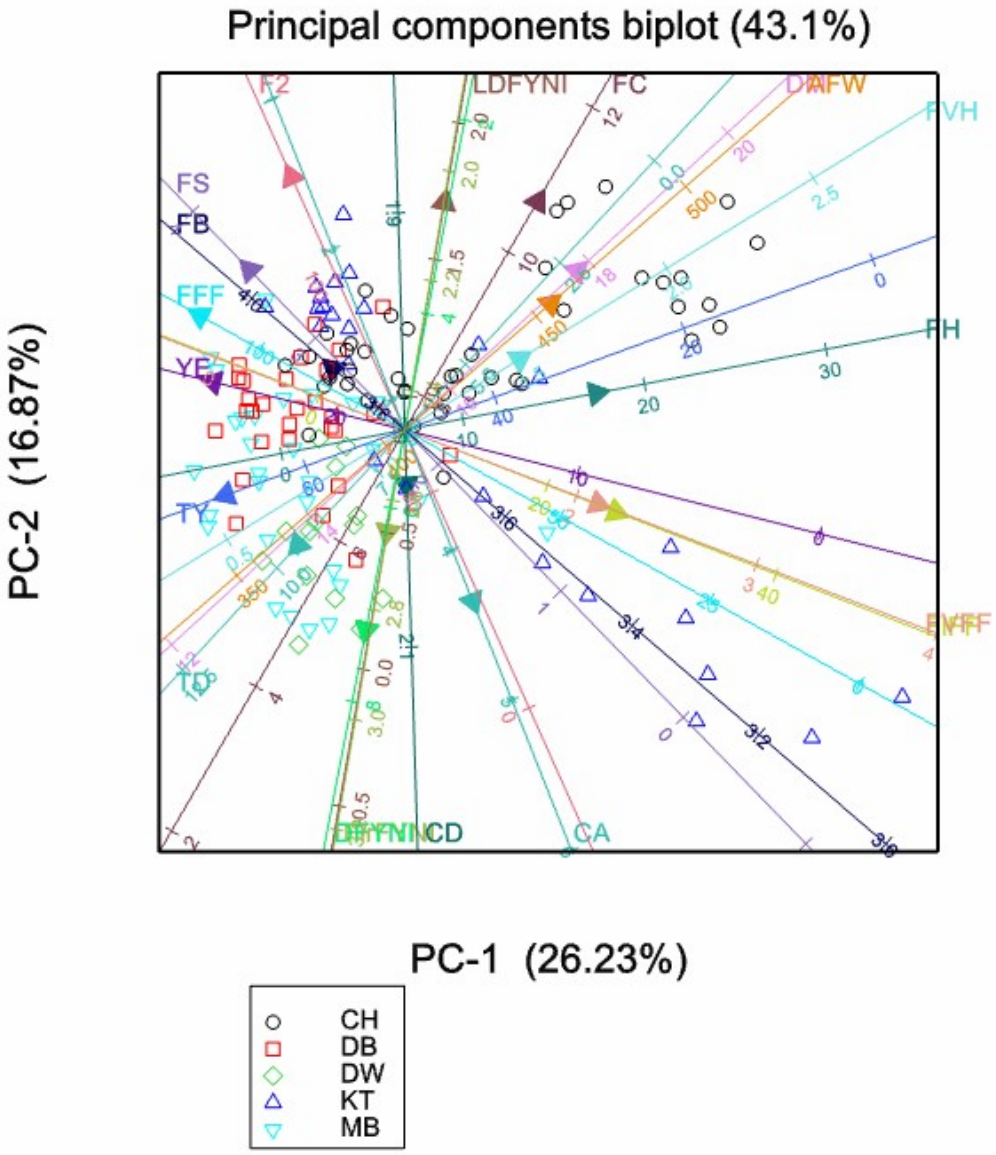
1	2
26.23	16.87

Trace

19.00

Latent vectors (loadings)

	1	2
AFW	0.23881	0.20694
CA	0.11128	-0.28064
CD	0.00274	-0.08905
DFYNI	-0.06332	-0.32751
DM	0.28284	0.26050
F2	-0.18544	0.41471
FB	-0.11717	0.10084
FC	0.21740	0.37387
FFF	-0.33529	0.18716
FH	0.31433	0.06019
FS	-0.25394	0.26204
FVFF	0.31655	-0.12100
FVH	0.18831	0.11612
FirFYNI	-0.02850	-0.16693
FIFF	0.34619	-0.13452
LDFYNI	0.06512	0.37281
TD	-0.17453	-0.18831
TY	-0.29290	-0.10672
YE	-0.31411	0.07865


Principal components biplot (43.1%)
PC-2 (16.87%)
PC-1 (26.23%)
F2
LDFYNI
FC
DAFW
FVH
FS
FB
FFF
YE
FH
TY
FVFF
TO
CA
CH
DB
DW
KT
MB

13.1. Lenticel damage in Calypso mangoes

2. Irradiated Fruit at Individual Years separately

Parameters codes:

Code	Parameter	Unit
TY	Tree yield	(kg)
YE	Yield efficiency	(Kg/m2)
CA	Canopy area	(m2)
CD	Canopy density	(1-3)
TD	Trunk differ.	(cm)
F2	Flowering -2 wk	(%)
FFF	Flowering FF	(%)
FS	Flower spread	(0-7)
FIFF	Flushing FF	(%)
FVFF	Flushing vigour FF	(0-5)
FH	Flushing harv.	(%)
FVH	Flushing vigour harv.	(0-5)
DM	Dry matter	(%)
FC	Flesh colour	(1-11)
AFW	Aver. fruit weight	(g)
FB	Fruit blush	(1-6)
	LD after irrad. (NI)	(0-5)
	LD after irrad. (I)	(0-5)
DFYNI	Days to FY (NI)	
DFYI	Days to FY (I)	
FirFYNI	Firmness FY(NI)	(0-4)
FirmFYI	Firmness FY (I)	(0-4)
LDFYNI	LD at FY (NI)	(0-5)
LDFYI	LD at FY (I)	(0-5)

Data:
Variables have been measured on Calypso Mango trees and fruit (averages per tree) from 5 farms over 3 years. A sample of 36 fruit were taken from each tree with 15 trees per farm. Half of these fruit were Irradiated and the other half not. The main trait of interest is the fruit Lenticel Damage rating (0-5) at Full Yellow (LDFYI and LDFYNI).

Aim:
The aim is to identify variables that may predict lenticel damage in Calypso fruit.

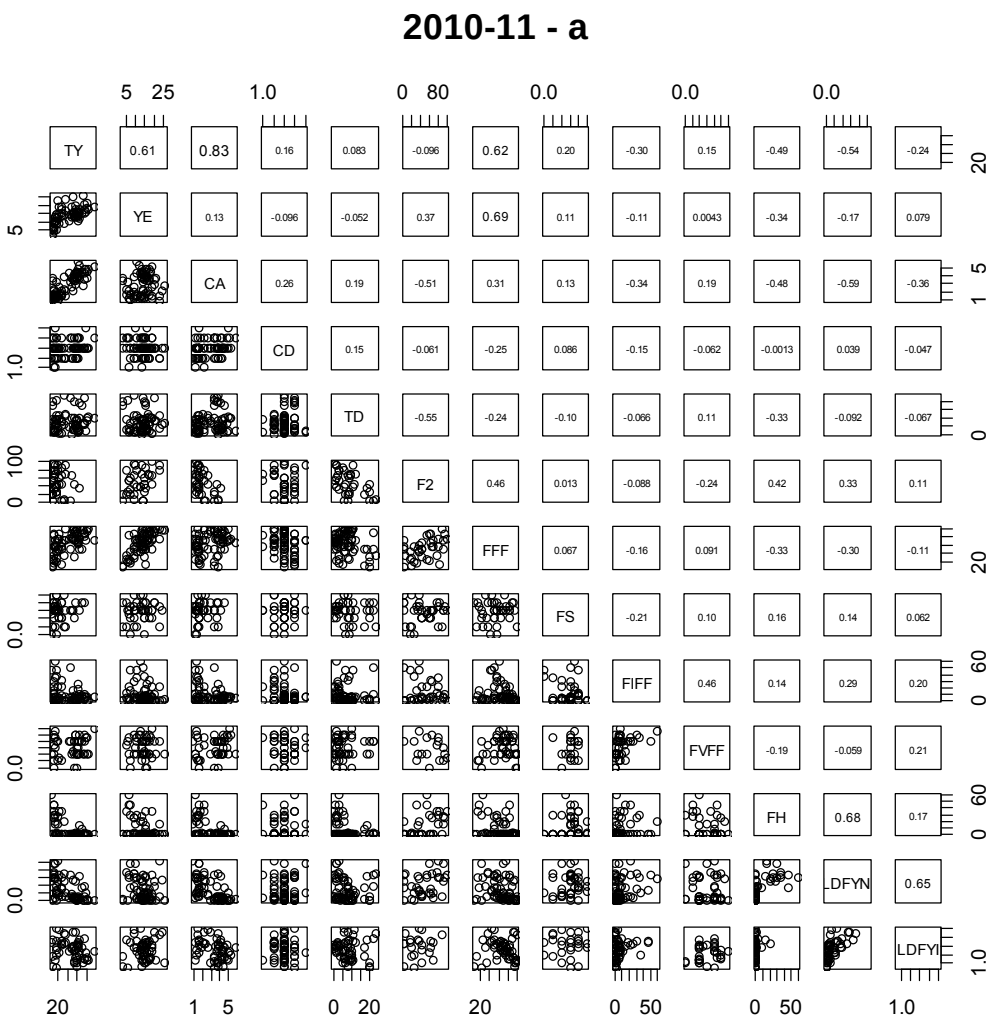
Statistical Methods:
A number of multivariate and regression approaches have been performed in order to try and find predictors of lenticel damage (at Full Yellow) in Calypso mangoes, including:
- multiple linear regression
- canonical variates analysis
- principal components analysis

- regression / classification trees

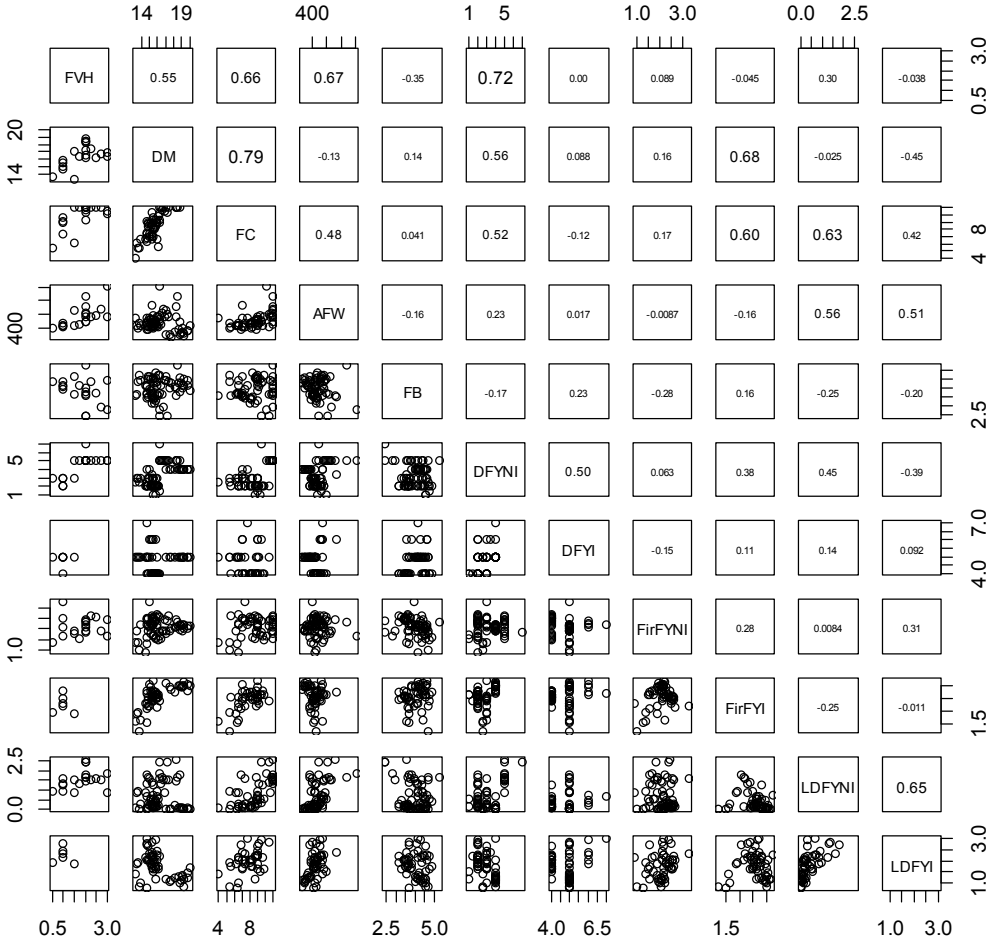
A further analysis has investigated the lenticel damage response over time (LD measured at time of irradiation, Full Yellow and 7 days after full yellow) and tested individual covariates to see if they have a significant impact on the lenticel damage response.

Analyses have been performed for each year (2010-11, 2011-12, 2012-13) and for Irradiated and Non-irradiated seperately.

Results:
Plots relating LDFYI and LDFYNI to other traits for each year separately (across all 5 farms). Correlations between variables are given in the upper half of plot.
2010-11



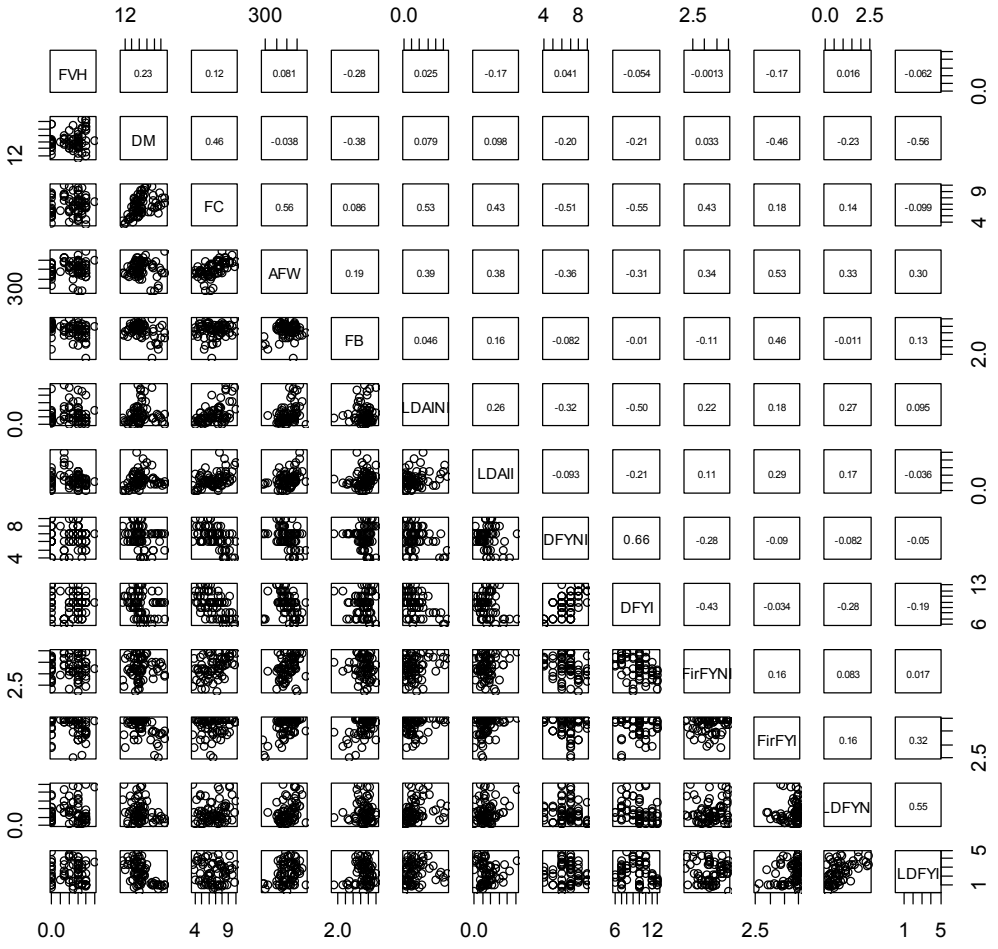
2010-11 - b



2011-12

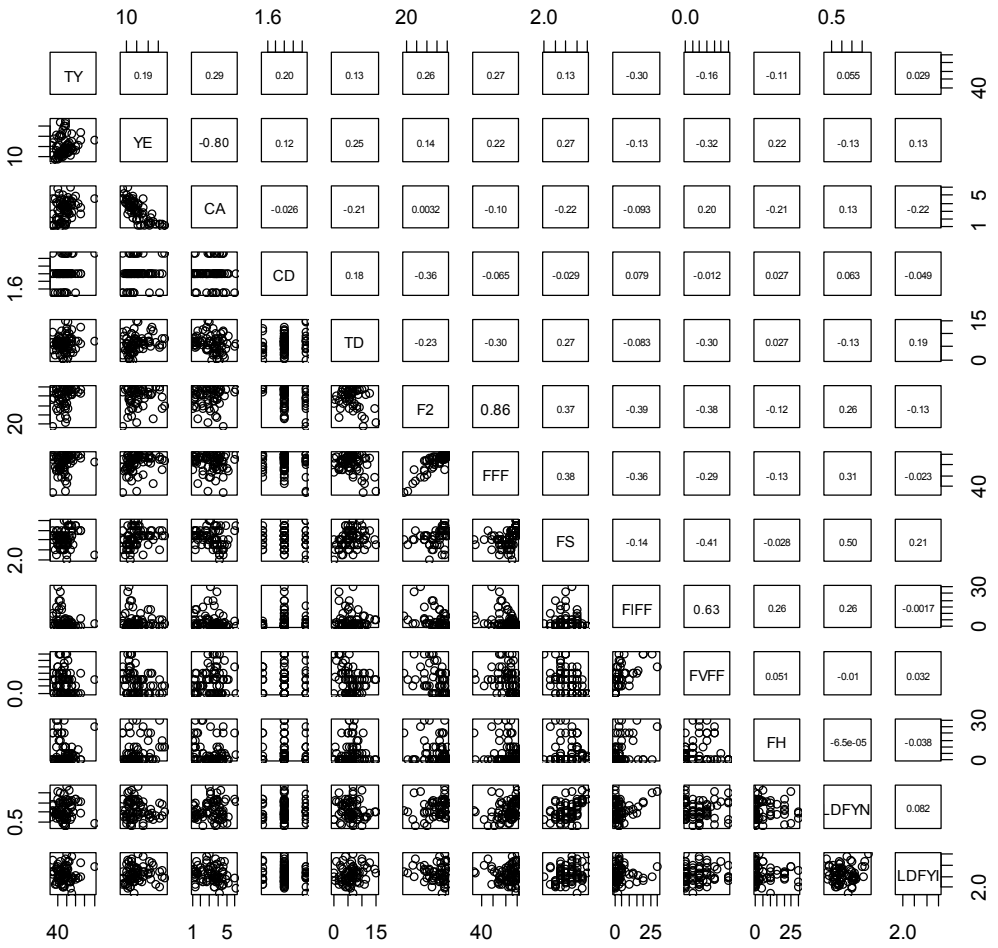
2011-12 - a

2011-12 - b



2012-13

2012-13 - a



2012-13 - b

1.Multiple regression for LD at Full Yellow for Irradiated fruit for each year separately

Have used forward stepwise multiple regression for LDFYI based on 13 variables (some variables excluded as too many missing values). The 13 explanatory variables investigated for 2010-11 were : `AFW + CA + CD + DM + FB + FFF + FH + FirFYI + FlFF + TD + TY + YE`

2010-11

Lenticel Damage at Full Yellow for Irradiated Fruit

Model selection

```
 Response variate: LDFYI
  Number of units: 55
     Forced terms: Constant
        Forced df: 1
      Free terms: AFW + CA + CD + DM + FB + FFF + FH + FirFYI +
                  FlFF + TD + TY + YE
```

Stepwise (forward) analysis of variance

Regression analysis

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ AFW	1	4.6305	4.6305	23.70	<.001
+ YE	1	1.1434	1.1434	5.85	0.019
+ DM	1	1.0355	1.0355	5.30	0.026
+ FirFYI	1	0.6945	0.6945	3.55	0.066
+ FB	1	0.4075	0.4075	2.09	0.155
+ FH	1	0.3116	0.3116	1.59	0.213
+ CA	1	0.3329	0.3329	1.70	0.198
Residual	47	9.1830	0.1954		
Total	54	17.7389	0.3285		

Final model: Constant + AFW + YE + DM + FirFYI + FB + FH + CA

Then fitted a multiple regression based on AFW + YE + DM + FirFYI to get regression equation etc... the regression coefficients are given below:

Regression analysis

Response variate: LDFYI
Fitted terms: Constant, AFW, YE, DM, FirFYI

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	4	7.50	1.8760	9.16	<.001
Residual	50	10.23	0.2047		
Total	54	17.74	0.3285		

Percentage variance accounted for 37.7
Standard error of observations is estimated to be 0.452.

Message: the following units have large standardized residuals.

Unit	Response	Residual
42	2.974	2.41
58	2.730	2.68

Message: the following units have high leverage.

Unit	Response	Leverage
26	1.684	0.21
43	2.361	0.31
60	0.810	0.25

Estimates of parameters

Parameter	estimate	s.e.	t(50)	t pr.

Constant	1.50	1.37	1.10	0.278
AFW	0.00408	0.00192	2.12	0.039
YE	0.0204	0.0163	1.25	0.217
DM	-0.1787	0.0621	-2.88	0.006
FirFYI	0.459	0.249	1.84	0.071

Correlations between parameter estimates

Parameter	ref	correlations				
Constant	1	1.000				
AFW	2	-0.912	1.000			
YE	3	-0.389	0.400	1.000		
DM	4	-0.822	0.649	0.318	1.000	
FirFYI	5	0.439	-0.468	-0.536	-0.738	1.000
		1	2	3	4	5

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ AFW	1	4.6305	4.6305	22.62	<.001
+ YE	1	1.1434	1.1434	5.59	0.022
+ DM	1	1.0355	1.0355	5.06	0.029
+ FirFYI	1	0.6945	0.6945	3.39	0.071
Residual	50	10.2349	0.2047		
Total	54	17.7389	0.3285		

```
408  RWALD
```

Wald tests for dropping terms

Term	Wald statistic	d.f.	F statistic	F pr.
AFW	4.510	1	4.51	0.039
YE	1.562	1	1.56	0.217
DM	8.276	1	8.28	0.006
FirFYI	3.393	1	3.39	0.071

Residual d.f. 50

Including Farm in the model?

Regression analysis

Response variate: LDFYI
Fitted terms: Constant + AFW + YE + DM + FirFYI + Farm + AFW.Farm + YE.Farm + DM.Farm + FirFYI.Farm

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	19	13.343	0.7023	5.59	<.001
Residual	35	4.396	0.1256		

Total	54	17.739	0.3285		
Change	-12	-3.367	0.2805	2.23	0.032

Percentage variance accounted for 61.8
Standard error of observations is estimated to be 0.354.

Message: the following units have large standardized residuals.

Unit	Response	Residual
34	1.176	-2.78
35	2.684	2.38
50	2.160	-2.56
58	2.730	2.60

Message: the following units have high leverage.

Unit	Response	Leverage
43	2.361	0.80

Estimates of parameters

Parameter	estimate	s.e.	t(35)	t pr.
Constant	21.41	6.20	3.45	0.001
AFW	-0.00522	0.00406	-1.29	0.207
YE	-0.0749	0.0313	-2.39	0.022
DM	-0.967	0.289	-3.35	0.002
FirFYI	-0.288	0.550	-0.52	0.604
Farm CH	0	*	*	*
Farm DW	-17.18	9.16	-1.88	0.069
Farm KT	-21.92	7.59	-2.89	0.007
Farm MB	-18.22	7.10	-2.57	0.015
AFW.Farm CH	0	*	*	*
AFW.Farm DW	0.00896	0.00758	1.18	0.246
AFW.Farm KT	0.01407	0.00761	1.85	0.073
AFW.Farm MB	0.00104	0.00659	0.16	0.876
YE.Farm CH	0	*	*	*
YE.Farm DW	0.0745	0.0620	1.20	0.238
YE.Farm KT	0.0593	0.0570	1.04	0.305
YE.Farm MB	0.0967	0.0386	2.51	0.017
DM.Farm CH	0	*	*	*
DM.Farm DW	0.751	0.413	1.82	0.078
DM.Farm KT	0.891	0.368	2.42	0.021
DM.Farm MB	0.800	0.343	2.34	0.025
FirFYI.Farm CH	0	*	*	*
FirFYI.Farm DW	0.072	0.938	0.08	0.939
FirFYI.Farm KT	0.36	1.08	0.33	0.744
FirFYI.Farm MB	1.574	0.683	2.30	0.027

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Farm	DB

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ AFW	1	4.6305	4.6305	36.87	<.001
+ YE	1	1.1434	1.1434	9.10	0.005
+ DM	1	1.0355	1.0355	8.24	0.007
+ FirFYI	1	0.6945	0.6945	5.53	0.024
+ Farm	3	2.4729	0.8243	6.56	0.001

+ AFW.Farm	3	0.4709	0.1570	1.25	0.307
+ YE.Farm	3	1.0478	0.3493	2.78	0.055
+ DM.Farm	3	0.9799	0.3266	2.60	0.068
+ FirFYI.Farm	3	0.8679	0.2893	2.30	0.094
Residual	35	4.3956	0.1256		
Total	54	17.7389	0.3285		

```
 434  RWALD
```

Wald tests for dropping terms

Term	Wald statistic	d.f.	F statistic	F pr.
AFW.Farm	4.339	3	1.45	0.246
YE.Farm	6.339	3	2.11	0.116
DM.Farm	6.880	3	2.29	0.095
FirFYI.Farm	6.910	3	2.30	0.094

Residual d.f. 35

2011-12
LDFYI
Multiple regression for LDFYI based on 18 explanatory variables
(AFW,CA,CD,DFYI,DM,F2,FB,FC,FFF,FH,FS,FVFF,FVH,FirFYI,FlFF,TD,TY,YE)
73 trees (out of 75) included

```
317  MODEL LDFYI
 318  RSEARCH [PRINT=model,results; METHOD=fstepwise; CONSTANT=estimate;
FACTORIAL=3; DENOMINATOR=ss;\
 319   INRATIO=1; OUTRATIO=1; MAXCYCLE=50; AFACTORIAL=2;
CRITERION=adjusted; EXTRA=cp; NTERMS=16;\
 320   NBESTMODELS=8]
AFW,CA,CD,DFYI,DM,F2,FB,FC,FFF,FH,FS,FVFF,FVH,FirFYI,FlFF,TD,TY,YE
```

Model selection

```
 Response variate: LDFYI
  Number of units: 73
     Forced terms: Constant
        Forced df: 1
      Free terms: AFW + CA + CD + DFYI + DM + F2 + FB + FC +
                  FFF + FH + FS + FVFF + FVH + FirFYI + FlFF +
                  TD + TY + YE
```

Stepwise (forward) analysis of variance

Regression analysis

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ FS	1	37.9367	37.9367	65.50	<.001
+ TD	1	19.1315	19.1315	33.03	<.001
+ FB	1	1.6945	1.6945	2.93	0.092

+ DM	1	2.0201	2.0201	3.49	0.067
+ DFYI	1	2.1103	2.1103	3.64	0.061
+ TY	1	3.5619	3.5619	6.15	0.016
+ FC	1	1.5712	1.5712	2.71	0.105
+ FirFYI	1	0.6632	0.6632	1.14	0.289
+ AFW	1	0.8680	0.8680	1.50	0.226
+ F2	1	0.9085	0.9085	1.57	0.215
+ FFF	1	0.5901	0.5901	1.02	0.317
+ FlFF	1	0.8186	0.8186	1.41	0.239
Residual	60	34.7518	0.5792		
Total	72	106.6264	1.4809		

Final model: Constant + FS + TD + FB + DM + DFYI + TY + FC + FirFYI + AFW + F2 + FFF + FlFF

```
 321  MODEL LDFYI
 322  RSEARCH [PRINT=model,results; METHOD=fstepwise; CONSTANT=estimate;
FACTORIAL=3; DENOMINATOR=ss;\
 323   INRATIO=1; OUTRATIO=1; MAXCYCLE=50; AFACTORIAL=2;
CRITERION=adjusted; EXTRA=cp; NTERMS=16;\
 324   NBESTMODELS=8; FORCED=Farm]
AFW,CA,CD,DFYI,DM,F2,FB,FC,FFF,FH,FS,FVFF,FVH,FirFYI,\
 325  FlFF,TD,TY,YE
```

Model selection

```
 Response variate: LDFYI
  Number of units: 73
     Forced terms: Constant + Farm
        Forced df: 5
       Free terms: AFW + CA + CD + DFYI + DM + F2 + FB + FC +
                   FFF + FH + FS + FVFF + FVH + FirFYI + FlFF +
                   TD + TY + YE
```

Stepwise (forward) analysis of variance

Regression analysis

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ Farm	4	68.6165	17.1541	39.53	<.001
+ TY	1	2.8321	2.8321	6.53	0.013
+ FB	1	2.4845	2.4845	5.73	0.020
+ DFYI	1	4.4848	4.4848	10.33	0.002
Residual	65	28.2084	0.4340		
Total	72	106.6264	1.4809		

Final model: Constant + Farm + TY + FB + DFYI

```
 326  "Multiple Linear Regression"
 327  MODEL LDFYI
 328  TERMS [FACT=9] FS,TD,FB,DM,DFYI,TY
 329  FIT [PRINT=model,summary,correlations,estimates,accumulated;
CONSTANT=estimate; FPROB=yes;\
 330   TPROB=yes; FACT=9] FS,TD,FB,DM,DFYI,TY
```

Regression analysis

Response variate: LDFYI
Fitted terms: Constant, FS, TD, FB, DM, DFYI, TY

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	6	66.45	11.0758	18.20	<.001
Residual	66	40.17	0.6087		
Total	72	106.63	1.4809		

Percentage variance accounted for 58.9
Standard error of observations is estimated to be 0.780.

Message: the following units have large standardized residuals.

Unit	Response	Residual
42	0.625	-2.60
45	0.222	-2.90
47	4.844	3.50

Message: the error variance does not appear to be constant: intermediate responses are more variable than small or large responses.

Message: the following units have high leverage.

Unit	Response	Leverage
21	0.969	0.27

Estimates of parameters

Parameter	estimate	s.e.	t(66)	t pr.
Constant	8.33	2.31	3.60	<.001
FS	0.410	0.141	2.90	0.005
TD	0.0670	0.0255	2.62	0.011
FB	-0.578	0.225	-2.57	0.013
DM	-0.310	0.108	-2.88	0.005
DFYI	-0.1520	0.0584	-2.60	0.011
TY	0.01505	0.00622	2.42	0.018

Correlations between parameter estimates

Parameter	ref	correlations						
Constant	1	1.000						
FS	2	-0.562	1.000					
TD	3	-0.480	0.240	1.000				
FB	4	-0.477	-0.156	0.262	1.000			
DM	5	-0.930	0.603	0.451	0.218	1.000		
DFYI	6	-0.604	0.542	0.207	0.062	0.470	1.000	
TY	7	0.252	-0.330	-0.422	-0.305	-0.195	-0.355	1.000
		1	2	3	4	5	6	7

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ FS	1	37.9367	37.9367	62.33	<.001
+ TD	1	19.1315	19.1315	31.43	<.001
+ FB	1	1.6945	1.6945	2.78	0.100
+ DM	1	2.0201	2.0201	3.32	0.073
+ DFYI	1	2.1103	2.1103	3.47	0.067
+ TY	1	3.5619	3.5619	5.85	0.018
Residual	66	40.1714	0.6087		
Total	72	106.6264	1.4809		

```
331  RWALD
```

Wald tests for dropping terms

Term	Wald statistic	d.f.	F statistic	F pr.
FS	8.388	1	8.39	0.005
TD	6.878	1	6.88	0.011
FB	6.587	1	6.59	0.013
DM	8.280	1	8.28	0.005
DFYI	6.761	1	6.76	0.011
TY	5.852	1	5.85	0.018

Residual d.f. 66

2012-13

Model selection

```
 Response variate: LDFYI
  Number of units: 72
     Forced terms: Constant
        Forced df: 1
      Free terms: AFW + CA + CD + DFYI + DM + FB + FFF + FH +
                  FS + FVFF + FVH + FirFYI + FlFF + TD + TY +
                  YE
```

Stepwise (forward) analysis of variance

Regression analysis

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ CA	1	0.4729	0.4729	3.84	0.054
+ FS	1	0.2634	0.2634	2.14	0.148
+ FVFF	1	0.2235	0.2235	1.82	0.183
+ TD	1	0.2213	0.2213	1.80	0.185
+ FlFF	1	0.1927	0.1927	1.56	0.215
+ YE	1	0.1431	0.1431	1.16	0.285
+ TY	1	0.3118	0.3118	2.53	0.116
Residual	64	7.8807	0.1231		
Total	71	9.7095	0.1368		

Final model: Constant + CA + FS + FVFF + TD + FIFF + YE +
TY

Regression analysis

Response variate: LDFYI
Fitted terms: Constant, CA, FS, FVFF, TD, FIFF, YE, TY

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	7	1.829	0.2613	2.12	0.054
Residual	64	7.881	0.1231		
Total	71	9.710	0.1368		

Percentage variance accounted for 10.0
Standard error of observations is estimated to be 0.351.

Message: the following units have large standardized residuals.

Unit	Response	Residual
17	1.750	-2.47

Message: the following units have high leverage.

Unit	Response	Leverage
1	2.944	0.36
25	2.950	0.33
28	2.425	0.29

Estimates of parameters

Parameter	estimate	s.e.	t(64)	t pr.
Constant	2.603	0.501	5.20	<.001
CA	-0.2160	0.0859	-2.52	0.014
FS	0.173	0.103	1.69	0.097
FVFF	0.1352	0.0705	1.92	0.060
TD	0.0206	0.0144	1.43	0.157
FIFF	-0.01290	0.00950	-1.36	0.179
YE	-0.0209	0.0111	-1.89	0.064
TY	0.00649	0.00408	1.59	0.116

Regression explains very little of variance. Really only CA sig anyway.

2. Canonical variates analysis

If we consider the data as being grouped into LD class groups (eg 0-1, 1-2, 2-3, 3-4, 4-5) we can perform canonical variates analysis to find linear combinations of the data variates that represent most of the variation between the groups. The aim is to maximize the ratio of between group to within group variation thereby giving functions of the variates that can be used to discriminate between the groups.

[May be best to scale the data so coefficients can be interpreted]

2010-11
(Based on standardized data)

Canonical variate analysis

Latent roots

1	2
1.0422	0.3283

Percentage variation

1	2
64.70	20.38

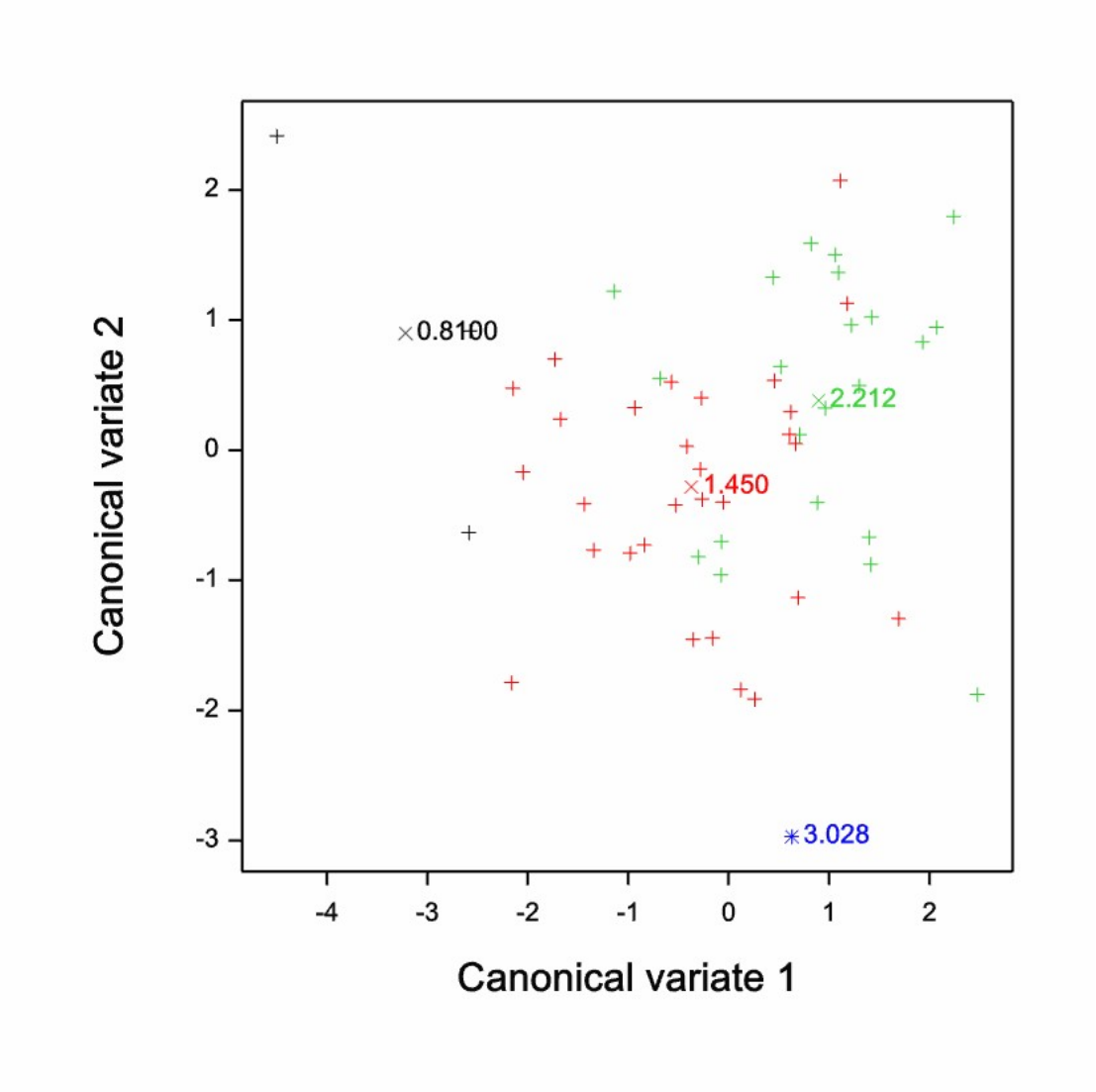
Trace

1.611

Latent vectors (loadings)

	1	2
AFW	0.6018	0.4105
CA	-1.5432	2.1667
CD	-0.3611	-0.7386
DM	-0.6590	0.2325
FB	-0.7884	-0.1778
FFF	-0.0364	0.4208
FH	0.9922	0.9921
FirFYI	0.7406	-0.7920
FIFF	0.2511	0.8768
TD	0.1010	0.3961
TY	1.7118	-2.2273
YE	-0.3870	1.2761

Can see that 64.70% of the between group variation is in the direction of the first canonical variate given by
1.7*TY -1.5*CA + 0.99*FH -0.78*FB + 0.74*FirFYI -0.65*DM + 0.6*AFW +...



2011-12

Canonical variate analysis

Latent roots

1	2
2.326	0.714

Percentage variation

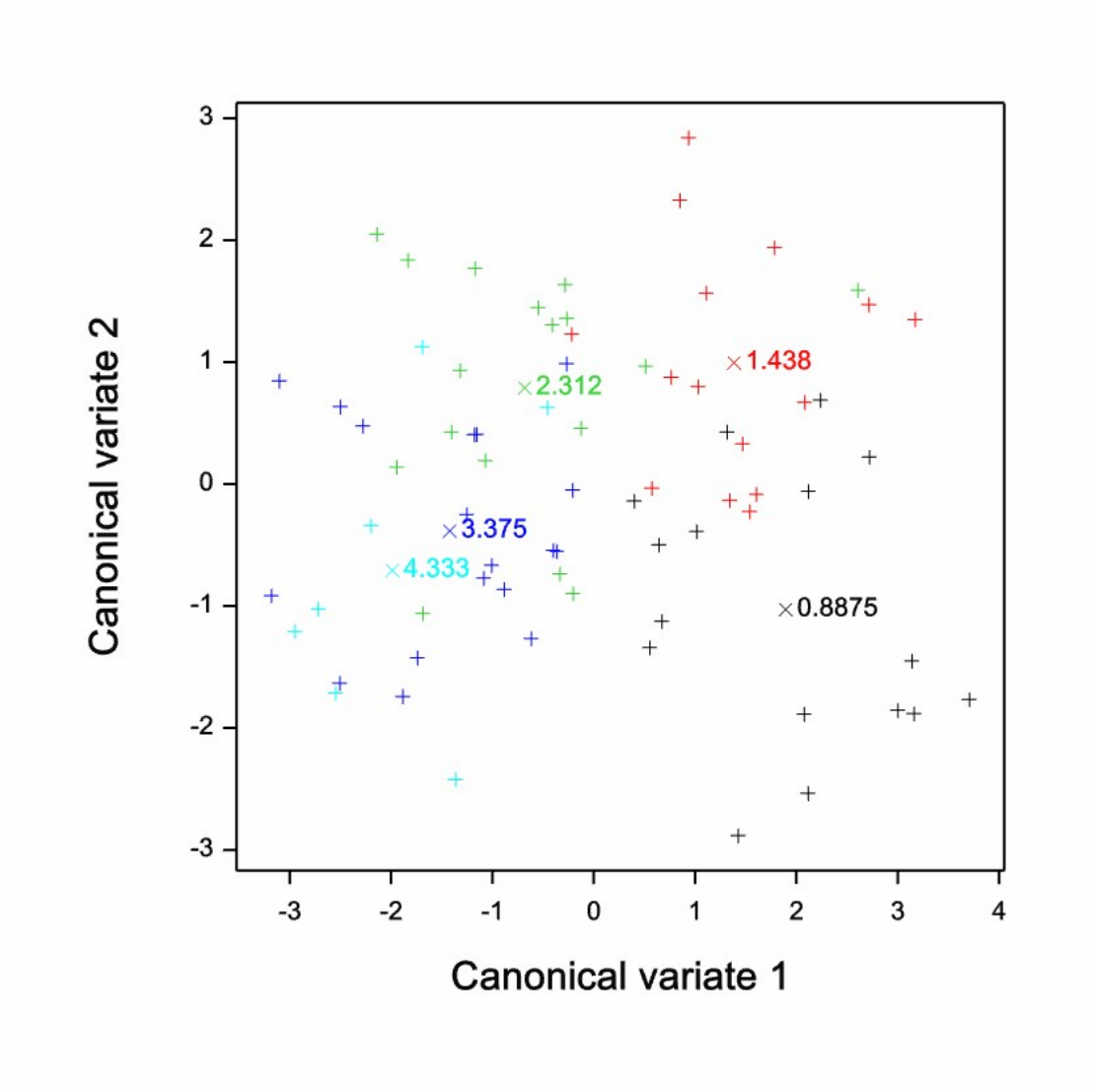
1	2
64.01	19.65

Trace

3.634

Latent vectors (loadings)

	1	2
CA	1.3100	0.7842
CD	0.0938	-0.2548
AFW	-0.2375	0.0515
DFYI	0.4445	-0.3119
DM	0.6668	-0.8329
F2	-0.7895	-0.3826
FB	0.3081	0.2265
FC	0.5030	-0.2661
FFF	1.0664	0.8999
FH	0.0283	-0.5729
FS	-0.7344	0.5651
FVFF	-0.2001	0.2696
FVH	-0.2941	0.3702
FirFYI	0.2890	0.4787
FlFF	0.1901	0.9829
TD	-0.1468	-0.6912
TY	-1.4155	-0.4740
YE	1.2043	-0.0782



2012-13

Canonical variate analysis

Latent roots

1	2
0.3819	0.3231

Percentage variation

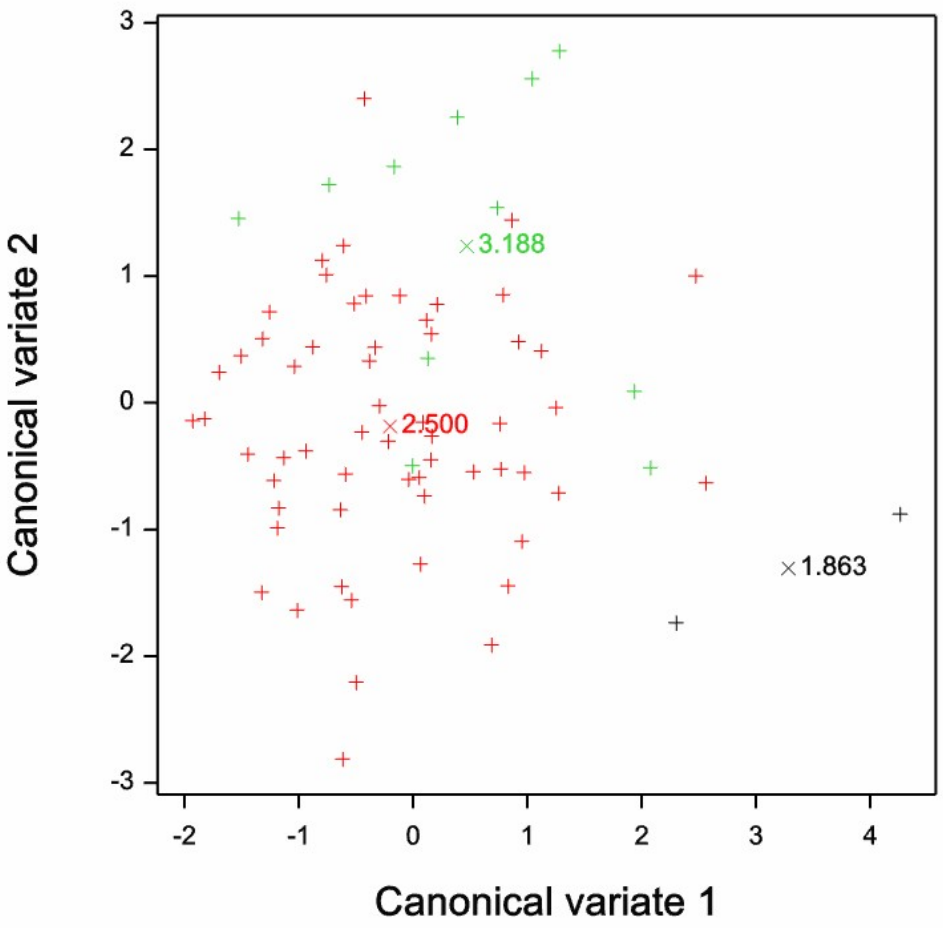
1	2
54.17	45.83

Trace

0.7050

Latent vectors (loadings)

	1	2
AFW	0.0189	0.5858
CA	1.5858	-0.7217
CD	0.1307	-0.4401
DFYI	-0.4392	0.4145
DM	-1.2975	-0.4356
FB	0.2777	0.2586
FFF	0.3735	0.0317
FH	-0.0905	-0.0842
FS	0.7395	0.7291
FVFF	0.0045	0.4454
FVH	0.0171	-0.4530
FirFYI	-0.1520	0.1071
FlFF	0.0723	-0.5122
TD	0.0564	0.1224
TY	-0.2418	0.4817
YE	0.2610	-0.9147



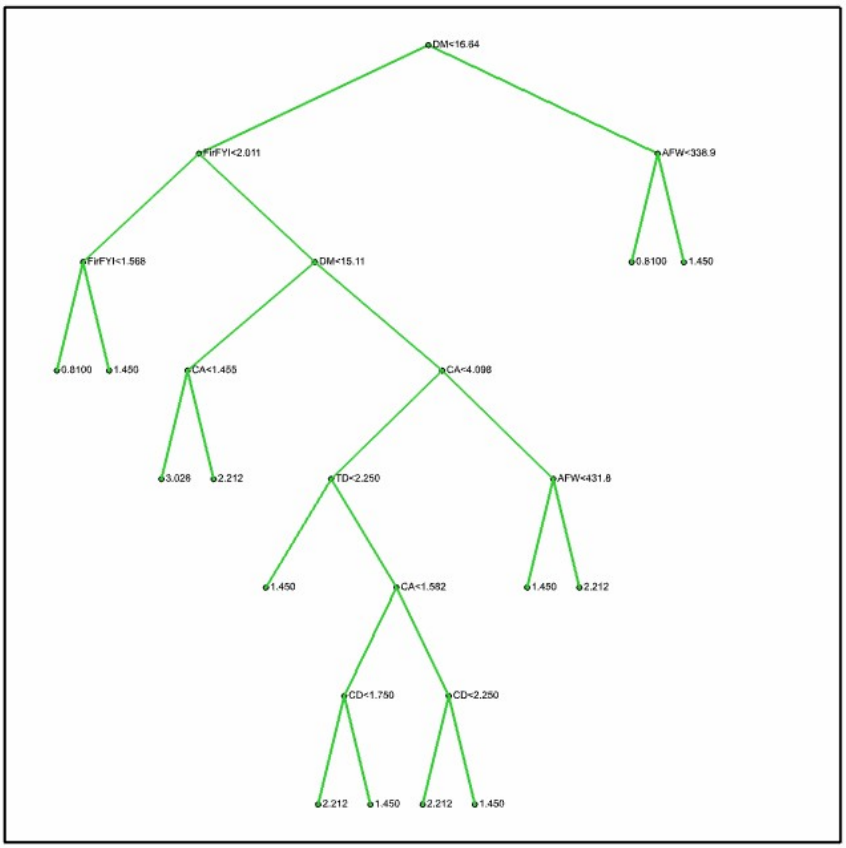
3.Regression or Classification Tree analysis

If we consider the LD response as a grouped rating (as above) and take these groups as factor levels of LDFYI then we can use the other variates to create a classification tree to predict or identify the groups to which an unidentified fruit (or tree's fruit) belongs. This way we can see which variates are most important in predicting what group (or LD rating) a tree's fruit will belong to.

2010-11

Summary of classification tree: LDtree

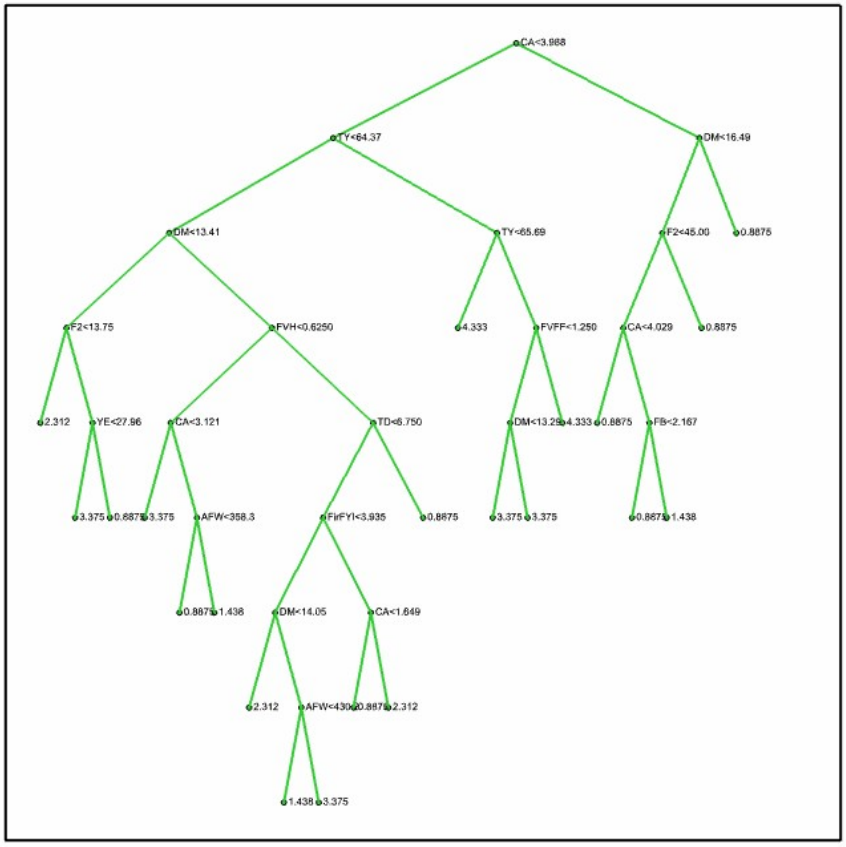
Number of nodes: 25
Number of terminal nodes: 13
Misclassification rate: 0.027
Variables in the tree: DM, FirFYI, AFW, CA, TD, CD.



2011-12

Summary of classification tree: Tree_yr2

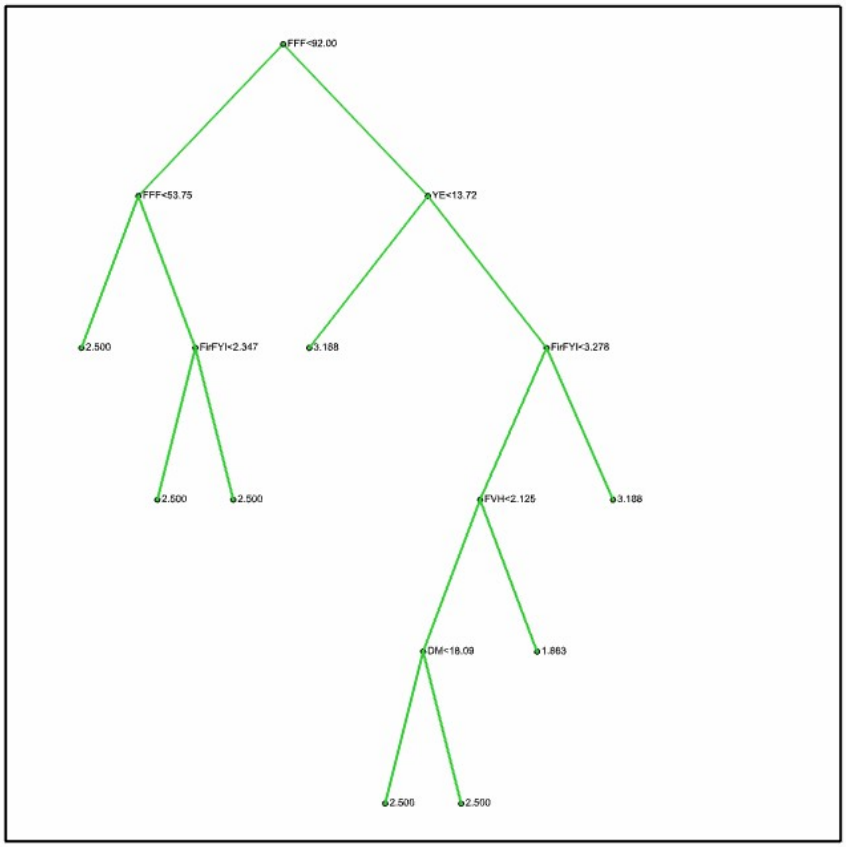
Number of nodes: 41
Number of terminal nodes: 21
Misclassification rate: 0.080
Variables in the tree: CA, TY, DM, F2, FVH, FVFF, YE, TD, FB, AFW, FirFYI.



2012-13

Summary of classification tree: Tree_yr3

Number of nodes: 15
Number of terminal nodes: 8
Misclassification rate: 0.093
Variables in the tree: FFF, YE, FirFYI, FVH, DM.



---

4. Principal components analysis and biplot

Principal components analysis based on correlation scale.

Principal components analysis

Latent roots

1	2
4.201	2.431

Percentage variation

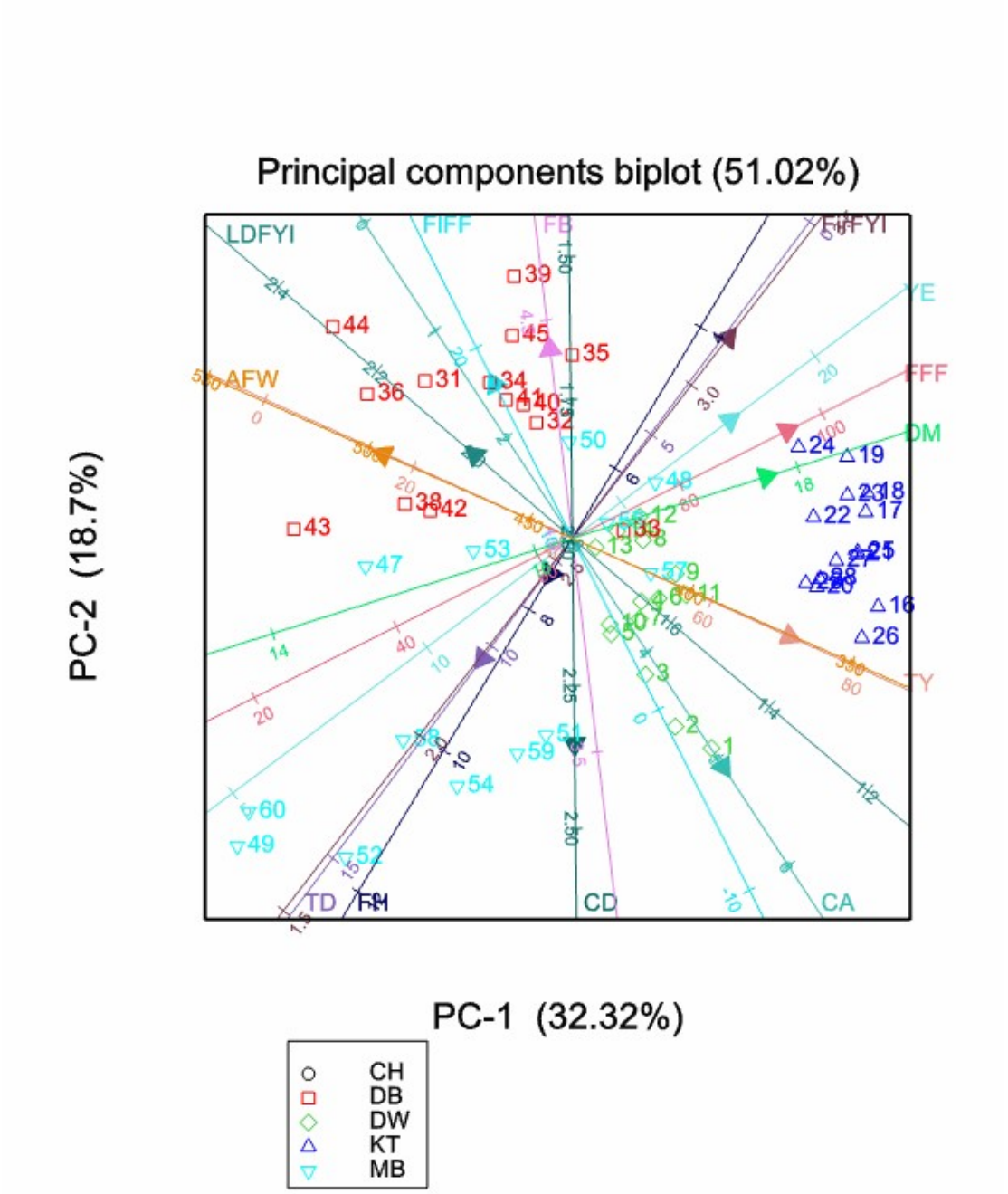
1	2
32.32	18.70

Trace

13.00

Latent vectors (loadings)

	1	2
AFW	-0.36868	0.16354
CA	0.29422	-0.44576
CD	0.00444	-0.40127
DM	0.37809	0.12014
FB	-0.04343	0.36920
FFF	0.41906	0.20975
FH	-0.04207	-0.06933
FirFYI	0.30372	0.39114
FIFF	-0.15140	0.30064
LDFYI	-0.19027	0.16258
TD	-0.17530	-0.23401
TY	0.42304	-0.19124
YE	0.30692	0.22989




```
359  DELETE [REDEFINE=yes] _lrv
 360  DELETE [REDEFINE=yes] _scores
 361  PCP [PRINT=roots,loadings; NROOTS=2; METHOD=correlation]
!p(AFW,CA,CD,DFYI,DM,F2,\
 362  FB,FC,FFF,FH,FS,FVFF,FVH,FirFYI,FlFF,LDFYI,TD,TY,YE); SCORES=_scores;
SAVE=_pcpsave;\
 363   LRV=_lrv
```

Principal components analysis

Latent roots

1	2
7.798	2.665

Percentage variation

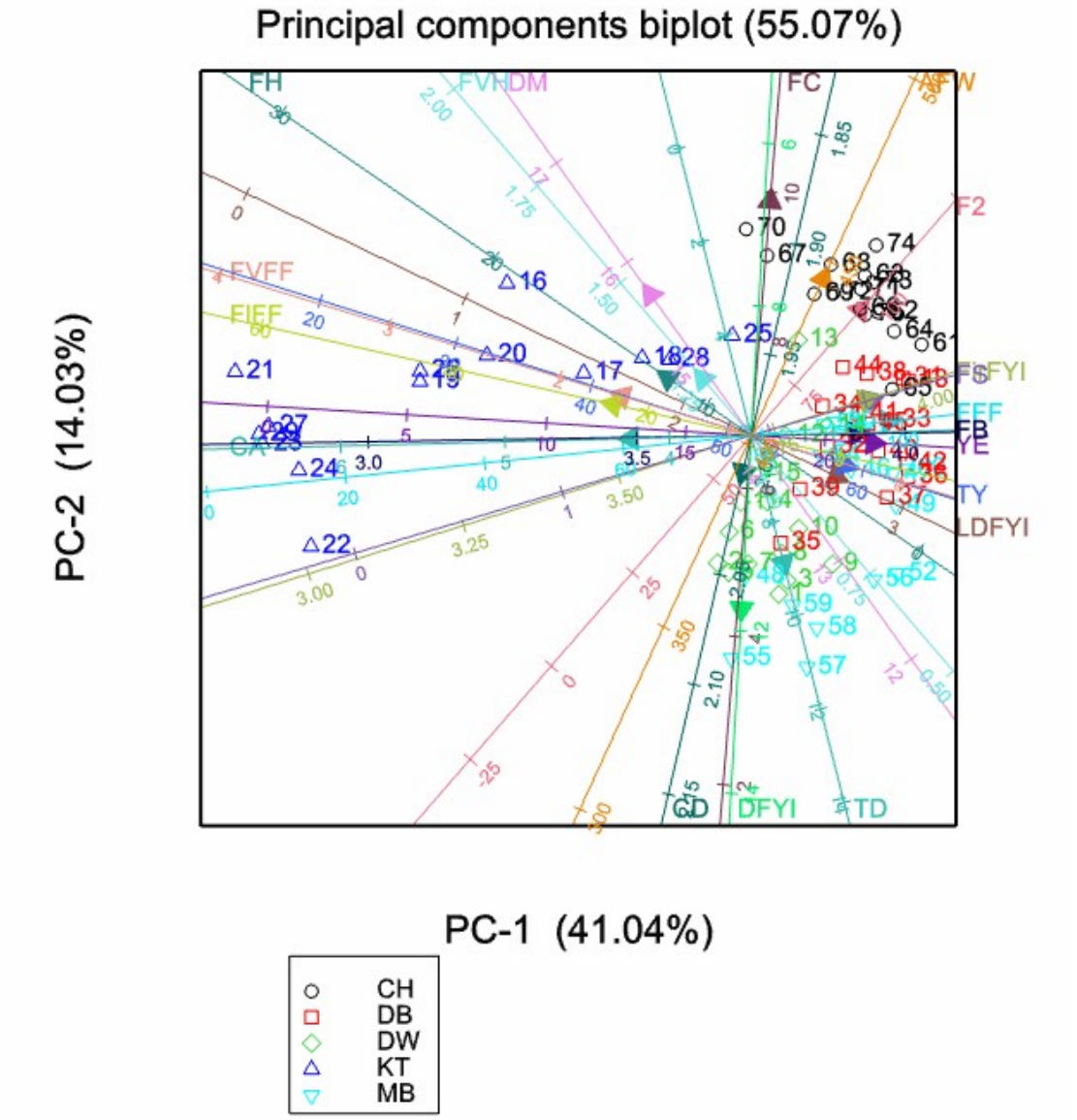
1	2
41.04	14.03

Trace

19.00

Latent vectors (loadings)

	1	2
AFW	0.17081	0.37234
CA	-0.28596	-0.00730
CD	-0.02011	-0.08687
DFYI	-0.02351	-0.41235
DM	-0.23937	0.33449
F2	0.25914	0.29839
FB	0.24940	0.00405
FC	0.04484	0.55674
FFF	0.32605	0.03359
FH	-0.19798	0.13609
FS	0.28591	0.08479
FVFF	-0.30746	0.09364
FVH	-0.11515	0.13427
FirFYI	0.27405	0.08570
FIFF	-0.32874	0.07363
LDFYI	0.20192	-0.09683
TD	0.07665	-0.30301
TY	0.22442	-0.07033
YE	0.29019	-0.01751



2012-13

Principal components analysis

Latent roots

1	2
3.786	2.717

Percentage variation

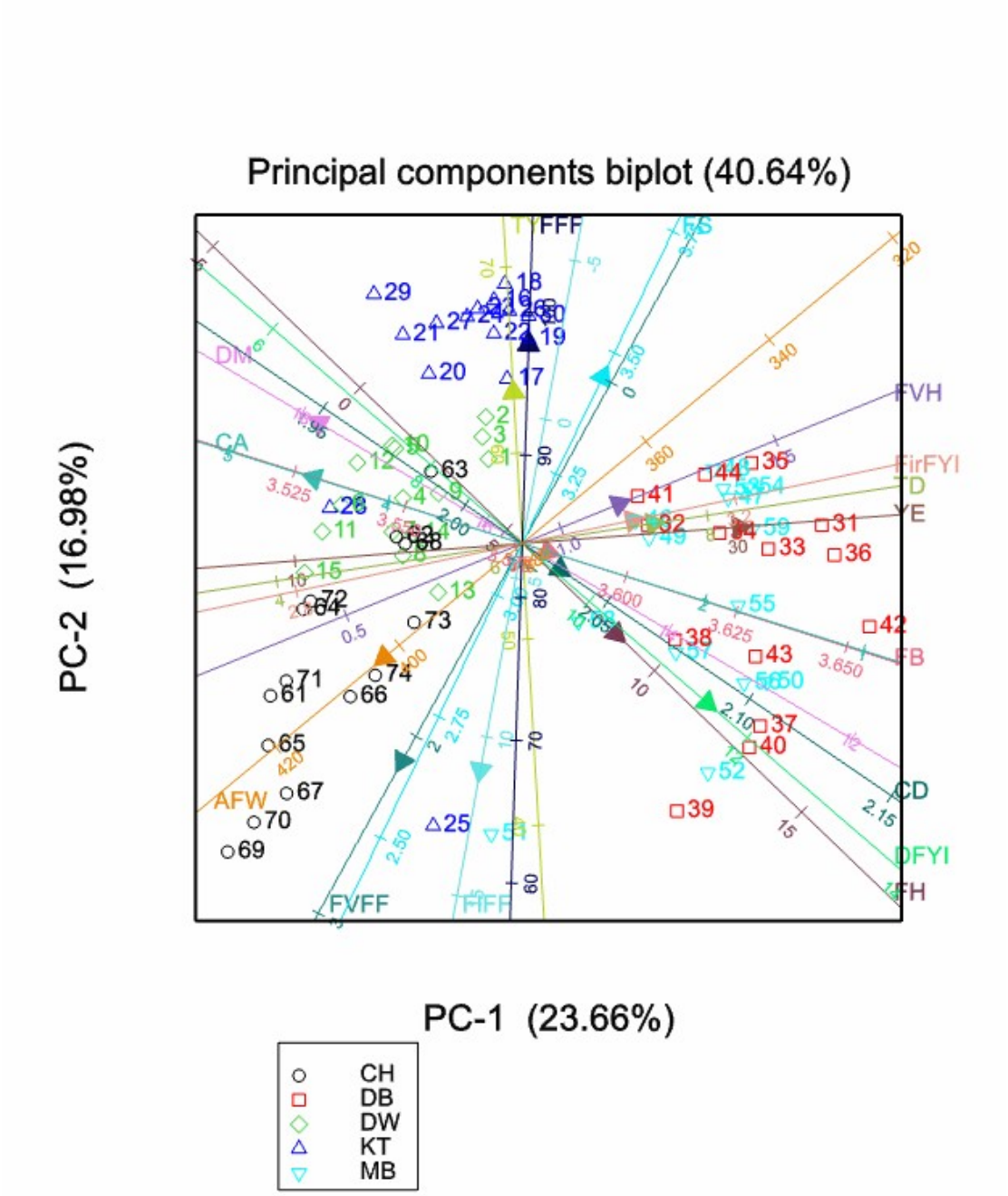
1	2
23.66	16.98

Trace

16.00

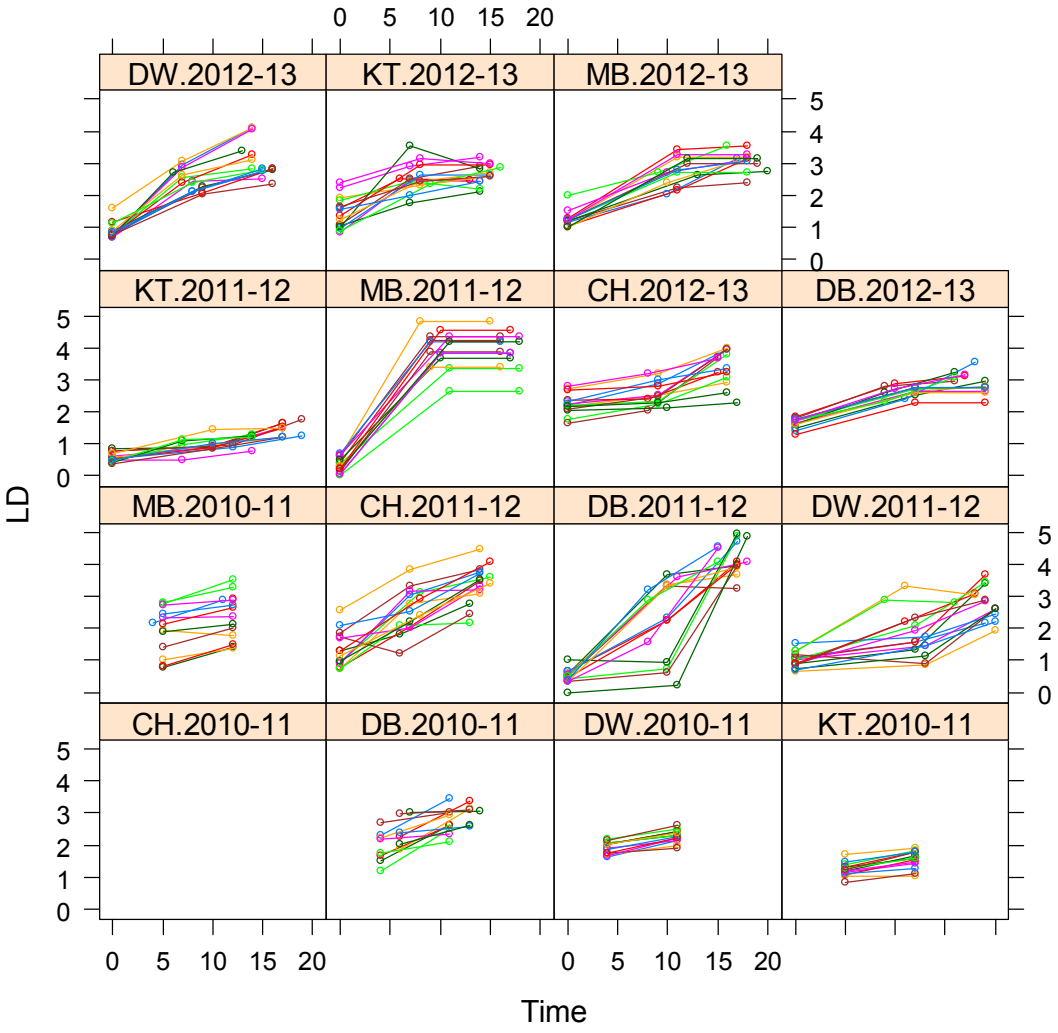
Latent vectors (loadings)

	1	2
AFW	-0.27198	-0.22444
CA	-0.41071	0.12905
CD	0.07406	-0.05058
DFYI	0.36443	-0.31393
DM	-0.40053	0.23742
FB	0.05120	-0.01628
FFF	0.01263	0.39194
FH	0.18492	-0.17771
FS	0.15845	0.32812
FVFF	-0.23650	-0.42849
FVH	0.19109	0.07774
FirFYI	0.21771	0.04588
FIFF	-0.08253	-0.44617
TD	0.25826	0.03931
TY	-0.01730	0.29822
YE	0.42766	0.03264

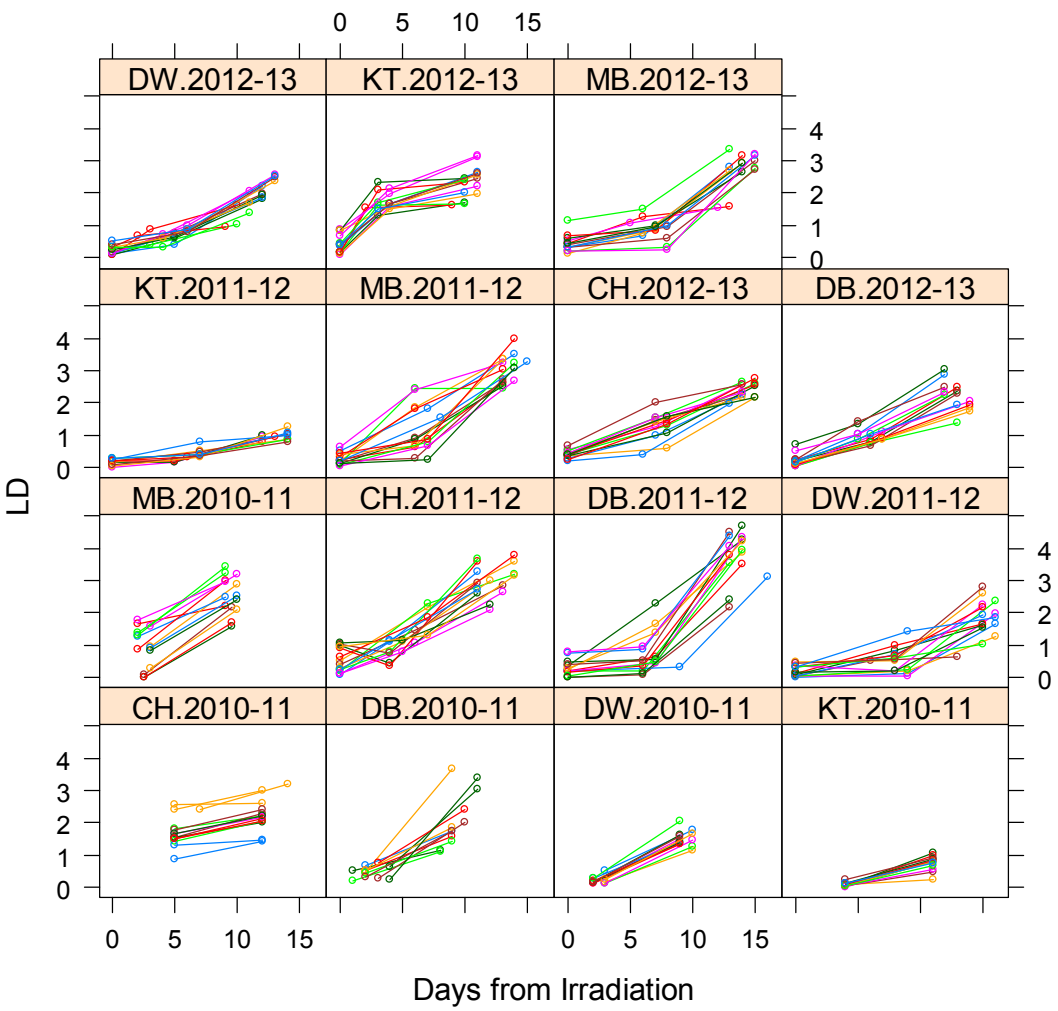


5. Consider the LD response over time (at Irradiation, at Full Yellow and 7 days after Full Yellow) and investigate which covariates impact significantly on the response

Plot of LD on Irradiated fruit at 3 assessment times (at Irradiation, at Full Yellow based on days to full yellow and 7 days post full yellow) for each Farm by Year.



LD over assessment times for non-irradiated Fruit



An analysis fitting a random regression model (linear mixed model) for the LD response over time (measured at 3 time points – at Irradiation, at Full Yellow (given at average time of Full Yellow which differs between trees) and 7 days after Full yellow) for Irradiated data was performed for each year separately . The model allows for a linear trend for LD across time with random intercepts and slopes for each tree. Covariates were added one at a time to the linear mixed model and their significance tested using Wald tests.

```
Model: LD.asr ~ asreml( LD ~ Farm + Farm:Time + Covariate,
Random =~str(~Tree + Time:Tree, ~corh(2):id(75)) ,
Rcov =~ diag(Timef):Tree, data=....)
```

2012-13 results: The following terms were found to be significant or close to significant on their own: FS (P=0.079), DM (P=0.071), DFYI (P=0.017). When including the most significant term (DFYI) in the model the addition of the other two terms did not give a significant improvement.
The coefficients for each of these terms in the individual models were:
FS 0.215
DM 0.088
DFYI -0.084

3. Irradiated fruit all years combined

Parameters codes:

Code	Parameter	Unit
TY	Tree yield	(kg)
YE	Yield efficiency	(Kg/m2)
CA	Canopy area	(m2)
CD	Canopy density	(1-3)
TD	Trunk differ.	(cm)
F2	Flowering -2 wk	(%)
FFF	Flowering FF	(%)
FS	Flower spread	(0-7)
FIFF	Flushing FF	(%)
FVFF	Flushing vigour FF	(0-5)
FH	Flushing harv.	(%)
FVH	Flushing vigour harv.	(0-5)
DM	Dry matter	(%)
FC	Flesh colour	(1-11)
AFW	Aver. fruit weight	(g)
FB	Fruit blush	(1-6)
	LD after irrad. (NI)	(0-5)
	LD after irrad. (I)	(0-5)
DFYNI	Days to FY (NI)	
DFYI	Days to FY (I)	
FirFYNI	Firmness FY(NI)	(0-4)
FirmFYI	Firmness FY (I)	(0-4)
LDFYNI	LD at FY (NI)	(0-5)
LDFYI	LD at FY (I)	(0-5)

Data:
Variables have been measured on Calypso Mango trees and fruit (averages per tree) from 5 farms over 3 years. A sample of 36 fruit were taken from each tree with 15 trees per farm. Half of these fruit were Irradiated and the other half not. The main trait of interest is the fruit Lenticel Damage rating (0-5) at Full Yellow (LDFYI and LDFYNI).

Aim:
The aim is to identify variables that may predict lenticel damage in Calypso fruit.

Statistical Methods:
A number of multivariate and regression approaches have been performed in order to try and find predictors of lenticel damage (at Full Yellow) in Calypso mangoes, including:
- multiple linear regression
- canonical variates analysis
- principal components analysis
- regression / classification trees

Results:

Model selection

```
 Response variate: LDFYI
  Number of units: 131
     Forced terms: Constant
        Forced df: 1
      Free terms: AFW + CA + CD + DFYI + DM + F2 + FB + FC +
                  FFF + FH + FS + FVFF + FVH + FirFYI + FlFF +
                  TD + TY + YE
```

Stepwise (forward) analysis of variance

Regression analysis

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ FS	1	32.5999	32.5999	67.38	<.001
+ TD	1	14.6244	14.6244	30.23	<.001
+ AFW	1	5.8635	5.8635	12.12	<.001
+ DM	1	3.5142	3.5142	7.26	0.008
Residual	126	60.9587	0.4838		
Total	130	117.5607	0.9043		

Final model: Constant + FS + TD + AFW + DM

Regression analysis

Response variate: LDFYI
Fitted terms: Constant, FS, TD, AFW, DM

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	4	48.84	12.2091	24.18	<.001
Residual	167	84.34	0.5050		
Total	171	133.18	0.7788		

Percentage variance accounted for 35.2
Standard error of observations is estimated to be 0.711.

Message: the following units have large standardized residuals.

Unit	Response	Residual
60	0.810	-3.01
120	0.222	-2.96
122	4.844	3.82

Message: the error variance does not appear to be constant; large responses are more variable than small responses.

Message: the following units have high leverage.

Unit	Response	Leverage
43	2.361	0.101
57	2.450	0.083
58	2.730	0.100
97	0.900	0.087
98	0.500	0.092
99	1.083	0.100
102	1.111	0.098

Estimates of parameters

Parameter	estimate	s.e.	t(167)	t pr.
Constant	1.041	0.801	1.30	0.196
FS	0.4793	0.0542	8.84	<.001
TD	0.0344	0.0120	2.86	0.005
AFW	0.00368	0.00131	2.80	0.006
DM	-0.0996	0.0351	-2.84	0.005

```
Response variate: LDFYI
  Number of units: 200
     Forced terms: Constant
        Forced df: 1
       Free terms: AFW + CA + CD + DM + FB + FFF + FH + FlFF +
                   TD + TY + YE
```

Stepwise (forward) analysis of variance

Regression analysis

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ DM	1	20.0318	20.0318	37.54	<.001
+ FFF	1	10.6282	10.6282	19.92	<.001
+ TD	1	4.5981	4.5981	8.62	0.004
+ FB	1	6.7643	6.7643	12.68	<.001
+ CA	1	5.4265	5.4265	10.17	0.002
+ AFW	1	3.0218	3.0218	5.66	0.018
Residual	193	102.9783	0.5336		
Total	199	153.4489	0.7711		

Final model: Constant + DM + FFF + TD + FB + CA + AFW

Regression analysis

Response variate: LDFYI
Fitted terms: Constant, DM, FFF, TD, FB, CA, AFW

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	6	50.5	8.4118	15.77	<.001
Residual	193	103.0	0.5336		
Total	199	153.4	0.7711		

Percentage variance accounted for 30.8
Standard error of observations is estimated to be 0.730.

Message: the following units have large standardized residuals.

Unit	Response	Residual
109	0.719	-2.89
117	0.625	-2.85
120	0.222	-3.69
122	4.844	3.19

Message: the following units have high leverage.

Unit	Response	Leverage
43	2.361	0.126
60	0.810	0.113
96	0.969	0.123
97	0.900	0.114
98	0.500	0.110
99	1.083	0.114
102	1.111	0.130

Estimates of parameters

Parameter	estimate	s.e.	t(193)	t pr.
Constant	3.785	0.825	4.59	<.001
DM	-0.0904	0.0345	-2.62	0.009
FFF	0.01339	0.00244	5.49	<.001
TD	0.0494	0.0123	4.01	<.001
FB	-0.600	0.126	-4.78	<.001
CA	-0.1485	0.0512	-2.90	0.004
AFW	0.00309	0.00130	2.38	0.018

Regression or Classification Tree:

Summary of classification tree: t2

Number of nodes: 85
Number of terminal nodes: 43
Misclassification rate: 0.044
Variables in the tree: DFYI, DM, FS, FC, AFW, FirFYI, CA, FB, TD, YE, FFF, CD, TY, FVFF, FH.

Details of classification tree: t2

1 Current prediction: 2.411
Number of observations: 200

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.095	0.230	0.490	0.150	0.035

```
Test: DFYI<5.500
      Next nodes:  2  3

2 Current prediction: 1.459
 Number of observations: 49
           LDFYIgr    0.875    1.459    2.411    3.312    4.333
       Proportions    0.061    0.571    0.367    0.000    0.000
 Test: DM<16.64
       Next nodes:  4  5

4 Current prediction: 2.411
 Number of observations: 35
           LDFYIgr    0.875    1.459    2.411    3.312    4.333
       Proportions    0.057    0.429    0.514    0.000    0.000
 Test: FC<6.500
       Next nodes:  6  7

 6 Current prediction: 1.459
  Number of observations: 6
            LDFYIgr    0.875    1.459    2.411    3.312    4.333
        Proportions    0.333    0.667    0.000    0.000    0.000
  Test: FirFYI<1.568
        Next nodes:  8  9

  8 Current prediction: 0.8750
   Number of observations: 2
            LDFYIgr    0.875    1.459    2.411    3.312    4.333
        Proportions    1.000    0.000    0.000    0.000    0.000
   Conclusion: 0.8750

  9 Current prediction:  1.459
   Number of observations: 4
            LDFYIgr    0.875    1.459    2.411    3.312    4.333
        Proportions    0.000    1.000    0.000    0.000    0.000
   Conclusion: 1.459

 7 Current prediction: 2.411
  Number of observations: 29
            LDFYIgr    0.875    1.459    2.411    3.312    4.333
        Proportions    0.000    0.379    0.621    0.000    0.000
  Test: DM<15.25
        Next nodes: 10 11

 10 Current prediction:  2.411
   Number of observations: 10
            LDFYIgr    0.875    1.459    2.411    3.312    4.333
        Proportions    0.000    0.100    0.900    0.000    0.000
   Test: DM<14.15
         Next nodes: 12 13

  12 Current prediction:   1.459
    Number of observations: 1
             LDFYIgr    0.875    1.459    2.411    3.312    4.333
         Proportions    0.000    1.000    0.000    0.000    0.000
    Conclusion: 1.459

  13 Current prediction:   2.411
    Number of observations: 9
             LDFYIgr    0.875    1.459    2.411    3.312    4.333
         Proportions    0.000    0.000    1.000    0.000    0.000
```

Conclusion: 2.411

11 Current prediction: 1.459
Number of observations: 19

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.526	0.474	0.000	0.000

Test: FirFYI<2.476
Next nodes: 14 15

14 Current prediction: 2.411
Number of observations: 6

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.167	0.833	0.000	0.000

Test: TD<2.250
Next nodes: 16 17

16 Current prediction: 1.459
Number of observations: 1

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	1.000	0.000	0.000	0.000

Conclusion: 1.459

17 Current prediction: 2.411
Number of observations: 5

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	1.000	0.000	0.000

Conclusion: 2.411

15 Current prediction: 1.459
Number of observations: 13

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.692	0.308	0.000	0.000

Test: CA<1.608
Next nodes: 18 19

18 Current prediction: 1.459
Number of observations: 4

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	1.000	0.000	0.000	0.000

Conclusion: 1.459

19 Current prediction: 1.459
Number of observations: 9

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.556	0.444	0.000	0.000

Test: CA<3.646
Next nodes: 20 21

20 Current prediction: 2.411
Number of observations: 3

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	1.000	0.000	0.000

Conclusion: 2.411

21 Current prediction: 1.459
Number of observations: 6

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.833	0.167	0.000	0.000

Test: AFW<431.8
Next nodes: 22 23

22 Current prediction: 1.459
Number of observations: 5

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	1.000	0.000	0.000	0.000

Conclusion: 1.459

23 Current prediction: 2.411
Number of observations: 1

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	1.000	0.000	0.000

Conclusion: 2.411

5 Current prediction: 1.459
Number of observations: 14

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.071	0.929	0.000	0.000	0.000

Test: AFW<338.9
Next nodes: 24 25

24 Current prediction: 0.8750
Number of observations: 2

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.500	0.500	0.000	0.000	0.000

Conclusion: 0.8750

25 Current prediction: 1.459
Number of observations: 12

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	1.000	0.000	0.000	0.000

Conclusion: 1.459

3 Current prediction: 2.411
Number of observations: 151

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.106	0.119	0.530	0.199	0.046

Test: FS<2.375
Next nodes: 26 27

26 Current prediction: 0.8750
Number of observations: 53

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.302	0.245	0.283	0.113	0.057

Test: DM<16.49
Next nodes: 28 29

28 Current prediction: 2.411
Number of observations: 45

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.200	0.289	0.311	0.133	0.067

Test: FirFYI<3.284
Next nodes: 30 31

30 Current prediction: 2.411
Number of observations: 14

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.286	0.643	0.071	0.000

Test: AFW<369.9
Next nodes: 32 33

32 Current prediction: 1.459
Number of observations: 3

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	1.000	0.000	0.000	0.000

Conclusion: 1.459

33 Current prediction: 2.411
Number of observations: 11

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.091	0.818	0.091	0.000

Test: YE<18.19
Next nodes: 34 35

34 Current prediction: 2.411
Number of observations: 8

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	1.000	0.000	0.000

Conclusion: 2.411

35 Current prediction: 1.459
Number of observations: 3

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.333	0.333	0.333	0.000

Conclusion: 1.459

31 Current prediction: 0.8750
Number of observations: 31

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.290	0.290	0.161	0.161	0.097

Test: FC<6.300
Next nodes: 36 37

36 Current prediction: 1.459
Number of observations: 18

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.111	0.389	0.278	0.056	0.167

Test: DFYI<11.50
Next nodes: 38 39

38 Current prediction: 2.411
Number of observations: 8

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	0.500	0.125	0.375

Test: YE<22.67
Next nodes: 40 41

40 Current prediction: 4.333
Number of observations: 4

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	0.000	0.250	0.750

Conclusion: 4.333

41 Current prediction: 2.411
Number of observations: 4

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	1.000	0.000	0.000

Conclusion: 2.411

39 Current prediction: 1.459
Number of observations: 10

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.200	0.700	0.100	0.000	0.000

Test: YE<12.69
Next nodes: 42 43

42 Current prediction: 0.8750
Number of observations: 2

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	1.000	0.000	0.000	0.000	0.000

Conclusion: 0.8750

43 Current prediction: 1.459
Number of observations: 8

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.875	0.125	0.000	0.000

Test: FS<1.125
Next nodes: 44 45

44 Current prediction: 2.411
Number of observations: 1

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	1.000	0.000	0.000

Conclusion: 2.411

45 Current prediction: 1.459
Number of observations: 7

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	1.000	0.000	0.000	0.000

Conclusion: 1.459

37 Current prediction: 0.8750
Number of observations: 13

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.538	0.154	0.000	0.308	0.000

Test: AFW<414.3
Next nodes: 46 47

46 Current prediction: 0.8750
Number of observations: 4

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	1.000	0.000	0.000	0.000	0.000

Conclusion: 0.8750

47 Current prediction: 3.312
Number of observations: 9

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.333	0.222	0.000	0.444	0.000

Test: FS<1.375
Next nodes: 48 49

48 Current prediction: 0.8750
Number of observations: 4

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.750	0.250	0.000	0.000	0.000

Conclusion: 0.8750

49 Current prediction: 3.312
Number of observations: 5

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.200	0.000	0.800	0.000

Conclusion: 3.312

29 Current prediction: 0.8750
Number of observations: 8

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.875	0.000	0.125	0.000	0.000

Test: FS<1.375
Next nodes: 50 51

50 Current prediction: 0.8750
Number of observations: 7

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	1.000	0.000	0.000	0.000	0.000

Conclusion: 0.8750

51 Current prediction: 2.411
Number of observations: 1

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	1.000	0.000	0.000

Conclusion: 2.411

27 Current prediction: 2.411
Number of observations: 98

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.051	0.663	0.245	0.041

Test: FirFYI<3.264
Next nodes: 52 53

52 Current prediction: 2.411
Number of observations: 52

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.038	0.846	0.115	0.000

Test: FirFYI<2.882
Next nodes: 54 55

54 Current prediction: 2.411
Number of observations: 24

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.083	0.667	0.250	0.000

Test: CA<2.694
Next nodes: 56 57

56 Current prediction: 3.312
Number of observations: 2

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	0.000	1.000	0.000

Conclusion: 3.312

57 Current prediction: 2.411
Number of observations: 22

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.091	0.727	0.182	0.000

Test: FFF<99.25
Next nodes: 58 59

58 Current prediction: 2.411
Number of observations: 20

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.100	0.800	0.100	0.000

Test: CA<5.978
Next nodes: 60 61

60 Current prediction: 2.411
Number of observations: 19

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.053	0.842	0.105	0.000

Test: DM<16.45
Next nodes: 62 63

62 Current prediction: 2.411
Number of observations: 7

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.143	0.571	0.286	0.000

Test: FVFF<2.000
Next nodes: 64 65

64 Current prediction: 2.411
Number of observations: 5

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.200	0.800	0.000	0.000

Conclusion: 2.411

65 Current prediction: 3.312
Number of observations: 2

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	0.000	1.000	0.000

Conclusion: 3.312

63 Current prediction: 2.411
Number of observations: 12

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	1.000	0.000	0.000

Conclusion: 2.411

61 Current prediction: 1.459
Number of observations: 1

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	1.000	0.000	0.000	0.000

Conclusion: 1.459

59 Current prediction: 3.312
Number of observations: 2

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	0.000	1.000	0.000

Conclusion: 3.312

55 Current prediction: 2.411
Number of observations: 28

LDFYlgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	1.000	0.000	0.000

Conclusion: 2.411

53 Current prediction: 2.411
Number of observations: 46

LDFYlgr	0.875	1.459	2.411	3.312	4.333

```
          Proportions     0.000     0.065     0.457     0.391     0.087
 Test: DM<14.41
          Next nodes: 66 67

 66 Current prediction:  3.312
   Number of observations: 28
                LDFYlgr     0.875     1.459     2.411     3.312     4.333
             Proportions     0.000     0.071     0.214     0.571     0.143
   Test: FB<3.188
            Next nodes: 68 69

  68 Current prediction:   2.411
    Number of observations: 3
                  LDFYlgr     0.875     1.459     2.411     3.312     4.333
               Proportions     0.000     0.000     1.000     0.000     0.000
    Conclusion: 2.411

  69 Current prediction:   3.312
    Number of observations: 25
                  LDFYlgr     0.875     1.459     2.411     3.312     4.333
               Proportions     0.000     0.080     0.120     0.640     0.160
    Test: AFW<437.3
             Next nodes: 70 71

   70 Current prediction:    3.312
     Number of observations: 19
                   LDFYlgr     0.875     1.459     2.411     3.312     4.333
                Proportions     0.000     0.105     0.000     0.789     0.105
     Test: YE<14.06
              Next nodes: 72 73

    72 Current prediction:     1.459
      Number of observations: 2
                    LDFYlgr     0.875     1.459     2.411     3.312     4.333
                 Proportions     0.000     1.000     0.000     0.000     0.000
      Conclusion: 1.459

    73 Current prediction:     3.312
      Number of observations: 17
                    LDFYlgr     0.875     1.459     2.411     3.312     4.333
                 Proportions     0.000     0.000     0.000     0.882     0.118
      Test: TY<94.19
               Next nodes: 74 75

     74 Current prediction:      3.312
       Number of observations: 16
                     LDFYlgr     0.875     1.459     2.411     3.312     4.333
                  Proportions     0.000     0.000     0.000     0.938     0.063
       Test:  FH<8.750
                Next nodes: 76 77

      76 Current prediction:       3.312
        Number of observations: 13
                      LDFYlgr     0.875     1.459     2.411     3.312     4.333
                   Proportions     0.000     0.000     0.000     1.000     0.000
        Conclusion: 3.312

      77 Current prediction:       3.312
        Number of observations: 3
                      LDFYlgr     0.875     1.459     2.411     3.312     4.333
```

Proportions 0.000 0.000 0.000 0.667 0.333
Conclusion: 3.312

75 Current prediction: 4.333
Number of observations: 1
LDFYlgr 0.875 1.459 2.411 3.312 4.333
Proportions 0.000 0.000 0.000 0.000 1.000
Conclusion: 4.333

71 Current prediction: 2.411
Number of observations: 6
LDFYlgr 0.875 1.459 2.411 3.312 4.333
Proportions 0.000 0.000 0.500 0.167 0.333
Test: AFW<445.1
Next nodes: 78 79

78 Current prediction: 4.333
Number of observations: 2
LDFYlgr 0.875 1.459 2.411 3.312 4.333
Proportions 0.000 0.000 0.000 0.000 1.000
Conclusion: 4.333

79 Current prediction: 2.411
Number of observations: 4
LDFYlgr 0.875 1.459 2.411 3.312 4.333
Proportions 0.000 0.000 0.750 0.250 0.000
Conclusion: 2.411

67 Current prediction: 2.411
Number of observations: 18
LDFYlgr 0.875 1.459 2.411 3.312 4.333
Proportions 0.000 0.056 0.833 0.111 0.000
Test: FB<4.306
Next nodes: 80 81

80 Current prediction: 2.411
Number of observations: 17
LDFYlgr 0.875 1.459 2.411 3.312 4.333
Proportions 0.000 0.000 0.882 0.118 0.000
Test: FirFYI<3.319
Next nodes: 82 83

82 Current prediction: 3.312
Number of observations: 1
LDFYlgr 0.875 1.459 2.411 3.312 4.333
Proportions 0.000 0.000 0.000 1.000 0.000
Conclusion: 3.312

83 Current prediction: 2.411
Number of observations: 16
LDFYlgr 0.875 1.459 2.411 3.312 4.333
Proportions 0.000 0.000 0.938 0.063 0.000
Test: CD<2.250
Next nodes: 84 85

84 Current prediction: 2.411
Number of observations: 14
LDFYlgr 0.875 1.459 2.411 3.312 4.333
Proportions 0.000 0.000 1.000 0.000 0.000

Conclusion: 2.411

85 Current prediction: 2.411
Number of observations: 2

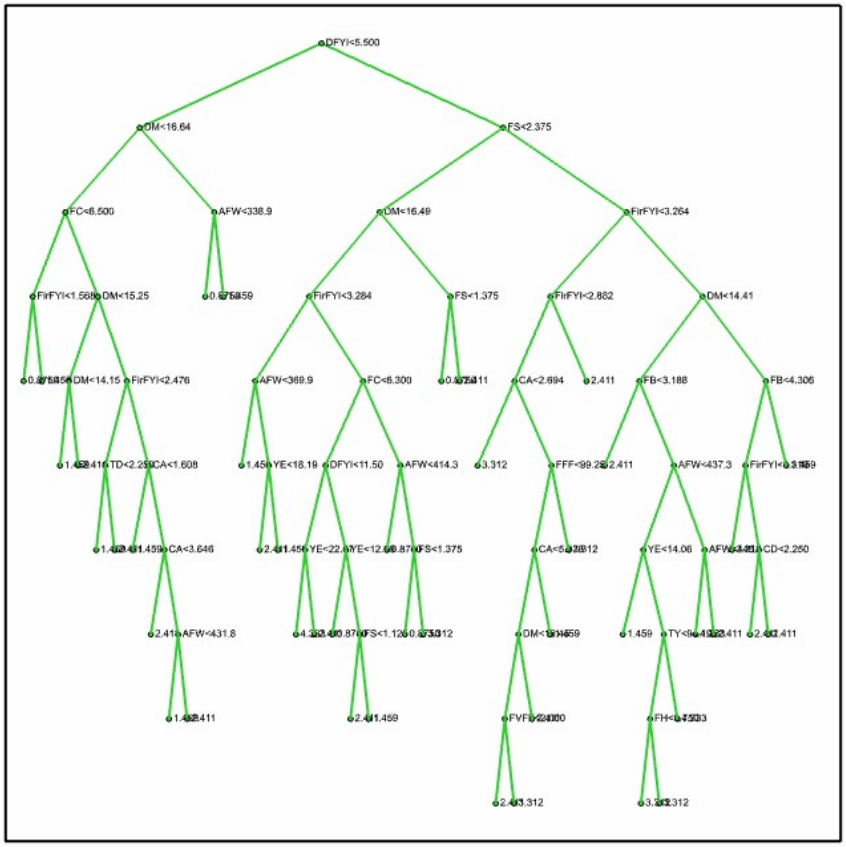
LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	0.000	0.500	0.500	0.000

Conclusion: 2.411

81 Current prediction: 1.459
Number of observations: 1

LDFYIgr	0.875	1.459	2.411	3.312	4.333
Proportions	0.000	1.000	0.000	0.000	0.000

Conclusion: 1.459



Principal components analysis and biplot

Principal components analysis

Latent roots

1	2
5.601	3.055

Percentage variation

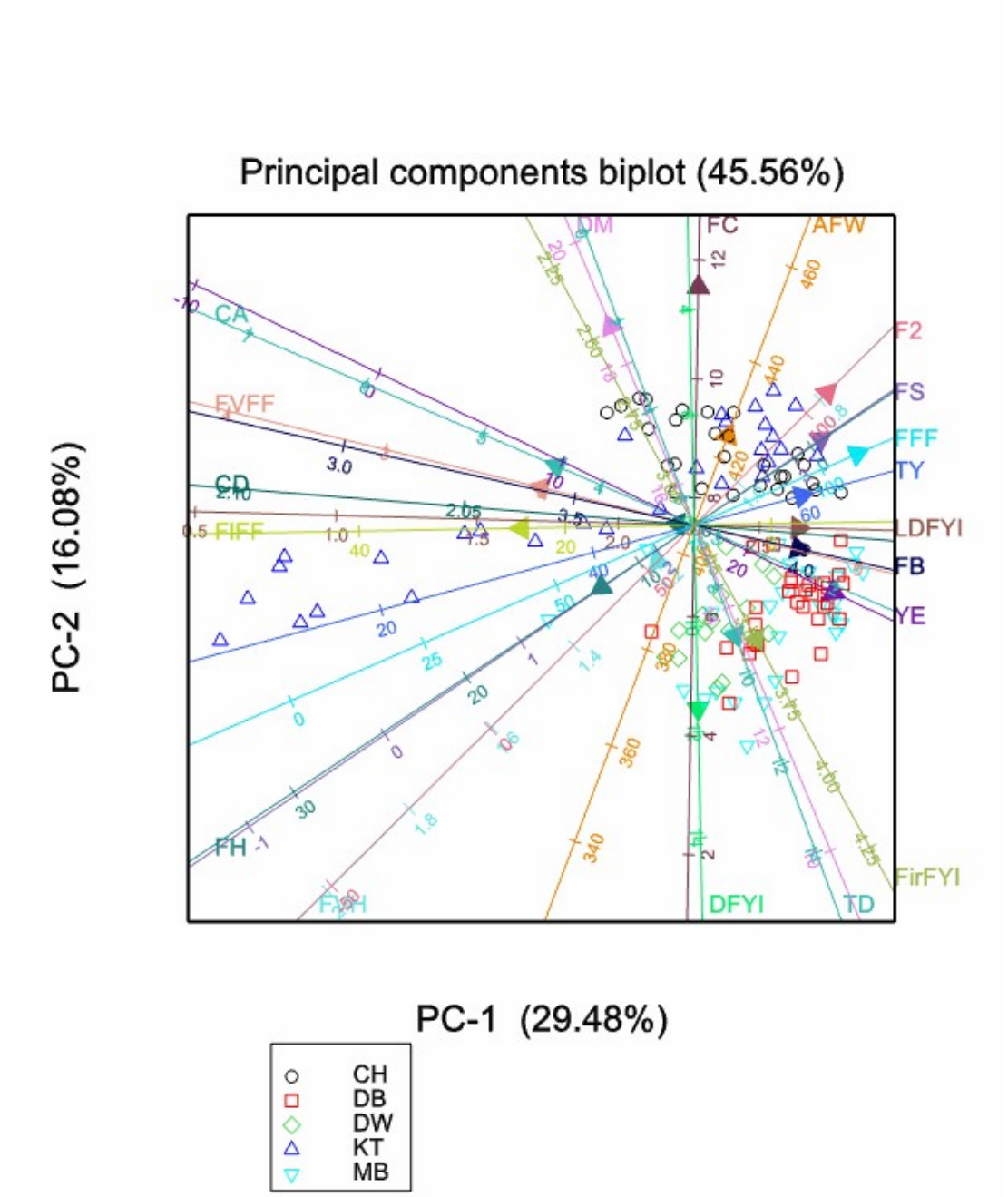
1	2
29.48	16.08

Trace

19.00

Latent vectors (loadings)

	1	2
AFW	0.07386	0.19580
CA	-0.30060	0.12910
CD	-0.03572	0.00283
DFYI	0.00829	-0.40187
DM	-0.18224	0.43452
F2	0.29127	0.28960
FB	0.22786	-0.05130
FC	0.00928	0.52047
FFF	0.35684	0.15549
FH	-0.20294	-0.13527
FS	0.27687	0.18756
FVFF	-0.33410	0.08166
FVH	-0.07959	-0.07937
FirFYI	0.13630	-0.25012
FIFF	-0.37764	-0.00681
LDFYI	0.22771	-0.00616
TD	0.08999	-0.24157
TY	0.23561	0.06385
YE	0.30496	-0.14694



Canonical variates analysis

Canonical variate analysis

Latent roots

1	2
1.745	0.427

Percentage variation

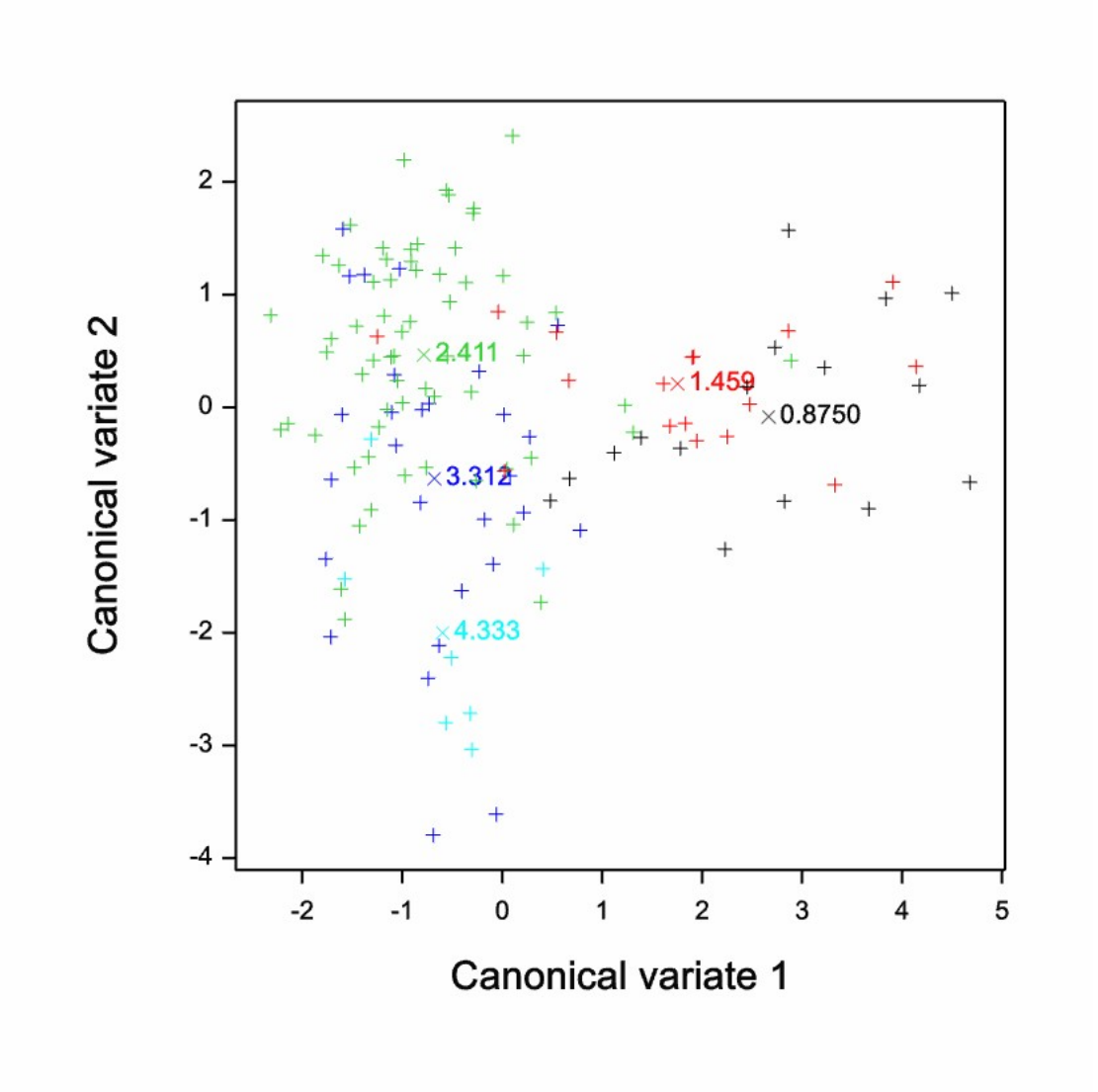
1	2
66.72	16.32

Trace

2.616

Latent vectors (loadings)

	1	2
AFW	-0.3858	-0.3113
CA	1.0140	0.0486
CD	0.1730	0.1825
DFYI	0.4331	0.0403
DM	0.7605	0.1352
F2	-0.2253	-0.5203
FB	0.1371	0.1257
FC	0.1431	0.2736
FFF	0.4686	0.3715
FH	0.0510	0.1333
FS	-0.9809	0.1107
FVFF	-0.3105	-0.2071
FVH	-0.1111	0.1224
FirFYI	0.7641	-0.6581
FIFF	0.2015	0.1220
TD	-0.0780	-0.7890
TY	-0.6967	-0.4853
YE	0.5845	0.4261


Canonical variate 2
Canonical variate 1
2.411
1.459
0.8750
3.312
4.333
2
1
0
-1
-2
-3
-4
-2
-1
0
1
2
3
4
5