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PRE-HARVEST AND POSTHARVEST FACTORS AFFECTING SKIN COLOUR AND OTHER QUALITY ATTRIBUTES OF 'KENSINGTON PRIDE' MANGO (*MANGIFERA INDICA* LINN.)

by

Ha Xuan Nguyen

B.Agr.Eng. (Hon)

University of Technology, Rouse, Bulgaria

M.Sc.

Massey University, New Zealand

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School of Land Water and Crop Sciences

The University of Sydney

New South Wales

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ABSTRACT

The retention of green colour on the skin of ripe fruit has a significant negative impact on the saleability and value of 'Kensington Pride' mangoes (*Mangifera indica* Linn.) in Australia. Previous, preliminary work on mango, and more detailed work on other commodities, indicated that pre-harvest factors such as nitrogen, and postharvest practices such as ripening conditions, can be a major factor in the skin colour of ripe fruit.

The aim of this thesis was to investigate how production practices, in particular nitrogen, and postharvest practices (ripening temperature, ethylene and carbon dioxide concentration) influence the skin colour of ripe 'Kensington Pride' fruit. In addition, the effects of deviations from the ideal ripening and holding conditions were investigated to understand how commercial holding and transport conditions can affect skin colour and quality. The key skin pigments concentrations were quantified to understand how these external factors affect skin colour. Other quality parameters such as diseases and skin blemishes were also measured to obtain a more complete understanding of the effects of these factors on fruit quality.

To understand the extent of the green, ripe fruit problem, and conditions that may affect fruit colour, a survey of 10 commercial orchards was conducted in the Burdekin district (north Queensland, Australia). The survey indicated that green, ripe fruit were produced by most of the orchards evaluated. Circumstantial evidence suggested that nitrogen may be involved. Therefore, the effect of pre-harvest nitrogen application on skin colour and other quality attributes was investigated in the following two seasons. Pre-harvest nitrogen applications of

150 g per tree or more mainly at pre-flowering, and 275 g per tree or more mainly just after harvest, increased the % of the skin area on the ripe fruit with green colour. In addition, nitrogen application can increase anthracnose severity. However, there were very few effects on days from harvest to ripe (DTR), flesh total soluble solids or acidity, or stem-end rot severity. There were also no significant effects on tree yield, although more work is required in this area to confirm the balance between quality and yield.

The effects of ripening temperature, ethylene concentration and duration, and carbon dioxide (CO_2) concentration, on skin colour and other quality attributes were also investigated. Ethylene concentrations of 100 $\mu L\ L^{-1}$ or higher for 72 h at 15°C increased the % green area on the skin of ripe fruit and the flesh acidity, due to softening occurring quicker than other ripening changes at 15°C compared with ripening at higher temperatures up to 25°C. However, ripening at 25°C, resulted in an uneven colour (blotchiness). Ethylene concentration of 10 $\mu L\ L^{-1}$ for 72 hours at 20°C resulted in the least green colour on ripe fruit, with no beneficial effects of higher concentrations of ethylene up to 1000 $\mu L\ L^{-1}$ at the same temperature and duration.

The % green area of the skin and other quality attributes were also affected by CO_2 concentration and short term exposure to low or high temperatures. Carbon dioxide concentrations of 4% and 6% resulted in higher % green colour, and the yellow skin colour had a more dull appearance, than those ripened without additional CO_2. Fruit at the mid-climacteric stage of ripening placed at 24°C until ripe or at 28°C for 3 days or more then back to 20°C, had more green colour on the skin than fruit ripened at a constant 20°C. A higher % green colour was also observed if mid-climacteric fruit were exposed to 7°C for 3 days or

more, or to 10°C for 7 days or more. In addition, fruit exposed to these higher or lower temperatures showed disorders such as blotchiness and skin chilling injury, respectively.

The present results demonstrate that green, ripe fruit can be a problem with the 'Kensington Pride' mango. Skin colour and disease severity of ripe fruit can be reduced by reducing pre-harvest nitrogen application without major changes in other quality attributes or a negative yield response in the orchards tested. In addition, skin colour and other quality attributes of fruit can be maintained by ripening at about 20°C with 3 days treatment with 10 $\mu L\ L^{-1}$ ethylene. Conditions that slow the ripening, such as ripening temperatures of 15°C and not using ethylene, can increase green skin colour and diseases. In addition, adequate ventilation of ripening rooms is required to prevent CO_2 concentrations above 4%. Therefore, this study has demonstrated that skin colour of mango fruit can be improved by both fertilizer management and postharvest practices.

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LIST OF ABBREVIATIONS

ACC – 1-aminocyclopropane-1-carboxylate

AOA – aminooxyacetic

AVG – amino ethoxyvinylglycine

Ca – calcium

CA – controlled atmosphere

Chl-*a* – chlorophyll *a*

Chl-*b* – chlorophyll *b*

CI – chilling injury

CO_2 – carbon dioxide

DTR – days to ripe

GC – gas chromatograph

H – high (N status)

HCL soil – heavy clay loam soil

HG, HG* orchard – high green colour orchard

HHHA – high humidity hot air

H° – hue angle

K – potassium

L – low (N status)

LG, LG* orchard – low green colour orchard

LHCP II – light-harvesting complex II

LS – lenticel spotting

LSD – least significant difference

M – moderate (N status)

MA – modified atmosphere

MAP – modified atmosphere packaging

Mg – magnesium

M1, 7, 26 – Malling 1, 7, 26

MM106 – Malling Merton 106

N – nitrogen

O_2 – oxygen

NCCs – nonfluorescent chlorophyll catabolites

P – phosphorous

PaO – pheophorbide a oxygenase

PE – pectinesterase

PEPC – phosphoenolpyruvate carboxylase

pFCC – primary fluorescent chlorophyll catabolite

PG – polygalacturonase

RCC – red chlorophyll catabolite

RH – relative humidity

RuBP – ribulose-1,5-bisphosphate

SAM – S-adenosylmethionine

SER – stem-end rots

SL soil – sandy loam soil

SPS – sucrose-phosphate synthase

TSS – total soluble solids

1-MCP – 1-methylcyclopropane

DECLARATION OF ORIGINALITY

This thesis contains no material that has been submitted for the award of any other degree or diploma in any university. Some of the results of this research have been published and presented at several national and international conferences (Appendix 1).

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CHAPTER ONE

INTRODUCTION

Mango (*Mangifera indica* Linn.) is one of the most important fruits of the tropical and subtropical regions of the world (Medlicott *et al.*, 1986a; Chadha and Pal, 1993). The mango is believed to have originated in the Indo-Burma region (Singh, 1960; Chadha and Pal, 1993; Kostermans and Bompard, 1993), but is now being grown in more than 100 countries spread over five continents, including relatively cold subtropical environments such as Israel and Spain (Sauco, 1993). Although there are many mango cultivars, only a few of them are commercially important (Krishnamurthy and Subramanyam, 1973). For example, there are nearly 1000 mango cultivars in India, but only about 20 cultivars are commercially grown. There are generally only one or two cultivars exported from each country, for instance: Alphonso from India; Carabao from the Philippines; Haden, Keitt and Zill from South Africa; Haden and Maya from Israel; Julie from Trinidad; Apple, Boribo and Nqowe from Kenya; Haden and Manila from Mexico; Okrong from Thailand; and Madame Francis from Haiti (Chadha, 1989).

In Australia, 'Kensington Pride' is the most popular mango cultivar and accounts for 80% of the total crop production (Bally *et al.*, 2000). 'Kensington Pride' fruit is known for its unique flavour and taste, however, it is also has a reputation for inconsistency in quality (Ledger, 1996). Although 'Kensington Pride' has inconsistent quality, if quality is high then it is a very popular fruit with Australian consumers. Quality of fresh fruit and vegetables is defined by two aspects, product-oriented and consumer-oriented quality (Shewfelt, 1999). Product-

oriented quality is determined by a series of measurable attributes, while consumer-oriented quality is based on either of acceptability and or willingness to purchase by consumers. The product-oriented quality assessment is useful for grading, however, consumer-oriented quality is very subjective since the consumer will take the final decision to purchase or not. Because of the importance of consumer-oriented quality, most postharvest researchers, producers and marketers have agreed that the appearance of the commodity is one of the most important quality attributes (Kays, 1999). For 'Kensington Pride' mango fruit, the most important postharvest quality attributes are the amount of green colour remaining on the skin when ripe as well as the amount of sapburn, skin browning, diseases and internal disorders (Hofman *et al.*, 1995a). The retention of green colour on ripe fruit has become a major quality issue for the 'Kensington Pride' cultivar and was highlighted as a priority research area at the Australian Mango Conference in Townsville in 1995. However, since then, there has been little investigation into the cause of the problem.

Pre-harvest factors such as rootstock, climate, weather, soils, water relations, light intensity, nutrition imbalances and maturity are believed to influence the appearance as well as other quality attributes of harvested products (Pantastico *et al.*, 1975; Sharples, 1984; Kays, 1999; Sams, 1999). Appearance is determined by size, shape, form, colour and the absence of diseases and defects. Skin colour is considered as the most important single factor contributing to fruit quality assessment (Kays, 1999). Inappropriate fertiliser management can lead to a reduction in fruit appearance, particularly the colour of the skin. For instance, high nitrogen (N) results in poor colouration in nectarine (Daane *et al.*, 1995); potassium deficiency leads to poor colouration in peach (Reeves and Cummings, 1970), and excess magnesium causes apple discolouration (Ferree *et al.*, 1984). The colour of many apple

cultivars is thought to be influenced by pre-harvest N application in that high N rates normally result in fruit with high green colour (Neilsen *et al.*, 1984; Fallahi *et al.*, 1985a).

In addition to pre-harvest factors, postharvest factors such as ripening temperature, relative humidity, ethylene treatment and atmospheric composition also influence the final quality attributes, as well as determine storage life. An understanding of the impact of postharvest handling practices on the quality of tropical fruits such as mango is important to improve fruit quality and storage potential. Postharvest temperature is the environmental factor that most influences the deterioration rate of harvested commodities (Kader, 1992a). In apple, the de-greening rate (chlorophyll degradation rate) of fruit is thought to be a function of ripening temperature (Dixon and Hewett, 1998). The de-greening rate reaches a peak when temperature is in the optimum range.

The use of ethylene to stimulate ripening, particularly colour development, has been reported in many fruit (Knee, 1985; Saltveit, 1999). In addition, atmospheric composition, in particular oxygen and carbon dioxide concentration, may affect other processes associated with the quality of fruit such as pigment, phenolic and volatile compound metabolism (Beaudry, 1999), as well as influence ethylene responses and respiration (Burg and Burg, 1967).

The overall aim of this thesis was to investigate ways in which the retention of green colour on ripe mango fruit can be minimised. A survey of the quality of 'Kensington Pride' fruit was undertaken in the first year (Chapter 4) to understand the extent of green, ripe fruit in the Burdekin district, a major 'Kensington Pride' production area in Australia, and to relate it to

the history of N fertilisation for these orchards. In the following two years, the rate and timing of pre-harvest N application was investigated to ascertain whether high rates of N contributed to the retention of green colour on ripe fruit and disease incidence. The aim was to reduce the green, ripe problem of 'Kensington Pride' fruit and postharvest disease incidence without reducing other fruit quality attributes and fruit yield.

Commercially, mature green mango fruit are ripened by applying ethylene gas, however there are no clear guidelines for ethylene concentration, temperature or duration of application for 'Kensington Pride' mango. Defined ethylene application strategies may assist in reducing the % green area on the skin of ripe fruit as well as provide overall improved fruit quality. Ethylene treatments in combination with different temperatures were applied to mature green fruit harvested from orchards with a history of green, ripe fruit to determine the optimum ripening conditions (Chapter 5). A second series of low concentration ethylene applications at optimum temperature were conducted to confirm the result of the first experiment. In addition, the effect of CO_2 concentration in the ripening room on colour and other quality attributes was also investigated.

During transport, particularly over long distances, mangoes are often exposed to adverse high and low temperatures due to shipment delays or poor temperature management (Holmes and Kernot, 2002). High temperatures can promote uneven, blotchy colour development while temperatures below 12°C can cause chilling damage, both reducing appearance quality of the fruit. The effects of short term exposure to extreme temperatures (high or low) on mango quality were also studied to determine the tolerance of 'Kensington Pride' to these adverse conditions (Chapter 6).

CHAPTER TWO

LITERATURE REVIEW

2.1 MANGO IN AUSTRALIA – IMPORTANCE AND PROSPECTS

Mango (*Mangifera indica* Linn.) was introduced to Australia in the mid nineteenth century (Alexander, 1987; Department of Primary Industries, 1989). There are several mango cultivars grown in Australia such as 'Kensington Pride', 'R2E2', 'Keitt', 'Irwin' and 'Kent', however, 'Kensington Pride' is the most popular. 'Kensington Pride' is known under several different names such as Bowen, Bowen Special or Kensington, and was first produced in Bowen, north Queensland (Bally *et al.*, 2000). This cultivar makes up 80% of all trees and is grown throughout the sub-tropical and tropical regions of Australia (Bally *et al.*, 2000). R2E2 is the second most popular cultivar in Australia, making up about 10% of the trees (Bally *et al.*, 2000). R2E2 originated in Australia in the 1980's from a seedling of 'Kent' (Bally, 1998).

The major mango production areas in Australia are: Queensland dry tropics, Atherton Tablelands, central Queensland, south-east Queensland, northern New South Wales, Darwin and Katherine (Northern Territory), and Carnarvon (Western Australia). Mangoes are marketed from mid September to March with the early fruit coming from the Northern Territory, followed by the Queensland dry tropics (Burdekin), Mareeba, central Queensland, Bundaberg and south-east Queensland (Bally *et al.*, 2000).

Mango production in Australia has dramatically increased in the last decade. Data from the Queensland Horticulture Institute, Department of Primary Industries (2000) shows that there were 716,021 trees in 91/92 producing 17,252 tonnes with a value of $AUD 32.8m. However, this figure doubled in 96/97 with 1,372,520 trees producing 32,403 tonnes worth $AUD 69.6m. Queensland produces about 80% of the mangoes in Australia. Approximately 90% of mango production is marketed within Australia and 10% is exported. Eighty percent is sold as ripe fruit and the remaining 20% is processed as puree, canned and frozen slices.

In general, international trade in mango has been limited by its highly perishable nature. The fruit are highly susceptible to diseases, physiological disorders, physical injury, as well as defects associated with extreme temperatures. As a result, transport to distant markets using sea freight is often difficult. In order to improve storage potential and access to international markets, appropriate postharvest technologies such as control of diseases, physiological disorders and injury due to low temperature storage, are required.

2.2 Tree and Fruit Development

Mango belongs to the *Anacardiaceae* family, which includes cashew nut and some other commercial fruit crops (Samson, 1986). It is cultivated through the tropics and subtropics. 'Kensington Pride' mango is a large vigorous tree that can reach heights of up to 8 m if left unpruned (Bally, 1998). The mango tree is well-branched with a dense spreading canopy and has a deep tap root (Whiley, 1984; Mukherjee, 1997). The new leaves are initially purple, turning green as they expand and mature. Leaves can stay on the tree for four to five years

(Whiley, 1984; Schaffer *et al.*, 1994). Mango such as 'Kensington Pride' can be planted from seed or grafted. Grafted trees will bear fruit in the second or third year after field planting, while seedlings can take six or more years to crop (Chadha and Pal, 1985). Grafting 'Kensington Pride' is more common in the tropical regions where tree vigour is a problem (Bally *et al*, 2000).

In the past, 'Kensington Pride' trees have been planted at relatively wide spacings at 10 x 10 metres or 12 x 12 metres, however, in recent years plant spacings of new orchards have been reduced to 6 x 9 metres (Bally *et al.*, 2000). Closely spaced trees require annual pruning to maintain a smaller canopy. Canopies generally touch within the row and heights are maintained at about 5 metres for ease of spraying and picking.

'Kensington Pride' mango trees grow rapidly in the first few years after planting and the trees are pruned twice a year in order to develop a well-branched frame capable of supporting heavy crops in later years. Pruning practices vary between growing areas. For instance, in tropical regions, 'Kensington Pride' trees require a large annual pruning to keep the tree manageable since the trees are excessively vigorous (Bally *et al.*, 2000).

Flowers are borne on terminal pyramidal, glabrous or pubescent panicles. Panicle length and width are about 30-35 cm and 20 cm, respectively. The flowers are small with a dark pink colour (Bally, 1998). Unlike most Indian monoembryonic cultivars, 'Kensington Pride' mangoes are polyembryonic and have a seed with a zygotic embryo plus up to 12 nucellar (asexual) embryos (Alexander, 1987; Schaffer *et al.*, 1994).

Mango fruit is a large, fleshy drupe (Mukherjee, 1997). It exhibits a simple single sigmoid pattern of growth where the mesocarp growth slows down during seed coat hardening (Bollard, 1970). Ripe 'Kensington Pride' fruit are yellow with an orange pink blush on the shoulder, round ovate with flattened base, with a slight-prominent beak. Mature fruit generally weighs from 300 to 600 g and are about 10 cm long, 8 cm broad and 8 cm thick. The skin is medium thick, tender and adherent. The flesh is yellow, soft and juicy, sweet and tangy, with medium fibre, and with a characteristic flavour that makes it the most popular cultivar in Australia (Knight, 1997; Bally, 1998). Sapburn is a particular problem for 'Kensington Pride' mangoes and it is caused by sap coming in contact with the fruit during havesting process. 'Kensington Pride' fruit are soft when ripe and have a relatively short shelf life. Fruit are harvested at the 'mature green' stage (fully mature, but still firm). This allows the harvesting, handling and transporting operations to be carried out before fruit soften. Fruit ready for harvest should have a minimum dry matter of 14% and have filled out at the beak and shoulders and has a slightly yellow pulp.

2.3 Fruit Maturity

Minimum horticultural maturity in climacteric fruit is defined as that stage of development when the fruit will ripen to at least the minimum acceptable consumer quality (Medlicott *et al.*, 1988; Reid, 1992a). Fruit maturity is known to influence the chemical composition and respiratory changes during ripening of several fruits and consequently influences storage life and consumer acceptability (Krishnamurthy and Subramanyam, 1970). Mangoes harvested at full maturity, however, do not store well, but if harvested immature, fail to ripen properly (Tucker, 1993).

It is not easy to determine the optimum harvest maturity for mango, as it can be different from cultivar to cultivar. Correlations between quality of the ripe fruit and physical and chemical attributes have been reported (de Leon and de Lima, 1968; Medlicott *et al.*, 1988; Mukherjee, 1972). The physical attributes relevant to mango maturity include specific gravity, shoulder "fullness", peel and flesh colour, firmness and dry matter. The chemical attributes include starch, titratable acidity, and total soluble solids (TSS).

2.3.1 Physical Parameters

Measurement of specific gravity appeared to be a simple and non-destructive test that can be used for determining mango fruit maturity (Mukherjee, 1960;1972; Salunkhe and Desai, 1984). The specific gravity was highly correlated with maturity in 'Bombay Yellow' mango (Jauhari and Tripathi, 1972) and 'Haden' mango cultivar (Pantastico, 1975). However, the changes in specific gravity were markedly different in different cultivars (Thangaraj and Irulappan, 1989).

In many fruits skin colour can be used as an indicator of fruit maturity. However, this method is not applicable to mango as fruit that have some yellow colour at harvest will ripen within a few days and therefore cannot be transported or stored for long periods (Pantastico, 1975). Furthermore, the initiation of external colour development may be related to 'jelly-seed' in 'Sensation' mango (van Lelyveld and Smith, 1979). Flesh colour at harvest changes from white to light yellow in more mature fruit. Ledger (1996) suggested that 'Kensington Pride' fruit are ready to harvest when the flesh colour near the seed is at least a light yellow.

2.3.2 Chemical Parameters

The maturity of mangoes is closely associated with an increase in starch (de Leon and de Lima, 1968; Jauhari and Tripathy, 1972; Krishnamurthy and Subramanyam, 1973). There is a continuous increase in starch as the fruit matures (Jauhari and Tripathy, 1972). In addition, the starch/acid ratio may be useful in judging the correct maturity for fruit picking (Jauhari and Tripathy, 1972; Lorh and Pantastico, 1975). Titratable acidity and the total soluble solids (TSS) to acid ratio were correlated with the number of days after flower induction, and were shown to have potential for use as maturity indicator (Del Mundo *et al.*, 1984).

Maturity indices for 'Kensington Pride' mangoes are similar to other cultivars. The % dry matter is used as a standard for maturity but it can be unreliable due to variations in weather conditions, orchard management and growing district (Ledger, 1996). For example, fruit from the Mareeba district (north Queensland, Australia) had a lower % dry matter than fruit from Burdekin district (north Queensland, Australia) for the same maturity stage. It has been proposed that flesh colour in conjunction with dry matter would be a better market maturity standard for 'Kensington Pride' mangoes (Ledger, 1996).

2.4 Ripening and Senescence

During ripening of mango, the most profound changes occurring in the fruit are changes in pigments, texture and flavour, but underlying these are changes in hormonal concentrations, respiration, and cell organization which bring about a series of biochemical reactions (de

Leon and de Lima, 1968). Both chemical and sensory properties of mango fruits change as they ripen and these changes vary among cultivars (Chaplin, 1989). Generally, ripening of mango fruit is associated with increased sugars and decreased fruit firmness and acidity (Medlicott and Thompson, 1985; Abu-Sarra and Abu-Goukh, 1992)

2.4.1 Respiration and Ethylene Production

The most significant postharvest change is in the respiration rate. Respiration is the process by which stored organic materials (carbohydrates, proteins, fat) are broken down into simple end products with a release of energy as heat. Oxygen (O_2) is used in this process and carbon dioxide (CO_2) is produced. In general, the loss of stored food reserves in the commodity during respiration means the hastening of senescence, reduced food values, and loss of saleable dry weight (Kader, 1992a). The respiration process affects postharvest technology considerations such as the estimation of refrigeration to remove heat and ventilation requirements to remove CO_2. Generally, the rate of deterioration of harvested commodities is proportional to the respiration rate or storage temperature.

Fruit can be classified as climacteric or non-climacteric according to their respiration and ethylene production pattern during ripening (Biale and Young, 1981; Knee, 1985; Kader, 1992a). Climacteric fruits show a large increase in CO_2 and ethylene production rates coincident with ripening. This sudden increase in respiratory activity is called *climacteric* (Solomos, 1988). Non-climacteric fruits show no appreciable change in CO_2 production during ripening (Knee *et al.*, 1977).

Mango is a climacteric fruit (Kays, 1991; Mitra & Baldwin, 1997) and has a high respiration rate with peak respiration rates exceeding 175 mg CO_2 $kg^{-1}h^{-1}$ at 25°C in some cultivars (Brown *et al.*, 1984; Cua and Lizada, 1989). The pattern of respiration and ripening behaviour varies among the cultivars, climatic conditions and where the fruit is grown (Krishnamurthy and Subramanyam, 1970). It exhibits a respiratory climacteric during ripening which is associated with the physical and chemical processes that take place as fruit goes from unripe to senescent. Therefore, the time from harvest to the onset of the climacteric can be used as a measure of the storage potential of mango (Lizada, 1991). With their high respiration rates, mango fruit are difficult to keep long for any purpose even with the use of refrigeration.

The respiration pattern of mango can be divided into four phases: 1) pre-climacteric, 2) climacteric rise, 3) climacteric peak and 4) senescent (Krishnamurthy and Subramanyam, 1973). In the pre-climacteric phase, fruit are green and firm and CO_2 is being produced at a low rate. Fruit remain green and firm during the climacteric rise but CO_2 production rapidly increases. Carbon dioxide production reaches a maximum at the climacteric peak and fruit at this point tend to change colour, become soft and develop an aroma. At the senescent phase, CO_2 production declines and fruit continue to develop an attractive colour and aroma as well as their softness. During the climacteric stage, fruit are more metabolically active than at the other stages. Therefore, fruit are probably more sensitive to extreme environmental conditions at this time. For example, in other species such as avocado and papaya, fruit at this stage have been reported to be more sensitive to chilling injury than fruit at later stages (Kosiyachinda and Young, 1976; Chen and Paull, 1986).

Ethylene production also declines as the fruit matures and it is undetectable for a time and reappears during the climacteric period (Akamine and Goo, 1973). The climacteric pattern of respiration is associated with the onset of ethylene production in ripening mangoes (Burg and Burg, 1962). The role of ethylene in fruit ripening is discussed further in the section 2.7.3.

2.4.2 Colour and Pigments Changes

Unripe mango fruit have a light to dark green skin colour. During ripening, the skin colour changes to a perceptibly lighter green colour, followed by changes to a yellow and yellow-orange colour (de Leon and de Lima, 1968). Chloroplasts in the skin are transformed into chromoplasts containing yellow and sometimes red pigments (Lizada, 1993). Several researchers (Krishnamurthy and Subramanyam, 1973; Medlicott *et al.*, 1986a; Medlicott *et al.*,1992) have reported that the loss of green colour and the development of yellow colouration was associated with an almost complete loss of chlorophylls and an increase in carotenoids. However, it is not clear whether the loss of chlorophylls or the gain of carotenoids is more important in the de-greening process of mango. Significant colour changes may be mediated in some fruits during ripening through the degradation of chlorophyll and the exposure of pre-existing carotenoids (Kays, 1991). However, there may be differences between mango cultivars in the role of pigments in colour changes during ripening. For example, total chlorophylls and ß-carotene in the skin of green 'Tongdum' fruit (skin colour remains green when ripe) were higher than those in yellow 'Nam Dokmai' (skin colour turns yellow when fruit ripe), however, the ripe 'Tongdum' did not have a yellow skin colour (Ketsa *et al.*, 1999a). This suggests that high chlorophyll levels in green-ripe fruit may mask the expression of ß-carotene and the yellow colour.

In addition to chlorophylls and carotenoids, anthocyanin is also found in mango skin. During ripening, anthocyanin concentration in the skin of 'Tommy Atkins' mangoes decreases slightly (Medlicott *et al.*, 1986a). Therefore, the development of the red blush was suggested to be due to the unmasking of red pigments by chlorophyll degradation rather than by the synthesis of anthocyanin.

2.4.2.1 Chlorophylls

Chlorophylls are pigments of photosynthesis and all green plants contain chlorophylls *a* and *b*. Chlorophyll *a* (Chl-*a*) has a blue-green colour while chlorophyll *b* (Chl-*b*) has yellow-green colour (Gross, 1987). Both chlorophylls are porphyrins and the basic skeleton is a tetrapyrrole ring obtained by the joining together of four pyrrole residues by methane groups (-C=). In chlorophyll *a* the four nitrogen atoms are coordinated with the Mg^{+2} ion. Chl-*b* differs from Chl-*a* only in having an aldehyde group (-COH) in place of the methyl group at position 3 (Gross, 1987).

The chlorophylls are contained within chloroplasts as complexes with protein, but the nature of the binding is not well understood. Chloroplasts are found not only in leaves, but also in other green tissues such as immature fruits. The chloroplast is filled with a matrix called stoma. Embedded within the stoma is a complex system of membranes or lamellae. The basic sub-units of the lamellae are double membranes closed in on themselves to form thylakoids which occur in regular packed stacks called grana (Gross, 1987).

During the early cell multiplication phases of fruit development, chlorophylls are intensively synthesized (Sanger, 1971). Generally, immature fruits contain chloroplasts in higher amounts in skin than in the pulp. After fruit growth ceases, the chloroplast pigments begin to decrease. During ripening the chloroplasts are gradually disorganized as the thylakoids are destroyed and chlorophyll breaks down (Gross, 1987). The destruction of chlorophylls during ripening has been related to increased activity of chlorophyllase (Rhodes and Wooltorton, 1967).

While the biosynthetic pathway of chlorophyll is quite clearly understood, knowledge of chlorophyll degradation is limited (Brown *et al*, 1991; Gross, 1987). The reason for the lack of experimental data is that products of cleavage of the tetrapyrole ring system remain undetected. The hypothetical pathway of chlorophyll breakdown in senescing leaves and ripening fruits is described by Hortensteiner (1999) and Takamiya *et al.* (2000). The pathways of chlorophyll breakdown includes de-phytylation, Mg de-chelation, porphyrin ring cleavage and transition of primary fluorescent chlorophyll catabolite (pFCC) into nonfluorescent chlorophyll catabolites (NCCs).

Chlorophyll is first dephytylated to chlorophyllide by action of the chlorophyllase enzyme. Chlorophyllase is believed to act as the first enzyme in chlorophyll breakdown (Matile *et al.*, 1997), but it has also been assumed to catalyze the phytylation step of chlorophyll biosynthesis (Fiedor *et al.*, 1992). Chlorophyllase has been identified as a component of membranes of chloroplasts (Brandis *et al.*, 1996; Matile *et al.*, 1997) as well as of chlorophyll-free chromoplasts (Hirschfeld and Goldschmidt, 1983). Recently, chlorophyllase genes have been identified from *Arabidopsis, Citrus* and *Chenopodium* and all deduced

proteins are soluble (Jakob-Wilk *et al.*, 1999; Tsuchiya *et al.*, 1999). To date the reports on chlorophyll degradation relating to fruit colour are limited and most of them focused on the early stage, catalyzed by chlorophyllase (Rhodes and Wooltorton, 1967; Ketsa *et al.*, 1999a; Ihl *et al.*, 2000). The change of chlorophyllase activity during fruit ripening may vary between cultivars and the storage conditions. In 'Cox's Orange Pippin' apple fruit stored at 12°C, chlorophyllase activity in the skin increased before the commencement of the climacteric rise in respiration and continued beyond the peak (Rhodes and Wooltorton, 1967).

At the Mg de-chelation stage, the central Mg atom is removed by Mg dechelatase. The product of this reaction, pheophorbide *a*, is the last coloured (green) intermediary catabolite of the pathway. The third reaction in the chlorophyll catabolic pathway is very important in relation to the loss of green colour (Yamauchi and Watada, 1991). This key step is catalyzed by two enzymes, pheophorbide *a* oxygenase (PaO) and red chlorophyll catabolite (RCC) reductase. PaO opens the porphyrin macrocycle by the introduction of oxygen and transforms to RCC. RCC is released from PaO after a site-specific reduction catalysed by RCC reductase (Rodoni *et al.*, 1997). PaO is located in the inner envelop membrane (Matile and Schellenberg, 1996), while RCC reductase are found in roots and in the stoma at all stages of leaf development (Rodoni *et al.*, 1997; Wuthrich *et al.*, 2000).

2.4.2.2 Carotenoids

The yellow, orange and red colours of many fruits are due to the presence of carotenoids and together with chlorophylls are found in all organisms capable of photosynthesis. Carotenoids

are isoprenoid polyenes formed by the joining together of eight C_5 isoprene units (Gross, 1987). They can be divided into carotenes, which are hydrocarbons ($C_{40}H_{56}$) and their oxygenated derivatives, xanthophylls. The carotenoids are located in plastids, chloroplasts and chromoplasts.

Total carotenoids in the mango peel increase during ripening (Ketsa *et al.*, 1999a; Medlicott *et al.*, 1992). In the pulp, carotenoids also increase during ripening and the rate of carotenoids synthesis is influenced by ripening temperature (O'Hare, 1995). Changes in carotenoid pigments during ripening differ between mango cultivars (Roy, 1973). Chloroplasts are capable of synthesizing chloroplast carotenoids using photosynthetically fixed CO_2 (Gross, 1987). The synthesis of carotenoids in mango involves mevalonic acid and geraniol as precursors (Mattoo *et al.*, 1968). There is evidence to suggest that chlorophyll degradation may play a more important role than carotenoids synthesis in the de-greening process (Ketsa *et al.*, 1999a). Treatment of mangoes at high temperature (50-55°C) such as hot water dip and vapour heat, often results in the enhancement of peel colour intensity and a detectable increase in total carotenoids (Medlicott *et al.*, 1986a; Lizada, 1993).

The findings of biochemistry of fruit colour indicate that colour change during fruit ripening is related to skin pigment changes, especially chlorophylls and chlorophyllase is an important enzyme that can catalyze chlorophyll degradation. It is important to find which pre-harvest and postharvest practices can reduce green colour n the skin of ripe 'Kensington Pride' mangoes to improve its quality.

4.2.3 Softening and Cell Wall Constituents

Ripening of the mango fruit is characterized by softening of the flesh (Mitra and Baldwin, 1997). In most fruit, the main changes in relation to softening are loosening of the primary cell walls and the loss of cell cohesion due to structural changes in the middle lamella (Ben-Arie *et al.*, 1979), rather than cell wall degradation by cellulases (Ferguson, 1984; Fischer and Bennett, 1991).

Softening of mango fruit is associated with an increase in the solubility of cell wall pectins (Lazan *et al.*, 1986; Roe and Bruemmer, 1981). Pectins are composed of polygalacturonides with non-uronide carbohydrates covalently bound to the unbranched chain of α(1-4) linked galacturonic acid units. The carboxyl groups of the polygalacturonic acids may be partly esterified by methyl groups and partly or completely neutralised by one or more bases (Pilnik and Voragen, 1970).

Pectinesterase (PE) is one of the enzymes involved in the softening process in mango fruit and PE activity decreases during the ripening of mangoes (Abu-Sarra and Abu-Goukh, 1992). The softening process is thought to be a result of de-esterification of pectin catalysed by PE, followed by pectin depolymerization catalysed by polygalacturonase (PG) (Roe and Bruemmer, 1981; Tandon and Kalra, 1984).

Polygalacturonase is believed to be another one of the major enzymes involved in mango fruit softening (Tucker and Seymour, 1991; Abu-Sarra and Abu-Goukh, 1992; Chaimanee, 1992; Gomez-Lim, 1993; Lazan *et al.*, 1993). PG acts on the pectin molecule and its activity

is promoted by ethylene (Tucker and Grierson, 1982; Grierson, 1992). PG activity is reported increase at the onset of ripening in pears (Ahmed and Labavitch, 1980) as well as in mangoes (Abu-Sarra and Abu-Goukh, 1992).

In mangoes, the softening changes do not proceed uniformly since the inner mesocarp tissue softens before the outer mesocarp tissue (Chaplin, 1989; Lazan *et al.*, 1986; Lazan and Ali, 1993). This occurs due to more pectin depolymerization and solubilization in the inner and outer mesocarp tissues (Lazan *et al.*, 1986).

2.4.4 Other Bio-Chemical Changes

The changes in respiration rate during mango fruit ripening have been shown to be accompanied by many other chemical and biological changes including changes in concentrations of starch, sugars, acidity, proteins, vitamins, tannins and volatile substances.

During ripening of mango fruit, it is believed that there is a breakdown of starch to sugars (Krishnamurthy and Subramanyam, 1973). Starch in 'Haden' mangoes disappears after 3 or 4 days of ripening at 27°C (Soule and Harding, 1957). The starch is converted to sugars. The sugars then form a high proportion of the soluble solids in ripe mango fruit. At the commencement of ripening, the majority of the sugars are reducing sugars (fructose and glucose), but the ripe fruit contains more non-reducing sugars, in the form of sucrose (Krishnamurthy and Subramanyam, 1973). Glucose, fructose and sucrose are all increased during 'Keitt' mango ripening and the concentration of sucrose is higher than the concentration of glucose and fructose (Medlicott and Thompson, 1985).

The three major organic acids present in mangoes in descending order are succinate, citrate and malate (Lazan *et al*, 1993). In contrast to sugars, these three acids along with the titratable acidity level decrease during ripening. Titratable acidity and citrate concentration in the inner mesocarp are reported to be higher than in the outer mesocarp, but they decline more rapidly in the inner mesocarp during fruit ripening (Lazan *et al*, 1993). The decline in titratable acidity and total acid concentration is accompanied by a corresponding increase in pH of the respective tissues. Malate acidity which appears in the unripe fruit in lower levels, also decreases during ripening (Medlicott and Thompson, 1985). The malate enzyme activity increases with a peak activity occurring at about the climacteric peak (Dubery *et al.*, 1984).

Changes in protein concentration during ripening may be different between mango cultivars. For example, during ripening the protein concentration in 'Alphonso' mangoes at 28°C remains more or less unchanged (Krishnamurthy and Subramanyam, 1973). However, it is almost doubled in 'Dashehari' mangoes during ripening (Kalra and Tandon, 1983). The increase in protein concentration suggests that additional enzymes are being synthesized to accelerate changes during ripening. Ripening requires the synthesis of mRNA novel proteins and new pigments and flavour compounds (Tucker, 1993).

Vitamins, tannins and volatile substances also change during mango ripening. The concentration of ascorbic acid (vitamin C) decreases during ripening (de Leon and de Lima, 1968; Kalra and Tandon, 1983; Vazquez-Salinas and Lakshminarayana, 1985). The level of vitamin A, increases with ripening and the values are related to the change in the β-carotene concentration (Godoy and Rodfiguez-Amaya, 1989). As fruit ripen, the concentration of

tannins decreases (Kalra and Tandon, 1983). Aromatic substances are produced during normal mango ripening. Two novel unsaturated acid esters (2-butenoic and 3-butenoic acid esters) are thought to be responsible for the characteristic aroma of mango (Ackerman and Torline, 1984). The absence of aroma in mangoes is believed related to a fibreless fruit flesh (Kostermans and Bompard, 1994); however, 'Kensington Pride' fruit with low fibre has a typical flavour and aroma profile, which is associated with high fruit levels of α-terpinolene (Bartley and Schwede, 1987; Macleod *et al.*, 1988).

2.5 FRUIT QUALITY

2.5.1 Quality Attributes

According to Kader (1992b), the quality of fresh horticultural commodities is defined, "as a combination of characteristics, attributes and properties of the value for food and enjoyment". Consumers assess quality based on appearance, texture, flavour and nutritive value. In most fresh produce, the rate of deterioration in nutritional quality occurs faster than flavour quality, but the flavour quality is lost faster than textural quality and appearance (Kader, 1992b). Appearance factors are often the most important quality attributes. The external colour of fruits contributes more to the assessment of quality than any other single factor (Abbott, 1999; Kays, 1999). Consumers normally correlate the colour to the overall quality of specific products. For instance, ripe tomatoes should be red, ripe bananas should be yellow.

The external quality of 'Kensington Pride' mangoes (skin colour, sapburn, skin browning and diseases) strongly influences saleability and the price of fruit (Hofman *et al.*, 1995a). Green, ripe fruit is a major quality problem for 'Kensington Pride' mangoes because consumers always expect ripe fruit to have a yellow colour, preferably with some pink/red blush (Hofman *et al.*, 1997a). 'Kensington Pride' fruit are also known to have a short shelf life (Whiley and Saranah, 1995) and inconsistent quality (Ledger, 1996).

2.5.2 Postharvest Diseases and Disorders

2.5.2.1 Diseases

The unripe mango has a high acid content and is not easily attacked by bacteria and fungi; the acid concentration falls rapidly as the fruit ripens and become more susceptible to fungal and bacterial growth (Salunkhe and Desai, 1984). The onset of ripening in fruits, and senescence in all commodities, renders them susceptible to infection by pathogens. Stress, such as mechanical injuries, chilling injury, and sunscald, lowers fruit resistance to pathogens (Kader, 1992a).

One of the most serious causes of spoilage of mango fruit is from fungal infection occurring at different stages of fruit development. There are several fungal diseases in mango, they include anthracnose, stem-end rot, alternaria rot and black mould rot (Johnson *et al.*, 1995a; Dodd *et al.*, 1997), however, anthracnose and stem-end rots are the major postharvest fungal diseases in Australian mangoes. The causal organisms usually infect fruit during

development on the tree. The fruit appear normal at harvest but become spoiled during and after ripening.

Anthracnose is a serious pre-harvest and postharvest disease of mango, and is caused by the fungus *Collectrichum gloeosporrioides* Penz (Coates *et al*, 1995; Dodd *et al.*, 1997). During fruit development an infection peg and appressorium is formed and there is usually limited colonization of the host tissue by infection hyphae at this stage (Peterson, 1986). Further development in unripe fruit is limited, with no visible signs of infection. Pathogen growth commences again when the fruit begin to ripen. Small, dark specks enlarge to irregular, dark-brown to black areas, with the lesion no more than 10 mm beneath the skin (Coates *et al*, 1995; Dodd *et al.*, 1997). Anthracnose is associated with high rainfall and humidity during the growing season which is required for inoculum spread (Fitzell and Peak, 1984; Dodd *et al.*, 1992). In Australia, a spray in field with mancozeb (800 g kg^{-1}) at the rate of 2 g L^{-1} weekly during blossoming and then monthly until harvest was reported to control anthracnose (Johnson and Muirhead, 1988).

Stem-end rots in mango are caused by a number of fungi, including *Dothiorella dominicana* (anamorph of *Botryosphaeria dothidea*), *Dothiorella mangiferae, Lasiodiplodia theobromae* (syn. *Botrydiplodia theobromae*), *Phomopsis mangiferae* and *Pestalotiopsis mangiferae* (Johnson *et al.*, 1992; Huang and Liu, 1995; Dodd *et al.*, 1997). In Australia, *Dothiorella dominicana* is the most important fungus (Johnson *et al.*, 1995a). Stem-end rot symptoms appear as the fruit ripen. A brown, soft decay starts at the stem end and rapidly spreads through the whole fruit (Johnson *et al.*, 1995a). The affected fruit rapidly becomes inedible and the flesh of affected fruit has an 'off' flavour. Fungi that cause stem-end rots live within

mango tree twigs and branches and grow inside the flower stalks, reaching the stem end of the fruit a few weeks before harvest (Johnson *et al.*, 1995b). They also occur in the soil and can infect the fruit at harvest when fruit are inverted in soil to facilitate sap removal (Johnson *et al.*, 1995b; Dodd *et al.*, 1997). Furthermore, the disease may spread to healthy fruit that are touching infected fruit (Johnson *et al.*, 1995a). Stem-end rot builds up in an orchard as trees age. Water stress, field temperature and tree canopy management may influence growth rate of the fungi and fruit infection levels (Johnson *et al.*, 1995b)

Both anthracnose and stem-end rots are difficult to control. Control often involves pre-harvest sprays (McMillan, 1984) followed by careful harvesting and handling to avoid infection by stem-end rots in the field (Johnson and Coates, 1993). In addition, postharvest treatments are also very important. Anthracnose and stem-end rots can be reduced by hot water dipping with or without fungicide, and vapour heat. However, treatment efficiency may differ between mango cultivars. Treatment at 46°C for 20-60 min can reduce anthracnose in 'Keitt' mangoes and stem-end rot in both 'Keitt' and 'Tommy Atkins' mangoes (Spalding *et al.*, 1988). Dipping fruit in hot water at 55°C for 5 min has been shown to give good control of stem-end rot of 'Nam Dokmai' mangoes without causing heat injury (Sangchote, 1989). Immersion in hot water (52°C for 5min) plus benomyl has also been found to reduce stem-end rot on mango fruit (Johnson *et al.*, 1990a).

In addition to hot water, the mango diseases are also controlled by a high humidity hot air (HHHA) treatment. A combination treatment consisting of HHHA followed by either hot benomyl or unheated prochloraz can give good control of anthracnose in cool-stored 'Kensington Pride' mangoes (Coates *et al.*, 1993).

In addition, hot water and vapour heat, biological control and irradiation can also reduce mango diseases (Johnson *et al.*, 1990b; Koomen and Jefferies, 1993). Some bacteria have been shown to be active antagonistic against mango isolates of *C. gloeosporioides* and stem-end rot pathogens (Coates *et al*, 1995; Jefferies and Koomen, 1992). Hot benomyl immediately followed by irradiation has also been reported to provide effective control of anthracnose and stem-end rots of 'Kensington Pride' mangoes during storage (Johnson *et al.*, 1990b).

2.5.2.2 Physiological Disorders

Mangoes are susceptible to several physiological disorders such as chilling injury, 'spongy tissue', 'soft nose' and 'jelly seed' which influence fruit quality. Such disorders can be considered as either induced or inherent. Chilling injury is an induced disorder of mangoes after exposure to low temperatures and impairs normal ripening of fruit (Chaplin, 1989) (see section 2.7). 'Spongy tissue' (or internal flesh breakdown) and 'soft-nose' are important inherent disorders in mango (Katrodia, 1989).

'Spongy tissue', is considered to be a ripening disorder, characterised by fruit with a soft centre, with white corky tissue or internal breakdown. The peculiarity of this disorder is that external symptoms of 'spongy tissue' in affected fruits are not apparent either at the time of picking or at the ripe stage. The affected tissue is visible only when the ripe fruit is cut open. Flesh pulp affected by 'spongy tissue' has been shown to contain higher acidity and starch

concentrations but lower pH, carotene concentration, sugars, ascorbic acid, and enzyme activities (amylase and invertase) than non-affected fruits (Katrodia and Sheth, 1989)

The incidence of the disorder increases with exposure time to high temperature before harvest (Katrodia, 1989). Recommendations for controlling 'spongy tissue' include: breeding for resistant cultivars, reducing growing temperatures through cultural practices such as mulching and sod-culture, reducing exposure of fruits after harvest to high temperature and direct sunlight (Katrodia, 1989; Katrodia and Sheth, 1989) and the single and double pre-harvest dips of fruit in calcium solution (Gunjate *et al.*, 1979; Wainwright and Burbage, 1989).

'Soft-nose' is another physiological disorder in mango often found in the fruit which are allowed to start ripening on the tree (Subramaryam *et al.*, 1971). This disorder occurs as fruit approaches maturity and always starts on the tree (Young and Miner, 1961). Unless already initiated at picking, typical and pronounced 'soft nose' does not develop after picking. Affected tissue is generally pale yellow in colour compared to the surrounding tissue, and has a fermented odour and air pockets (Subramanyam *et al.*, 1971). Affected tissue also has low pH, higher acidity and lower soluble solids and sucrose concentration compared to the normal tissue. Total carotenoids and β-carotene are also lower in the affected tissue but vitamin C is high in the affected tissue compared to the healthy tissue (Young and Miner, 1961). The incidence of 'soft-nose' is thought to be related to high N application and may be reduced by Ca fertilization (Young and Miner, 1961).

2.6 PRE-HARVEST FACTORS AFFECTING FRUIT QUALITY

Quality of mango and other fruit is related to pre-harvest conditions and management. It could also be affected by pest and disease management. Quality is thought to be a function of energy, water and nutrient flows through the trees (Beverly *et al.*, 1993). An imbalance of this flow can affect fruit quality. In New Zealand and the U.K., prediction systems of postharvest quality in temperate fruit based on pre-harvest factors are in commercial use (Bramlage *et al.*, 1985; Fallahi *et al.*, 1985a; Autio *et al.*, 1986). Pre-harvest factors including nutrition and other cultural practices such as variety, production locality, soil type, climate and irrigation can influence postharvest quality in many fruit (Monsenlise and Goren, 1987; Hofman and Smith, 1994). In 'Kensington Pride' mango, the retention of green skin colour when ripe and the incidence of disease development are two of the most important undesirable quality attributes (Hofman *et al.*, 1995a). These two factors are reported to be affected by pre-harvest N fertilizer in other fruits (Neilsen *et al.*, 1984; Fallahi *et al.*, 1985a; Daane *et al.*, 1995); however, there is limited information on tropical fruits, especially on mangoes.

2.6.1 Nitrogen

Nitrogen is a constituent of a large number of important compounds found in all living cells, such as amino acids, proteins (enzymes) and nucleic acids (RNA and DNA) (Lea, 1993). Among nutrients, N has the greatest influence on growth and development of a plant, promoting production of branches, leaves and fruits (Samra and Arora, 1997). Generally, deficiency in N can lead to poor growth and low productivity. However, excessive use of N

increases crop production costs and may lead to reduction of fruit quality and contamination of groundwater.

2.6.1.1 Nitrogen Uptake and Remobilization

In the majority of plants, nitrate (NO_3^-) is the sole source of N taken up from the soil by the roots (Lea, 1993). Ammonia (NH_4^+) is present in certain acidic anaerobic soils and may be taken up directly, but normally it is rapidly oxidized to nitrate by nitrifying bacteria. After the uptake of nitrate, N is transported to the growing parts of the plant such as leaf, fruit and shoot tissue.

Nitrogen uptake by the plant is affected by the availability of this element in the soil solution at the root surface, the state and morphology of the root and the concentration of N inside the plant (Jeuffroy *et al*., 2002; Karrou and Maranville, 1994). The uptake rate of N depends on the shoot : root ratio, with a high ratio resulting in increased xylem transport and plasmalemma influx (Jeschke, 1980). Nitrogen uptake is also influenced by root growth (Cure *et al.*, 1988). The uptake and partitioning of N are influenced by the N level and CO_2 intake by leaves. As the amount of N application is increased, leaf area, photosynthesis and accumulation of dry matter in the shoot are also increased. As the result, there is sufficient energy to allow further nitrate reduction and N uptake allowing rapid growth the highly active shoot (Karrou and Maranville, 1994).

Nitrate is reduced to ammonia by the enzymes nitrate reductase and nitrite reductase. Nitrate reductase is a soluble enzyme located in the cytoplasm of both leaf and root tissue (Lee,

1980), while nitrite reductase is located in the chloroplast or plastid (Lea, 1993; Ritenour *et al.*, 1967). Further assimilation of ammonia is catalyzed by the enzymes glutamine synthetase and glutamate synthase (Schrader and Thomas, 1981). Glutamine synthetase is located both within the plastids and cytosol in green and non-green tissues and appears to be present to detoxify ammonia formed by nitrite reduction in chloroplasts and proplastids, and ammonia released during photorespiration and other deamination reactions (Keys *et al*, 1978). Glutamine synthetase is the sole port of entry of ammonia into amino acids in higher plants (Lea *et al.*, 1990). Glutamate synthase is located in the chloroplasts and is responsable for transfer of the amide group of glutamine to 2-oxoglutarate to yield two molecules of glutamate (Lea, 1993).

There are situations when the plant needs to transport N from one organ to another, for instance, N-fixing root nodules to the leaves and fruit, senescing leaves to the young leaves and fruit and cotyledons of germinating seed to expanding shoots and root tips (Lea, 1993). Nitrogen metabolism changes at the onset of senescence from assimilation to remobilization (Patterson *et al.*, 1980). The metabolic change from nitrogen assimilation to remobilization is based on a sequential loss of macromolecules with anabolic or catabolic functions. For example, some enzymes are inactivated early, some remain active longer and others reach highest activities during senescence (Patterson *et al.*, 1980). Enzyme and membrane proteins serve, after hydrolysis, as nitrogen sources, and the amino acids produced are remobilized to nitrogen sinks within the same plant, such as the seed (Feller, 1990). The rate of senescence and the remobilization of leaf N are related to the N status of the plant and the N difference between the source and the sink (Crafts-Brandner *et al.*, 1996).

For mango trees, most of the N uptake goes to the leaves and fruit (Stassen *et al.*, 2000). The level of N in fruit (seed and flesh) declines as the trees get older while it increases in the wood and young shoots (Stassen *et al.*, 2000). Nitrogen concentration in mango leaves increases during the rapid phase of fruit growth, but decreases during the later stages of development. This increase may relate to the demand placed on nutrients by the growing fruit and nutrient translocation from remote sources within the plant, and the reduction in late stages might be seen as depletion by the fruit (Oosthuyse, 2000). In other species such as apple (Neilsen *et al.*, 2001) and citrus (Lea-Cox and Syvertsen, 2001), N is also thought to be remobilized from senescent leaves to fruit.

2.6.1.2 Effect on Fruit Skin Colour

Pre-harvest N application can influence postharvest colour of many fruit and vegetables (Kays, 1999). The background colour of several apple and pear cultivars at harvest and post storage is affected by N fertilisation (Neilsen *et al.*, 1984; Fallahi *et al.*, 1985a; Olivier *et al.*, 1994; Raese and Drake, 1997; Raese, 1998). Generally, mature apple fruit from trees treated with high pre-harvest N rates have more green colour (Neilsen *et al.*, 1984; Fallahi *et al.*, 1985a; Raese and Drake, 1997). However, sometimes there is no fruit colour response to high N fertilization (Meheriuk *et al.*, 1996). Nitrogen concentration in apple leaves is highly correlated to fruit colour and has been used as a predictor of fruit green colour development (Marsh *et al.*, 1996; Raese and Williams, 1974). It is likely that the same relationship may be true for mangoes.

The ripening process in many fruit involves the degradation of some pigments and the synthesis of others (Goldschmidt, 1980). In mango, the role of pre-harvest N application on postharvest colour change of fruit is largely unknown. Carbon and nitrogen metabolism in photosynthetic tissues are thought to be integrated (Imsande, 1998). Higher concentrations of nitrate stimulate the phosphorylation of phosphoenolpyruvate carboxylase (PEPC) and sucrose-phosphate synthase (SPS) (Champigny, 1995; Scheible *et al.*,1997). Phosphorylation activates PEPC, promotes ammonia assimilation and aspartate synthesis, and also reduces SPS activity which can inhibit sucrose synthesis. Therefore, more nitrate availability can help direct the flow of photosynthate towards protein synthesis and away from starch accumulation (Champigny, 1995; Scheible *et al.*, 1997). In addition, N stress is believed to enhance thermal energy dissipation and lower photosynthetic efficiency (Verhoeven *et al.*, 1997).

In leaves, there is a direct correlation between increased N nutrition and increased photosynthetic activity (Evans, 1989). Nitrogen is either partitioned into CO_2 fixing enzymes such as ribulose-1,5-bisphosphate (RuBP) or proteins associated with the thylakoid membranes of the chloroplast. In senescing chloroplasts, the contact between grana stacks is loosened and the membranes disappear. At the same time, the membrane constituents such as lipids, proteins and chlorophyll are degraded (Matile, 1992). Most important in terms of N content are the thylakoid-bound apoproteins of chlorophyll, mainly light-harvesting complex II (LHCP II) (Hortensteiner and Feller, 2002). A net loss of aproproteins during senescence only occurs after the removal and degradation of chlorophyll (White and Green, 1987). Chlorophyll is largely responsible for the dark-green coloration of plants (Imsande, 1998). Therefore, increased N fertilisation can be seen as increased green colour due to higher

chlorophyll concentration. Such strong correlations between the chlorophyll and N concentration in leaves exist because a large proportion of the leaf N is bound up in the proteins that complex the chlorophyll pigments (Evans, 1989). In perennial plants, fruits and seeds are strong sinks for N, often drawing N from the soil, senescent leaves and other reserves in the plant (Lea, 1993). Fruit with photosynthetic capability for all or part of their development would therefore be responsive to N fertilisation through the mechanisms described by Evans (1989). Therefore, a relationship between pre-harvest N application and chlorophyll in fruit peel is observed. For example, higher N rates result in more chlorophyll in the skin of 'Gala' apple fruit (Reay *et al.*, 1998).

In mangoes, fruit colour may relate to pre-harvest N fertilisation (McKenzie, 1994; Oosthuyse, 1993). A survey of grower nutritional practices conducted by Oosthuyse (1993) and McKenzie (1994) on South African 'Sensation' mangoes suggested that there was a link between the skin colour of fruit and the amount of pre-harvest N application. Fruit from orchards with a low soil N status de-greened completely when ripe while those from orchards with moderate or high soil N either failed to de-green appreciably or did not de-green at all.

2.6.1.4 Effect on Diseases and Disorders

Pre-harvest N application increases fruit susceptibility to rots in a number of crops including pear (Sugar *et al.*, 1992), grape (R'-Houma *et al.*, 1998), avocado (Abou Aziz *et al.*, 1975) and nectarine (Daane *et al*, 1995). In avocado, higher N applications increases body rots severity in 'Pinkerton' fruit (Penter and Stassen, 2000). Excess N fertilization does not increase nectarine fruit yield or improve fruit quality; but rather, fruit from high N trees are

more susceptible to attack from brown rot (Daane *et al.*, 1995). Furthermore, high N rates are correlated with increased soft rot in tomato (Bartz *et al.*, 1979; Segall *et al.*, 1977). There are limited reports on the relationship between N application and diseases in mangoes. However, N application on 'Keitt' trees resulted in more disease in the ripe fruit (Bally, 2002). There are occasions when increased pre-harvest N application may not lead to increase postharvest diseases (Carballo *et al.*, 1994). This suggests that postharvest disease severity is not only influenced by pre-harvest N application, but also other pre-harvest factors such as variety (host resistance to fungus) and growing conditions. This is supported by the investigation conducted by Canaday (1992) on broccoli. Application of N increased the incidence and severity of bacteria soft rot in susceptible cultivars, but had no effect on a resistant one.

There is little understanding of the N effect on fruit diseases, but several mechanisms may be involved. High N can decrease the concentration of some phenolics, lignin, and silicon in plant tissues, and consequently reduce the host resistance to fungal growth in crops such as rice and cucumber (Matsuyama and Dimond, 1973; Menzies *et al.*, 1991). In addition, the high N effect may be through calcium (Ca). The ammonium ion is antagonistic to the absorption of Ca since, as the proportion of ammonium in nutrient solution increases, the absorption of Ca decreases (Young *et al.*, 1965). This is confirmed by the fact that the ammonium ion is thought to affect Ca absorption and transport within tomato and sweet corn plants (Quebedeaux and Ozbun, 1973; Wilcox *et al.*, 1973). Also, N application during early fruit development tends to stimulate shoot growth in avocado, which provides a preferential sink for Ca through the transpiration stream and may further reduce Ca transport into fruit (Witney *et al.*, 1990). Calcium deficiency due to ammonium ions was also reported in cucumber fruit (Alan, 1989; Kotsiras *et al.*, 2002). High Ca concentration in the fruit has

been related to reduced diseases in a number of fruit (Hofman *et al.*, 2002). Furthermore, pre-harvest N application increases the susceptibility to the physiological disorders such as 'soft nose' due to the reduction of Ca in the fruit (Young *et al.*, 1965).

2.6.1.3 Effect on Other Quality Attributes and Yield

In addition to fruit colour, pre-harvest N application can also influence other postharvest quality attributes such as fruit maturity, fruit firmness, chemical composition, and fruit yield. High N fertilization is believed to decrease firmness in several crops (Reeve, 1970; Blanpied *et al.*, 1978, Prasad *et al.*, 1988). In mango, increasing pre-harvest application of N can reduce flesh acidity (Singh *et al.*, 1979) and increase sugars (Singh, 1977). Fruit of the same age from trees treated with high N can be less mature than low N fruit which is an important attribute for postharvest ripening (Samra *et al.*, 1977).

Increasing N application can increase the average number of fruits per tree and fruit weight (Samra *et al.*, 1977; Singh, 1977). In 'Hass' avocado, time and rate of N application are important factors increasing yield and fruit size (Lovatt, 2001). In addition, N concentration in leaves increases with increased N application rates (Samra *et al.*, 1977). Mango fruit yield is correlated to leaf N concentration before flowering and after harvest (Rao and Mukherjee, 1988). However, in contrast, Lovatt (2001) suggests that yield is not correlated with leaf N in avocado.

2.6.2 Interaction of Nitrogen with Other Macro- and Micronutrients

Generally, the balance between N and other elements such as Ca, potassium (K) and phosphorous (P) is more important in relation to yield and fruit quality than the concentration of the individual elements. Calcium has numerous important roles in plants such as the activation of many membrane bound enzymes (Rensing and Cornelius, 1980), structure of the middle lamella of cell walls (Burstrom, 1968) and permeability and maintenance of membrane stability and cell integrity (Wieneke, 1995). It is also important in reducing diseases and disorders. Low fruit Ca increases the bitter pit incidence in apple (Ferguson and Watkins, 1992). Ca is also important for fruit ripening and senescence. It can retard the rate of fruit ripening and prolong the shelf life of fruit (Sharples and Johnson, 1977). This is achieved by decreasing the respiration rate, ethylene production, tissue softening and colour changes (Cooksey *et al.*, 1994; Schirra *et al.*, 1997). In addition, Ca deficiency can induce a loss of membrane integrity, which reduces concentration of K and Mg and influences the function of chloroplasts and mitochondria (Pill and Lambert, 1977). High N can reduce fruit Ca (Alan, 1989; Kotsiras, 2002).

An excess or deficiency of K and P may also affect the quality of fruit. Potassium deficiency can cause poor colouration in peach fruit (Reeves and Cummings, 1970) and increase blotchy ripening of tomato (Picha and Hall, 1981). Potassium fertilization can also decrease firmness of mature fruit (Peck and van Buren, 1975). Like N, high K can cause Ca deficiency and increase the occurrence of fruit disorders associated with undesirable texture (Bramlage *et al.*, 1983; Sharples, 1984). The green colour of 'Mutsu' apples is believed to have a positive correlation with leaf N, but a negative correlation with leaf K. In contrast, yellow colour is

positively correlated with leaf K, but negatively correlated with leaf N (Daugaard and Grauslund, 1999).

High P results in poor cranberry colour development (Francis and Atwood, 1965), while low P causes a loss in fruit firmness, particularly in fruit that are low in Ca (Sharples, 1980). Leaf P is positively correlated with yellow colour in 'Mutsu' apple, but not correlated with green colour (Daugaard and Grauslund, 1999).

2.6.3 Other Production Factors

Other pre-harvest factors such as rootstocks, vegetation management, light, field temperature and water stress also influence fruit quality. Rootstocks have been shown to affect fruit quality, maturity and fruit yield in apple (Daugaard and Callesen, 2002; Drake *et al.*, 1991; Lord *et al.*, 1985). For example, 'Starkspur Golden Delicious' apples from OAR 1 rootstock have greater total soluble solids, more yellow colour at harvest and after storage and firmer fruit at harvest than those fruit from other rootstocks such as Seedling, Malling 1 (M1), Malling Merton 106 (MM106), M7 and M26 (Fallahi *et al.*, 1985a). In avocado, rootstocks can have a significant impact on avocado fruit quality (Hofman *et al.*, 2002).

Under-tree vegetation management is thought to influence Ca, Mg and K concentrations in fruit flesh of 'Golden Delicious' apple at harvest. For instance, there was a significant reduction in leaf N and trunk diameter, and better fruit skin colour and firmness at commercial harvest when the orchard floor was sod (Neilsen *et al.*, 1984). However, up to

date the orchard floor management has not been used by Australian growers for 'Kensington Pride'.

Intensity of solar radiation affects postharvest quality, via fruit appearance and texture (Kays, 1999; Sams, 1999). Exposure to excessive light causes sun-scald of the fruit because of pigment destruction (bleaching). The prolonged exposure to high light intensity causes cellular death and collapse of the fruit tissue (Kays, 1999). On the other hand, fruit quality can also be reduced if there is insufficient light by reducing fruit size (strawberry; Osman and Dodd, 1994 and apple; Rom, 1990) and colour development (apples; Campbell and Marini, 1992 and peach; Corelli-Grappadelli and Coston, 1991). In tomato, low light was reported to increase the incidence of puffy fruit and blotchy ripening (Rylski *et al.*, 1994).

Field temperatures influence fruit growth and development by its direct influence on metabolism. Exposure of tropical and subtropical tree species such as mangoes to chilling temperatures results in the inhibition of photosynthesis and other metabolic processes (Graham and Patterson, 1982). For example, the young cucumbers exposed to chilling temperatures can be susceptible to disorders such as curvature and scarring (Howard *et al.*, 1994). In Australian subtropical regions, low temperatures (below 12°C) during flowering period in July to August may reduce production by reducing pollen growth (Whiley *et al.*, 1988). High field temperature can also reduce fruit quality by damage to cellular membranes, proteins, nucleic acids or indirect effects such as inhibited pigment synthesis (Kays, 1999).

In mango, fruit position on the tree and flowering time affected 'Kensington Pride' mango skin colour at eating soft mainly due to the effect of irradiation exposure (Hofman *et al.*,

1995b). Fruit from the south and west side, and from the upper layer in the canopy are less green when ripe compared with fruit from the north-east side, or lower layer. Bagging of individual mango fruit can reduce the incidence of anthracnose and stem-end rots and inhibit red colour development (Hofman *et al.*, 1997b).

Good management of irrigation can improve fruit quality by influencing mineral uptake (Simmons, 1998). Waters tress can reduce mineral uptake by decreasing the diffusion of nutrients to the root surface (Mengel and Kirkby, 1982). Water stress at the late stage of fruit development can significantly reduce 'Kensington Pride' fruit quality due to high incidence of diseases (Simmons *et al.*, 1995).

2.7 Postharvest Factors Affecting Fruit Quality

Harvested fruit are living organs and they continue to respire and lose water after harvest (Kays, 1991). However, their losses are not replaced as with fruit still attached to the parent plant. Therefore, they can easy suffer detrimental changes after harvest. These changes affect respiration, biochemical composition, texture and ethylene production associated with the ripening of climacteric fruit. The rate at which changes occur in harvested fruit may be influenced by a range of environment factors including temperature, relative humidity (RH), atmospheric compositions and postharvest treatments.

2.7.1 Temperature

2.7.1.1 The Role of Temperature on Fruit Ripening

Postharvest temperature is the environmental factor that most influences the deterioration rate of harvested commodities (Kader, 1992a). Above the optimum temperature, they respire at unacceptably high rates and are more susceptible to disease (Thompson, 1992). As product temperature increases, reaction rates increase often resulting in a shorter shelf life. However, not all reactions in the tissue have the same relative rates of change. For example, photosynthesis has an optimum temperature lower than respiration (Kays, 1991). In the case of climacteric fruit, low temperature can be used to delay the onset of ripening (Wills *et al.*, 1998). A lower storage temperature not only reduces ethylene production, but also the response of the tissue to ethylene and consequently, delays the fruit ripening.

In addition to shelf life, temperature is believed to influence fruit appearance and other quality attributes such as texture, nutrition, flavour and aroma (Paull, 1999). Postharvest colour change in 'Cox's Orange Pippin' and 'Granny Smith' apples is thought to be a function of ripening temperatures (Dixon and Hewett, 1998). The rate of change of chlorophyll and skin colour intensity (hue angle), increases from 0 to 20°C, reaches a peak at the optimum ripening temperature (20-24°C), and then declines at higher temperatures. Warm fruit are more plastic than cold fruit and are more susceptible to vibration injury (Somner *et al.*, 1960). High storage temperature can significantly reduce fruit firmness. The rate of loss in firmness increases with storage time (Miccolis and Saltveit, 1995; Paull, 1999). The loss of firmness is associated with an increase in PG activity (Abu-Sarra and Abu-

Goukh, 1992). Nutrition, flavour and aroma are also important quality attributes. Higher ripening temperature can reduce the amount of vitamin C (Watada, 1987) and volatiles (butyl, isopentyl and hexyl acetates and alcohols) produced (Wills and McGlasson, 1971).

The storage and ripening temperature is also believed to influence postharvest disease development (Hopkirk *et al.*, 1994; Reyes and Paull, 1995). In avocado, fruit stored at 4 to 6°C had less disease severity than those stored at 0, 2 or 10°C and ripened at 20°C. In addition, avocado ripened at 25 or 30°C had a higher disease severity than those ripened at 15 or 20°C (Hopkirk *et al.*, 1994). The severity of diseases on guava fruit stored at 20°C is reported to be higher compared to those stored at 10, 12.5 and 15°C and ripened at 22°C (Reyes and Paull, 1995). This suggests disease severity can be reduced if storage and ripening temperatures are kept in the optimum range.

'Kensington Pride' mangoes achieved the highest quality scores based on skin and pulp colour, and eating quality when ripened between 18-22°C (O'Hare, 1995). Fruit ripened at 13°C or 30°C had lower skin quality scores because of poor carotenoid development and higher chlorophyll retention. In addition, mango fruit ripened at less than 18°C had higher acidity (Vazquez-Salinas and Lakshminarayana, 1985), less yellow colour development (Thomas, 1975) and reduced sugar development (Thomas, 1975; Veloz *et al.*, 1977). Total carotenoids and β-carotenoids increased with increased temperatures (Vazquez-Salinas and Lakshminarayana, 1985). However, the effect on vitamin C is inconsistent between cultivars, since higher temperatures can increase in vitamin C concentration in 'Haden', 'Iwrin', and 'Keitt' mangoes, but it declines in 'Kent' mangoes (Vazquez-Salinas and Lakshminarayana, 1985).

2.7.1.2 Temperature stress

As indicated above, the temperature at which the fruit ripens can affect ripe fruit quality. However, fruit can also be damaged by exposure to too low or too higher temperatures for excessive times after harvest. 'Kensington Pride' fruit may suffer temperature stress since they can be exposure to extreme temperature during postharvest handling process. Fruit are easy to be at extreme high temperature when they are in the field, during quarantine treatments or transportation and extreme low temperature when they are in cold storage.

Chilling injury

Refrigeration is a widely used technology to delay the ripening or postharvest deterioration of fresh horticultural commodities. However, many tropical commodities cannot be stored at low temperatures because they are very sensitive to chilling injury (CI). The degree of CI depends on storage temperature, duration of exposure and the sensitivity of the variety (Kays, 1991) as well as the physiological condition of the commodities. For example, chilling sensitivity of avocado fruit increases up to the climacteric peak, then declines thereafter (Kosiyachinda and Young, 1976). In addition, harvest maturity is an important factor with immature mango fruit being more susceptible to CI than mature fruit (Medlicott *et al.*, 1990).

Chilling injury occurs at low temperatures that are above the freezing point of the produce. Injury is the result of a primary response in the physical properties of cellular membranes (Steponkus, 1984). The changes in membranes leads to a number of possible secondary

responses such as loss of membrane integrity, leakage of solutes, loss of compartmentation and changes in enzyme activity (Wang, 1982).

In mango, CI symptoms include rind discolouration, pitting, uneven ripening, poor colour and flavour development, and increased susceptibility to decay (Veloz *et al*., 1977; Kane *et al*., 1982; Thomas and Oke, 1983). Storage temperatures below 13°C for several weeks are generally not suitable for mango because of the risk of CI (Hatton *et al*. 1965, Thompson, 1971). However, mangoes stored at non-chilling temperatures, that is 13°C and above, generally have a storage life of less than 3 weeks due to ripening of the fruits. Thus the potential to export fresh fruit is generally limited to those production regions from which the shipping time to market does not exceed about 2 weeks. Therefore, determining the optimum cold storage temperature, and other factors that affect fruit response to low temperature, is important. In 'Kensington Pride' mango, fruit can be held at 10°C for 3 weeks with no visual symptoms of chilling injury (Chaplin *et al*., 1991). Heat and ethylene treatments are believed to increase the resistance of mango fruit to chilling injury (Mohammed and Brecht, 2000; McCollum *et al*., 1993).

High-Temperature Stress

Exposure of harvested produce to direct sunlight, high ambient temperatures and heat treatments can result in heat injury. The primary direct injuries are membrane damage, lipid liquidification and protein and nucleic acid denaturation (Kays, 1999). Disruption of the integrity of the membrane structure and function can lead to many secondary responses.

Heat treatments are currently used for disease control and disinfestation of many fruit. However, these treatments can result in hyperthermal injury. Vapour heat treatment of 'Carabao' mangoes at a pulp temperature of 46°C for 10 min induced internal breakdown in the inner mesocarp of the fruit (Esguerra *et al.*, 1990). The injury is characterised by the presence of white, starchy lesions. Heat-related injuries have been observed in 'Kensington Pride' mango. Symptoms include rupturing of the patterned culticle and exocarp and exposing the underlying cells and hollow cavities that are randomly distributed within the mesocarp beneath the skin. The cell walls of damaged mesocarp parenchyma cells are convoluted and thickened in places (Jacobi and Gowanlock, 1995). In addition, fruit treated with hot water or vapour heat showed accelerated ripening (Salunkhe and Desai, 1984; Jacobi and Wong, 1992). This may be due to removal of some of the natural waxes on the fruit surface by hot water and thus an easier and faster exchange of respiratory gases is facilitated by the increased cell wall permeability. Immature fruit treated with hot water usually did not ripen and became shrivelled. Overripe mangoes treated with hot water occasionally had darkened and depressed lenticels on the skin (Jacobi *et al.*, 1994).

Continuous exposure of some climacteric fruit to high temperature allows the flesh to ripen but inhibits pigment synthesis. Papaya fails to ripen normally at 32.5°C which is characterised by poor colour development, abnormal softening, surface pitting, and an occasional off-flavour (An and Paull, 1990). De-greening in 'Cavendish' banana is also inhibited at high ripening temperatures due to the retention of chlorophyll and the associated thylakoid lamellae (Blackbourn and John, 1989). The failure to de-green is more likely associated with the enhanced breakdown of galactolipids at high temperatures, which leads to the loss of membrane structure, disrupting the spatial arrangement between the degradative

system and chlorophyll complexes. Inhibition of ripening at high temperature may relate to the inhibition of ethylene production (Lurie and Klein, 1991; Ketsa *et al.*, 1999b). High temperature stress has been reported to inhibit 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) more than 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) (Yu *et al.*, 1980; Field, 1985; Antunes and Sfakiotakis, 2000). However, ACC synthase is thought to recover faster than ACC oxidase activity after removal from high temperature stress (Biggs *et al.*, 1988).

In mangoes, ripening temperatures greater than 24°C may cause mottled skin (Hatton *et al.*, 1965; Medlicott *et al.*, 1986b) and strong flavours (Hatton, *et al.*, 1965). Temperatures exceeding 25°C result in softer fruit in 'Haden', 'Maya', and 'Mabruka' mango (Fuchs *et al.*, 1975). 'Kensington Pride' mangoes also have more green colour retained when ripened at 30°C (O'Hare, 1995).

2.7.2 Relative Humidity

The humidity of the air in storage rooms can directly affect the keeping quality of stored products. If it is too low, wilting or shrivelling due to water loss is likely to occur in most commodities. The rate of water loss depends upon the vapour pressure deficit between the fruit and the surrounding ambient air, which is influenced by temperature and relative humidity. At a given relative humidity, water loss increases with increasing in temperature (Kader, 1992a; Thompson, 1992; Wills *et al.*, 1998). Vazquez-Salinas and Lakshminarayana (1985) recommended that the relative humidity should be in the range of 85-90% for mangoes held at 16-22°C.

Weight loss results from water loss due to transpiration and respiration; however, weight loss by respiration is small and often negligible. Transpiration is the main cause of weight loss and it can reduce appearance, textural and nutritional quality (Kader, 1992a). Weight loss of mango increases with the length of storage and is influenced by cultivar and storage temperature (Dietz *et al.* 1989; Vazquez-Salinas and Lakshminarayana, 1985). Excessive humidity can cause condensation on fruit which can facilitate invasion of fruit by wound pathogens such as *Rhizopus* spp. (Joyce and Patterson, 1994).

2.7.3 Ethylene

2.7.3.1 The Role of Ethylene in Fruit Ripening

Ethylene is a naturally produced, simple two carbon gaseous plant growth regulator that has numerous effects on the growth, development and storage life of many fruits (Saltveit, 1999). It is well known both as a product of ripening fruit and as a hormone promoting ripening (Knee, 1985). The effects of ethylene on harvested horticultural commodities can be desirable or undesirable; thus it is of major concern to all produce handlers. Ethylene can be used to promote faster and more uniform ripening of fruits picked at the mature-green stage. On the other hand, exposure to ethylene can be detrimental to the quality of fruit causing effects such as excessive softening and promotion of discolouration (e.g. browning) (Saltveit, 1999). If ethylene synthesis or ethylene action are inhibited the ripening of climacteric fruits can be delayed (Knee, 1985). Therefore, ethylene could be a benefit for storage of horticultural produce by addition to stimulate, or removal to delay the ripening process.

Ethylene applied at a concentration as low as 0.1-1.0 μL L^{-1} for 1 day, is normally sufficient to stimulate ripening of climacteric fruit (Wills *et al.*, 1998). However, the magnitude of the climacteric is relatively independent of the concentration of applied ethylene. In contrast, ethylene application to non-climacteric fruit results in a transient increase in the respiration rate with the magnitude of the increase depending on the concentration of ethylene. The rise in respiration rate in response to ethylene may occur more than once in non-climacteric fruit, while only a single respiration increase occurs in climacteric fruit.

Despite the fact that ethylene treatment stimulates fruit ripening, it is unclear whether its role is continuing or only a switch for initiation of the process (Knee, 1985). This is an important issue since it can influence the concentration and duration of ethylene application treatment. Unfortunately there is limited information on this topic. Ethylene treatment for wild tomato fruit was required for at least 6 days to overcome the inhibition of ripening (Picton *et al.*, 1995). This suggests that ethylene plays a continuing role in regulating the process of ripening rather than simply being a trigger for the process. In addition, the rate of stimulation of ripening process by ethylene treatment may be different such as colour development, softening and other chemical and biochemical changes.

2.7.3.2 Ethylene Synthesis and Action

Biosynthesis of ethylene in higher plants from S-adenosylmethionine (SAM) via 1-amimo-cyclopropane-1-carboxylic acid (ACC) has been well studied (Picton *et al.*, 1995; Saltveit, 1999). The amino acid methionine is converted to SAM by addition of adenine, and SAM is

then converted to ACC by the enzyme ACC synthase. In the final step, ACC is oxidized by the enzyme ACC oxidase (formerly referred to as the ethylene-forming enzyme) to form ethylene.

ACC synthase, is the main site of control of ethylene biosynthesis (Yang, 1985). ACC synthase seems to be a pyridoxal enzyme, because the enzyme requires pyridoxal phosphate for maximal activity (Yang, 1985). It is strongly inhibited by amino ethoxyvinylglycine (AVG) and aminooxyacetic acid (AOA) which are well known inhibitors of pyridoxal phosphate-dependent enzymes. ACC synthase activity increases markedly during fruit ripening (Boller *et al.*, 1979). This enzyme appears to be developmentally regulated and acts as the pacemaker for ethylene biosynthesis. Therefore, the reaction sequence it catalyzes is a key point for metabolic regulation of ethylene production (Matoo and Aharoni, 1988).

Conversion of ACC to ethylene by plant tissues is dependent on O_2 (Saltveit, 1999). Low O_2 not only inhibited ethylene production in climacteric fruits such as apple (Burg and Thimann, 1959) and bananas (Mapson and Robinson, 1966), but also in the non-climacteric fruit like strawberry (Li and Kader, 1989). Cobalt iron is another inhibitor of ACC oxidase enzyme (Yu and Yang, 1979; Reid, 1992b). CO_2 can inhibit, promote or have no effect on ethylene production depending on the tissue (Abeles *et al.*, 1992). Increased CO_2 concentration in the storage atmosphere is used to prolong the storage of climacteric and other commodities. High CO_2 resulted in a decreased ethylene production and respiration in apple (Burg and Thimann, 1959) and in tomato (Mathooko *et al.*, 1995). However, ACC oxidase enzyme activity seems to be activated by CO_2 and consequently, promotes ethylene production (Imaseki, 1991).

2.7.3.3 Effects of Ethylene Treatment on Fruit Colour

Ethylene enhances fruit colour by stimulating ripening. Rapid development of the characteristic colour can produce a higher quality fruit (Salveit, 1999). Ethylene treatment can improve the skin colour of citrus (Trebitsh *et al.*, 1993; Yamauchi *et al.*, 1997), papaya (An and Paull, 1990) and mango (Burg and Burg, 1962; Fuchs *et al.*, 1975; Medlicott *et al.*, 1987). 'Haden' mango treated with 100 μL L^{-1} for 48 h had full colour developed earlier and also had a more uniform colour than the control fruit (Fushs *et al.*, 1975). The skin colour of ripe 'Tommy Atkins' mangoes is better if treated with 10 μL L^{-1} ethylene for 24 h at 25°C compared to 1.2 μL L^{-1} or lower. However, if the ethylene concentration increase to 100 μL L^{-1} or 1000 μL L^{-1} there is no significant improvement in the colour score compared to 10 μL L^{-1} (Medlicott *et al.*, 1987).

As mentioned earlier (section 2.4.2) chlorophyllase and peroxidase are involved in the de-greening process. Ethylene treatment is believed to promote the activity of chlorophyllase and peroxidase enzymes (Mattoo and Modi, 1969; Trebitsh *et al*, 1993). Ethylene application increases chlorophyllase activity in citrus peel and improves the de-greening process (Trebitsh *et al.*, 1993). It also promotes peroxidase activity in slices of pre-climacteric mango (Mattoo and Modi, 1969).

There are also some difficulties in explaining the role of chlorophyllase in chlorophyll degradation. For example, when fruit are removed from ethylene, the chlorophyllase activity does not change even though chlorophyll degradation has stopped (Purvis and Barmore, 1981). Therefore, the level of chlorophyllase is not the controlling factor in chlorophyll

degradation. Ethylene may interact with the thylakoid membranes to release chlorophyll molecules or induce another enzyme outside the chloroplast whose activity precedes and influences that of chlorophyllase.

2.7.3.4 Effects of Ethylene on Fruit Texture and Other Quality Attributes

Ethylene treatment not only promotes colour development, but also promotes the softening process. The firmness of many fruit decreases with ethylene treatment. This is usually beneficial for some fruits, but if applied at too high concentration and/or too long, ripening can progress into senescence and the flesh can become too soft (Saltveit, 1999). Even quite low concentrations of ethylene can affect fruit firmness. For instance, kiwifruit are very sensitive to ethylene and exposure to concentration of 30 nL L^{-1} can cause unacceptable softening in storage (Saltveit, 1999). 'Tommy Atkins' mangoes treated with ethylene at 10 μL L^{-1} were significantly softer on day 4 compared with those treated at 1.2 μL L^{-1} or below, however, there was no significant differences between treatments on day 8 (Meddlicott *et al.*, 1987).

In addition to affecting fruit colour and firmness, ethylene enhances the taste and flavour (Watada, 1986). However, Stern *et al.* (1994) indicated that the total volatile development in tomatoes picked mature-green and ripened with ethylene never attained the levels produced by fruit ripened on the plant.

Furthermore, ethylene may also affect postharvest diseases. The effects of ethylene on fungal growth and disease development are still unclear. Ethylene is thought to stimulate

stem-end rot development in citrus fruit (Barmore and Brown, 1983), but it also inhibits the development of *Gloeosporium album* rot in apple (Lockhart *et al.*, 1968).

2.7.3.5 Methods of Ethylene Treatment

There are three potential sources of ethylene for commercial use: liquid, gas, and ripening fruit (Sherman, 1985). Liquid sources are ethylene-releasing chemicals such as ethephon. Ethephon (2-chloroethane phosphonic acid) is strongly acidic in aqueous solution, but when the pH rises above 5, the ethephon molecule spontaneously hydrolyses, liberating ethylene (Reid, 1992b). Ethylene gas can be generated *in situ* or purchased in compressed cylinders (Sherman, 1985).

There are two methods for ethylene gas treatment: shot and trickle (or flow-through) (Reid, 1992b; Sherman, 1985). Shot methods employ the rapid injection of ethylene into the ripening room atmosphere every 12-24 h. This method has the advantage of simplicity. However, the disadvantage is that, because the room containing the product being ripened is sealed, CO_2 accumulates in the room and may inhibit the ripening process. The trickle or flow-though methods introduce ethylene into the room continuously rather than intermittently. To prevent a buildup in either CO_2 or ethylene, fresh air is drawn into the ripening room. As the flow of ethylene is very small, it has to be regulated carefully.

2.7.4 Atmospheric Composition

Normal air has 78.08% N_2, 20.95% O_2 and 0.03% CO_2 (Kader, 1992c). Changes in the concentrations of the respiratory gases, O_2 and CO_2 in the storage atmosphere, may extend fruit storage life. Respiration can be slowed by limiting O_2 and/or by raising CO_2 concentrations in the storage atmosphere. There are two methods of manipulating O_2 and CO_2 concentrations, controlled atmosphere (CA) storage and modified atmosphere (MA) packaging. CA and MA differ only in the degree of control in that CA is more exact (Kader, 1992c). The potential benefits of CA and MA are the retardation of senescence (ripening), reduction of fruit sensitivity to ethylene action at low O_2 or high CO_2 , alleviation of certain physiological disorders such as CI, and control of postharvest diseases and insects in some commodities (Kader, 1992c). However, there are also potential negatives of too low O_2 and/or too high CO_2 concentration including initiation and/or aggravation of certain physiological disorders, irregular ripening of fruits, off-flavour as a result of anaerobic respiration, and susceptibility to decay.

Reduced partial pressures of O_2 and enhanced partial pressures of CO_2 resulted in inhibition of ethylene responses (Burg and Burg, 1967). Apart from the effects on ethylene response and the respiratory pathway, O_2 and CO_2 may affect other processes associated with the quality of produce such as pigment metabolism, phenolic metabolism and volatile compound metabolism (Beaudry, 1999). In climacteric fruit, chlorophyll degradation can be inhibited by low O_2 and elevated CO_2 primarily by virtue of the effects of these molecules on ethylene sensitivity (Beaudry, 1999). Low O_2 can cause irreversible inhibition of mango ripening, especially chlorophyll degradation (Bender *et al.*, 2000). In addition, too low O_2 and/or too

high CO_2 concentration may inhibit the production of ethylene by the fruit in an irreversible manner by inhibiting the enzymes ACC synthase and ACC oxidase, respectively (Bender *et al.*, 2000).

For temperate fruit, notably apples and pears, control or modification of storage atmosphere has enabled the storage life extension with minimal reduction in product quality. However, mangoes have been notoriously difficult to store under controlled atmosphere (CA), with most workers reporting only slight increases in storage life (Spalding and Reeder, 1974; Spalding, 1977). The application of CA to mango storage has been studied for different cultivars, but there is no recommended atmospheric composition applicable for all cultivars. Optimum atmosphere conditions may vary between cultivars. Preferred conditions for 'Keitt' mangoes have been reported as 5% O_2 and 5% CO_2 (Hatton and Reeder, 1966). 'Chok Anan' mangoes can be stored at 2-5% O_2 (Abd-Shukor *et al.*, 2000), while 'Tommy Atkins', 'Haden', 'Keitt' and 'Kent' mangoes can benefit from 3-4% O_2 plus 25% CO_2. In 'Kensington Pride' mango, an optimum atmosphere of 2-4% O_2 and 4% CO_2 has been reported (McLauchlan and Barker, 1994). Colour development is linearly retarded by decreasing O_2 from 10 to 2% and increasing CO_2 from 0 to 4 %. Storage of 'Kent' and 'Tommy Atkins' mangoes in elevated CO_2 atmospheres results in increased respiration rates, increased ethanol and reduced ethylene production rates (Bender and Brecht, 1994). Reduction of O_2 levels to 3 % in the presence of elevated CO_2 has little effect on ethanol and ethylene production. During the storage, high levels of CO_2 treatments significantly delayed chlorophyll breakdown compared to the control fruit (Bender and Brecht, 1994).

The concentration of O_2 and CO_2 in the storage atmosphere may influence postharvest diseases. Avocados stored under 2% O_2 + 10% CO_2 for 3-4 weeks at 7°C, prevented the development of anthracnose (Spalding and Reeder, 1975). In apples, CO_2 at 2.8% or above (O_2 about 16-17%) reduced disease severity (Sitton and Patterson, 1992). Avocado fruit exposed to high CO_2 had less disease incidences as since high CO_2 stimulates the antifungal compound (1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene) in the skin and flesh (Prusky *et al.*, 1991).

Modified atmospheres are generated by the fruit itself by relying on product respiration to use O_2 and generate CO_2 in a sealed package or coating. To reduce gas exchange of fruit the physical barriers such as modified atmosphere packaging (MAP) and surface coating are used. The final atmospheric composition is not controlled as for CA storage, but is dependent on the interaction between produce, barrier, and the environment.

MAP is a common technique to prevent weight loss and extend storage life of perishable products (Ben-Yehoshua *et al.*, 1994). Sealing fruit in a plastic film reduced shrivelling, improved appearance and extended the storage life of 'Tommy Atkins' and 'Nam Dokmai' mangoes stored at 13°C (Rodov *et al.*, 1994).

Perforated films enable MAP to maintain the advantages of sealed-packaging without the possibility of anaerobiosis, and avoid poor ripening found in unperforated packaged fruits held at ambient temperature (Yantarasri *et al.*, 1995). Mangoes in perforated plastic bags had lower weight loss and shrivelling compared to nonsealed fruit and had better firmness compared to nonsealed and unperforated fruit. The degree of perforation significantly affects

the rate of colour development of mangoes inside the packages, with more holes producing less green fruit. In addition, it is believed that mangoes are better packed in low and high density polyethylene films (Gonzalez *et al.*, 1990).

Surface coating such as waxes can also be used as a physical barrier around the fruit. It reduces the movement of gases into and out of the fruit, thereby modifying the atmospheric composition in the fruit. The degree of efficiency depends on the permeability of the surface coatings (Wills *et al.*, 1998). Many studies indicated that waxing of mangoes reduced the rate of weight loss during storage (Parmar and Chundawat, 1989; Shivarana Reddy and Thimma Raju, 1989). Coated 'Manila' mangoes have less mesocarp softening which is associated with low PG activity (Diaz-Sobac *et al.*, 1997). Furthermore, coating can reduce 70% of anthracnose incidence in 'Manila' mangoes (Diaz-Sobac *et al.*, 2000).

2.8 CONCLUSIONS

Mango is a climacteric fruit which has a high respiration rate. Therefore, fruit are highly perishable and susceptible to physiological disorders and diseases. Acceptable fruit appearance including skin colour, absence of rots and blemishes of ripening and ripe fruit, is an important factor for determining the rapid sale and consumption. The retention of green colour of ripe mango fruit is the most important aspect of skin colour affecting sales and can be affected by production practices. Nitrogen appears to be the most important factor for skin colour quality. In addition, N application can also influence postharvest diseases. It is also important to get the right balance of N nutrition for good yield and quality. However, there is limited information on tropical and subtropical fruits, particularly for mango on how

to manipulate N nutrition to reduce the problem of green, ripe fruit. Fruit quality is also reliant on several postharvest factors such as ripening temperature, ethylene and CO_2 levels. The most important aspects of fruit ripening are colour change and softening. To fit the marketing purpose retailers prefer that fruit develop colour faster than they soften, so that they can hold fruit for a longer time with satisfactory quality. This concept has not been studied well in mango. It is evident that there was a need to investigate the pre-harvest N application and postharvest factors to establish their effect with 'Kensington Pride' mango quality, especially skin colour.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

The materials and methods which apply to more than one chapter of the thesis are presented in this chapter. In addition, the flow-through systems which were designed and set up for the postharvest treatment of ethylene and carbon dioxide are presented. Other methods specifically relevant to individual chapter, are included in those chapters.

3.1 FRUIT SOURCES

'Kensington Pride' mango fruit were obtained from three main districts:

- the Burdekin district in north Queensland (dry tropics; latitude 19-21°S) for pre-harvest treatments,
- the Bundaberg district in south-east Queensland (sub tropics, latitude 24-25°S) for postharvest treatments, and
- the Gympie district in south-east Queensland (sub tropics, latitude 26-27°S) for postharvest treatments.

Fruit were normally picked at the green, mature stage and from the northern, outer canopy sector of the trees. After harvest, the fruit were transported by air overnight from the Burdekin district, or within 8 h or 4 h of harvest by car from the Bundaberg or Gympie districts, to the postharvest laboratory at Maroochy Research Station, Nambour, south-east Queensland. In the laboratory, fruit samples were examined for the presence of mechanical injuries, pests and diseases and were sorted accordingly. Some fruit samples were dipped in

hot Spin Flo® (prochloraz) (1g L^{-1}) at 52°C for 5 minutes (min) as required. Dipped samples were dried and cooled under ambient conditions before applying any treatments.

3.2 Flow-through Systems for Gas Treatment

Three flow-through systems (called system A, B and C) were designed and set up in the postharvest laboratory at Nambour for the gas treatment experiments, using the concepts described by Smith *et al.* (1997).

3.2.1 System A

The treatments applied with System A were:

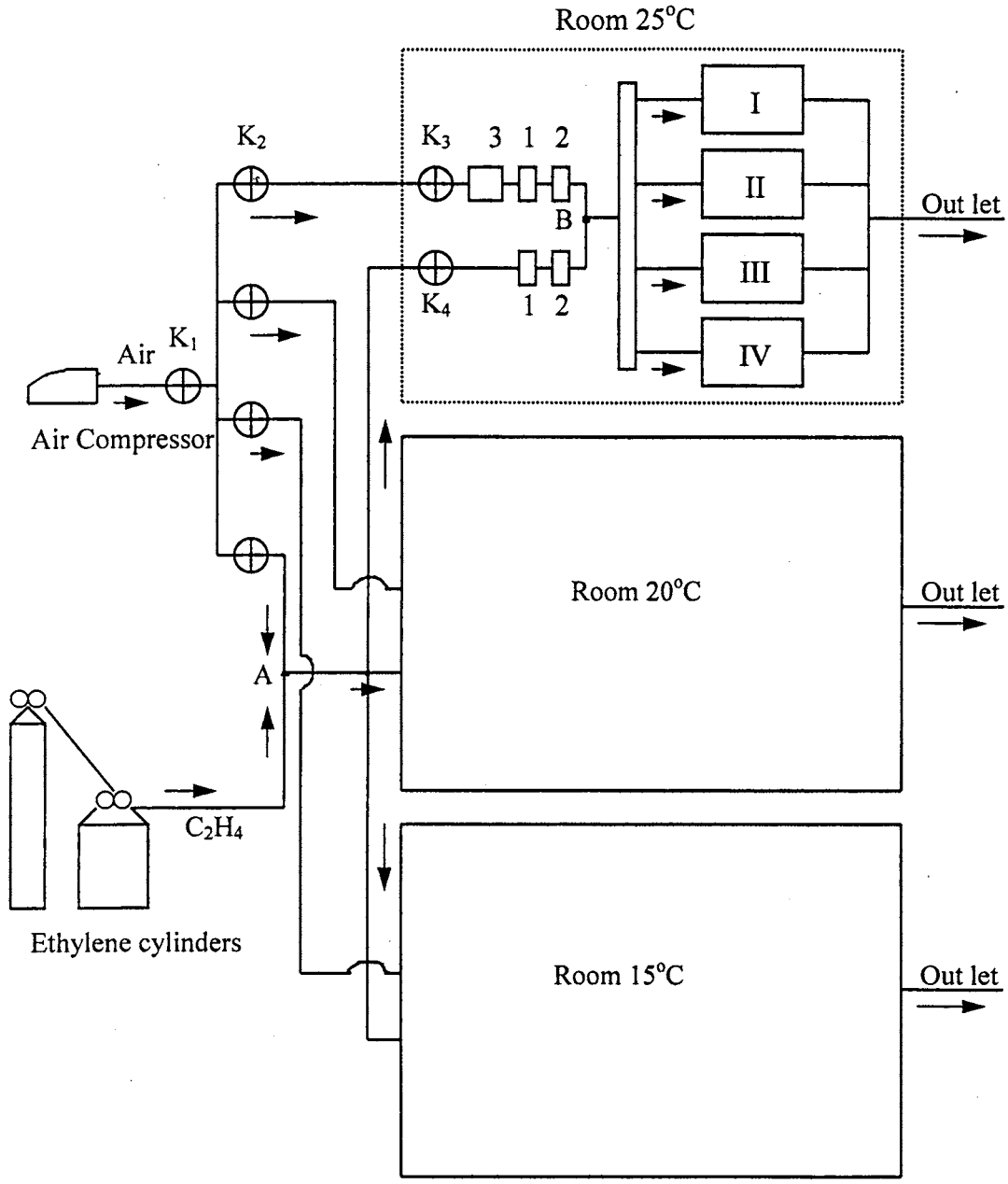
- three ripening temperatures (15, 20, and 25°C)
- four ethylene concentrations (0, 10, 100, and 1000 μL L^{-1})
- two durations of ethylene treatment (24 and 72 h).

Fourteen fruits were placed in a 30-L plastic container representing a single experimental unit (replicate). There were 24 containers in each cool room. Six containers had the same ethylene concentration (total of four ethylene concentrations), however, three containers per concentration were for the 24 h treatment and the other three for the 72 h treatment. After 24 h, the ethylene supply to three of the containers was turned off, but the fruit remained in the containers with the same air flow and conditions. After 72 h, the ethylene supply to the remaining three containers per concentration (for 72 h treatment fruit) was removed. The fruit were immediately removed from all containers then transferred to another cold room

free from additional ethylene, and held at the same treatment temperature until ripe. A flow rate of 1 L min^{-1} per container and a relative humidity (RH) of between 90-95% were used.

The treatment system was fabricated using capillary bore glass tubing and plastic irrigation manifolds and fittings to produce the four concentrations of ethylene in each room (Figure 3.1). The length and diameter of the glass tubing were adjusted to produce the required ethylene concentrations and flow rates for each container. Compressed air from a rotary vane compressor and pure ethylene from an ethylene cylinder were first mixed at point (A) outside the cool rooms to a 3000 μL L^{-1} ethylene concentration by the gas cylinder regulators. The mix was then humidified to 90-95 % RH and diluted with air from the same compressor as above (also humidified to 90-95%) at the second mixing point (B). The appropriate internal diameter and length of glass capillary tubing was used to give the required flow rate and ethylene concentrations for each container. The air used for the 0 μL L^{-1} treatment was passed through a filter of Purafil to remove ethylene.

Ethylene concentrations in the containers were checked three times daily by withdrawing a 1 mL sample and injecting it into a gas chromatograph (GC) (Shimadzu GC-8A, Shimadzu Corp., Kyoto, Japan) with a flame ionization detector and a 600 mm x 5 mm glass column containing 100 to 120 mesh activated alumina (Alltech Associated Pty. Ltd., Sydney, Australia). Nitrogen was used as the carrier gas and the injector, column and detector temperatures were 120, 80 and 120°C, respectively. The ethylene concentration in the containers was adjusted when required by altering the head pressures of air and/or ethylene.



1 - Humidified
2 - Head pressure control
3 - Purafil
A - First mix
B - Second mix
K_1, K_2, K_3, K_4 - flow rate control
I, II, III, IV - 6 parallels of 0, 10, 100, 1000 $\mu L\ L^{-1}$

Figure 3.1 Diagram of flow-through system A for ethylene treatment

3.2.2 System B

The treatments applied using system B were:

- five ethylene concentrations (0, 5, 10, 20, and 50 μL L^{-1})
- two durations of ethylene treatment (24 and 72 h).

Fifteen fruits were placed in a 30-L plastic container (experimental unit). The design of system B was similar to system A; however, there were five ethylene concentrations in only one cool room (20°C). As for system A, ethylene was turned off after 24 h, with air at the same flow rate continuing the pass through these containers until all fruit were removed after 72 h. Ethylene concentrations were checked three times daily by GC as above, and were adjusted by altering the head pressures of air/or ethylene.

3.2.3 System C

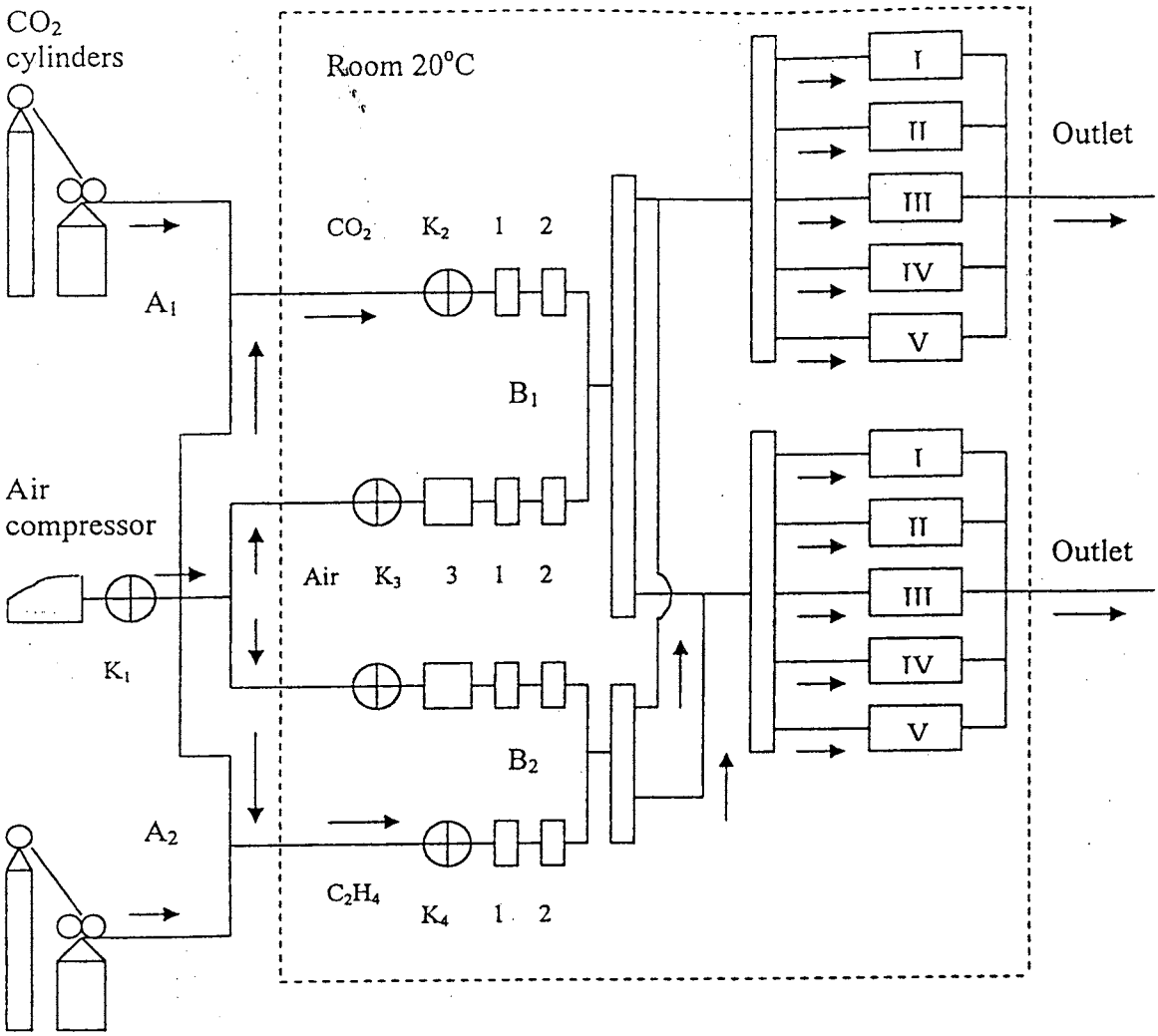
The treatments applied using system C were:

- five CO_2 concentrations (0, 1, 2, 4, and 6 %)
- two ethylene concentrations (0 and 10 μL L^{-1})
- ethylene and CO_2 treatment were for 72 h.

Fifteen fruits were each placed in a 30-L plastic container (experimental unit). Six containers had the same carbon dioxide concentration. However, three containers were without ethylene and other three were supplied with 10 μL L^{-1} of ethylene. After 72 h, fruit from all six containers were removed and transferred to a cool room (20°C) in the absence of ethylene for

ripening. The flow rate and other conditions for each individual container were similar to those used in system A.

The design of system C was similar to that of systems A and B. However, a CO_2 cylinder parallel to the ethylene cylinder was included to the system (Figure 3.2). Air and CO_2 were first mixed (A_1) outside the cool room, and then humidified to 90-95 % RH. After that the mix was diluted with air (also humidified to 90-95%) at the second mixing point (B_1) to give the required flow rates and CO_2 concentrations before joining with the ethylene-air flowing through the containers. Air was mixed with ethylene at points A_2 and B_2. Ethylene and CO_2 concentrations in the containers were also checked by GC three times daily, and were adjusted by altering the head pressures of air and/or ethylene or CO_2.



1 - Humidified
2 - Head pressure
3 - Purafil
A_1 - First mix of air and CO_2, A_2 - First mix of air and C_2H_4
B_1, B_2 - Second mixes
K_1, K_2, K_3, K_4 – flow rate control
I, II, III, IV, V – 3 parallels of 0, 1, 2, 3, 6% CO_2

Figure 3.2 Diagram of flow-through system C for ethylene and carbon dioxide treatment

3.3 FRUIT QUALITY ASSESSMENT

Skin colour: The skin colour of ripe 'Kensington Pride' mangoes was visually assessed by estimating the % of the skin surface area with green, yellow and red blush colour (called % green, yellow and red blush colour). To ensure consistency, only the author, trained prior to the experiments, conducted the ratings at all times. The colour of the greenest and the most yellow parts of the skin was measured by a Minolta Colorimeter (Model CR-200/CR-210, Japan) and the results expressed as hue angle. The hue angle (0-360°) is represented by a colour circle divided by four colours - red, green, yellow and blue with purplish red = 0° (or 360°), yellow = 90°, bluish green = 180° and blue = 270° (McGuire, 1992; Voss, 1992). The other descriptors in this system are the lightness (L) value from black to white, and the chroma (C), which characterises the intensity of each hue from pure grey to the maximum colour intensity.

The typical colour development of a 'Kensington Pride' mango fruit during ripening is shown in Plate 3.1. Generally, the yellow colour appears first on the fruit shoulders, with the yellow colour progressing toward the "nose" of the fruit. The fruit in Plate 3.1 shows full yellow colour development, but some fruit can retain some green colour (mainly around the nose of the fruit) when ripe. Generally, during ripening the colour of 'Kensington Pride' mango changes from green to green yellow, light yellow, yellow or yellow orange and the hue angle changes from about 110-120° (green mature at harvest) to 80-90° (ripe fruit with yellow or yellow orange colour) (unpublished data).

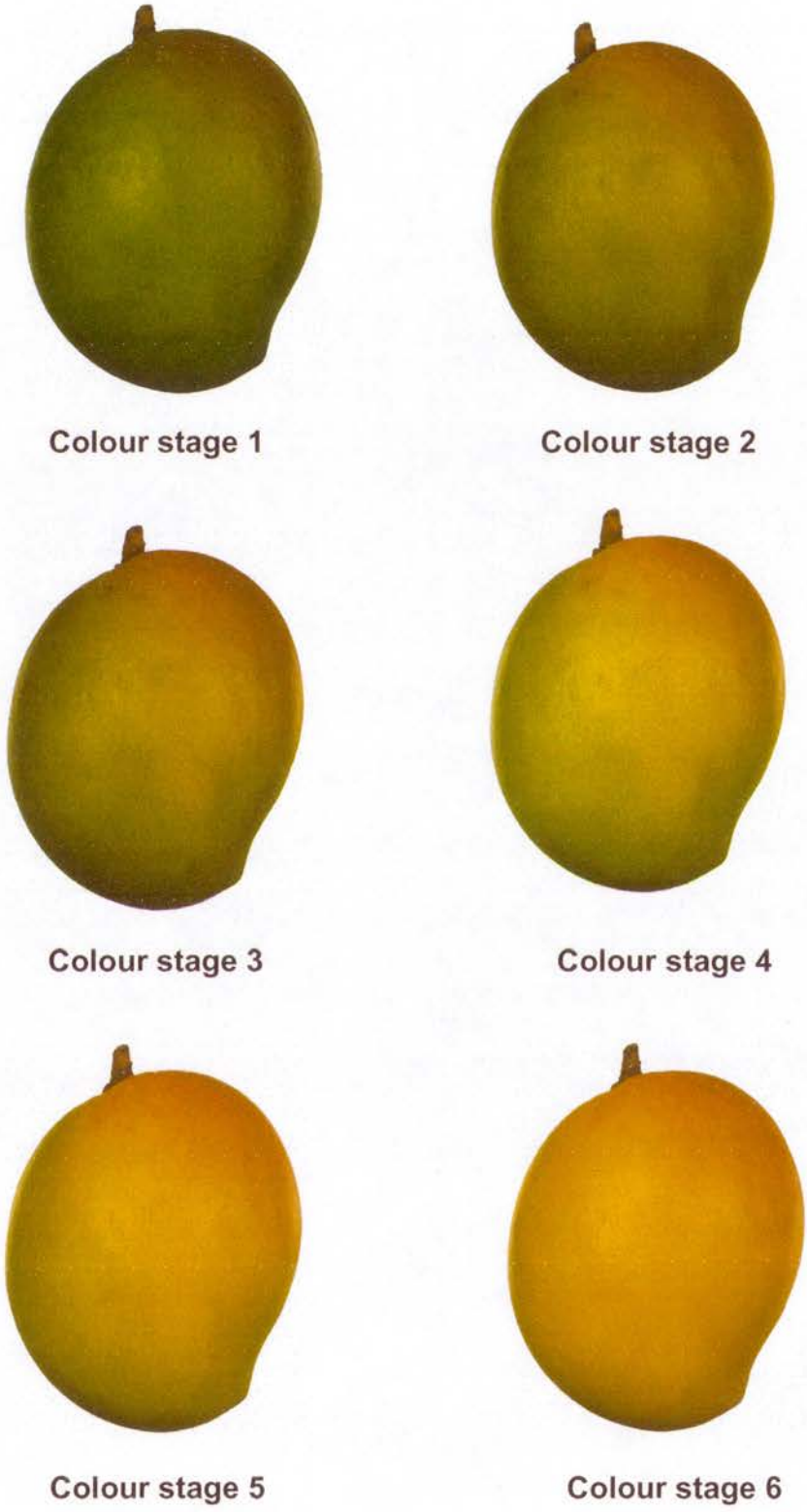


Plate 3.1 Skin colour development of 'Kensington Pride' mango during ripening

Fruit firmness: Fruit firmness was assessed by gentle hand pressure and rated using a scale of 1-6 where 1 = firm, 2 = sprung (can feel the flesh deform under extreme finger force), 3 = softening (can feel 2-3 mm deformation with moderate finger pressure), 4 = near ripe (deformation achieved with very little finger pressure), 5 = ripe or eating soft (whole fruit deforms with moderate hand pressure) and 6 = over ripe (whole fruit deforms with slight hand pressure). The firmness rating of 5 was equivalent to approximately 4 Newtons (N) which is the force required to push an 8 mm hemispherical probe 2 mm into the flesh at a rate of 2 mm min^{-1} using an Instron Universal Testing Machine model 1122 (Instron Ltd, UK). Regular reference was made to the Instron to ensure consistency with the hand firmness assessment.

Days to ripe: The number of days from harvest to ripe (DTR) was recorded as the number of days from harvest for each fruit to reach a firmness rating of 5.

Diseases: Anthracnose (caused mainly by *Colletotrichum* spp.; Plate 3.2A) and stem-end rots (caused mainly by *Dothoriella* spp.; Plate 3.2B) were characterised based on the appearance of the lesions as described by Coates *et al.* (1995). Occasional isolations of the affected areas were conducted to confirm the pathogens. However, extensive isolations were not conducted, so the results should be interpreted based on the appearance of either anthracnose (firm rots with diffuse margins either at the stem end or on the body of the fruit) or of stem-end rots (watery rots starting at the stem end). For disease identification, skin and flesh samples were taken from the advancing margin of representative lesions and incubated on potato dextrose agar with and without streptomycin (approx 0.5%) at 25°C for 7-10 days. The severity of the

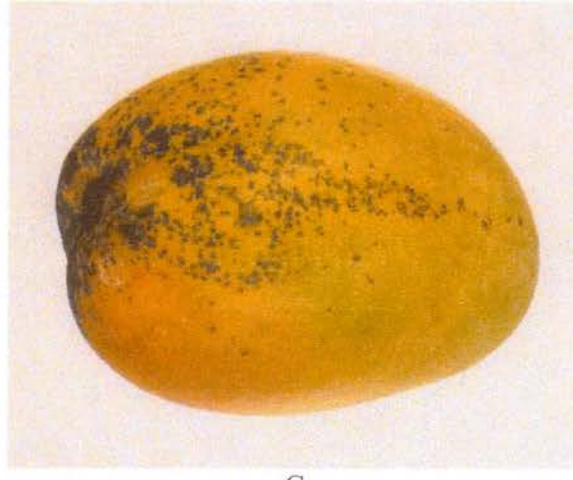
anthracnose was rated as the % of the skin surface area affected, and for stem-end rots (SER), as the % of the flesh volume affected. In addition dendritic spot (caused by *Dothoriella* spp.; Department of Primary Industries, 1989) was noted on occasion as small, black lesions with irregular, angular margins (Plate 3.2C). The severity was rated as a scale of 0-4 (0 = none, 1 = few small spots, 2 = moderate number of small spots or few large spots, 3 = large number of small spots or moderate number of large spots and 4 = large number of large spots).

Lenticel spotting: Lenticel spotting (LS) was assessed using a scale of 0-4 (0 = none, 1 = few small spots, 2 = moderate number of small spots or few large spots, 3 = large number of small spots or moderate number of large spots and 4 = large number of large spots).

Flesh total soluble solids and acidity: Flesh samples (excluding skin) were taken from the middle equatorial section of each fruit. All fruit samples from each tree were pooled and blended to provide one representative sample per tree. Total soluble solids (TSS) expressed as °Brix was measured on the blended sample with an Atago (Model 3T, Japan) bench refractometer, adjusted to 20°C. Titratable acidity (expressed as % citric acid) was measured on 10 g fruit pulp by titrating with 0.1 M sodium hydroxide to pH 8.2 using a Metrohm Titrino (Model 719S) with a sample changer (Model 730).

A

B



C

Plate 3.2 Postharvest diseases of 'Kensington Pride' mangoes: anthracnose (A), stem-end rots (B) and dendritic spot (C).

3.4 PIGMENT AND ENZYME ANALYSES

Pigment Analyses: Total skin chlorophylls and carotenoids concentrations were determined by taking one skin disc (12 mm diameter) with pulp removed from the greenest area of ripe fruit. The discs were homogenised with 2 ml of 80% acetone and centrifuged at 2000 x *g* for 5 min. The residue was washed with another 2 ml of 80% acetone and centrifuged again. The combined supernatants were made up to 5 ml with 80% acetone. Total chlorophylls (*a* and *b*) and carotenoids (xanthophylls and ß-carotene) were determined by measuring the absorbance at 663, 646 and 470 nm and the concentrations (expressed by $\mu g\ cm^{-2}$) calculated according to the method of Lichtenthaler (1987):

$$C_a = 12.5\ A_{663} - 2.79\ A_{646}$$

$$C_b = 21.50\ A_{646} - 5.10\ A_{663}$$

$$C_{x+c} = (1000\ A_{470} - 1.82\ C_a - 85.02\ C_b)/198$$

Where C_a, C_b are chlorophyll a and b,

Total chlorophylls $(a + b) = C_a + C_b$

C_{x+c} is total carotenoids (xanthophylls and ß-carotene)

A_{663}, A_{646} and A_{470} is absorbance at 663, 646, and 470 nm

Chlorophyllase activity: An acetone powder was prepared as described by Fernaldez-Lopez *et al.* (1992). Frozen (-80°C) mango skin (5 g) was homogenized in a blender with 10 ml of 100% cold acetone (-20°C). The homogenate was filtered with filter paper and the residue repeatedly extracted with the same solvent until colourless. The final residue was dried *in vacuo* and stored at -20°C prior to analysis.

The chlorophyllase assay method was modified slightly from that used by Trebitsh *et al.* (1993). Acetone powder (150 mg) was stirred with 5 ml of buffer containing 5 mM potassium phosphate (pH = 7.0), 50 mM KCl and 0.24% Triton X-100 for 60 min at 30°C. The extract was filtered through cheesecloth and centrifuged at 12,000 x *g* for 15 min at -15°C. The supernatant (0.5 ml) was added to 0.5 ml of a buffer of 100 mM sodium phosphate (pH = 7.0)/0.24 % Triton X-100, and 0.2 ml of chlorophyll *a* (from spinach leaves; Sigma, Germany) dissolved in 100% acetone (100 μg/ml). This was incubated in a water bath at 37°C for 30 min, and the reaction was stopped by transferring 0.5 ml of the reaction mixture to a centrifuge tube containing 7.5 ml of acetone/hexane/10 mM KOH (3:4:0.5 vol/vol). The mixture was well shaken and centrifuged at 10,000 x *g* for 10 min to separate the phases. Activity was based on a decrease in absorbance by chlorophyll *a* at 663 nm of upper phase. One unit of chlorophyllase activity was defined as a change of 0.01 absorbance unit per mg protein per min. The protein concentration of the enzyme extracts were determined by the method of Bradford (1976).

3.5 Leaf and Soil Analyses

Leaf samples were collected before N application at pre-flowering (early July), at panicle emergence (late August) and at harvest (late November). Twenty newly mature leaves were randomly picked, washed with deionised water and dried at 60°C for 2 days in a forced-draught oven (Catchpoole and Bally, 1996). After drying, leaf samples were sent to the laboratory at the University of Sydney for N analysis. Leaf N concentration was determined by the Dumas total-combustion method using a Leco CHN-1000 elemental analyser (Leco Inc., St. Joseph, MI, USA), and the results expressed as % N in the leaf. Soil samples were

taken from four positions (depth of 15 cm) under the canopy within the irrigated zone of each tree just before the pre-flowering N treatment on 04/07/00, and the samples pooled for each orchard. After drying at 60°C for 4 days, soil samples were sent to a commercial laboratory (Incitec Ltd., Brisbane) for N analysis.

CHAPTER FOUR

EFFECT OF PRE-HARVEST NITROGEN APPLICATION ON SKIN COLOUR AND OTHER QUALITY ATTRIBUTES

4.1 INTRODUCTION

Skin colour is an important quality parameter for Australian 'Kensington Pride' mangoes. During typical ripening, the skin colour of these fruit changes from green to yellow. Ripening also results in flesh softening, conversion of starch to sugars, loss of acidity and the development of flavours and aromas. However, in some fruit, the loss of green colour occurs at a slower rate than the other ripening changes, resulting in green, soft fruit (Hofman *et al.*, 1997a). These fruit sell more slowly at the wholesale and retail markets, and are often sold at a lower price. Green, ripe fruit is now one of the major quality problems with 'Kensington Pride' mangoes on the Australian market.

Several factors can affect fruit skin colour. McKenzie (1994) reported that the mango cultivars 'Heidi', 'Haden', and 'Tommy Atkins' had little green skin colour when soft, however, 'Sensation', 'Keitt' and especially 'Kent' had significant numbers of fruit with green skin colour when ripe. Pre-harvest nitrogen (N) application can influence the background colour of apples at harvest and after storage (Neilsen *et al.*, 1984; Fallahi *et al.*, 1985a; Raese and Drake, 1997), with high rates of N significantly increasing the amount of green colour on the fruit. In mango, a survey of grower fertiliser practices in South Africa suggested there was a link between the green skin colour of ripe 'Sensation' mangoes and the amount of pre-harvest N application (Oosthuyse, 1993; McKenzie, 1994). Fruit from

orchards with a low soil N status de-greened completely when ripe while those from orchards with moderate or high soil N either failed to de-green appreciably or did not de-green at all. These results suggest that N fertiliser regimes can be manipulated to improve the skin colour of ripe 'Kensington Pride' mango. Beside fruit colour, high N rates have also been associated with increased fruit rots in avocado (Abou Aziz *et al.*, 1975), nectarine (Daane *et al.*, 1995), and tomato (Segall *et al.*, 1977; Bartz *et al.*, 1979). Furthermore, other pre-harvest factors such as light exposure, position in the canopy and fruit maturity at harvest can also affect skin colour (Seymour *et al.*, 1990; Hofman *et al.*, 1995b; Hofman *et al.*, 1997b).

The aim of the following three experiments was to investigate the effects of pre-harvest N on skin colour and other quality attributes of ripe 'Kensington Pride' mango. Fruit yield was also measured to determine whether yield can be manipulated without affecting fruit quality. In the first experiment, ten orchards in a large mango production district were surveyed to gain some understanding of the extent of the green fruit colour problem, and the production factors that may affect the retention of green colour in ripe fruit. In the second experiment, more detailed investigations were carried out on the fruit responses to soil and foliar N applied mainly at pre-flowering, a period with high nutrient demand from the trees. This experiment aimed to establish a clear relationship between N application and fruit quality, particularly the retention of green colour on ripe fruit. In the third experiment, the effect of N application to the soil, mainly after harvest, on skin colour and other quality attributes of ripe fruit, was studied. The aim of the third experiment was to study whether N application at different times can also influence skin colour and other postharvest parameters. A smaller, fourth experiment was carried out to get some preliminary idea of the colour and firmness changes that occur during ripening in response to pre-harvest N application.

4.2 MATERIALS AND METHODS

4.2.1 Experiment 1: Survey of 'Kensington Pride' Mango Quality

This experiment was conducted during the 99/00 cropping season in the Burdekin district (north Queensland). The climate of this region is dry tropics with a well defined wet and dry season (Donnollan, 1991). Ten orchards around this area with a range of soil types and history of green, ripe fruit were selected for the survey (Table 4.1). The soil type and tree age are recorded for each orchard. The N status was described as low, medium or high based on pre-harvest N fertiliser history as supplied by growers (Table 4.1).

Ten 'green mature' 'Kensington Pride' fruits were harvested from the northern, outer canopy sector from each of six representative trees in each orchard on 25/11/99 (commercial maturity). The fruit were transported by air to the postharvest laboratory at Nambour within 24 h after harvest. In this experiment, no postharvest fungicide or ethylene treatment was applied. The fruit were ripened at 20°C. Fruit firmness was tested by gentle hand pressure (Section 3.3). At the eating soft stage (firmness rating of 5), fruit were assessed DTR, % of skin area with green and red blush colour (% green and red blush colour), hue angle of the greenest part on the skin (hue angle of green skin), rots severity (anthracnose and stem-end rots), lenticel spotting, flesh colour (hue angle) and the flesh TSS and acidity as described in Section 3.3. In addition, skin pigments (chlorophylls and carotenoids) were analysed (Section 3.4).

Table 4.1 Experiment 1. Soil type, tree age and N status of 'Kensington Pride' mango trees in the orchards used for the survey in 99/00 in the Burdekin district.

Orchard	Soil type	Tree age (years)	N status	
			Pre-harvest N fertiliser, (g/tree)	N classification[1]
1	SL	50	0	L
2	SL	10	160	H[2]
3	HCL	15	305	H
4	HCL	15	89	M
5	HCL	20	392	H
6	HCL	15	347	H
7	HCL	20	143	M
8	HCL	20	0	L
9	HCL	20	0	L
10	SL	7	150	M

[1]N classification was based on pre-harvest N fertiliser treatments supplied by the growers.
[2]Orchard 2 is classified as high N status because of heavy N applied in previous years (with indication of very high leaf and soil N).
L, M and H = low, moderate and high N status.
SL = sandy loam, HCL = heavy clay loam.

4.2.2 Experiment 2: Pre-flowering Nitrogen Application

This experiment was conducted during the 00/01 cropping season in the Burdekin district. Three orchards from the previous survey were selected. The survey results showed that one orchard produced ripe fruit with high green skin colour (called HG orchard) and two other orchards produced ripe fruit with little green skin colour (called LG1 and LG2 orchard). HG and LG1 were on sandy loam soil (11 year old trees), while LG2 was on a heavy clay loam soil (21 year old trees). The trees in all three orchards were planted at the relatively wide spacing at 8 x 10 m. LG2 trees were about 25% taller and wider than those from HG or LG1.

In all three orchards, treatments were randomly assigned to each of six trees per treatment (each tree as a replicate), within five rows in each orchard. A buffer tree was used between each datum tree in the same row. A total of 30 datum trees (five treatments and six single tree replications) were used in each orchard. No N was applied to the buffer trees. Other macro and micro minerals (except N) were applied according to standard commercial practice to all trees (included buffer trees). The 10 m spacing between the rows, and the small size of the trees resulted in minimal root or canopy overlap of trees between rows.

In each orchard, four soil N treatments and one foliar spray treatment was applied. The soil treatments (as ammonium nitrate) were applied at pre-flowering and at panicle emergence, as described in Table 4.2. The total N application to soil for HG orchard was 0, 75, 150 and 300 g per tree, while for LG orchards was 0, 150, 300 and 450 g per tree. Soil applications were spread evenly under the canopy of each tree on 04/07 and 28/08/00. Trees were irrigated by under tree sprinklers immediately after application and then regularly thereafter based on standard commercial practice. The foliar treatment (as ammonium nitrate at 0.75%) was applied by spraying trees four times (12.5 g N per tree per spray) on 04, 18/07 and 14, 28/08/00 with a low pressure backpack spray in the absence of wind, to eliminate spray drift to non-treated trees. The N concentrations in leaves just before the first treatment on 04/07/00 (pre-flowering) were 1.37, 1.07 and 1.12% for HG, LG1 and LG2 orchards, respectively, and soil N (as nitrate) was 3.4, 1.4 and 1.3 mg kg^{-1}, respectively.

At commercial maturity (24/11/00 for HG and LG1 orchard, and 30/11/00 for LG2 orchard), 12 'green mature' 'Kensington Pride' fruits were harvested from the northern, outer canopy

sector of each tree (total of 72 fruit per treatment), and transported to the laboratory at Nambour within 24 h of harvest. Fruit were then treated with hot Spin Flo® (carbendazim 50% a.i.; 1g L^{-1}) at 52°C for 5 min and ripened 20°C. When ripe (firmness of 5), fruit were removed and assessed for quality as in Experiment 1.

The total fruit weight and fruit number per datum tree was recorded for the HG and LG2 orchards, and the average fruit weight per tree calculated by dividing the total fruit weight by the fruit number per tree. Yield data for LG1 was not available because a selected harvest by the grower was done before total tree yield could be recorded.

Chlorophyll and carotenoids concentration of the greenest part of the skin were determined as described in Section 3.4 with samples obtained from three typical fruits from each tree.

Leaf samples were collected just before N was applied to the soil at pre-flowering (04/07/00), before application at panicle emergence (28/07/00) and at harvest (24 and 30/11/00). Twenty most recently mature leaves from randomly selected branches around each tree were sampled (Section 3.5). After drying at 60°C for 2 days, leaf samples were sent to the laboratory at the University of Sydney (Chapter 3) for N analysis. Soil samples were taken and sent to Incitec Ltd. (Brisbane) for N analysis (Section 3.5).

Table 4.2 Experiment 2. Nitrogen treatments applied to the soil at pre-flowering (04/07/00) and panicle emergence (28/08/00) to 'Kensington Pride' mango trees. The N was applied as ammonium nitrate to the drip zone of each datum tree. Foliar sprays (at 0.75% ammonium nitrate) were applied on 04/07, 18/07, 14/08 and 28/08/00 as 12.5 g N in 5 L per tree per spray).

Treatment (total g N/tree)	N at pre-flowering (g/tree)	N at panicle emergence (g/tree)
HG orchard		
0	0	0
75	50	25
150	100	50
300	200	100
foliar sprays[1]	50	-
LG orchards		
0	0	0
150	100	50
300	200	100
450	300	150
foliar sprays[1]	50	-

[1] Total N of each treatment = 4 sprays x 12.5 g = 50g per tree.

4.2.3 Experiment 3a: Nitrogen Application after Harvest

This experiment was conducted during the 01/02 cropping season in the Burdekin district. Two orchards surveyed in 99/00 were selected; one orchard had ripe fruit with high green colour (HG*) and another had fruit with low green (LG*). The HG* orchard was on a sandy loam soil orchard (the HG orchard in Experiment 2, but on a different block), while the LG* orchard was a heavy clay loam soil (different to the LG orchards in Experiment 2). The trees in both orchards were planted at a relatively wide spacing of 8 x 10 m. The LG* trees (16 year old) were about 25% taller than those from HG*.

The experimental layout was a completely randomised design with eight treatments in each of the two orchards. Each treatment had six trees (each tree as a replicate) with a buffer tree in between each datum tree in the same row as in Experiment 2. Nitrogen treatments (as ammonium nitrate) were applied to the soil only, using the same rates in both orchards (Table 4.3) just after harvest (22/02/01), at pre-flowering (04/07/01) and at panicle emergence (28/08/01). Two additional treatments included; 375 g N applied just after harvest only (375b) and another with 275 g N plus potassium (275K). Additional potassium (K) was applied on the same day as the N treatments, as potassium sulphate at 330 g K per tree above that applied to all treatments as part of the standard fertiliser regime (extra above of standard commercial practice) on the same days with N applied. Fertilisers were spread evenly under the drip zone of each tree. Trees were irrigated by under tree sprinklers immediately after application and then regularly thereafter based on standard commercial practice. Other macro and micro minerals were applied according to standard commercial practice to all trees (Kernot *et al.*, 1998).

At commercial maturity (14 and 15/11/01 for HG* and LG*, respectively), 15 'green mature' 'Kensington Pride' fruits were harvested from the northern, outer canopy sector of each tree (total of 90 fruit per treatment) and transported to the postharvest laboratory as in Experiment 1. On arrival at the laboratory, the fruit were dipped in hot Spin Flo® (carbendazim 50% a.i.; 1g L^{-1}) at 52°C for 5 min then ripened at 20°C. Quality attributes of the ripe fruit were assessed as in experiment 1.

Table 4.3 Experiment 3a. Nitrogen treatments applied to soil after harvest (22/02/01), at pre-flowering (04/07/01) and at panicle emergence (28/08/01) to 'Kensington Pride' mango trees in LG* and LG* orchards. N was applied as ammonium nitrate to the drip zone of each datum tree. K as potassium sulphate was applied at 330 g K per tree on the same days with N.

Treatment (Total N g/tree)	N after harvest (g/tree)	N at pre-flowering, (g/tree)	N at panicle emergence, (g/tree)
0	0	0	0
75	0	50	25
175	100	50	25
275	200	50	25
375	300	50	25
575	500	50	25
375b[1]	375	0	0
275 K[2]	200	50	25

[1] 375 g N per tree applied just after harvest only.
[2] 275 g N plus 3 x 330 g K per tree.

The total fruit weight and fruit number per datum tree was recorded for the HG* and LG* orchards, and the average fruit weight per tree calculated by dividing the total fruit weight by the fruit number per tree.

4.2.4 Experiment 3b: Pre-harvest Nitrogen Application and Fruit Ripening

This experiment was part of Experiment 3a in the 01/02 cropping season in the Burdekin district. Fruit for Experiment 3b were harvested from the LG* orchard on the same day as those for Experiment 3a, but only from the 0 N (control) and the 575 g N (high N fruit) treatments. Forty five fruits were randomly harvested from three trees for each treatment (control and high N). The fruit were harvested and treated similarly to those in Experiment 3a.

Fruit were dipped in hot Spin Flo® (carbendazim 50% a.i.; 1g L^{-1}) at 52°C for 5 min in the laboratory, and then ripened at 20°C. Nine control and nine high N fruits (three from each tree) were randomly selected for quality assessment (firmness, % green, yellow and blush colour remaining on fruit skin, and average hue angle of two fruit cheeks) on days 1, 5, 9, 12 and 18 after harvest. Total skin chlorophyll and carotenoids concentrations of the fruit skin were analysed by the skin removed from opposite sides of the fruit (Section 3.4). Chlorophyllase activity was analysed according to the method described in Section 3.4, using skin samples pooled from three fruit per replication.

4.2.5 Statistical Analyses

Data were analysed with the Genstat 5® (Release 4.2) general analysis of variance model using a completely randomised design. Six replications (trees) per treatment for Experiment 1, 2 and 3a and three replications (trees) per time for Experiment 3b were used. There were 10, 12 and 15 fruits per replication for Experiment 1, 2 and 3a, respectively, and three fruits for Experiment 3b. The protected least significant difference (LSD) procedure at $P < 0.05$ was used to test for differences between treatment means. Only significant differences at $P < 0.05$ are discussed, unless otherwise stated. Skin colour, anthracnose and stem-end rot ratings with percentage data were angular transformed (in degrees) prior to analysis. Mean values and LSDs of these parameters are graphed on the transformed scales, with the corresponding non-transformed percentage values shown on the *y*-axis. In the tables, the back-transformed means are shown in brackets. Regression analysis was performed on the average data for each tree.

4.3 RESULTS

4.3.1 Experiment 1: Survey of 'Kensington Pride' Mango Quality

Skin colour and pigments: The % of skin area with green colour (% green colour) of ripe 'Kensington Pride' mango fruit was significantly different between the orchards (Figure 4.1A). Ripe fruit from orchards 2 and 5 (both with high N fertiliser history) had higher % green colour than those fruit from orchards 1 (low N) and 10 (medium N) (Figure 4.1A). Seven of the ten orchards had more than 25% green colour remaining on ripe fruit. High green colour fruit were observed in orchards on both sandy loam (orchard 2) and heavy clay loam (orchard 5) soils.

The pattern of hue angle of the greenest part of the skin (hue angle of green skin) across the ten orchards was similar to that of % green colour. Hue angle of green skin was higher (more green) in the fruit from orchard 2 than those from orchards 1 and 10 (Figure 4.1B).

The total chlorophyll concentration of the greenest part of the skin of ripe fruit was higher in fruit from orchards 2, 4 and 5 than fruit from orchards 1 and 10 (Figure 4.2A). Fruit from orchard 2, which had the highest % green colour and highest hue angle of green skin, also had the highest chlorophyll concentration (9.2 $\mu g\ cm^{-2}$) and was more than twice that in the fruit from orchard 1 (4.1 $\mu g\ cm^{-2}$).

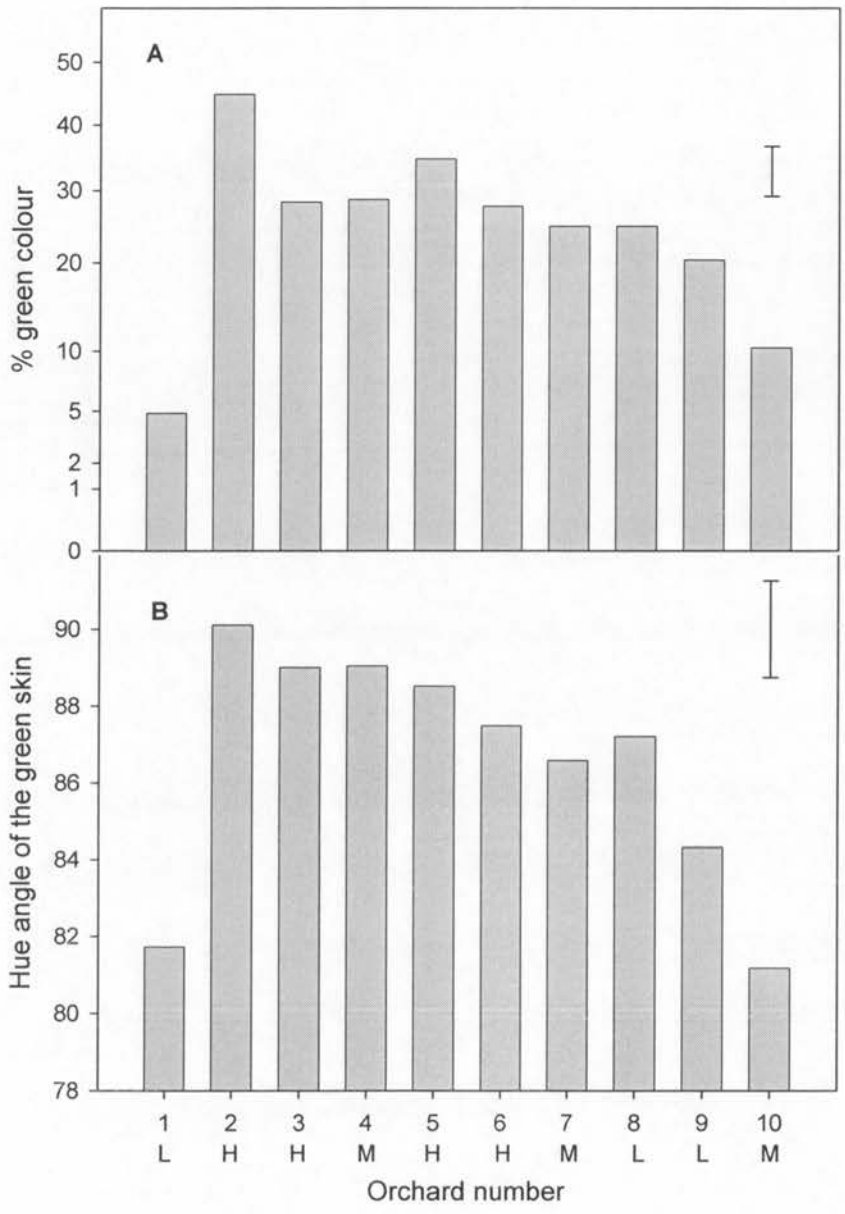


Figure 4.1 Experiment 1. The % green colour (A) and the hue angle of the green skin (B) of ripe 'Kensington Pride' mangoes obtained from different orchards in the Burdekin district when ripened at 20°C. The y-axis scale of % green colour is angular transformed. N status for each orchard is shown by the letters L (low), M (moderate) and H (high). Vertical bars represent LSDs ($P < 0.05$; $n = 60$).

Total carotenoids concentration was higher in fruit from orchards 5, 6 and 2 than from orchards 1 and 9 (Figure 4.2B). However, the differences in total carotenoids concentrations between orchards were not as great as for chlorophylls concentration.

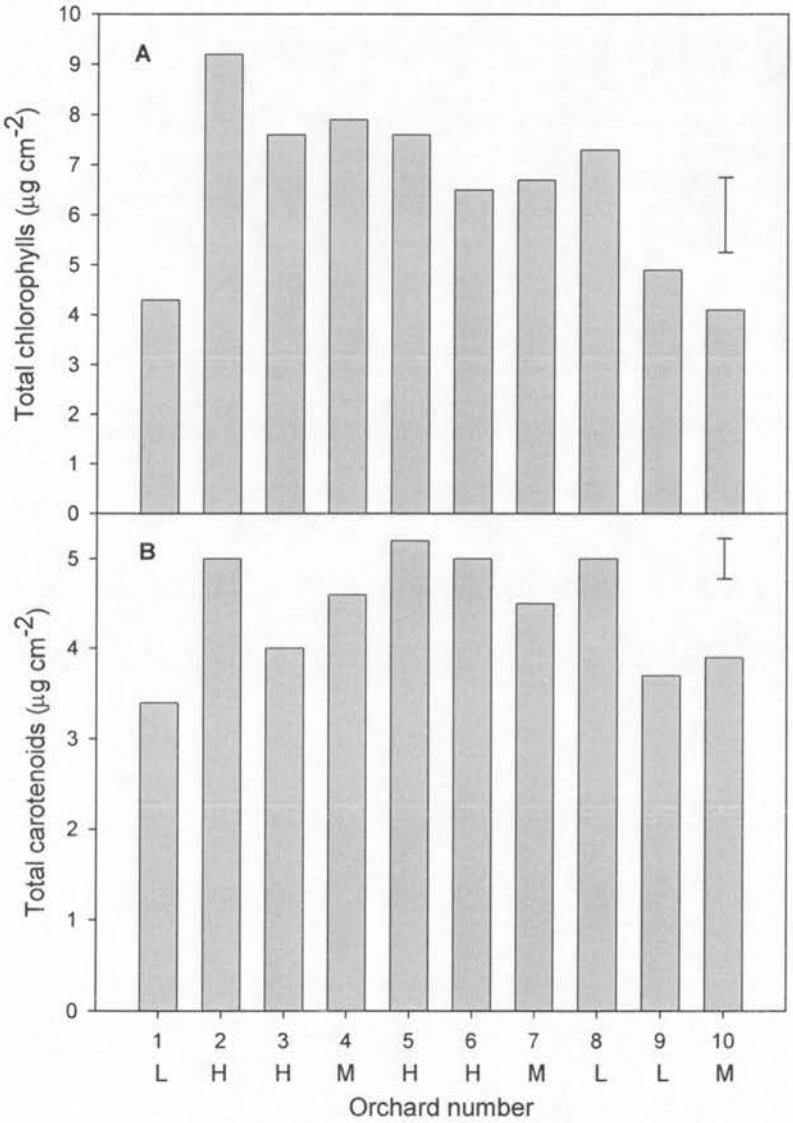


Figure 4.2 Experiment 1. Total chlorophylls (*a* and *b*; A) and total carotenoids (xanthophylls and ß-carotene; B) of the greenest part of the skin of ripe 'Kensington Pride' mangoes obtained from different orchards in the Burdekin district when ripened at 20°C. N status for each orchard is shown by the letters L (low), M (moderate) and H (high). Vertical bars represent LSDs ($P < 0.05$; $n = 60$).

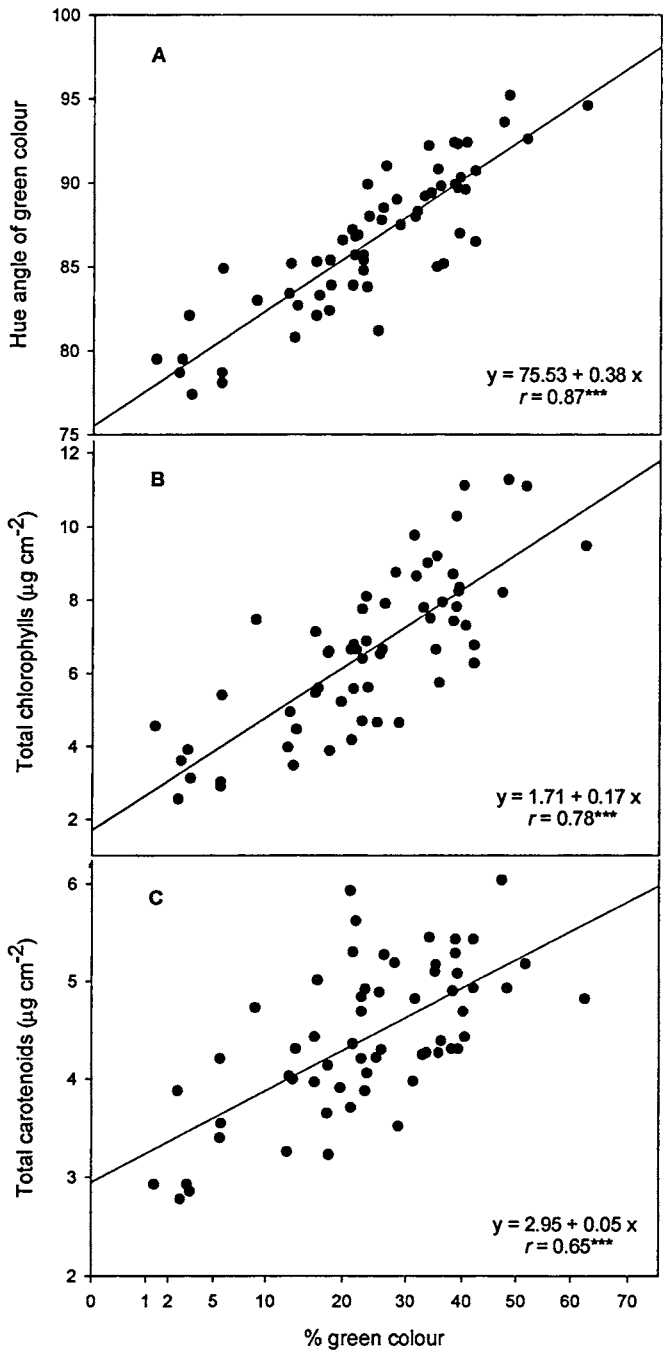


Figure 4.3 Experiment 1. Linear regression of % green colour of ripe 'Kensington Pride' mangoes and (A) hue angle of the greenest part of the skin for each tree, (B) total chlorophylls concentration (*a* and *b*) of the greenest part of the skin, and (C) total carotenoids concentration (xanthophylls and ß-carotene) of the greenest of the skin. The x-axis scale of % green colour is angular transformed. *** represents P = 0.001, n = 30.

Regression analyses demonstrated that there were significant positive relationships between % green colour, and hue angle (r = 0.87, P = 0.001) and skin chlorophylls concentration (r = 0.82, P = 0.001) (Figure 4.3 A & B), indicating that fruit with a higher % green colour also had more intense green colour on the greenest part of the skin. In addition, linear regression of % green colour and total carotenoids concentration was also significant (Figure 4.3C), but the regression coefficient was lower (r = 0.65, P = 0.001) than that between % green colour and total chlorophylls concentration.

Days to ripe: Fruit from orchards 6 and 7 ripened more quickly than those from the other orchards (Figure 4.4). There was no evidence of a significant relationship between % green colour and DTR (r = 0.14, n.s).

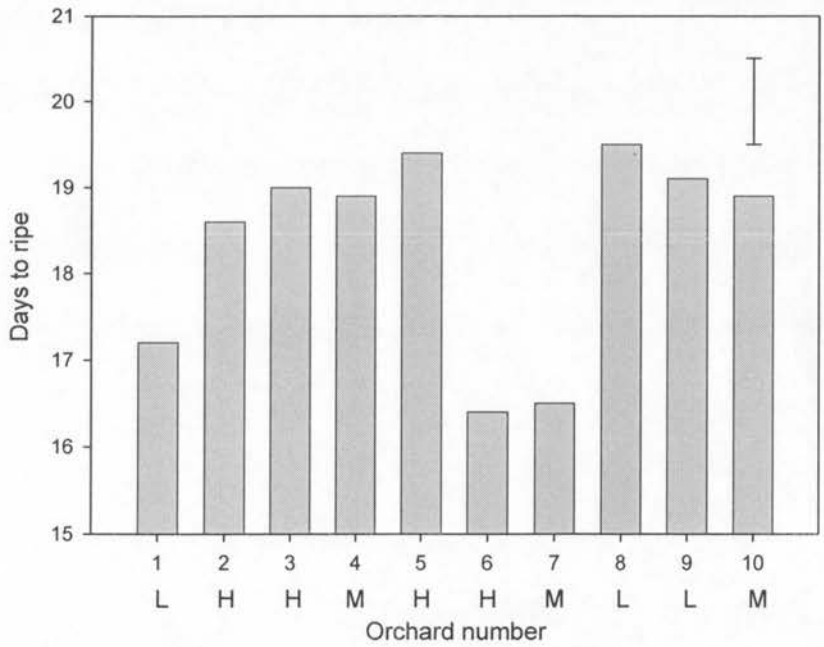


Figure 4.4 Experiment 1. Days from harvest to ripe (DTR) of 'Kensington Pride' mangoes obtained from different orchards in the Burdekin district when ripened at 20°C. N status for each orchard is shown by the letters L (low), M (moderate) and H (high). Vertical bar represents LSD (P < 0.05; n = 60).

Diseases: Generally, anthracnose severity was low in all the orchards (Figure 4.5A). Anthracnose severity was lower in fruit from orchards 1, 2 and 10 (all on sandy loam), compared to fruit from orchards 5, 8 and 9 (all on heavy clay loam soil). No anthracnose observed in fruit from orchards 1 and 10.

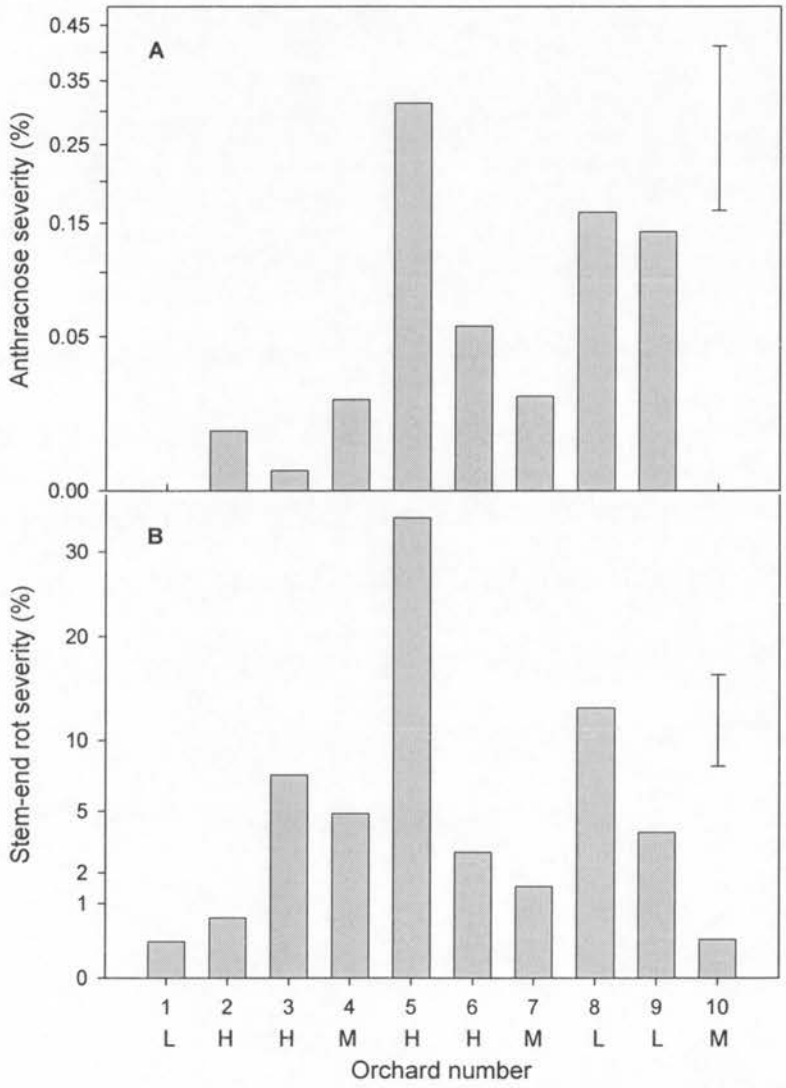


Figure 4.5 Experiment 1. Anthracnose (% skin area affected; A) and stem-end rot (% flesh volume affected; B) severity of ripe of 'Kensington Pride' mangoes obtained from different orchards in the Burdekin district when ripened at 20°C. The y-axis scales are angular transformed. N status for each orchard is shown by the letters L (low), M (moderate) and H (high). Vertical bars represent LSDs ($P < 0.05$; $n = 60$).

The severity of SER was greater than anthracnose. Fruit from orchards 1, 2 and 10 (on sandy soils) also had lower SER severity than fruit from most of the orchards on heavy clay loam, e.g. orchards 5 and 8 (Figure 4.5B). Fruit from orchard 5 had more than 30% of flesh volume affected by SER.

There was a significant relationship between SER severity and % green colour (r = 0.41, P = 0.01), and SER severity and DTR (r = 0.41, P = 0.01). However, there were no significant relations between anthracnose severity and % green colour (r = 0.24, n.s), or anthracnose severity and DTR (r = 0.17, n.s).

Other quality attributes: Fruit from orchards 3, 4 and 5 had a higher flesh hue angle (less yellow) than those from orchards 10 and 2 (Table 4.4). Fruit from orchards 1 and 10 (which had less % green colour) had less % red blush colour compared with those from other orchards (Table 4.4). Orchard 2 produced fruit which had the lowest lenticel spotting severity compared to the other orchards (Table 4.4). The flesh TSS was higher in fruit from orchards 1 and 6 compared to that in orchards 3 and 9, while flesh acidity was higher in orchards 1 and 7 compared to that in orchards 2, 4, 5 and 8 (Table 4.4).

Table 4.4 Experiment 1. The flesh hue angle (H°), % red blush colour (angular transformed), lenticel spotting (0 = no spotting to 4 = severe spotting) and the flesh TSS (°Brix) and acidity of ripe 'Kensington Pride' mangoes from different orchards in Burdekin district when ripened at 20°C.

Orchard	Flesh H°	Red blush (%)		Lenticel spotting	TSS (°Brix)	Acidity (%)
1	75.4^{bcd}	8.9^{a}	(2.4)	0.80^{b}	13.4^{e}	0.14^{c}
2	74.7^{ab}	33.3^{ef}	(30.2)	0.29^{a}	11.9^{bcd}	0.11^{a}
3	76.5^{ef}	29.8^{dc}	(24.7)	1.18^{c}	10.9^{a}	0.12^{ab}
4	76.5^{ef}	25.0^{c}	(17.8)	1.21^{c}	12.7^{de}	0.11^{a}
5	76.7^{f}	19.6^{b}	(11.2)	1.56^{de}	11.5^{abc}	0.11^{a}
6	75.2^{bc}	29.5^{de}	(24.0)	1.69^{e}	13.2^{e}	0.13^{bc}
7	75.0^{ab}	26.0^{cd}	(19.3)	1.75^{e}	11.9^{bcd}	0.14^{c}
8	75.9^{de}	35.6^{f}	(33.9)	1.62^{de}	12.2^{cd}	0.11^{a}
9	75.8^{cd}	32.3^{ef}	(28.6)	1.34^{cd}	11.3^{ab}	0.13^{bc}
10	74.5^{a}	17.8^{b}	(9.3)	1.82^{e}	12.6^{de}	0.13^{bc}
LSD	0.7	4.2		0.31	0.8	0.01

Red blush data are angular transformed, with back transformed means presented in brackets. Means followed by the same letter in each column are not significantly different at $P < 0.05$ ($n = 60$).

4.3.2 Experiment 2: Pre-flowering Nitrogen Application

Skin colour and pigments: In the HG orchard, fruit from trees treated with 150 g N per tree and above, or with foliar sprays, had more % green colour than those from trees treated with 0 or 75 g (Figure 4.6A). There was no significant difference in % green colour between the 150 and 300 g treatments. The foliar treatment resulted in the highest % green colour of all treatments.

In both LG orchards, the 0 g N treatment resulted in less than 1.5% of the ripe fruit skin with green colour (Figure 4.6A). N applications increased the % green colour, with more green

colour on the 150 g, 300g, 450 g, and foliar applications, compared to 0 N. In contrast to the HG orchard, fruit from the foliar N treatment had less % green colour than fruit from the 300 or 450 g soil N treatments.

In the HG orchard, the effect of N treatment on hue angle of the greenest part of the skin was very similar to that of % green colour (Figure 4.6B). However, in the LG orchards, there was no significant difference in hue angle between the 0 or 150 g N treatments. In addition, the foliar treatment in LG1 did not result in a lower hue angle than the 450 g treatment, as occurred in the % green colour for the same orchard.

Total chlorophylls and carotenoids concentrations of the greenest part of the skin of ripe fruit were determined only for the HG orchard (Figure 4.7). The total chlorophylls concentration was higher for treatments with N applied to soil □ 150 g, and with foliar sprays, compared with 0 N (Figure 4.7A). Regression analysis indicated there was a significant positive relationship between skin chlorophylls concentration and the amount of N applied to the soil (r = 0.72, P = 0.01). In contrast to chlorophylls concentration, carotenoids concentration was not affected by nitrogen application (Figure 4.7B).

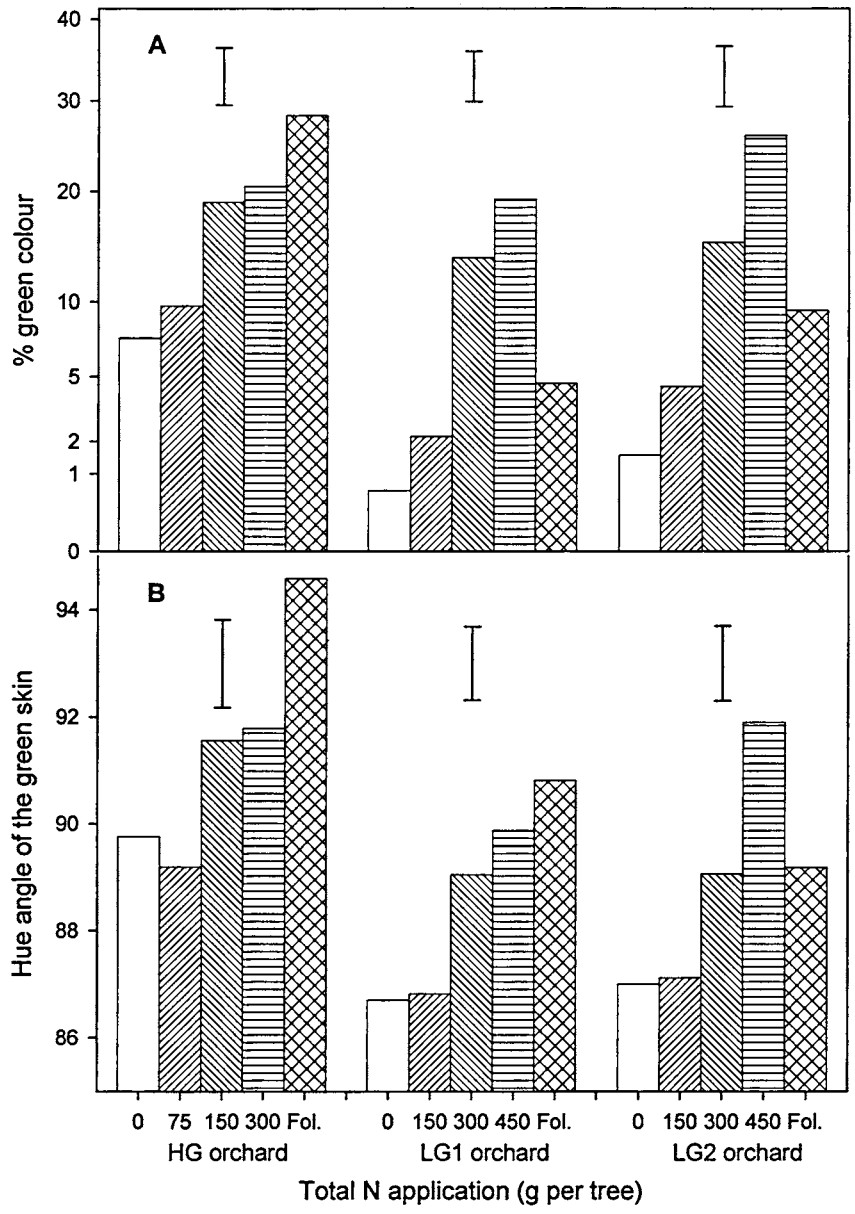


Figure 4.6 Experiment 2. Effect of N application to soil or as foliar sprays (total of 50 g per tree; abbreviated "Fol.") on the % green colour (A) and the hue angle of the greenest part of the skin (B) of ripe 'Kensington Pride' mangoes when ripened at 20°C without ethylene. The y-axis scale of % green colour is angular transformed. HG = orchard with high green colour and LG1, LG2 = orchards with low green colour. Vertical bars represent LSDs ($P < 0.05$, $n = 72$).

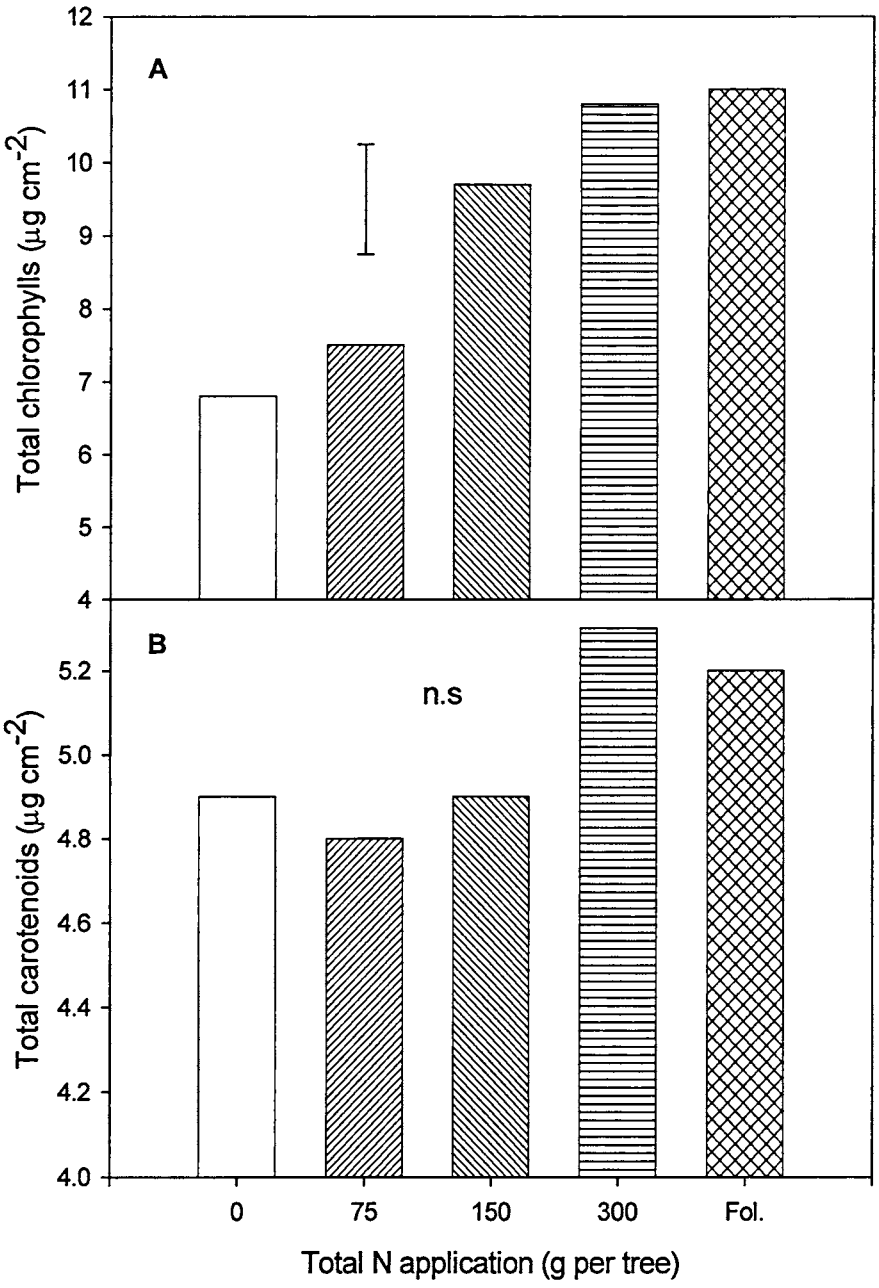


Figure 4.7 Experiment 2. Effect of N application to soil or as foliar sprays (total of 50 g per tree; abbreviated "Fol.") on (A) total chlorophylls (*a* and *b*) and (B) total carotenoids (ß-carotene and xanthophylls) concentration of the greenest part of the skin of ripe 'Kensington Pride' mangoes from the HG orchard when ripened at 20°C without ethylene. Vertical bar presents LSD ($P < 0.05$, $n = 72$) and n.s = not significant.

Plates 4.1 and 4.2 illustrates the effects of N application on skin colour of 'Kensington Pride' mangoes after 13 days at 20°C (each tray was typical for one treatment in one orchard; more yellow side of each fruit was showed). Control fruit (0 g per tree) and fruit from the trees treated with 75 g N per tree in the HG orchard appeared to have less green colour than fruit from the 150 and 300 g N treatments or foliar sprays (Plate 4.1). This is similar to the treatment effects on the % green colour on the ripe fruit (Figure 4.6A).

Similarities between skin colour at 13 days (based on Plate 4.2) and at ripe (Figure 4.6A) were also noted in LG1 fruit, although there was less green colour on the LG1 skin at this time, than on HG fruit. On day 13, there was little green colour remaining on the control fruit from LG1, while fruit from the 300 and 450 g treatments had visibly more green colour.

Days to ripe: The effect of N application on DTR was not consistent in the three orchards (Figure 4.8). There was no treatment effect on DTR in the HG orchard. In the LG1 orchard, the DTR was significantly higher in the 300 g N treatment compared with 0 g N, but not with 450 g N. In contrast, 450 g and foliar treatments in the LG2 orchard had shorter DTR than the 0 g N treatment.

Control fruit (0 g N per tree)

75 g N per tree

300 g N per tree

150 g N per tree

Foliar sprays (50 g N per tree)

Plate 4.1 Experiment 2. Effect of N application (mainly at pre-flowering) on skin colour of 'Kensington Pride' mangoes from HG orchard after 13 days ripened at 20°C without ethylene treatment.

Control fruit (0 g N per tree)

150 g N per tree

450 g N per tree

300 g N per tree

Foliar sprays (50 g N per tree)

Plate 4.2 Experiment 2. Effect of N application (mainly at pre-flowering) on skin colour of ' Kensington Pride' mangoes from LG1 orchard after 13 days ripened at 20°C without ethylene treatment.

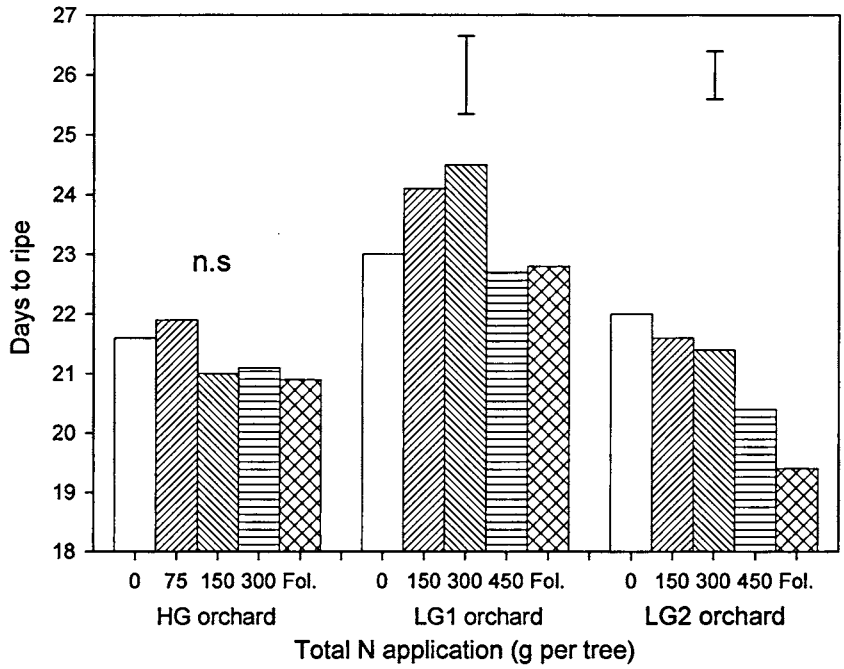


Figure 4.8 Experiment 2. Effect of N application to soil or as foliar sprays (total of 50 g per tree; abbreviated "Fol.") on the days from harvest to ripe (DTR) of 'Kensington Pride' mangoes when ripened at 20°C without ethylene. HG = orchard with high green colour and LG1, LG2 = orchards with low green colour. Vertical bars represent LSDs ($P < 0.05$, $n = 72$) and n.s = not significant.

Diseases: Anthracnose severity was higher in the 300 g and foliar treatments, compared with 0 g N in the HG orchard, but there were no treatment effects on anthracnose severity in the two LG orchards (Figure 4.9). SER was not affected by N treatments in all three orchards (data not presented) with means of 8.2, 3.4 and 3.1% of the flesh affected for HG, LG1 and LG2, respectively.

Regression analyses indicated that there was a significant positive relationship between % green colour and anthracnose severity ($r = 0.42$, $P = 0.05$) in the HG orchard only. In

addition, anthracnose and SER severity were also significantly positively related with DTR (r = 0.67, P = 0.001 and r = 0.44, P = 0.05, respectively), but only in the LG1 orchard.

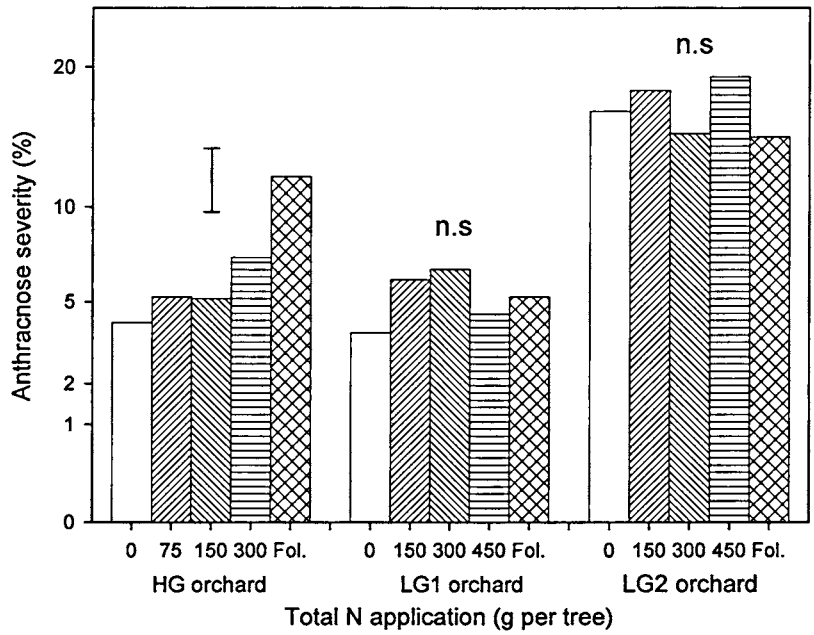


Figure 4.9 Experiment 2. Effect of N application to soil or as foliar sprays (total of 50 g per tree; abbreviated "Fol.") on anthracnose severity (% skin area affected) of ripe 'Kensington Pride' mangoes when ripened at 20°C without ethylene. The y-axis scale is angular transformed. HG = orchard with high green colour and LG1, LG2 = orchards with low green colour. Vertical bar presents LSD (P < 0.05, n = 72) and n.s = not significant.

Other quality attributes: In both LG orchards, fruit from the 450 g N treatments had lower hue angle of the flesh (less green) compared to control treatments (Table 4.5). In the HG orchard, the lowest hue angle was noted in the 150 g treatment.

Table 4.5 Experiment 2. Effect of N application to soil or as foliar sprays (total of 50 g per tree) on the flesh hue angle (H°), % red blush colour (angular transformed), lenticel spotting (0 = no spotting to 4 = severe spotting) and the flesh TSS (°Brix) and acidity of ripe 'Kensington Pride' mangoes when ripened at 20°C without ethylene. HG = orchard with high green colour and LG1, LG2 = orchards with low green colour.

Treatment	Flesh H°	Red blush (%)	Lenticel spotting	TSS (°Brix)	Acidity (%)
HG orchard					
0	85.4 [b]	29.0 (26.3)	1.64	10.7	0.13
75	85.4 [b]	32.8 (31.0)	1.44	10.5	0.13
150	84.8 [a]	28.2 (25.2)	1.68	10.6	0.14
300	85.3 [b]	28.9 (26.6)	1.72	10.5	0.13
Foliar	86.0 [b]	27.8 (26.3)	1.56	10.2	0.13
LSD	0.7	ns	ns	ns	ns
LG1 orchard					
0	85.0 [b]	20.6 (16.3)	1.79 [a]	10.8	0.16
150	85.5 [b]	17.5 (14.2)	1.69 [a]	10.1	0.15
300	85.8 [b]	18.2 (14.2)	1.82 [ab]	9.5	0.16
450	84.7 [a]	18.8 (16.2)	2.04 [b]	10.0	0.18
Foliar	85.6 [b]	22.0 (18.5)	1.70 [a]	10.3	0.16
LSD	0.7	n.s	0.24	n.s	n.s
LG2 orchard					
0	89.3 [c]	11.8 (9.4)	1.60 [a]	9.6 [a]	0.14
150	88.3 [b]	10.6 (8.3)	1.58 [a]	9.8 [a]	0.15
300	88.2 [b]	12.1 (9.9)	1.66 [a]	9.5 [a]	0.15
450	87.8 [ab]	6.7 (4.6)	1.82 [a]	9.8 [a]	0.15
Foliar	87.1 [a]	11.3 (9.3)	2.33 [b]	11.3 [b]	0.14
LSD	0.85	n.s	0.44	1.3	n.s

Red blush data are angular transformed, with back transformed means presented in brackets. Means followed by the same letter in each column for each parameter are not significantly different at $P < 0.05$ ($n = 72$). n.s = not significant.

Lenticel spotting severity was higher with 450 g N compared with 0 N in the LG1 orchard (Table 4.5). Lenticel spotting was higher in the foliar treatment compared with all other treatments in LG2. The % red blush colour and the flesh acidity were not affected by N application (Table 4.5). Treatment with foliar sprays in the LG2 orchard resulted in more TSS in fruit compared to 0 g N treatment (Table 4.5).

Leaf nitrogen: In the HG orchard there was no effect of N application on the leaf N either at panicle emergence or at harvest (Table 4.6). In contrast, leaf N concentration at both panicle emergence and at harvest in the LG orchards was higher in the 450g N treatment compared with untreated trees. The leaf N concentration was higher in the foliar treatment than 0 g N only at panicle emergence from LG2. In all other cases the leaf N concentration was similar to that in the other N treatments.

The leaf N concentration at panicle emergence, and SER severity were significantly positively related with SER severity in all three orchards (r = 0.45, 0.40 and 0.42, P = 0.05 for HG, LG1 and LG2, respectively), but there was no significant relationship between leaf N concentration at panicle emergence and anthracnose severity. In addition, leaf N concentration at panicle emergence was positive significantly related with % green colour on ripe fruit only in the LG1 orchard (r = 0.44, P = 0.05).

Table 4.6 Experiment 2. Effect of N application to soil or as foliar sprays (total of 50 g per tree) on leaf N of 'Kensington Pride' mango trees at panicle emergence (28/08/00) and at harvest (24/11/00 for the HG and LG1 orchard and 30/11/00 for the LG2 orchard). HG = orchard with high green colour and LG1, LG2 = orchards with low green colour.

Treatment (g N/tree)	Leaf nitrogen, %	
	Panicle emergence	Harvest
HG orchard		
0	1.19	1.12
75	1.19	1.16
150	1.19	1.12
300	1.22	1.12
Fol.	1.22	1.12
LSD	n.s	n.s
LG1 orchard		
0	0.87 [a]	0.92 [a]
150	0.93 [ab]	1.04 [ab]
300	0.96 [ab]	0.98 [ab]
450	0.97 [b]	1.05 [b]
Fol.	0.96 [ab]	0.94 [ab]
LSD	0.10	0.12
LG2 orchard		
0	1.04 [a]	0.96 [a]
150	1.11 [b]	1.06 [ab]
300	1.14 [b]	1.10 [ab]
450	1.12 [b]	1.14 [b]
Fol.	1.12 [b]	1.05 [ab]
LSD	0.07	0.16

Means followed by the same letter in each column for each orchard are not significantly different at $P < 0.05$ ($n = 6$), n.s = not significant.

Fruit yield: Yield was not affected by treatment in the HG orchard (Figure 4.10). In the LG2 orchard, the foliar treatment had a lower fruit yield compared to the 300 and 450 g N per tree treatment. However, there was no significant difference between the control and the soil treatments. The average fruit weight was also not affected by treatment in either orchard (data not presented), with averages of 352 g for HG and 366 g for LG2.

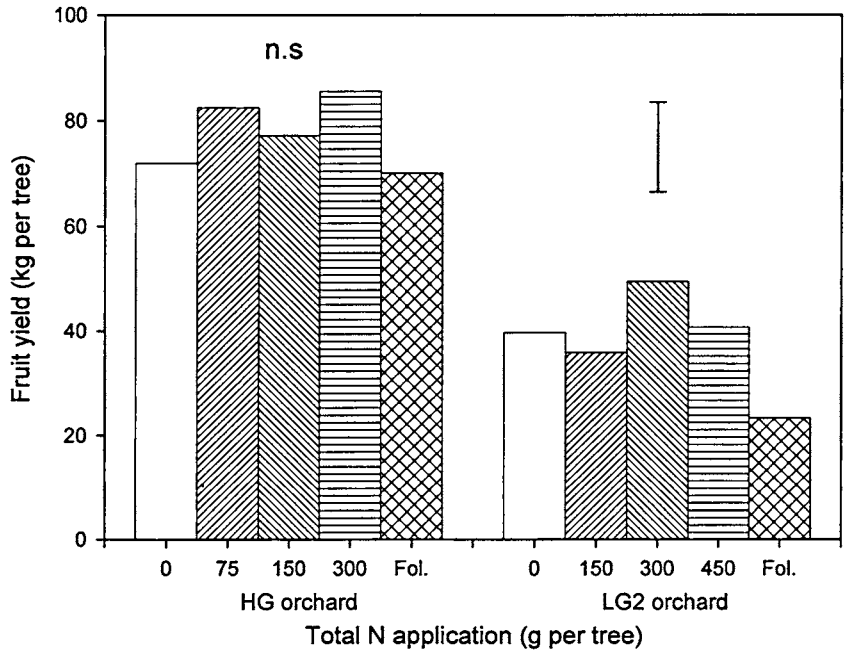


Figure 4.10 Experiment 2. Effect of N application to soil or as foliar sprays (total of 50 g per tree; abbreviated "Fol.") on fruit yield 'Kensington Pride' trees. HG = orchard with high green colour (11 year old trees) and LG2 = orchard with low green colour (21 year old trees). Vertical bar presents LSD ($P < 0.05$, $n = 6$), n.s = not significant.

4.3.3 Experiment 3a: Nitrogen Application after Harvest

Skin green colour. The % green colour of the ripe fruit was significantly increased when N applied increased from 0 g to 275 g for both orchards (Figure 4.11A). There was no significant effect of N on % green colour at rates lower than 275 g. The application of K with N (275 g N plus K) increased the green colour compared to 275 g N alone in the HG* orchard, but not in the LG* orchard. Applying all the N after harvest (375b) compared to split applications after harvest and around flowering, resulted in less green colour on the HG*fruit, but not on the LG* fruit.

The pattern of the treatment effects on hue angle of green skin in the HG* orchard was similar to that of % green colour (Figure 4.11B). However, the response of hue angle to N in HG* was greater than to % green colour, since significant increases in hue angle were noted between 0 and 75 g.

The effect of N application on the skin colour of the mangoes from the HG* orchard after 10 days ripened at 20°C is shown in Plate 4.3. By day 10, more than half the control fruit (0 g N per tree) had lost most of the green colour, with increasing green colour in the fruit with higher N applications. This reflects the % green colour on the skin when ripe (Figure 4.11A). The effect of N application on the skin colour of fruit from the LG* orchard 9 days after harvest was similar to that in the HG* fruit (Plate 4.4). Treatment of 275 g N per tree also resulted in greener fruit than the untreated fruit. Fruit treated with the highest N rate (575 g N per tree) still retained most of the green colour.

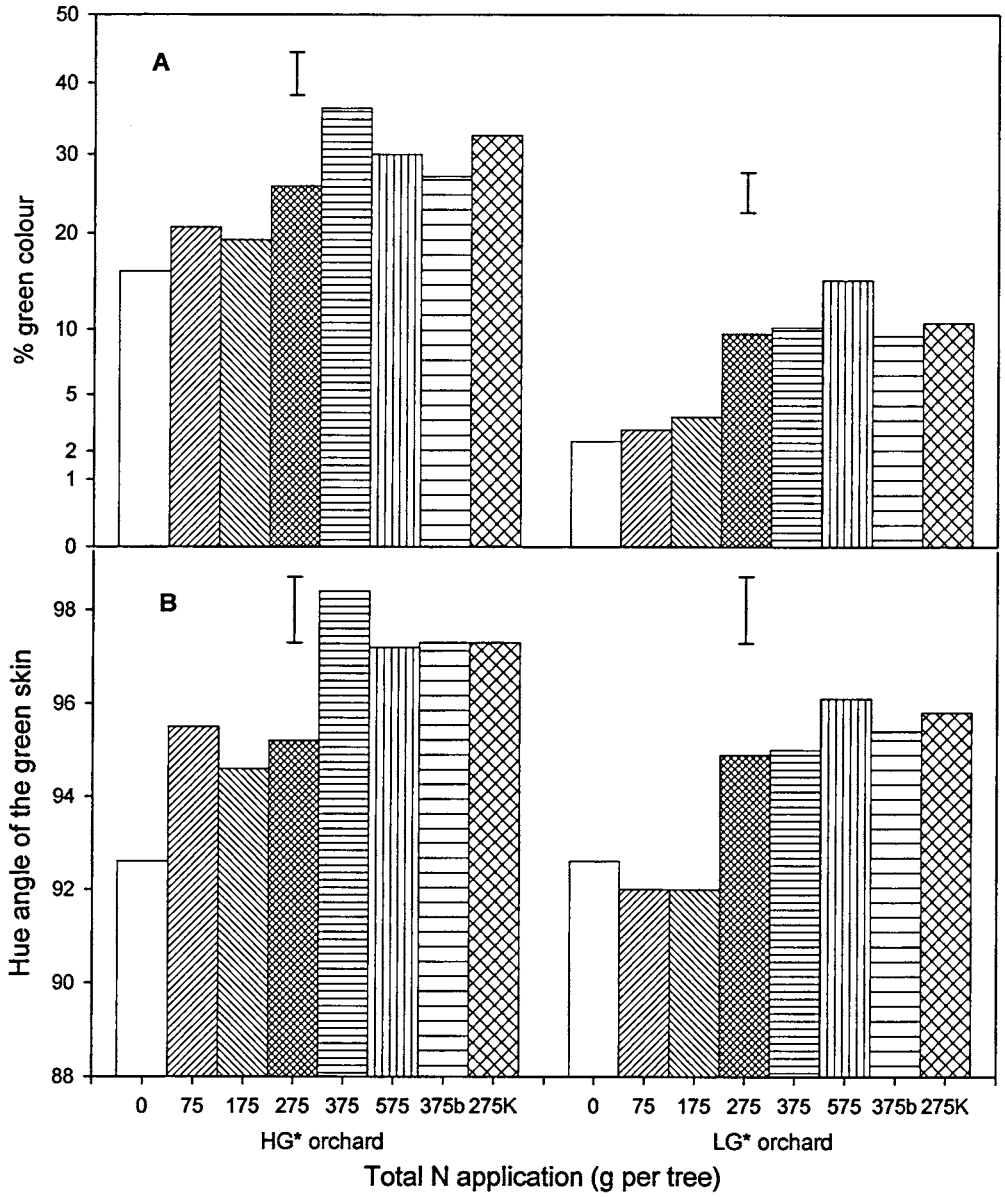


Figure 4.11 Experiment 3a. Effect of N application (mainly after harvest) on the % green colour (A) and the hue angle of the greenest part of the skin (B) of ripe 'Kensington Pride' mangoes when ripened at 20°C without ethylene. The y-axis scale of % green colour is angular transformed. Treatment 375b had all 375 g N applied after harvest (22/02/01). Treatment 275K had 275 g N plus 990 g K. HG* = orchard with high green colour and LG* = orchard with low green colour. Vertical bars represent LSDs ($P < 0.05$, $n = 90$).

Control fruit (0 g N per tree)

275 g N per tree

575 g N per tree

Plate 4.3 Experiment 3a. Effect of N application (mainly at after harvest) on skin colour of 'Kensington Pride' mangoes from HG* orchard after 10 days ripened at 20°C without ethylene treatment.

Control fruit (0 g N per tree)

275 g N per tree

575 g N per tree

Plate 4.4 Experiment 3a. Effect of N application (mainly at after harvest) on skin colour of 'Kensington Pride' mangoes from LG* orchard after 9 days ripened at 20°C without ethylene treatment.

Days to ripe: In the LG* orchard, there was a general decrease in DTR with higher N application, resulting in the 375 and 575 g N fruit ripening more quickly than untreated fruit (Figure 4.12). A similar trend was not observed in the HG* orchard. In the HG* orchard, the combination of N with K reduced the DTR compared to 275 g N alone, but this was not observed in the LG* orchard.

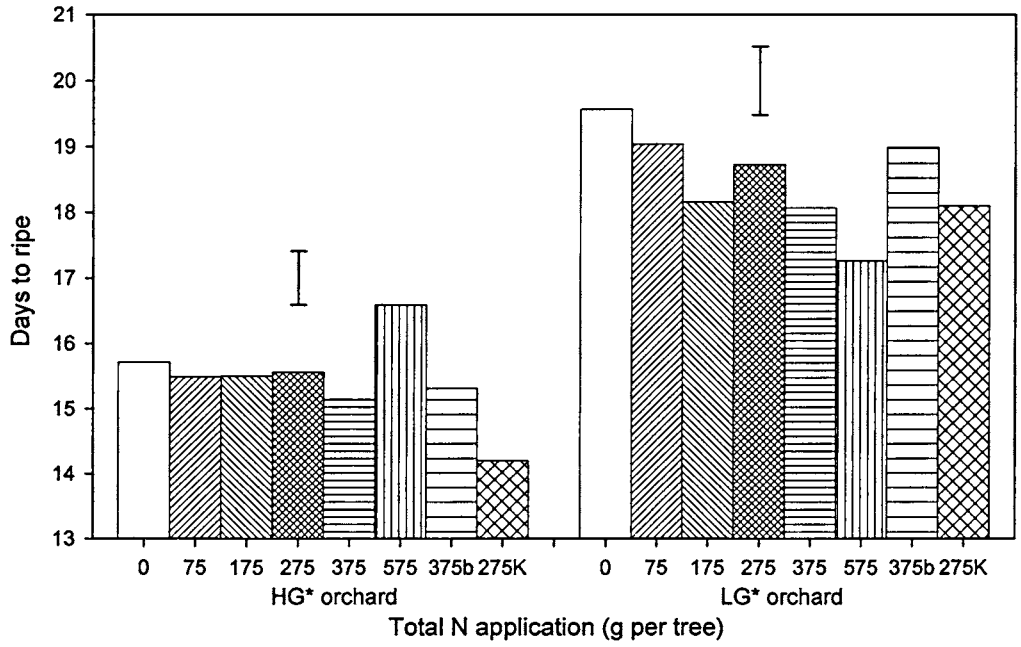


Figure 4.12 Experiment 3a. Effect of N application (mainly after harvest) on the days from harvest to ripe (DTR) of 'Kensington Pride' mangoes when ripened at 20°C without ethylene. Treatment 375b had all 375 g N applied after harvest (22/02/01). Treatment 275K had 275 g N plus 990 g K. HG* = orchard with high green colour and LG* = orchard with low green colour. Vertical bar represents LSD ($P < 0.05$, $n = 90$).

Diseases: Generally, the severity of anthracnose was low (less than 1%) in both orchards (Figure 4.13A). In the HG* orchard, N application increased the anthracnose severity compared to 0 g N. There was no significant difference between all N treatments, except that the addition of K with 275 g N reduced anthracnose severity compared with 275 g N alone.

There were no treatment effects in the LG* orchard. Generally, SER severity was also low (less than 1.5% of the flesh affected) in both orchards. There were no treatment effects on SER severity in both orchards (Figure 4.13B).

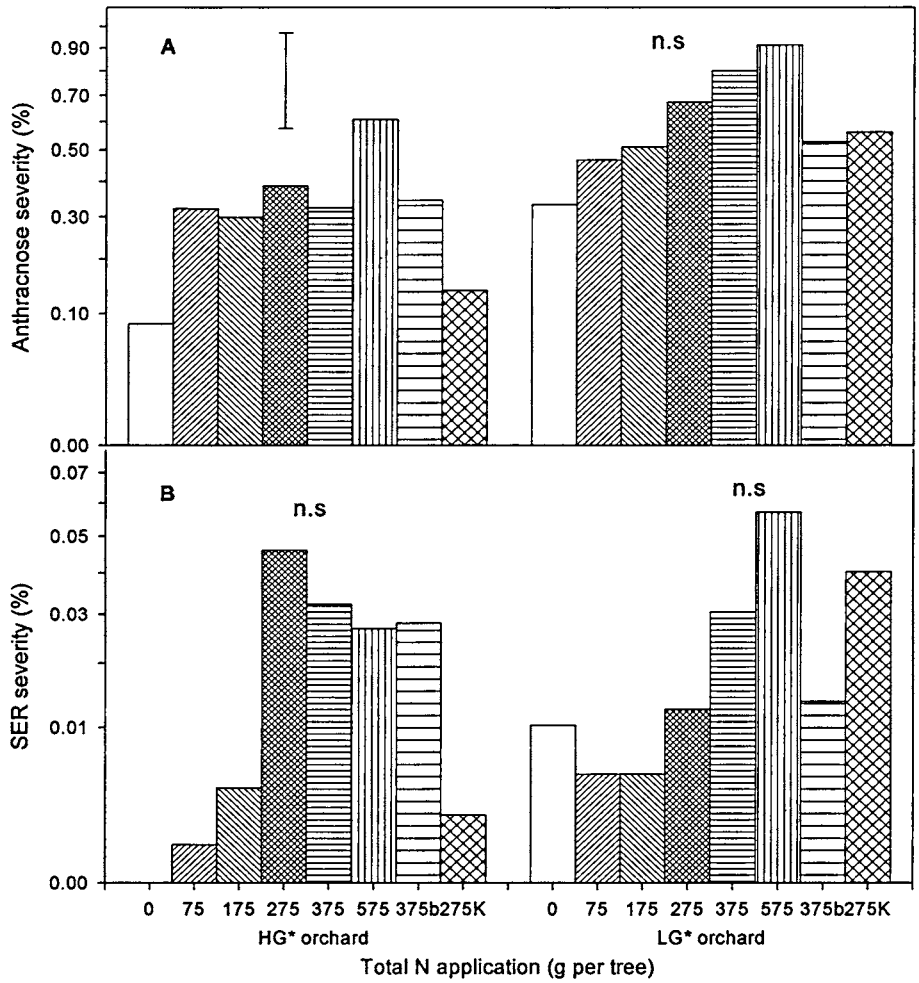


Figure 4.13 Experiment 3a. Effect of N application (mainly after harvest) on the severity of anthracnose (% skin area affected; A) and stem-end rots (% flesh volume affected; B) of ripe 'Kensington Pride' mangoes when ripened at 20°C without ethylene. The y-axis scales are angular transformed. Treatment 375b had all 375 g N applied after harvest (22/02/01). Treatment 275K had 275 g N plus 990 g K. HG* = orchard with high green colour and LG* = orchard with low green colour. Vertical bar presents LSD ($P < 0.05$, $n = 90$) and n.s = not significant.

Table 4.7 Experiment 3a. Effect of N application (mainly after harvest) on the flesh hue angle (H°), % red blush colour, lenticel spotting (0 = no spotting to 4 = severe spotting) and the flesh TSS (°Brix) and acidity of ripe 'Kensington Pride' mangoes when ripened at 20°C without ethylene. Treatment 375b had all 375 g N applied after harvest (22/02/01). Treatment 275K had 275 g N plus 990 g K. HG* = orchard with high green colour and LG* = orchard with low green colour.

Treatment	Flesh H°	Red blush (%)		Lenticel spotting	TSS (°Brix)	Acidity (%)
HG orchard*						
0	84.0 ^{ab}	30.0 ^{c}	(26.8)	1.82 ^{a}	14.2	0.12
75	84.3 ^{bc}	27.5 ^{abc}	(23.7)	1.83 ^{a}	13.9	0.13
175	83.9 ^{ab}	25.6 ^{ab}	(22.0)	2.06 ^{b}	14.5	0.12
275	84.6 ^{c}	27.0 ^{abc}	(23.4)	2.04 ^{b}	14.1	0.13
375	84.4 ^{bc}	26.3 ^{ab}	(22.7)	1.76 ^{a}	13.9	0.13
575	85.3 ^{d}	25.2 ^{a}	(21.6)	1.93 ^{ab}	13.4	0.13
375b	84.8 ^{cd}	25.0 ^{a}	(20.7)	1.87 ^{ab}	13.7	0.12
275K	83.6 ^{a}	28.9 ^{bc}	(26.8)	1.81 ^{a}	14.2	0.13
LSD	0.62	3.4		0.20	n.s	n.s
LG orchard*						
0	86.9 ^{bc}	24.1	(19.5)	2.42 ^{ab}	12.4	0.18
75	87.6 ^{c}	24.4	(19.6)	2.40 ^{a}	12.2	0.18
175	86.8 ^{abc}	24.7	(19.7)	2.42 ^{ab}	12.4	0.18
275	87.3 ^{c}	22.4	(17.9)	2.46 ^{ab}	11.8	0.17
375	86.2 ^{ab}	25.4	(21.5)	2.66 ^{abc}	13.4	0.18
575	86.0 ^{a}	24.5	(20.3)	2.81 ^{c}	12.8	0.18
375b	87.6 ^{c}	26.4	(22.0)	2.47 ^{ab}	12.1	0.17
275K	86.8 ^{abc}	26.4	(21.2)	2.68 ^{bc}	12.8	0.18
LSD	0.82	n.s		0.27	n.s	n.s

Red blush data are angular transformed, with back transformed means presented in brackets. Means followed by the same letter in each column for each orchard are not significantly different at $P < 0.05$ ($n = 90$).

Other quality attributes: In the HG* orchard, fruit from the trees treated with 575 g N had higher flesh hue angle than those from 0 N trees, while the addition of K with 275 g N

resulted in lower hue angle than 275 g N alone (Table 4.7). There was no clear effect of N treatments on the flesh hue angle in fruit from the LG* orchard. Lenticel spotting was more severe in fruit from the LG* orchard than the HG* orchard (Table 4.7). The 575 g N treatment in the LG* orchard resulted in higher severity of lenticel spotting than in the 0 N treatment, but there was no consistent N effect in the HG* orchard. There were very few treatment effects on the % red blush colour, or on the flesh TSS and acidity (Table 4.7).

Fruit Yield: Neither N or K treatments affected the fruit tree yield compared with 0 g N in both orchards (Figure 4.14). Average fruit weight was also not significantly affected by the treatments (data not presented), with means of 337 and 385 g per fruit for the HG* and LG* orchard, respectively.

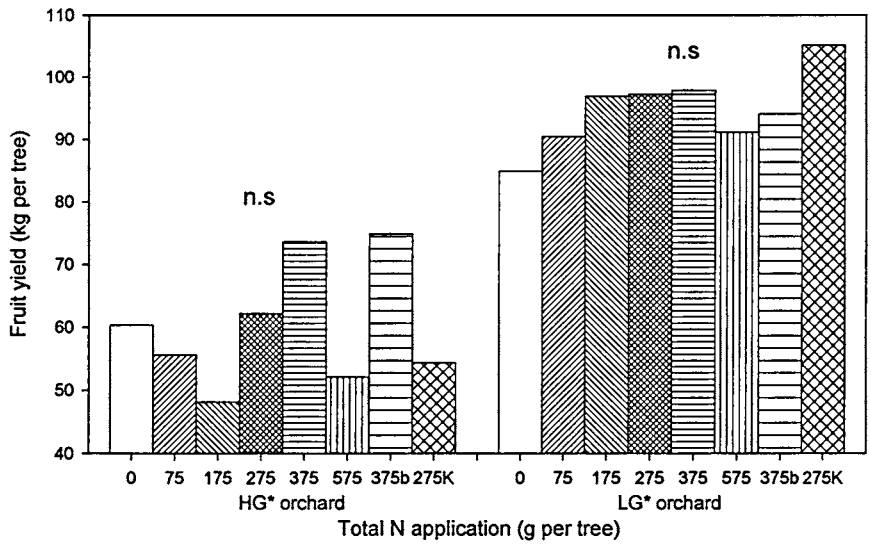


Figure 4.14 Experiment 3a. Effect of N application (mainly after harvest) to the soil on fruit yield (kg per tree) of 'Kensington Pride' mango trees. Treatment 375b had all 375 g N applied after harvest (22/02/01). Treatment 275K had 275 g N plus 990 g K. HG* = orchard with high green colour (12 year old trees) and LG* = orchard with low green colour (16 year old trees). n.s = not significant at $P < 0.05$.

4.3.4 Experiment 3b: Pre-harvest Nitrogen Application and Fruit Ripening

Fruit firmness: The firmness of both control (0 g N per tree) and high N (575 g per tree) fruit increased during ripening (Figure 4.15). However there was no significant difference in firmness between the control and high N treatments at any of the days assessed.

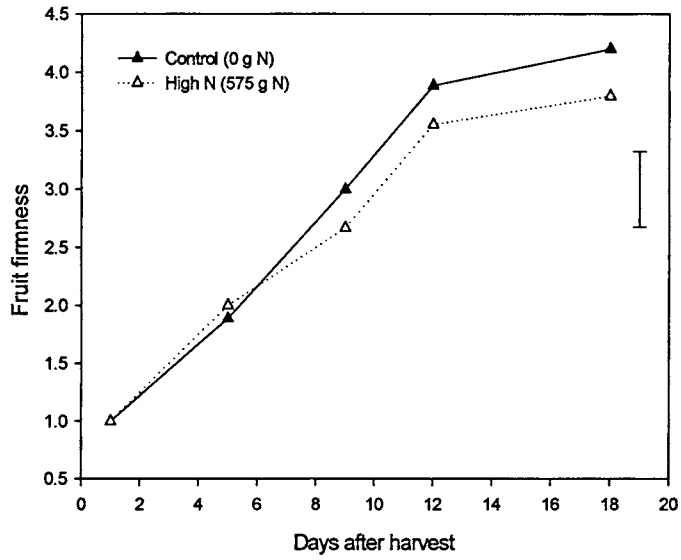


Figure 4.15 Experiment 3b. Effect of N application on firmness (rating scale of 1-6 of 1 = firm, 5 = ripe, 6 = over ripe) of 'Kensington Pride' mangoes during ripening at 20°C without ethylene. Control = fruit from trees treated with 0 g N per tree and high N = fruit from trees treated with 575 g N per tree. Vertical bar represents LSD ($P < 0.05$, $n = 9$).

Skin colour: As fruit ripened, the % green colour declined and the % yellow colour increased in both control and high N fruit (Figure 4.16A and B). After 5 days the high N fruit showed less decrease in green colour than the control fruit, resulting in more green colour after 9 days. At day 18, only 6 % of green colour remained on control fruit, but while it was 38% of the skin had green colour in the high N fruit.

The % red blush colour increased strongly from day 9 to day 12 for both treatments and then changed little thereafter (Figure 4.16C). There was no significant difference in % red blush colour between N treatments during ripening.

The average hue angle (H^{o}) of the skin of control and high N fruit declined (less green), while the C (chroma or saturation) and the L value (brightness) increased during ripening (Figure 4.17). From day 1 to day 9 after harvest there were no significant differences in H^{o} and L between control and high N fruit, while after day nine the H^{o} was lower in the control than the high N fruit. The C in the control fruit was higher than that of high N fruit from day 1 to day 18.

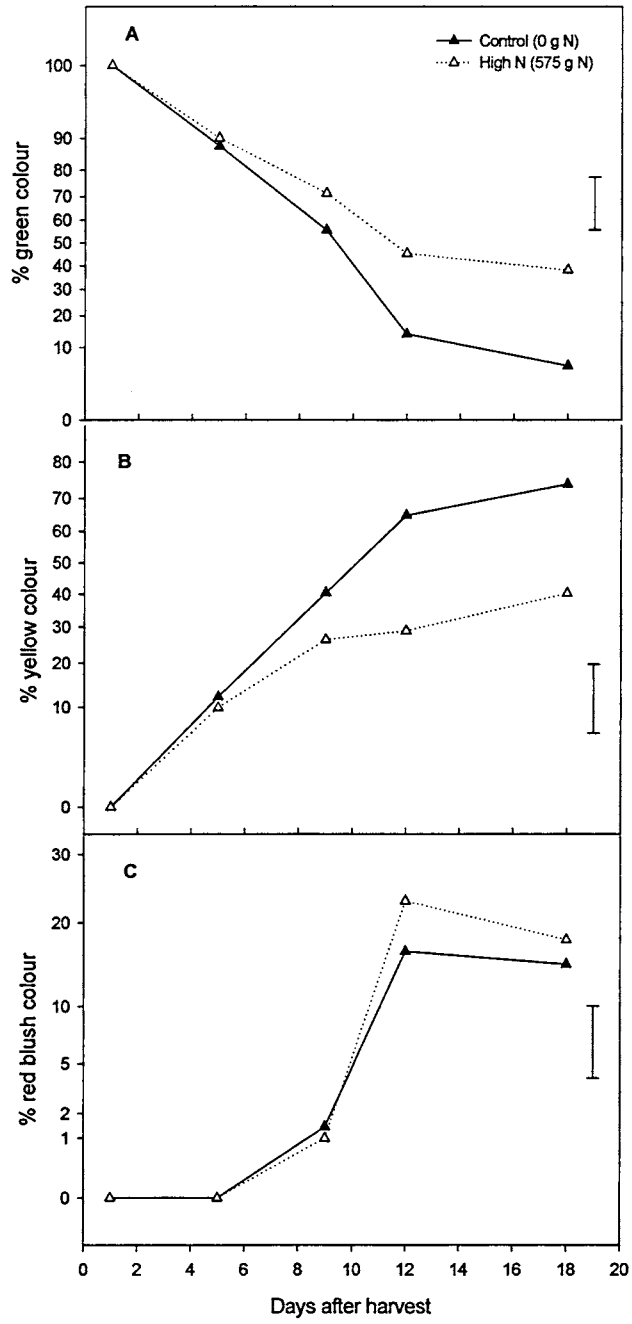


Figure 4.16 Experiment 3b. Effect of N application on the % green (A), the % yellow (B) and the % red blush (C) colour of 'Kensington Pride' mangoes during ripening at 20°C without ethylene. The y-axis scales are angular transformed. Control = fruit from trees treated with 0 g N per tree. High N fruit = fruit from trees treated with 575 g N per tree. Vertical bars represent LSDs ($P < 0.05$, $n = 9$).

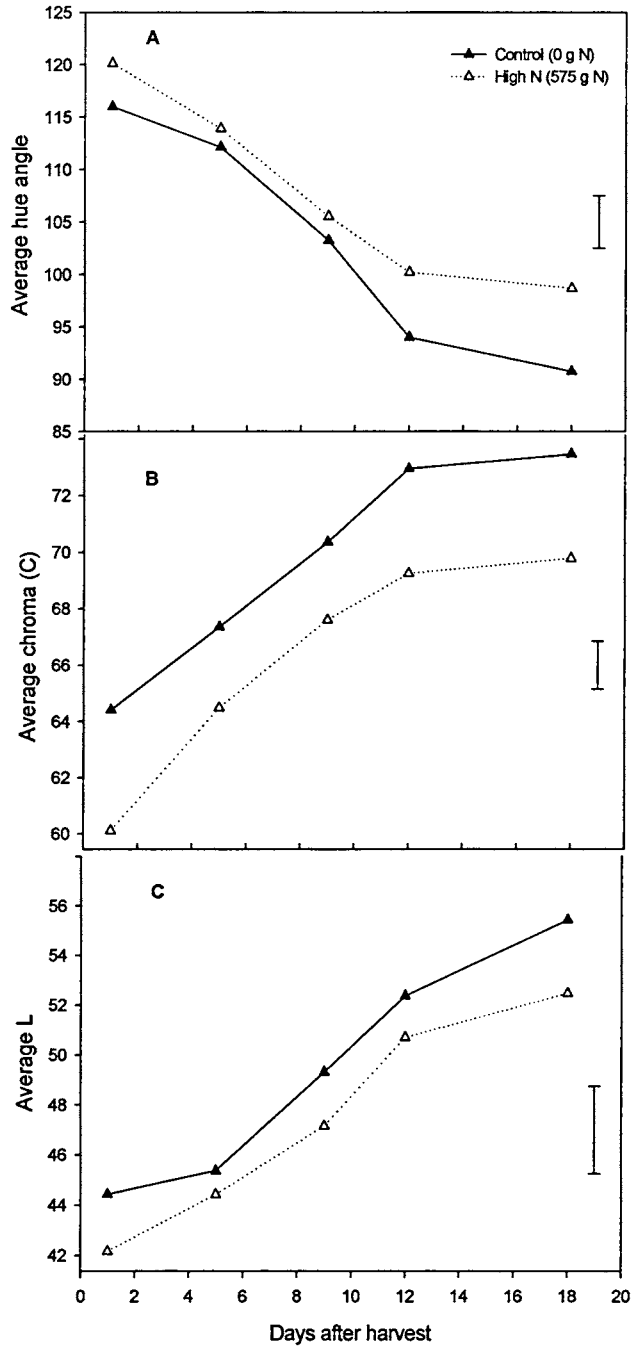


Figure 4.17 Experiment 3b. Effect of N application on average hue angle (A), chroma (B) and L value (C) of the skin of 'Kensington Pride' mangoes during ripening at 20°C without ethylene. Control = fruit from trees treated with 0 g N per tree. High N fruit = fruit from trees treated with 575 g N per tree. Vertical bars represent LSDs ($P < 0.05$, $n = 9$).

Skin pigments and chlorophyllase activity: The decrease in skin chlorophylls concentration during ripening was very similar to that of % green colour (Figure 4.18A). From day 1 to 5, there were no significant differences in total chlorophylls concentration in the skin between control and high N fruit, but after 5 days the concentration was higher in the high N fruit than the control fruit. On day 18 when some fruit were already ripe (firmness of 5), total chlorophylls concentration in the high N fruit skin was double that of the control fruit, despite the fact that the high N fruit was not significantly firmer than the control fruit (Figure 4.15).

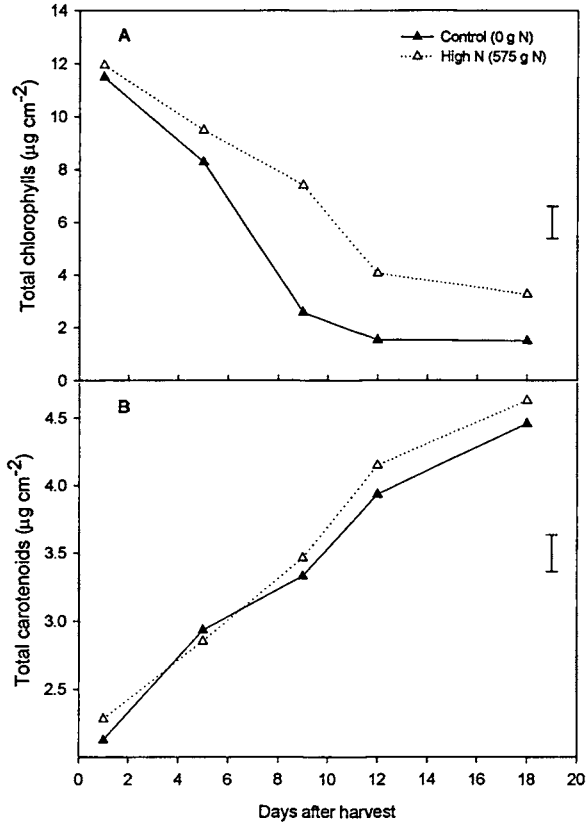


Figure 4.18 Experiment 3b. Effect of N application on skin total chlorophylls (*a* and *b*; A) and skin total carotenoids (ß-carotene and xanthophylls; B) concentration of 'Kensington Pride' mangoes during ripening at 20°C without ethylene. Control = fruit from trees treated with 0 g N per tree. High N fruit = fruit from trees treated with 575 g N per tree. Vertical bars represent LSDs ($P < 0.05$, $n = 9$).

In contrast to total chlorophylls, total carotenoids concentration increased during ripening (Figure 4.18B), and was not affected by N application at any stage.

Chlorophyllase activity of the skin of control and high N fruit one day after harvest was lower compared with activity at day 9 (Figure 4.19). High N fruit had lower activity than control fruit on both days.

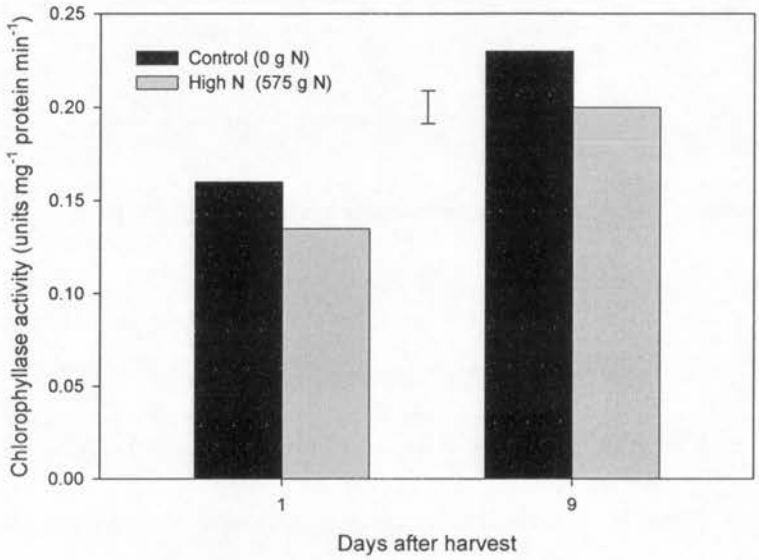


Figure 4.19 Experiment 3b. Effect of N application on chlorophyllase activity in the skin of 'Kensington Pride' mangoes during ripening at 20°C without ethylene. Control = fruit from trees treated with 0 g N per tree. High N fruit = fruit from trees treated with 575 g N per tree. Vertical bar represents LSD ($P < 0.05$, $n = 9$).

4.4 Discussion

The results from the survey suggests that growing practices, including N level, can have a significant effect on 'Kensington Pride' mango quality, including skin colour, DTR, flesh

TSS (°Brix) and acidity, and diseases. The fact that 70% of the orchards produced fruit with more than 25% of area of the skin with green colour confirms the significance of this problem in 'Kensington Pride'. The survey indicates that trees on sandy loam soil may be more likely to produce fruit with less green colour than those on higher clay soils. However, N history is also a significant factor since orchard 2, on sandy loam, was classified as a high N orchard based on pre-harvest N fertiliser application and soil analysis, and produced high % green fruit. It is likely that there are interacting factors, since orchard 8 had a low N status classification but was on clay loam soil, and produced ripe fruit with more than 25% green colour. This suggests that the response to N may be orchard specific. The survey results supported the need for further investigation into the relationship between N and green colour in ripe fruit.

The regression results indicate that there was a significant positive relationship between % green colour and hue angle of the greenest part of the skin of ripe fruit. This suggests that the higher % green colour on the fruit, the more intense is remaining green colour. A high % green colour remaining on the skin of ripe fruit is probably due to high retention of chlorophylls, since the regression coefficient between these two factors was significantly high.

The link between pre-harvest N and skin colour was further confirmed by the results of Experiments 2 and 3a. In all three orchards in Experiment 2, and the two orchards in Experiment 3a, high N application often resulted in more green colour on ripe fruit, irrespective of whether N was applied after harvest or around flowering. This finding has been reported in apple, with high pre-harvest N application resulting in fruit with more green

colour (Neilsen *et al.*, 1984; Fallahi *et al.*, 1985a; Raese and Drake, 1997). In mango, fruit from 'Sensation' mango trees fertilised with more N have a lesser ability to de-green during ripening than fruit from trees fertilised with less N (Oosthuyse, 1993; McKenzie, 1994).

Our results indicate that the previous N fertiliser regime and the history of green, ripe fruit needs to be considered when developing N application recommendations. In Experiment 2, the high % green colour in the fruit from HG control trees compared to the LG control trees was probably due to the higher soil and leaf N in the HG trees. In addition, the response to N application varied in accordance with the initial soil and leaf N and the history of green fruit. For example, both LG orchards had a greater response to 300 g N than the HG trees. In contrast to N application to the soil, the foliar treatment increased the % green in the HG orchard but not in the LG orchards. In apples, foliar N treatment was also found to increase fruit green colour more than soil N application (Meheriuk *et al.*, 1996a). The smaller response to foliar sprays in the LG orchards may be partly explained by the lower leaf N concentration in these orchards compared to HG.

It is unclear whether the addition of K caused more green colour on ripe fruit in Experiment 3a since the combination of N with K showed a response only in the HG* orchard. However, K has been related to skin colour in other fruit. In 'Mutsu' apple, the percentage of leaf K was negatively related to green fruit colour, but positively with yellow fruit colour (Daugaard and Grauslund, 1999). In addition, pre-harvest K application improved red skin colour in peach (Cummings, 1965; Reeves and Cummings, 1970). Further work on a number of representative orchards is required to determine the interaction between K and skin colour in mango.

The effect of timing of N application is also not clear. In all three orchards in Experiment 2 (N applied mainly pre-flowering), a significant % green colour response was observed with 150 g N per tree or above, while in both orchards in Experiment 3a (applied mainly after harvest), 275 g per tree was required for a significant response. This suggests that timing may play some part of the % green colour remaining on ripe fruit. However, seasonal or orchard block factors may have been involved in these responses indicated by the fact that timing of application did not affect the % green in the LG* orchard in experiment 3a. Therefore, there is more research required in this area.

The ripening process in many fruit involves the degradation of some pigments and the synthesis of others (Goldschmidt, 1980). In mango, the change in colour from green to yellow during ripening can be influenced by the rate of chlorophyll breakdown and carotenoids synthesis (Medlicott *et al.*, 1986a). The current results suggest that both chlorophylls and carotenoids play a role in the development of ripe fruit colour, since they both changed during ripening. However, chlorophylls concentration plays a greater role in determining ripe fruit skin colour in response to N, than carotenoids concentration. This is confirmed by the similar responses of % green and chlorophylls concentration to N treatment, and the stronger correlations between chlorophyll concentration and % green, than carotenoids concentration and % green. In addition, the N treatments that increased the chlorophylls concentration in Experiment 2 had no effect on the carotenoids concentration. The increase in carotenoids concentration during ripening in Experiment 3b also supports this suggestion. In relation to red blush, Medlicott *et al.* (1986a) suggested that the increase in red colour during ripening in 'Tommy Atkins' as observed in these experiments, was a result of

unmasking of the anthocyanin due to green colour loss, rather than anthocyanin production *per se*.

The effect of N fertilisation on the chlorophylls concentration in fruit skin is supported by the known roles of N in plants. In leaves, there is a direct correlation between increased N nutrition and increased photosynthetic activity (Evans, 1989). Nitrogen is either partitioned into CO_2 fixing enzymes such as ribulose 1,5-bisphosphate (RuBP) or proteins associated with the thylakoid membranes of the chloroplast. Visually, however, increased N fertilisation can be seen as increased green colour due to higher chlorophylls concentrations. Such strong correlations between the chlorophyll and N concentration in leaves exist because a large proportion of the leaf N is bound up in the proteins that complex the chlorophyll pigments (Evans, 1989). In perennial plants, fruit are strong sinks for N, often drawing N from leaves and other reserves in the plant (Lea, 1993). Fruit with photosynthetic capability for all or part of their development would therefore be responsive to N fertilisation through the mechanisms described by Evans (1989). Therefore, excess N applied to fruiting trees may be partly partitioned into the fruit skin, where it is visible in the photosynthetic apparatus (chloroplasts). In apples for example, foliar sprays of urea significantly increased the chlorophyll concentrations in the skin (Reay *et al.*, 1998). Our data indicate that chlorophylls concentration in the skin of 'Kensington Pride' mangoes is closely related to green skin colour, and that both are influenced by N fertilisation.

Pre-harvest production and fertiliser history can also affect other aspects of fruit quality. Experiment 1 results suggest that soil type may influence diseases, since anthracnose and SER severity were lower in orchards on sandy loam soils. This may be partly related to an N

effect. In Experiment 2 and 3a, high N rates increased fruit diseases, despite the fact that the fruit were treated with hot fungicide after harvest, and there was a significant positive relation between SER and green colour on ripe fruit in the survey. Similar effects of N on fruit diseases have been reported on avocado, nectarine and tomato (Abou Aziz *et al.*,1975; Segall *et al.*, 1977; Bartz *et a*l., 1979; Daane *et a*l., 1995). This N effect may be mediated through reducing the concentration of some phenolics, lignin and silicon in plant tissues (Matsuyama and Dimond, 1973; Menzies *et a*l., 1991). In addition to the effect on diseases, high N rates can also increase susceptibility to the physiological disorders of 'soft nose' (Young *et al.*, 1965) and 'necrosis' (Ram *et al.*, 1988) in mangoes. The form of N applied also needs to be considered. It is possible that the ammonium ion could be antagonistic to the absorption of Ca (Pill *et al.*, 1977; Witney *et al.*, 1990) leading to reduced Ca uptake and transport into fruit, and increased physiological disorders (Young *et al.*, 1965).

The poor correlation between leaf N and % green colour on ripe fruit suggests that using leaf N to predict fruit colour, as has been used on apples (Raese and Williams, 1974), may not be practical in 'Kensington Pride' mangoes since only one orchard showed a significant relationship. However, the significant positive relationships between skin chlorophylls concentration and pre-harvest N application, and skin chlorophylls concentration and % green colour, suggests they could be used as a tool to estimate tree N status and determine the following year's N fertilisation requirement. The assessments could be based on either % green colour or hue angle rather than chlorophylls concentration, because of the relatively good correlation between these parameters in ripe fruit.

The present results demonstrate clearly that increased N application can increase the green, ripe fruit problem in 'Kensington Pride' mangoes as well as increase fruit diseases. The small effect on yield and other quality attributes such as the flesh TSS and acidity suggest that N fertiliser levels can be reduced to improve skin colour without a negative yield response in these orchards. Yields overall were low, particularly considering the age of the trees. The absence of a clear effect on yield may result from the large variation in yield between datum trees or other factors masking/overriding the potential response to N. Nevertheless it is clear that N applications had a stronger effect on skin colour than on yield in these orchards. Application of N at pre-flowering may be more critical to the green, ripe fruit problem than after harvest, but this requires further investigation.

Pre-harvest measures to reduce green colour in ripe fruit need to be considered in conjunction with postharvest ripening and storage practices. Ripening temperature and the use of ethylene can also affect the skin colour of mangoes (Chapter 5 and 6), indicating that an integrated approach to reducing green ripe fruit is required.

CHAPTER FIVE

EFFECT OF TEMPERATURE, ETHYLENE AND CARBON DIOXIDE ON SKIN COLOUR AND OTHER QUALITY ATTRIBUTES

5.1 INTRODUCTION

Mangoes are normally harvested as green mature fruit then ripened naturally or with ethylene (O'Hare, 1995). For 'Kensington Pride' mango, the skin colour when ripe is a very important quality attribute. An Australian market survey conducted over 3 seasons (Ledger, 1995; unpublished results) indicated that a major contributor to poor appearance of saleable mango fruit was retention of green colour on near-ripe or ripe fruit. Our results in Chapter 4 show that the skin colour and other quality attributes of ripe 'Kensington Pride' mangoes can be affected by pre-harvest N application. In this chapter, we look at postharvest aspects that may influence skin colour and quality, such as ripening temperature, ethylene application and atmospheric compositions during ripening.

Postharvest temperature is the most important environmental factor influencing the deterioration rate of many fruits and vegetables (Kader, 1992a). Fruit ripened at high temperatures respire at unacceptably high rates resulting in a shorter shelf life. Additionally, high temperatures can result in abnormal ripening patterns (Blackbourn and John, 1989; An and Paull, 1990). To delay fruit ripening, low temperature is widely used. However, quality can be reduced in many tropical and subtropical fruits if temperatures are too low or exposure is too long (Kays, 1991).

Skin colour is a very important quality parameter for many fruits, since it is usually used by consumers as the primary criterion in determining fruit appeal and the stage of ripeness. The rate of green colour loss during ripening (often associated with chlorophylls degradation) is often affected by the ripening temperature (An and Paull, 1990; Dixon and Hewett, 1998). Ripening temperatures have been reported to play a major role in the relative rate of the ripening changes in mango (Thomas; 1975; Vazquez-Salinas and Lakshminarayana, 1985; O'Hare, 1995). Thomas (1975) indicated that mango cultivars ripened at less than 18°C had significantly more green skin colour than fruit ripened at the optimum temperatures and at temperatures greater than 24°C, many cultivars can develop a mottled green skin colour (Hatton *et al.*, 1965; Medlicott *et al.*, 1986b).

Ethylene is an important regulator of fruit ripening in climacteric fruits (Medlicott *et al.*, 1987; Brady and Speirs, 1991). The use of ethylene gas to initiate mango ripening has been investigated by several workers (Burg and Burg, 1962; Mattoo and Modi, 1969; Fuchs *et al.*, 1975; Medlicott *et al*, 1987; O'Hare, 1995), however very little is reported on the role of ethylene application in the development of ripe fruit skin colour. In addition, the optimum ethylene concentration and duration needed to stimulate mango ripening has not been well addressed, particularly for the Australian 'Kensington Pride' cultivar. Treatment with 10 μL L^{-1} ethylene for 24 h in a sealed container reduced the green colour remaining on the skin of ripe 'Tommy Atkins' mangoes, however, there was no further benefit at 100 μL L^{-1} ethylene or above (Medlicott *et al.*, 1987). In the 00/01 season, a market survey in Brisbane indicated that ethylene concentrations in commercial mango gassing rooms of many growers and ripeners were often higher than 100 μL L^{-1} , and in one instance, more than 500 μL L^{-1} (Lyn Smith, personal communication). In contrast to the potential benefits of ethylene in relation

to skin colour, there is the potential for negative effects, since ethylene can also accelerate the softening process and promote discolouration (e.g. browning) (Kays, 1991; Saltveit, 1999). Therefore, it is important to understand the impacts of ethylene concentration and duration on the main quality parameters of 'Kensington Pride' mango in order to achieve maximum benefit from ethylene treatment.

In CA or MA storage, reduced O_2 and/or increased CO_2 concentration can delay the ripening process in many horticultural products (Beaudry, 1999) mainly by reducing respiration and partly due in some instances to inhibition of ethylene production (Abeles *et al.*, 1992). However, inappropriate O_2 or CO_2 concentration may lead to poor colour development in mangoes (McLauchlan and Barker, 1994; Bender *et al.*, 2000). In practice, the CO_2 concentration in commercial ripening rooms can reach potentially damaging levels if the rooms have poor ventilation. Therefore, it is important to understand how CO_2 affects skin colour development as well as other quality attributes.

The main objective of the following four experiments was to investigate whether some of the major postharvest ripening conditions (temperature, ethylene and CO_2) affect the skin colour and other quality parameters of ripe 'Kensington Pride' fruit. In the first experiment, the effects of a combination of ripening temperatures, and a wide range of ethylene concentrations and durations were studied. The results of this trial indicated the possible range of ethylene concentrations at specific ripening temperatures that minimised the ripe fruit green colour. In the second experiment, the effect of ethylene concentrations over a narrower range was investigated to confirm the optimum concentrations and durations. In the third experiment, the effects of carbon dioxide on skin colour and other quality parameters

were studied. A smaller, fourth experiment was undertaken to obtain some preliminary data on the colour and firmness changes that occur during ripening in response to ethylene.

5.2 Materials and Methods

5.2.1 Experiment 1: Combination of Ripening Temperature and Ethylene Concentration

'Kensington Pride' mango trees from an orchard in the Bundaberg district with a history of green, ripe fruit were selected based on uniform tree size and crop load. Fifty six green mature fruit were harvested from the northern, outer canopy sector from each of 21 trees on 08/01/00 (total of 1176 fruit). After harvest, the fruit were transported by car to the laboratory at Nambour within 8 h. Fruit were then fungicide treated with hot Spin Flo® (carbendazim 50% a.i.; 1g L^{-1}) at 52°C for 5 min, and then randomly allocated to 24 treatments of 0, 10, 100, or 1000 μL L^{-1} ethylene, each at a temperatures of either 15, 20 or 25°C for either 24 h or 72 h. Fourteen fruits were each placed into each ethylene gassing container connected to the flow-through system, as described in Section 3.2. Each container was considered as a replicate, with three replicates (containers) used per treatment. Each temperature treatment was held in a different temperature controlled room [a total of 24 containers (four ethylene concentrations by two durations by three replications) per room]. After 24 h under ethylene, the ethylene supply to half of the containers was removed, but the fruit remained in the containers with the same air flow and conditions as the 72 h treatments. After 72 h all fruit were removed from the containers, and held at the same temperature (15, 20 or 25°C) until ripe.

At the eating soft stage (firmness of 5) the fruit were assessed for % of area of the skin with green colour (% green colour), % area of the skin with red blush colour (% red blush colour) and hue angle of the greenest and the most yellow parts of the skin (hue angle of the green and yellow skin), disease severity (anthracnose, SER and dendritic spot), DTR, lenticel spotting, flesh colour (hue angle) and the flesh TSS (°Brix) and acidity according to the methods described in Section 3.3. In addition, the colour blotchiness (mottled or uneven colour) was rated using a scale of 0 to 4 where 0 = no blotchiness; 1 = 1-5%; 2 = 6-20%; 3 = 21-40%; and 4 = more than 40% area of the skin affected with blotchiness.

5.2.2 Experiment 2: Low Ethylene Concentration

Commercially mature 'Kensington Pride' mango fruit were harvested from three orchards (replicates) in the Bundaberg district on 10/01/01. After harvest, the fruit were transported to the laboratory at Nambour and dipped in hot Spin Flo® (carbendazim 50% a.i.; 1g L^{-1}) at 52°C for 5 min. The fruit from each orchard (15 fruit per treatment from each orchard or replication) were randomly allocated to the 10 treatments of 0, 5, 10, 20, and 50 µL L^{-1} ethylene for 24 h or 72 h at 20°C. The fruit from each treatment and orchard were placed into separate 30-L plastic containers attached to the flow-through system B (Section 3.2). A total of 30 containers were used (10 treatments by three replications). After 24 h the ethylene was removed from the 24 h ethylene treatments as in Experiment 1. After 72 h all fruit were removed from the containers, and ripened at 20°C.

The ripe fruit were assessed for the quality as described in the Experiment 1, except for colour blotchiness.

5.2.3 Experiment 3: Carbon Dioxide Concentration

'Kensington Pride' mango fruit were harvested from three orchards (replicates) in the Gympie district on 25/01/02. The fruit were transported to the laboratory at Nambour within 4 h and dipped in hot Spin Flo® (carbendazim 50% a.i.; 1g L^{-1}) at 52°C for 5 min. The fruit from each orchard were randomly allocated to 10 treatments of either 0, 1, 2, 4 and 6 % CO_2 without ethylene or the same CO_2 concentrations with ethylene at 10 μL L^{-1}. Fifteen fruits from each orchard were placed in each container connected to the flow-through system C (Section 3.2), and the relevant CO_2 and ethylene treatments applied for 72 h at 20°C. After treatment, the fruit were removed from the containers and ripened at 20°C. Skin colour (% green, % red blush colour and hue angle of the green and yellow skin), disease severity (anthracnose, SER and dendritic spot), DTR, lenticel spotting, flesh colour (hue angle) and the flesh TSS (°Brix) and acidity were assessed according to the methods described in Section 3.2. In addition, the dullness of the skin colour (a lack of the glossy appearance) was rated using a scale of 0 to 4 where 0 = no dullness; 4 = very dull.

5.2.4 Experiment 4: Ethylene Application and Fruit Ripening

'Kensington Pride' mango fruit were collected from the packing line of three orchards (replicates) in the Bundaberg district on 17/01/02. The fruit from each orchard were randomly allocated to either the control (untreated) or the ethylene treatment (10 μL L^{-1}

ethylene for 48 h). All fruit were held at 20°C until ripe. Fifteen control fruits (five from each orchard) were removed on days 0, 4, 8, 10 and 14, and 15 ethylene-treated fruit (five from each orchard) removed on days 0, 4, 6, 8 and 10. The fruit were assessed for firmness, % green, yellow and red blush colour and hue angle of the skin (average of two readings taken on opposite sides of the fruit). The skin from opposite sides of the fruit were removed and kept at -80°C for pigment analyses and chlorophyllase assay. Total skin chlorophyll and carotenoids concentrations were analysed by using skin of three typical fruit (Section 3.4). Chlorophyllase activity was analysed according to the method described in Section 3.4, using skin samples pooled from five fruit per replication.

5.2.5 Statistical Analyses

Data were analysed with the Genstat 5® (Release 4.2) general analysis of variance using a factorial treatment design for Experiment 1, 2 and 3 and completely randomized design for Experiment 4. Three replications (containers) per treatment for Experiment 1, three replications (orchards) per treatment for Experiments 2 and 3, and three replications (orchards) per time for Experiment 4 were used. There were 14, 15 and 15 fruits per replication for Experiment 1, 2 and 3, respectively, and five fruits for Experiment 4. The protected least significant difference (LSD) procedure at $P < 0.05$ was used to test for differences between treatment means. Only significant differences at $P < 0.05$ are discussed, unless otherwise stated. Skin colour, anthracnose and stem-end rot ratings with percentage data were angular transformed (in degrees) prior to analysis. Mean values and LSDs of these parameters are graphed on the transformed scales, with the corresponding non-transformed

percentage values shown on the *y*-axis. In the tables, the back-transformed means are shown in brackets. Regression analysis was performed on the average data for each tree.

5.3 RESULTS

5.3.1 Experiment 1: Combination of Ripening Temperature and Ethylene Concentration

Skin colour: The analysis of variance (Appendix 3.1) indicates that temperature, ethylene concentration and treatment duration significantly affected % green colour ($P < 0.001$, 0.01 and 0.05, respectively). However, there were also significant interactions between temperature and ethylene concentration ($P < 0.001$), temperature and treatment duration ($P < 0.01$), ethylene concentration and treatment duration ($P < 0.001$), and temperature and ethylene concentration and treatment duration ($P < 0.01$). The interactions between the three treatment factors show that the effect of ethylene concentration on % green colour differs according to temperature and treatment duration. The results of analysis of variance for hue angle of the green and yellow skin were different from the results for % green colour (Appendix 3.1). There were no interactions between temperature and ethylene concentration for the hue angle of green skin and between ethylene concentration and treatment duration for the hue angle of yellow skin.

At 15°C, most of fruit had more than 30% of the skin area with green colour (% green colour) on the skin when ripe (Figure 5.1A). With 24 h ethylene treatment, the % green colour and the hue angle of the greenest part of the skin (hue angle of green skin) of ripe fruit were not significantly affected by ethylene concentration. However, with 72 h ethylene

treatment, the % green colour increased as ethylene concentration increased to 1000 μL L^{-1}. Similar trends were also noted with hue angle of the green and yellow skin (Figures 5B and C).

Fruit that were treated and ripened at 20°C had significantly less % green colour and hue angle of the green skin than fruit treated and ripened at 15°C (Figure 5.1A and B). In the 24 h treatment, 100 and 1000 μL L^{-1} ethylene also reduced the % green colour compared with control (untreated) fruit (Figure 5.1A), but a reduction in hue angle of the green skin was only observed with 1000 μL L^{-1} compared with the control (Figure 5.1B). With 72 h treatment, 10 or 100 μL L^{-1} ethylene resulted in less green colour than control fruit, while 1000 μL L^{-1} was not different to the control. Treatment at 10 μL L^{-1} ethylene for 72 h at 20°C produced fruit with the least green colour. The hue angle of the yellow skin was lower (more yellow) than those treated at 15°C for all treatments. Only 1000 μL L^{-1} ethylene for 72 h resulted in higher hue angle of the yellow skin than control fruit (Figure 5.1C).

At 25°C, the effect of ethylene on % green colour was similar to that at 20°C (Figure 5.1A). With 72h exposure to ethylene, the 10 μL L^{-1} treatment had the lowest % green colour of all 25°C treatments, while the 100 and 1000 μL L^{-1} treatments had less green colour than the control. The hue angle of the green skin of 72 h ethylene treated fruit was lower (less green) than those fruit treated for 24 h (Figure 5.1B). Ethylene treatment for 72 h reduced the hue angle compared with the control (Figure 5.1C).

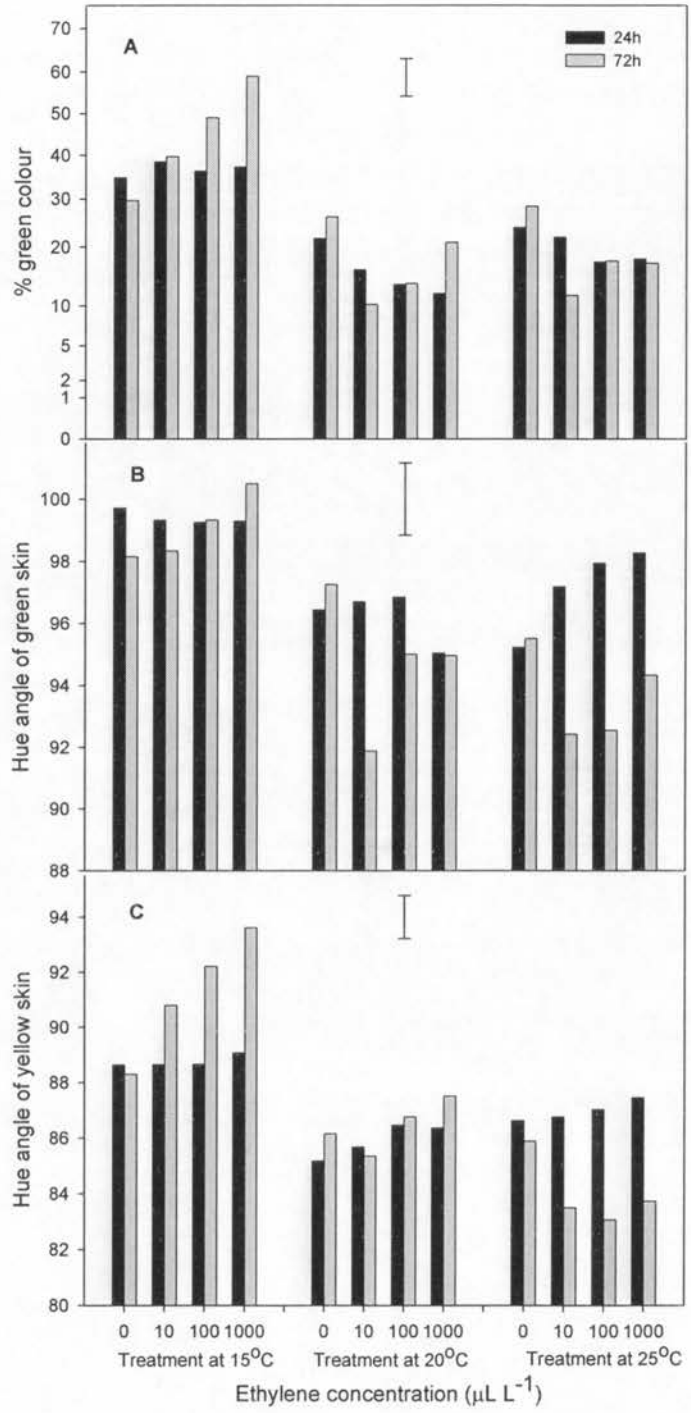


Figure 5.1 Experiment 1. Effect of ethylene concentration and duration (hours), and ripening temperature on the % green colour (A), the hue angle of the greenest part of the skin (B) and the hue angle of most yellow part of the skin (C) of ripe 'Kensington Pride' mangoes. The y-axis scale of % green colour is angular transformed. Vertical bars represent LSDs ($P < 0.05$, $n = 42$).

There was no significant treatment effects on the % red blush colour (data not presented) with means of 23, 24 and 26% for the 15, 20 and 25°C treatments, respectively. Colour blotchiness was significantly affected by the three treatment factors (Appendix 3.1), but there were no interactions between temperature and treatment duration. The colour blotchiness ratings were less than 0.5 with the 15°C treatments (Figure 5.2). At 20°C, treatment 1000 μL L^{-1} ethylene for 72 h resulted in higher blotchiness than control fruit. The pattern of blotchiness at 25°C was similar to 20°C, but with higher ratings. The higher ethylene concentration and longer durations (100 or 1000 μL L^{-1} for 72 h) resulted in the highest blotchiness ratings.

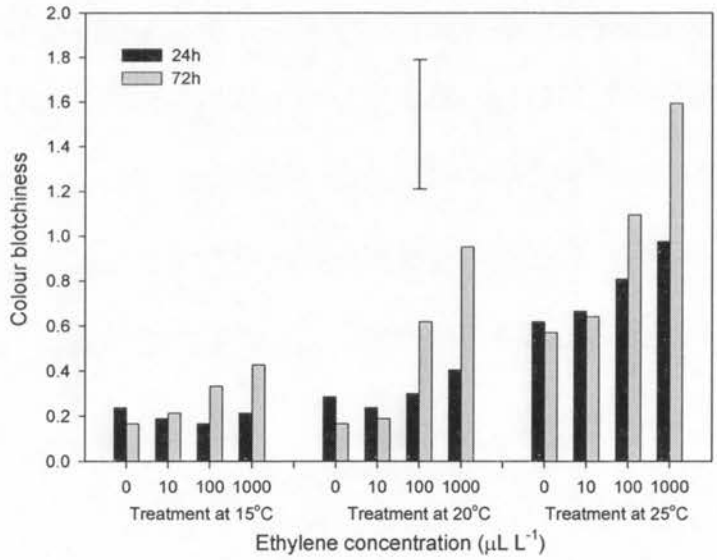


Figure 5.2 Experiment 1. Effect of ethylene concentration and duration (hours), and ripening temperature on skin colour blotchiness (0 = no blotchiness to 4 = severe blotchiness) of ripe 'Kensington Pride' mangoes. Vertical bar represents LSD ($P < 0.05$, $n = 42$).

Plates 5.1 to 5.6 show the skin colour of 'Kensington Pride' mangoes 6 days after harvest (24 h ethylene treatment + 5 days ripening or 72 h treatment + 3 days ripening, with each tray representing one replication for one treatment). Plate 5.1 confirms the absence of a treatment effect with 24 h ethylene, while Plate 5.2 suggests that the 1000 $\mu L\ L^{-1}$ treatment had more green colour after 6 days.

After 6 days at 20°C, both treatment durations showed ethylene-treated fruit had less green colour than control (untreated) fruit (Plates 5.3 and 5.4). With the 24 h treatment (Plate 5.3), increasing ethylene concentration resulted in less green colour, largely because the fruit were more ripe compared with the controls (Plate 5.4). Generally, fruit treated with ethylene for 72 h had more yellow colour than those treated for 24 h. However, 10 or 100 $\mu L\ L^{-1}$ treated fruit showed less green colour than those treated at 1000 $\mu L\ L^{-1}$ for 72 h. In addition, there were symptoms of colour blotchiness on some fruit treated with 100 and 1000 $\mu L\ L^{-1}$ for 72 h.

At 25°C, the ethylene treated fruit had less green colour than control fruit by day 6 (Plates 5.5 and 5.6). Fruit treated with 100 $\mu L\ L^{-1}$ ethylene for 24 h appeared to have less green colour than those treated with 10 or 1000 $\mu L\ L^{-1}$ (Plate 5.5). Blotchiness was evident on the skin of fruit treated with 100 and 1000 $\mu L\ L^{-1}$ ethylene. With 72 h ethylene treatment, the fruit had developed more yellow colour than those treated for 24 h, and 1000 $\mu L\ L^{-1}$ ethylene resulted in more green colour than 10 or 100 $\mu L\ L^{-1}$ ethylene (Plate 5.6). Colour blotchiness was observed on fruit treated with 100, and especially 1000 $\mu L\ L^{-1}$ ethylene.

Control fruit (no ethylene)

100 µL L^{-1} ethylene

10 µL L^{-1} ethylene

1000 µL L^{-1} ethylene

Plate 5.1 Experiment 1. Effect of ethylene concentration on skin colour of 'Kensington Pride' mangoes 6 days after harvest (24 h ethylene treatment + 5 days ripening) when treated and ripened at 15°C.

Control fruit (no ethylene)

100 µL L^{-1} ethylene

10 µL L^{-1} ethylene

1000 µL L^{-1} ethylene

Plate 5.2 Experiment 1. Effect of ethylene concentration on skin colour of 'Kensington Pride' mangoes 6 days after harvest (72 h ethylene treatment + 3 days ripening) when treated and ripened at 15°C.

Control fruit (no ethylene)

100 µL L^{-1} ethylene

10 µL L^{-1} ethylene

1000 µL L^{-1} ethylene

Plate 5.3 Experiment 1. Effect of ethylene concentration on skin colour of 'Kensington Pride' mangoes 6 days after harvest (24 h ethylene treatment + 5 days ripening) when treated and ripened at 20°C.

Control fruit (no ethylene)

100 µL L^{-1} ethylene

10 µL L^{-1} ethylene

1000 µL L^{-1} ethylene

Plate 5.4 Experiment 1. Effect of ethylene concentration on skin colour of 'Kensington Pride' mangoes 6 days after harvest (72 h ethylene treatment + 3 days ripening) when treated and ripened at 20°C.

Control fruit (no ethylene)

100 µL L^{-1} ethylene

10 µL L^{-1} ethylene

1000 µL L^{-1} ethylene

Plate 5.5 Experiment 1. Effect of ethylene concentration on skin colour of 'Kensington Pride' mangoes 6 days after harvest (24 h ethylene treatment + 5 days ripening) when treated and ripened at 25°C.

Control fruit (no ethylene)

100 µL L^{-1} ethylene

10 µL L^{-1} ethylene

1000 µL L^{-1} ethylene

Plate 5.6 Experiment 1. Effect of ethylene concentration on skin colour of 'Kensington Pride' mangoes 6 days after harvest (72 h ethylene treatment + 3 days ripening) when treated and ripened at 25°C.

Days to ripe: There was no interaction between temperature and ethylene concentration for DTR (Appendix 3.1). However, there were interactions between treatment duration with both temperature and ethylene concentration ($P < 0.001$). DTR was therefore significantly affected by temperature and ethylene concentration in relation to the duration of ethylene treatment. At 15°C, fruit took a longer time to reach the ripe stage compared to those treated at 20 or 25°C (Figure 5.3). With 24 h treatment, ethylene-treated fruit had less DTR than control fruit, but increasing ethylene concentration from 10 μL L^{-1} to 1000 μL L^{-1} did not affect the DTR. Ethylene treatment for 72 h resulted in lower DTR than 24 h treatment, and increasing ethylene concentrations for 72 h also reduced the DTR. At 20 and 25°C, the control fruit reached the ripe stage by 11 days compared to 18 days at 15°C (Figure 5.3). The relative effect of ethylene concentration and duration on DTR at these temperatures was similar to those at 15°C, but with different magnitudes.

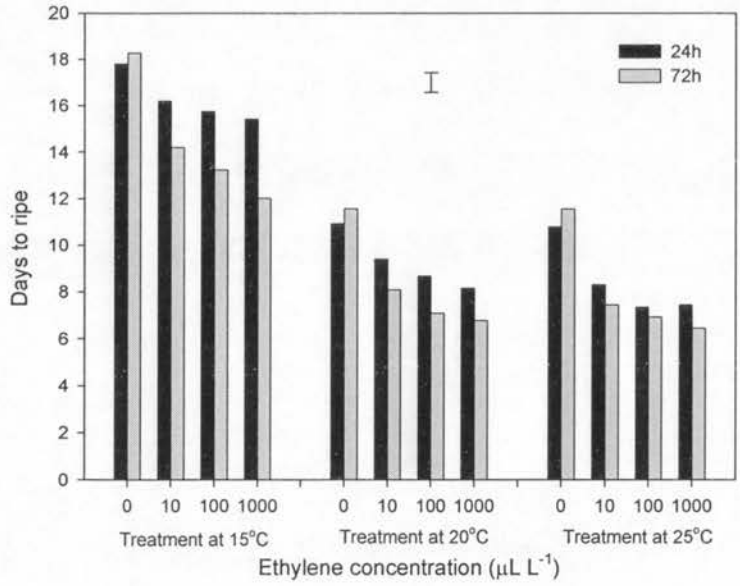


Figure 5.3 Experiment 1. Effect of ethylene concentration and duration (hours), and ripening temperature on the days from harvest to ripe (DTR) of 'Kensington Pride' mangoes. Vertical bar represents LSD ($P < 0.05$, $n = 42$).

Diseases: Generally, anthracnose and SER severity were very low (below 1%) in all treatments (Figure 5.4A and B). For both diseases, fruit that were treated and ripened at 15°C generally had a greater severity compared to those ripened at 20 and 25°C. As pathogen isolations were not made routinely, it is possible that some lesions that developed at lower temperatures may have been caused by *Alternaria alternata* rather than by *Colletotrichum* spp. Results of analysis of variance indicate that the severity of both diseases was significantly ($P < 0.001$ and 0.01 for anthracnose and SER, respectively) affected by temperature, but not by treatment duration. Ethylene significantly ($P < 0.05$) influenced the severity of anthracnose, but not SER severity. There were no interactions between three treatment factors for both anthracnose and SER severity.

In contrast to anthracnose and SER, dendritic spot was obvious in all treatments (Figure 5.4C). Dendritic spot severity was higher in the 15°C fruit than those ripened at the higher temperatures. There was no significant difference in severity between the control and 24 h ethylene treatments, however with 72 h treatment, increasing ethylene concentration reduced the severity. At 20°C, control fruit and fruit treated with 10 $\mu L\ L^{-1}$ ethylene for 24 or 72 h had similar dendritic spot severity, while 100 or 1000 $\mu L\ L^{-1}$ treatments reduced the severity compared to the controls.

Other quality attributes: Lenticel spotting was not significantly affected by ethylene treatment at 15°C (Figure 5.5). At 20°C and 24 h ethylene treatment, 1000 $\mu L\ L^{-1}$ ethylene resulted in more lenticel spotting compared with the control or 10 and 100 $\mu L\ L^{-1}$ ethylene treatments. With 72 h treatment, all ethylene concentrations resulted in more lenticel spotting than the control. At 25°C, there were no significant differences between control fruit and 24 h

ethylene treatment. However, with the 72 h treatment, the lenticel spotting severity was higher with the 100 and 1000 μL L^{-1} treatments than the control.

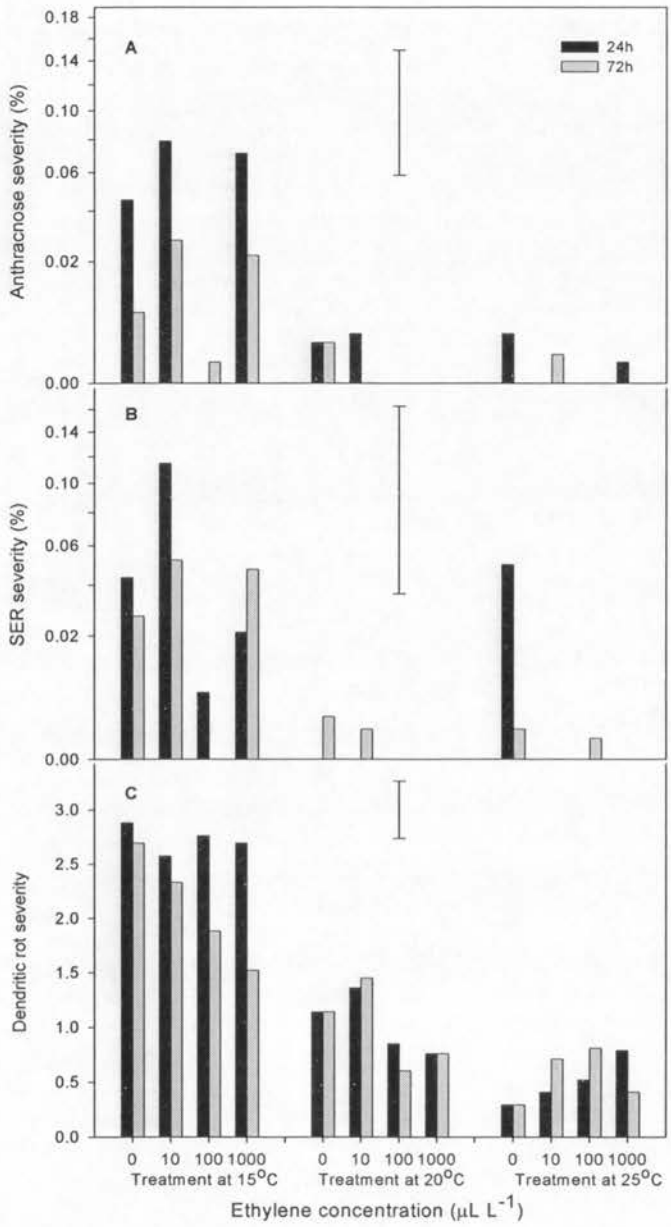


Figure 5.4 Experiment 1. Effect of ethylene concentration and duration (hours), and ripening temperature on the severity of anthracnose (% skin area affected; A), SER (% flesh volume affected; B) and dendritic spot (0 = no spot to 4 = severe spot; C) of ripe 'Kensington Pride' mangoes. Treatment with no bar is equivalent with severity of 0%. The y-axis scales of anthracnose and SER severity are angular transformed. Vertical bars represent LSDs ($P < 0.05$, $n = 42$).

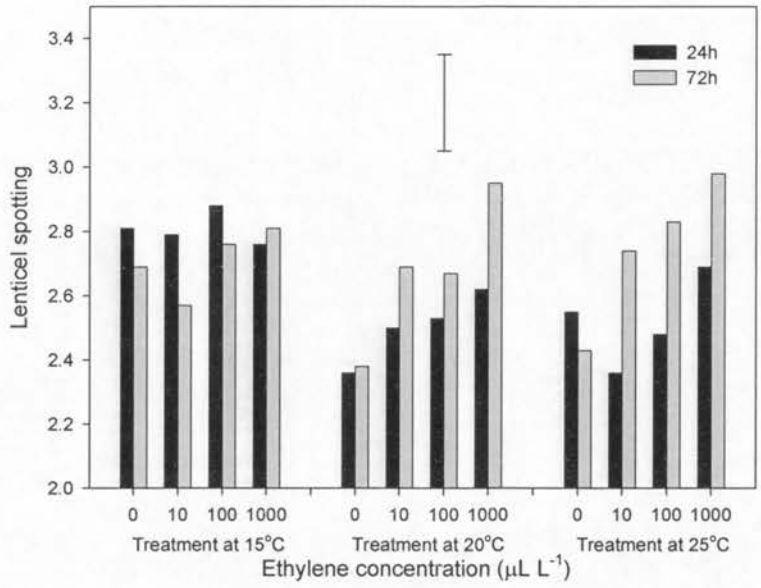


Figure 5.5 Experiment 1. Effect of ethylene concentration and duration (hours), and ripening temperature on lenticel spotting severity (0 = no spotting to 4 = severe spotting) on ripe 'Kensington Pride' mangoes. Vertical bar represents LSD ($P < 0.05$, $n = 42$).

The hue angle of the flesh was higher (less yellow) when ripened at 15°C compared to 20 and 25°C, while at low ethylene concentration (0 and 10 μL L^{-1}) fruit treated at 25°C had lower flesh hue angle than those treated at 20°C (Figure 5.6A). Ethylene treatment at 20 and 25°C increased the flesh hue angle, however there were no significant differences between fruit treated at higher ethylene concentrations (100 and 1000 μL L^{-1}). There was no ethylene concentration or duration effect at 15°C and no duration effects at 20 and 25°C.

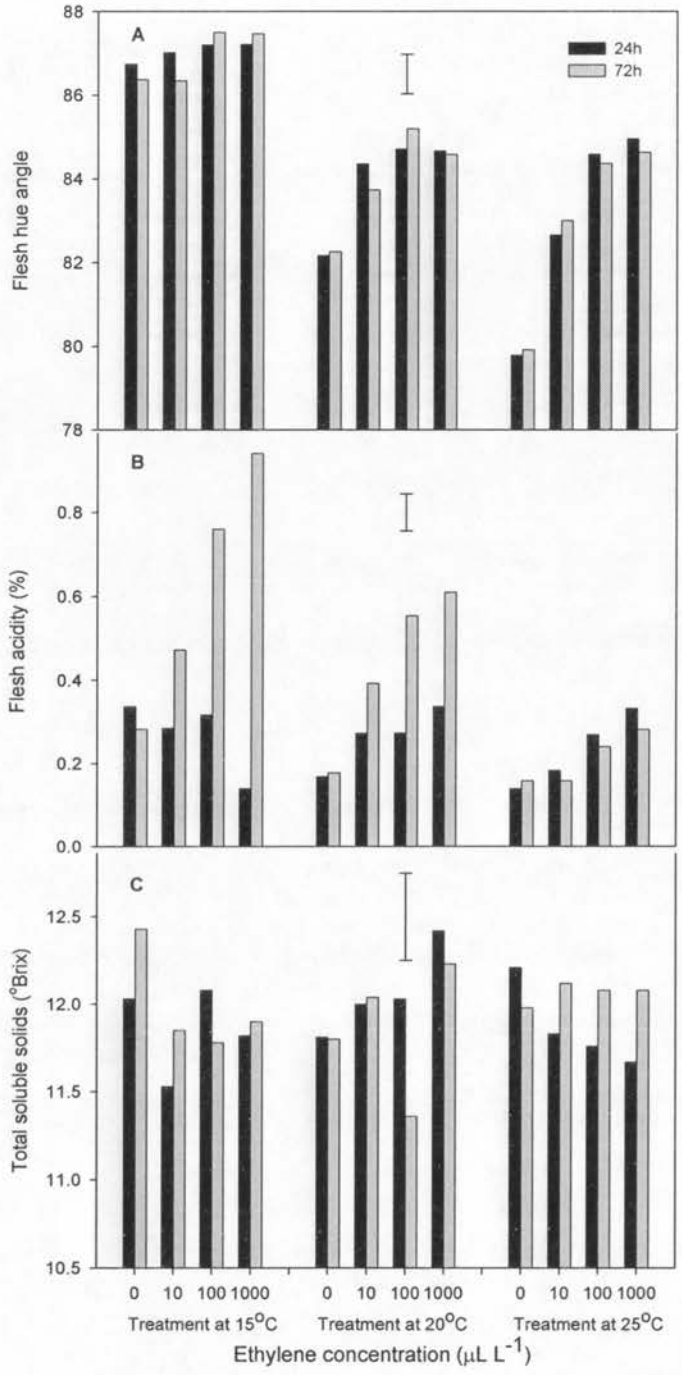


Figure 5.6 Experiment 1. Effect of low ethylene concentration and duration (hours), and ripening temperature on the flesh hue angle (A), the flesh acidity (B) and the flesh TSS (°Brix; C) of ripe 'Kensington Pride' mangoes. Vertical bars represent LSD ($P < 0.05$, $n = 42$).

Generally, control fruit treated and ripened at 15°C had higher flesh acidity than those held at 20 and 25°C (Figure 5.6B). With the 24 h treatment, increasing ethylene concentration increased the flesh acidity at 20 and 25°C but not at 15°C. With 72 h treatment, higher ethylene concentration caused higher acidity with all three temperatures, but with lesser effect at higher temperatures. There were significant interactions between both ethylene concentration and treatment duration and temperature, and between ethylene concentration and duration (Appendix 3.1).

Ripening temperature did not effect flesh TSS of ripe fruit since TSS was not significantly different in control fruit at the three temperatures (Figure 5.6C). The effect of ethylene application on TSS was inconsistent across the three temperatures. Ethylene application reduced the flesh TSS at 15°C for the 72 h treatment compared with the control, but had no effect with the 24 h treatment. At 20°C, 1000 μL L^{-1} ethylene for 24 or 72 h resulted in higher TSS compared with control fruit, but ethylene treatment at 100 μL L^{-1} for 72 h resulted in less TSS. At 25°C, TSS was lower in fruit treated with 1000 μL L^{-1} for 24 h compared to controls, but there were no significant differences between other treatments

5.3.2 Experiment 2: Low Ethylene Concentration

Skin colour: The analysis of variance (Appendix 3.3) indicates that ethylene concentration and treatment duration were significantly affected % green colour ($P < 0.001$). However, there was also an interaction between these two treatment factors ($P < 0.01$). The interaction between these two treatment factors indicates that the effect of ethylene concentration on % green colour differs according to treatment duration. For hue angle, there were no significant

interactions between ethylene concentration and treatment duration for the hue angle of green skin and yellow skin (Appendix 3.3).

The % green colour of ripe fruit was not affected by ethylene application for 24 h, when compared to the control (Figure 5.7A). However, ethylene treatment for 72 h resulted in significantly less green skin colour. There was little difference in % green colour between 5, 10, 20 or 50 μL L^{-1} ethylene treatments for 72 h. The treatment effect on the hue angle of the green skin was similar to that of the % green colour (Figure 5.7B), with 24 treatment having no effect and the 72 h treatment reducing the hue angle (less green).

Treatments affected the hue angle of the yellow skin differently to the hue angle of the green skin (Figure 5.7C). With 24 h treatment, 5 or 10 μL L^{-1} ethylene resulted in lower hue angles (more yellow) than 0, 20 or 50 μL L^{-1} ethylene. With 72 h treatment, all ethylene concentrations decreased the hue angle of the yellow skin, and 5 and 10 μL L^{-1} ethylene also resulted in lower hue angles than 20 and 50 μL L^{-1} ethylene.

The % red blush colour was not significantly affected by ethylene concentration or duration (data not presented) with a mean of 23% across all treatments. There were very few symptoms of colour blotchiness on ripe fruit; therefore the blotchiness severity was not recorded.

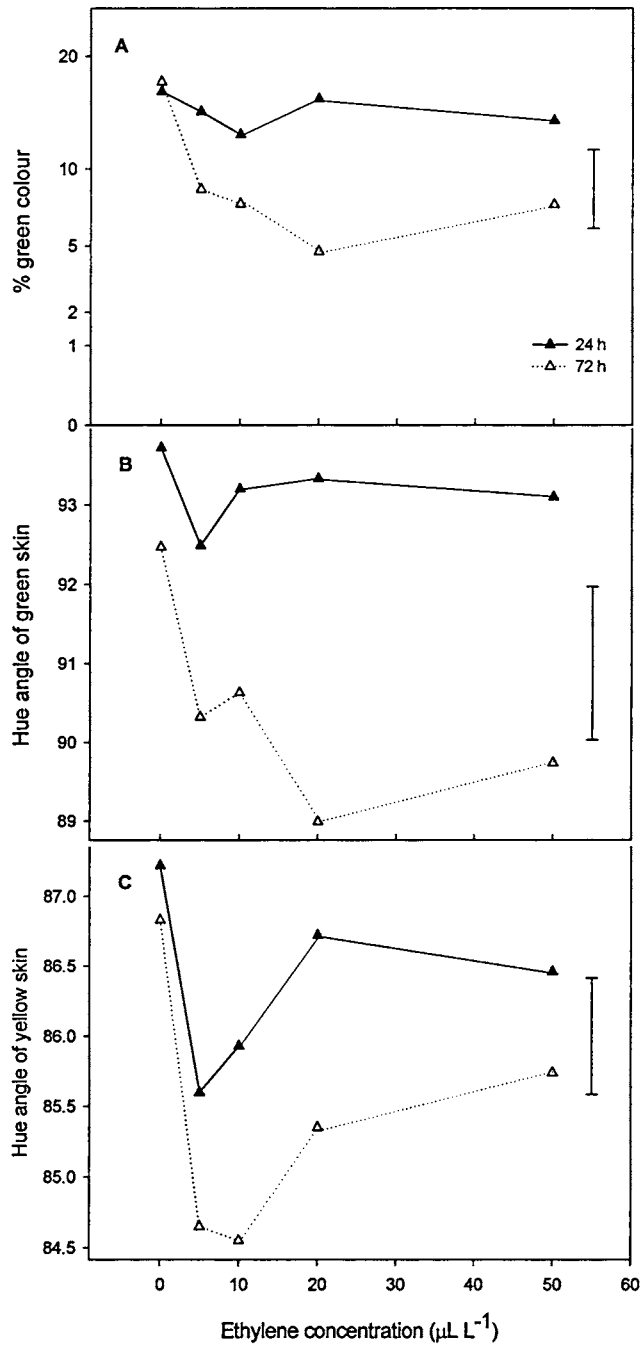


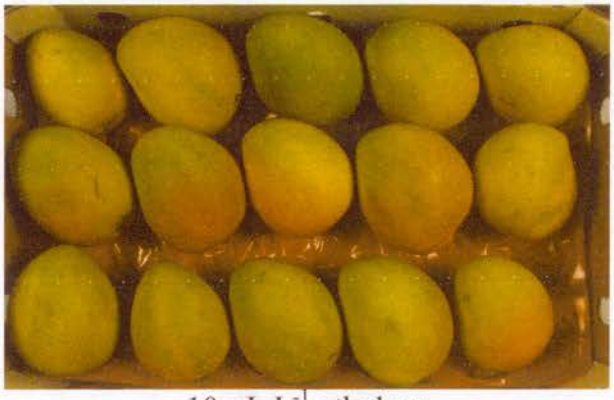
Figure 5.7 Experiment 2. Effect of low ethylene concentration and duration (hours) on the % green colour (A), the hue angle of the greenest part of the skin (B) and the hue colour of the most yellow part of the skin (C) of ripe 'Kensington Pride' mangoes. The y-axis scale of % green colour is angular transformed. Vertical bars represent LSDs ($P < 0.05$, $n = 45$).

The appearance of the fruit from one orchard (replicate) on the fourth (24 h ethylene treatment + 3 days ripening or 72 h ethylene treatment + 1 day ripening) and sixth day (24 h ethylene treatment + 5 days ripening or 72 h ethylene treatment + 3 days ripening) after harvest are shown in Plates 5.7 to 5.10. On the fourth day, some yellow colour had developed on the 24 h treatment fruit, but there was no obvious ethylene treatment effect (Plate 5.7). However, with 72 h treatment, there was less green colour on the ethylene treated fruit compared to control fruit, with less green colour with higher ethylene concentrations (Plate 5.8). By day 6, ethylene treatment for 24 h resulted in less green colour on the fruit (Plate 5.9), possibly because these fruit were more ripe (lower DTR in Figure 5.8). This was particularly obvious in fruit treated with 20 $\mu L\ L^{-1}$ ethylene. The effects of ethylene treatment for 72 h was also obvious at 6 days, but the differences between the different ethylene concentrations was less obvious (Plate 5.10).

Control (no ethylene)

5 µL L^{-1} ethylene

20 µL L^{-1} ethylene



10 µL L^{-1} ethylene

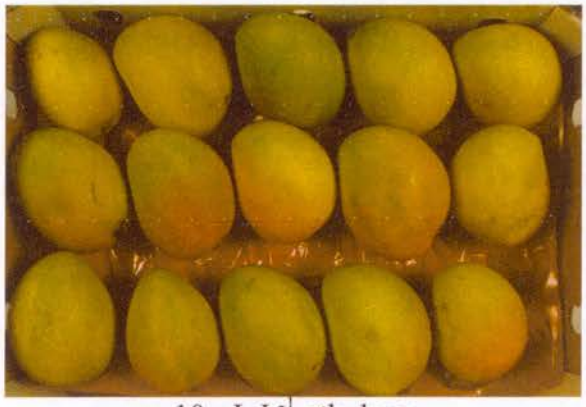
50 µL L^{-1} ethylene

Plate 5.7 Experiment 2. Effect of ethylene concentration on skin colour of 'Kensington Pride' mangoes 4 days after harvest (24 h ethylene treatment + 3 days ripening) when treated and ripened at 20°C.

Control fruit (no ethylene)

5 µL L^{-1} ethylene

20 µL L^{-1} ethylene



10 µL L^{-1} ethylene

50 µL L^{-1} ethylene

Plate 5.8 Experiment 2. Effect of ethylene concentration on skin colour of 'Kensington Pride' mangoes 4 days after harvest (72 h ethylene treatment + 1 day ripening) when treated and ripened at 20°C.

Control fruit (no ethylene)

5 µL L^{-1} ethylene

20 µL L^{-1} ethylene

10 µL L^{-1} ethylene

50 µL L^{-1} ethylene

Plate 5.9 Experiment 2. Effect of ethylene concentration on skin colour of 'Kensington Pride' mangoes 6 days after harvest (24 h ethylene treatment + 5 days ripening) when treated and ripened at 20°C.

Control fruit (no ethylene)

5 µL L^{-1} ethylene

20 µL L^{-1} ethylene

10 µL L^{-1} ethylene

50 µL L^{-1} ethylene

Plate 5.10 Experiment 2. Effect of ethylene concentration on skin colour of 'Kensington Pride' mangoes 6 days after harvest (72 h ethylene treatment + 3 days ripening) when treated and ripened at 20°C.

Days to ripe: DTR was significantly affected by ethylene concentration and treatment duration and there was a significant interaction between these two factors (Appendix 3.3). All ethylene treatments reduced the DTR (Figure 5.8). There was slight difference in DTR between ethylene treatments with different concentrations for 24 h. However, higher ethylene concentrations (20 or 50 μL L^{-1}) for 72 h resulted in lower DTR than 5 or 10 μL L^{-1}.

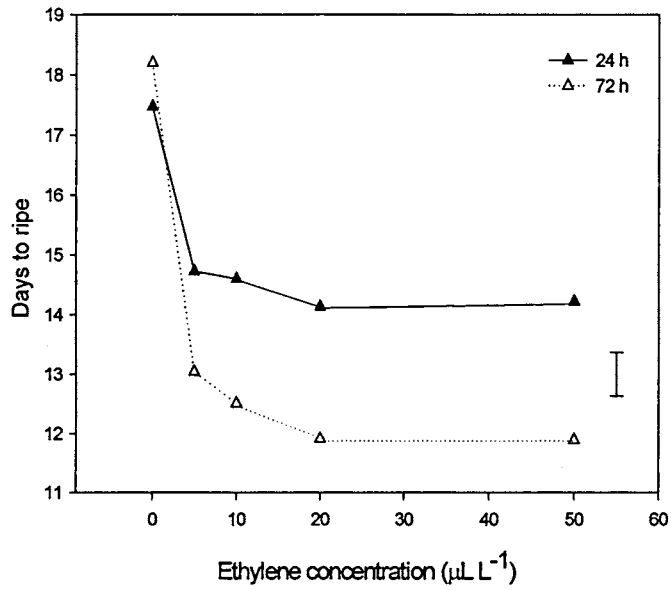


Figure 5.8 Experiment 2. Effect of low ethylene concentration and duration (hours) on the days from harvest to ripe (DTR) of 'Kensington Pride' mangoes. Vertical bar represents LSD ($P < 0.05$, $n = 45$).

Diseases: The analysis of variance indicates that anthracnose and SER severity were not significantly affected by ethylene concentration (Appendix 3.3). However, treatment duration can significantly influence anthracnose severity. There was little difference in anthracnose severity between ethylene treatments for 24h (Figure 5.9). With 72 h treatment, the 50 μL L^{-1} ethylene treatment reduced the anthracnose severity compared with control fruit, but there were no differences between ethylene treatments. In general, SER severity was low (less than 1%) in all treatments and there were no significant treatment effects (data not presented). The

severity of dendritic spot was also not affected by ethylene treatment (data not presented), with a mean rating of 1.1 across all treatments.

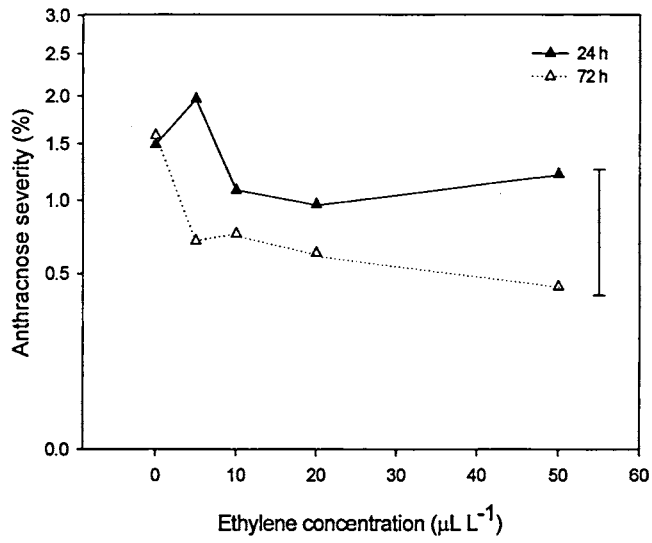


Figure 5.9 Experiment 2. Effect of low ethylene concentration and duration (hours) on anthracnose severity (% skin area affected) of ripe 'Kensington Pride' mangoes. The y-axis scale of anthracnose severity is angular transformed. Vertical bar represents LSD ($P < 0.05$, $n = 45$).

Other quality attributes: All ethylene treatments for 24 h reduced lenticel spotting severity compared to the control, however, there was no significant effect between ethylene treatments (Figure 5.10). With the 72 h treatment, lenticel spotting was not significantly different in all treatments. Generally, the duration of ethylene treatment did not significantly affect lenticel spotting, except 50 µL L^{-1} treatment in which 24 h treatment resulted in less severity than the 72 h treatment.

The flesh hue angle was significantly affected by both treatment factors ($P < 0.001$) and there was an interaction between ethylene concentration and treatment duration ($P < 0.001$) (Appendix 3.3). Treatment with ethylene for 24 h did not affect the hue angle of the flesh

(Figure 5.11A). However, with the 72 h treatment, all ethylene-treated fruit had lower flesh hue angle (more yellow) than control fruit. There was also no treatment effect for 24 h ethylene application on flesh acidity (Figure 5.11B). However, 10, 20 and 50 μL L^{-1} ethylene for 72 h resulted in higher acidity compared with the controls. There was no treatment effect on the flesh TSS (data not presented), with a mean of 11.6 °Brix across all treatments.

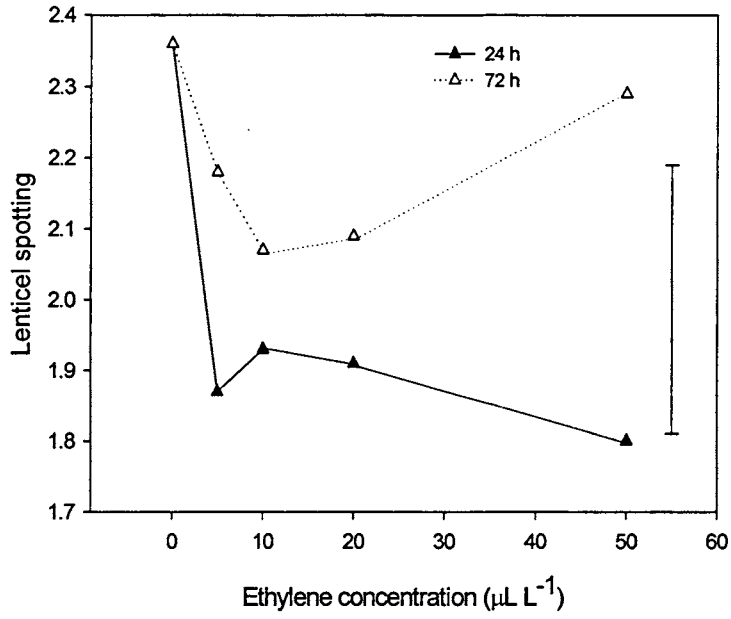


Figure 5.10 Experiment 2. Effect of low ethylene concentrations and duration (hours) on lenticel spotting (0 = no spotting to 4 = severe spotting) of ripe 'Kensington Pride' mangoes. Vertical bar represents LSD ($P < 0.05$, $n = 45$).

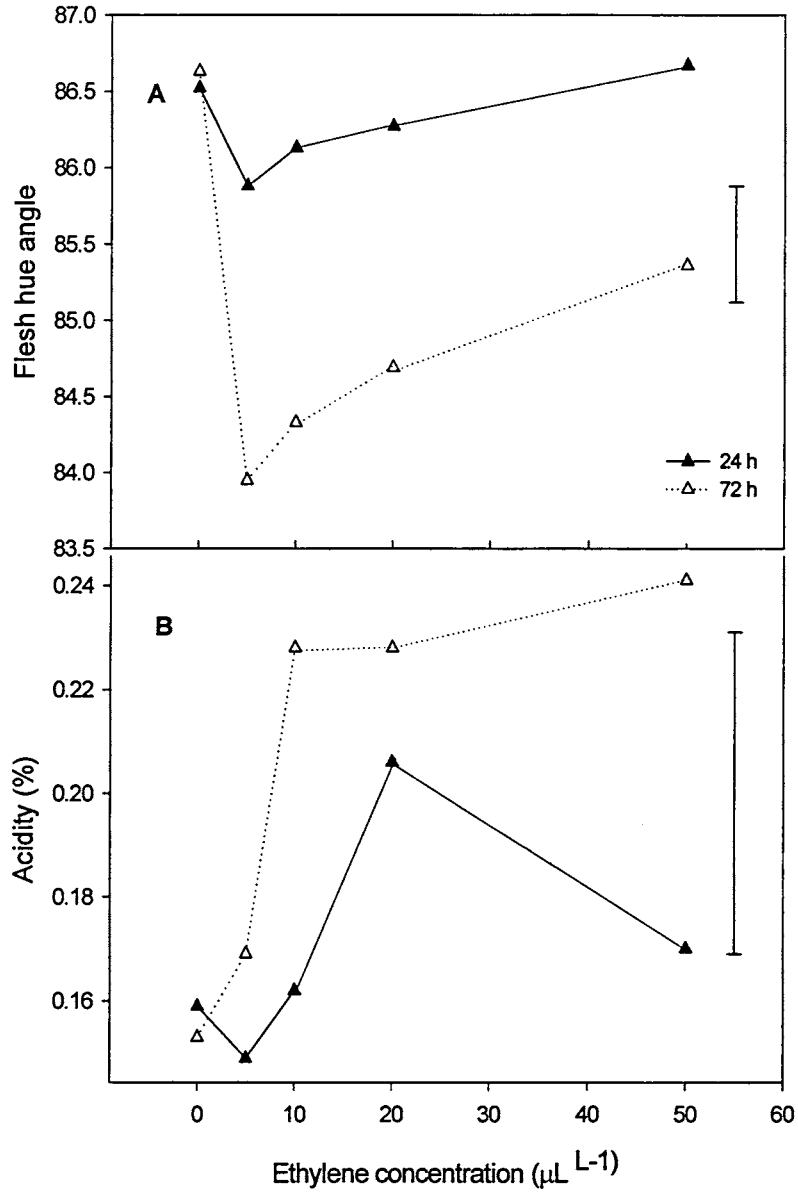


Figure 5.11 Experiment 2. Effect of low ethylene concentrations and duration (hours) on the flesh hue angle (A) and total soluble solids (°Brix; B) of ripe 'Kensington Pride' mangoes. Vertical bars represent LSDs ($P < 0.05$, $n = 45$).

5.3.3 Experiment 3: Carbon Dioxide Concentration

Skin colour: The analysis of variance indicates that % green colour was significantly affected by CO_2 concentration ($P < 0.05$), but not ethylene concentration ($P > 0.05$) (Appendix 3.5). However, there was an interaction between CO_2 and ethylene concentration ($P < 0.001$). This interaction shows that the effect of CO_2 concentration on % green colour differs according to ethylene concentration. Only CO_2 concentration significantly influenced hue angle of green skin ($P < 0.05$) and there was no interaction between the two treatment factors. Hue angle of the yellow skin was not significantly affected by both treatment factors (Appendix 3.5).

Generally, % green colour of ripe fruit treated with 10 $\mu L\ L^{-1}$ ethylene was lower than that of the control fruit (non-ethylene treated) at 0, 1 and 6% CO_2 (Figure 5.12A). In both ethylene and control fruit, there was a general trend for % green colour to increase with increasing CO_2 concentration, resulting in the % green colour being higher at 6% CO_2 compared to 0% CO_2.

The ethylene treated fruit had a lower hue angle of the green skin (less green) than the control fruit at 0, 1 and 2% CO_2 (Figure 5.12B). There was slight difference between ethylene treatment effects on hue angle at 4 and 6% CO_2. In ethylene treated fruit, fruit treated with 4 or 6% CO_2 had higher hue angle (more green) compared to fruit at treated at 0% CO_2.

There was no treatment effect on % red blush colour or hue angle of the yellow skin (data not presented). However, increasing CO_2 concentration increased the colour dullness rating in

both ethylene and control fruit (Figure 5.13). At 0% CO_2 only, ethylene fruit had a less dull colour than the control fruit. The analysis of variance indicates that ethylene did not affect colour dullness, and that there was an interaction between CO_2 and ethylene concentration.

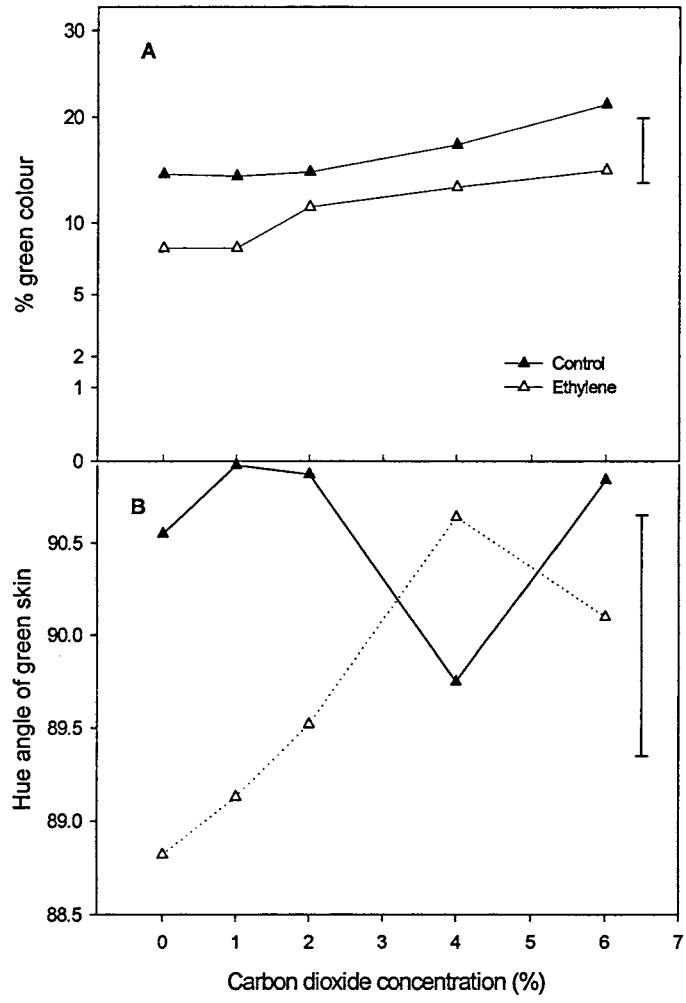


Figure 5.12 Experiment 3. Effect of carbon dioxide concentration, either with no ethylene treatment (control) or with 10 μL L^{-1} ethylene for 72 h, on the % green colour (A) and the hue angle of the greenest part of the skin (B) of ripe 'Kensington Pride' mangoes. The y-axis scale of % green colour is angular transformed. Vertical bars represent LSDs ($P < 0.05$, $n = 45$).

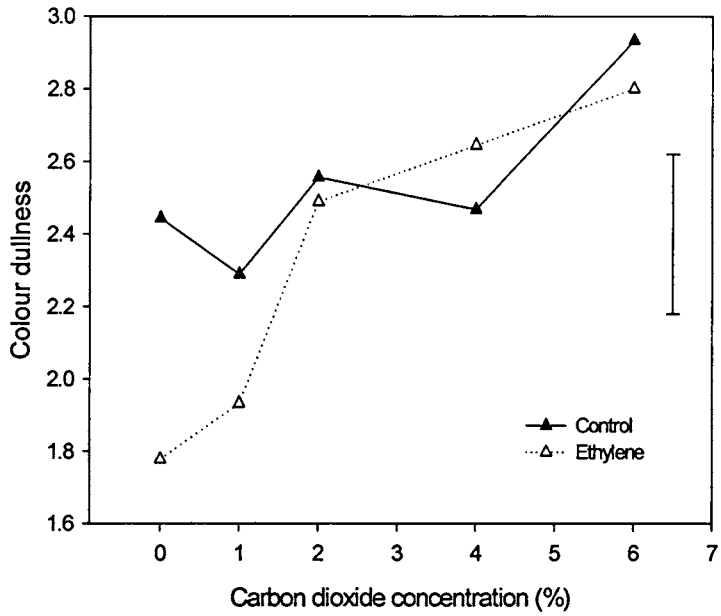


Figure 5.13 Experiment 3. Effect of carbon dioxide concentration, either with no ethylene treatment (control) or with 10 μL L^{-1} ethylene for 72 h, on colour dullness (0 = no dullness to 4 = full dull) of ripe 'Kensington Pride' mangoes. Vertical bar presents LSD ($P < 0.05$, $n = 45$).

Plates 5.11 and 5.12 illustrate the appearance of 'Kensington Pride' mangoes after 7 days of harvest (72 h ethylene and CO_2 treatment + 4 days ripening) at 20°C. Considerable green colour was still present on the skin of the control (non-ethylene treated) fruit (Plate 5.11) with 0% CO_2 fruit having the least green colour. In contrast, there was little green colour remaining in ethylene-treated fruit (Plate 5.12). Ethylene-treated fruit held under 4 and 6% CO_2 had more green colour than fruit from lower concentrations.

0% CO_2

1% CO_2

4% CO_2

2% CO_2

6% CO_2

Plate 5.11 Experiment 3. Effect of CO_2 concentration on skin colour of 'Kensington Pride' mangoes 7 days after harvest when treated for 72 h at 20°C (no ethylene applied).

0% CO_2

1% CO_2

4% CO_2

2% CO_2

6% CO_2

Plate 5.12 Experiment 3. Effect of CO_2 concentration on skin colour of 'Kensington Pride' mangoes 7 days after harvest when treated for 72 h at 20°C (with 10 µL L^{-1} ethylene applied for 72 h).

Days to ripe: The analysis of variance shows that DTR was significantly affected by CO_2 and ethylene concentration ($P < 0.001$) (Appendix 3.5). However, there was also an interaction between these two treatment factors ($P < 0.001$). Fruit treated with ethylene ripened faster than the control fruit (Figure 5.14). Carbon dioxide treatment increased the DTR, with 4% in ethylene fruit and 6% in control fruit resulting in significantly greater DTR than 0%.

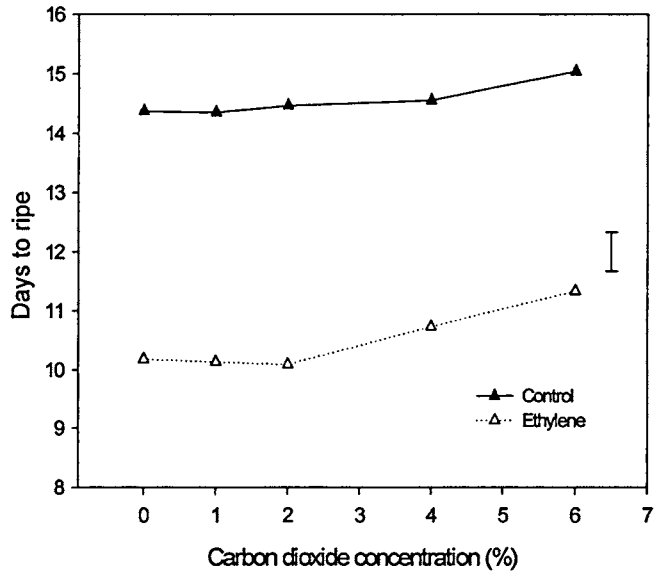


Figure 5.14 Experiment 3. Effect of carbon dioxide concentration, either with no ethylene treatment (control) or with 10 μL L^{-1} ethylene for 72 h, on the days from harvest to ripe (DTR) of 'Kensington Pride' mangoes. Vertical bar presents LSD ($P < 0.05$, $n = 45$).

Diseases: Anthracnose severity was significantly influenced by CO_2 concentration ($P < 0.01$), but not ethylene concentration (Appendix 3.5). There was an interaction between these two treatment factors that indicates the effect of CO_2 concentration on anthracnose severity differs in relation to ethylene concentration. The severity of anthracnose was low (less than 1.5%) in all fruit. There was little difference in anthracnose severity between CO_2 treatments

in ethylene treated fruit or in non ethylene treated fruit (Figure 5.15). Although, ethylene treatment reduced severity at most CO_2 concentrations, the disease levels were too low to draw any meaningful findings about the likely impact of ethylene on anthracnose severity. There were no significant treatment effects on SER and dendritic spot severity (data not presented).

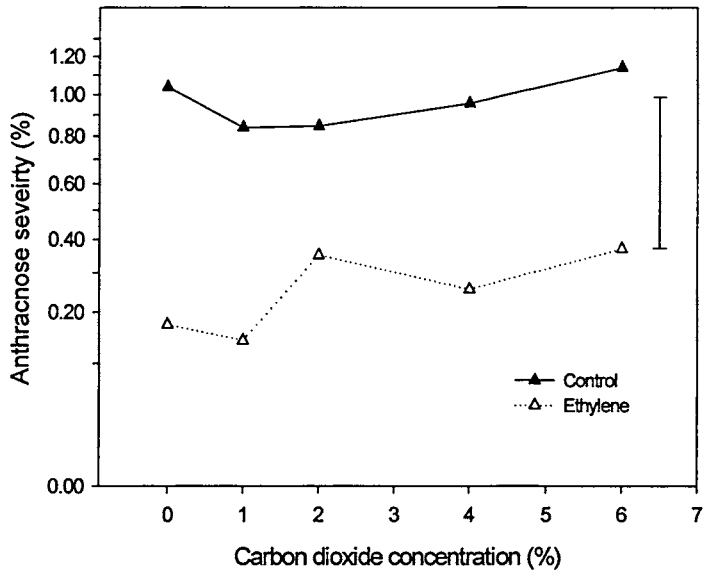


Figure 5.15 Experiment 3. Effect of carbon dioxide concentration, either with no ethylene treatment (control) or with 10 μL L^{-1} ethylene for 72 h, on anthracnose severity (% skin area affected) of ripe 'Kensington Pride' mangoes. The y-axis scale of anthracnose severity is angular transformed. Vertical bar presents LSD ($P < 0.05$, $n = 45$).

Other quality attributes: Lenticel spotting was significantly affected by CO_2 concentration ($P < 0.001$), but not ethylene (Appendix 3.5). However, there was a significant interaction between CO_2 and ethylene concentration. There was no significant difference in lenticel spotting between CO_2 treatments in ethylene treated fruit or in control fruit (Figure 5.16). However, at 0 to 2% CO_2, ethylene fruit had more lenticel spotting than the control fruit, but not at higher CO_2 concentrations.

The flesh hue angle was not significantly affected by CO_2 in ethylene treated fruit (Figure 5.17). However, in the control fruit, the hue angle of the flesh was lower with 6% CO_2 compared to 0% CO_2. Also, at 6% CO_2, the flesh hue angle of ethylene fruit was higher than that in the control fruit. There was no significant treatment effect on flesh acidity and TSS (data not presented).

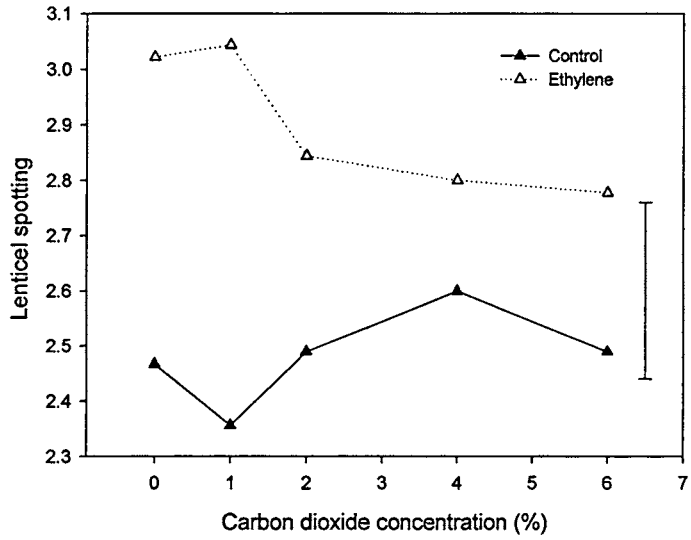


Figure 5.16 Experiment 3. Effect of carbon dioxide concentration, either with no ethylene treatment (control) or with 10 μL L^{-1} ethylene for 72 h, on lenticel spotting (0 = no spotting to 4 = severe spotting) of ripe 'Kensington Pride' mangoes. Vertical bar presents LSD ($P < 0.05$, $n = 45$).

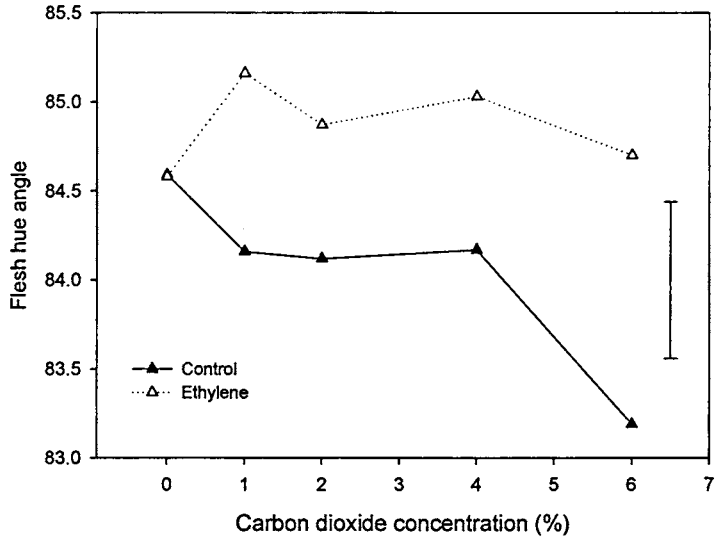


Figure 5.17 Experiment 3. Effect of carbon dioxide concentration, either with no ethylene treatment (control) or with 10 μL L^{-1} ethylene for 72 h, on the flesh hue angle of ripe 'Kensington Pride' mangoes. Vertical bar presents LSD ($P < 0.05$, $n = 45$). n.s = not significant.

5.3.4 Experiment 4: Ethylene Application and Fruit Ripening.

5.3.3.1 Fruit Firmness

Fruit firmness decreased (rating increased) during ripening (Figure 5.18). By day 4 (2 days under ethylene + 2 days at 20°C), ethylene-treated fruit were softer than the control (non-ethylene treated) fruit. Eighty percent of the ethylene-treated fruit were ripe (rating of 5) by 10 days after harvest (average rating of 4.8), while only 50% of the control fruit were ripe by day 14 (average rating of 4.5).

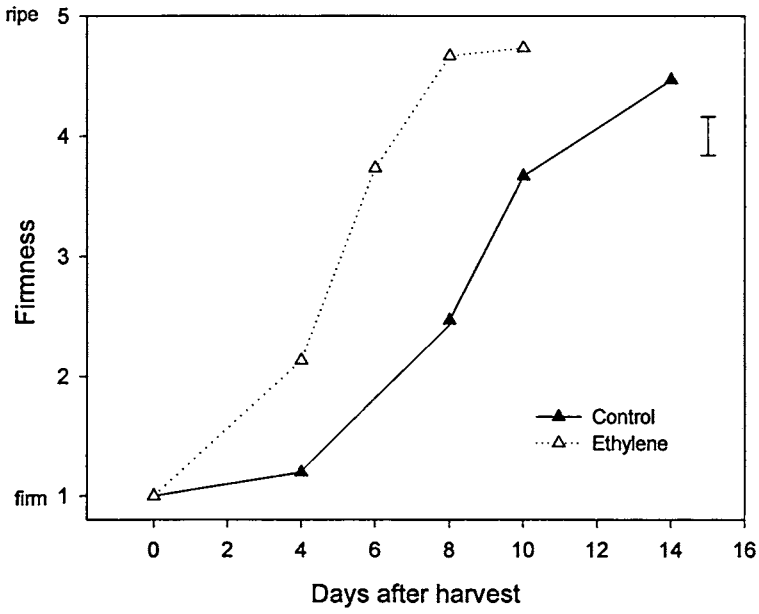


Figure 5.18 Experiment 4. Effect of ethylene application (10 μL L^{-1} for 2 days) on firmness (1-6 scale of 1 = firm, 5 = ripe fruit, 6 = over ripe) of 'Kensington Pride' mango during ripening at 20°C. Vertical bar represents LSD ($P < 0.05$, n = 15).

5.3.3.2 Skin Colour

Fruit colour: The % of the skin area with green colour decreased during ripening in both ethylene-treated and control fruit (Figure 5.19A). However, the de-greening process occurred more rapidly in ethylene-treated fruit; these fruit reached 10% green colour after 8 days, while control fruit required 14 days. In contrast, the % yellow colour increased during ripening in both categories of fruit (Figure 5.19B). The % yellow colour increased more rapidly in ethylene-treated fruit, reaching more than 70% within 8 days after harvest, while the control fruit reached about 70% yellow after 14 days.

The % red blush colour increased from day 0 to day 4, but there were no significant change thereafter (Figure 5.19C). In addition, the % blush colour was not affected by ethylene treatment.

The change in the average hue angle of the skin during ripening was similar to the changes in % green colour (Figure 5.20A). The decrease in the hue angle also occurred faster in ethylene-treated fruit compared with the control fruit. The effect of time after harvest and ethylene application on the flesh hue angle was also similar to the effect on skin hue angle (Figure 5.20B), decreasing during ripening, and with a greater effect with ethylene treatment.

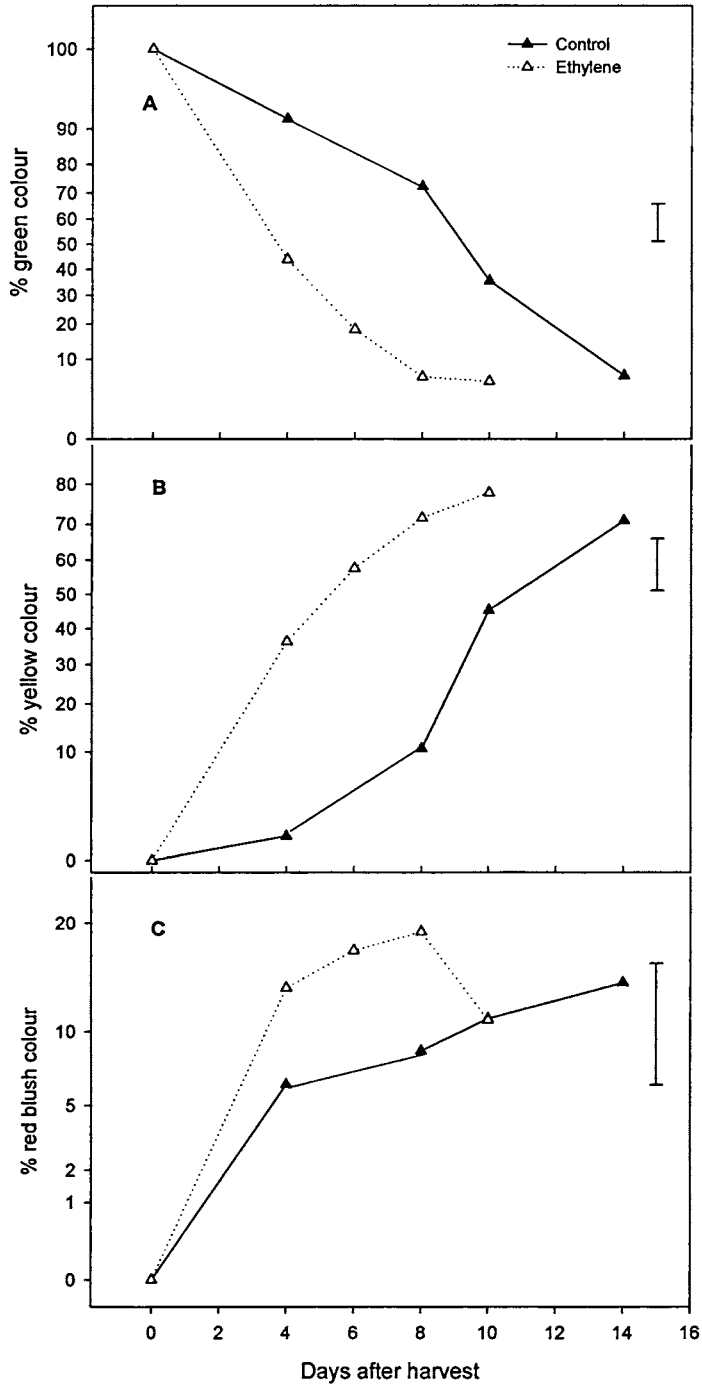


Figure 5.19 Experiment 4. Effect of ethylene application (10 μL L^{-1} for 2 days) on the % green colour (A), the % yellow colour (B) and the % red blush colour (C) of 'Kensington Pride' mango during ripening at 20°C. The y-axis scales are angular transformed. Vertical bars represent LSDs ($P < 0.05$, $n = 15$).

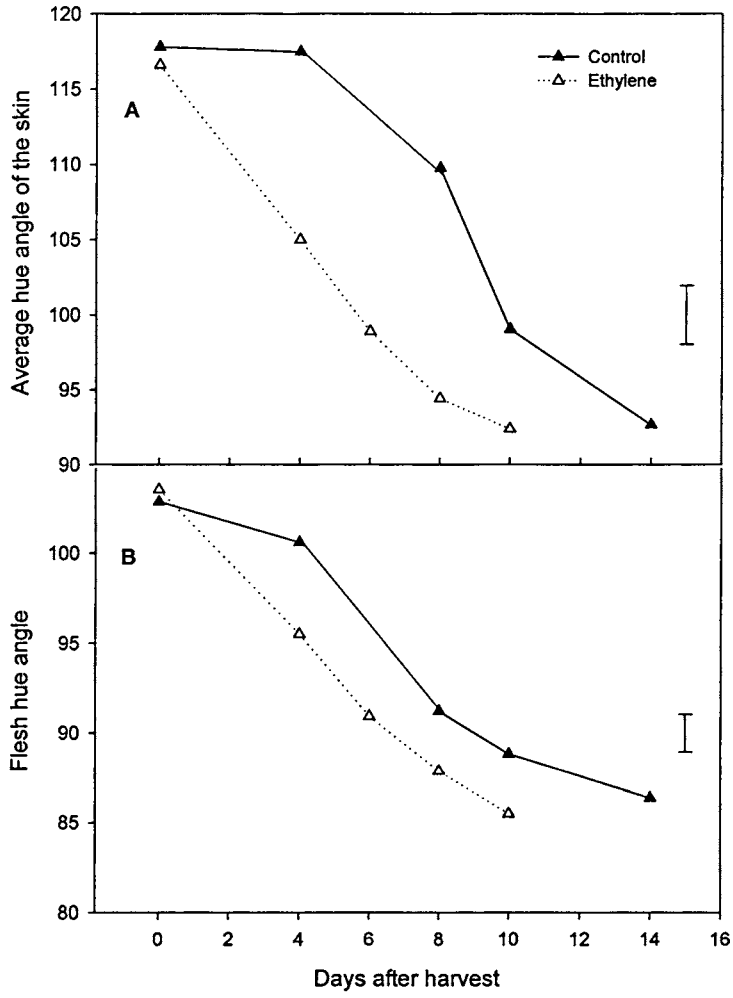


Figure 5.20 Experiment 4. Effect of ethylene application (10 μL L^{-1} for 2 days) on the average hue angle of the skin (A) and the flesh hue angle (B) of 'Kensington Pride' mangoes during ripening at 20°C. Vertical bars represent LSDs ($P < 0.05$, $n = 15$).

Skin pigments and chlorophyllase activity: The change of chlorophyll concentration in fruit skin was very similar to the change of the % green colour and average hue angle of the skin during ripening (Figure 5.21A). The chlorophyll concentration was lower in ethylene-treated fruit from day 4 onwards. In contrast, the carotenoids concentration increased during ripening (Figure 5.21B), with a higher carotenoids concentration in ethylene-treated fruit from day 4

after harvest. There was no significant difference in the carotenoids concentration between control fruit on day 14 and ethylene-treated fruit on day 10. However, when the fruit were almost ripe (days 14 and 10 for control and ethylene-treated fruit, respectively) the chlorophylls concentration in the control fruit was higher than in the ethylene-treated fruit.

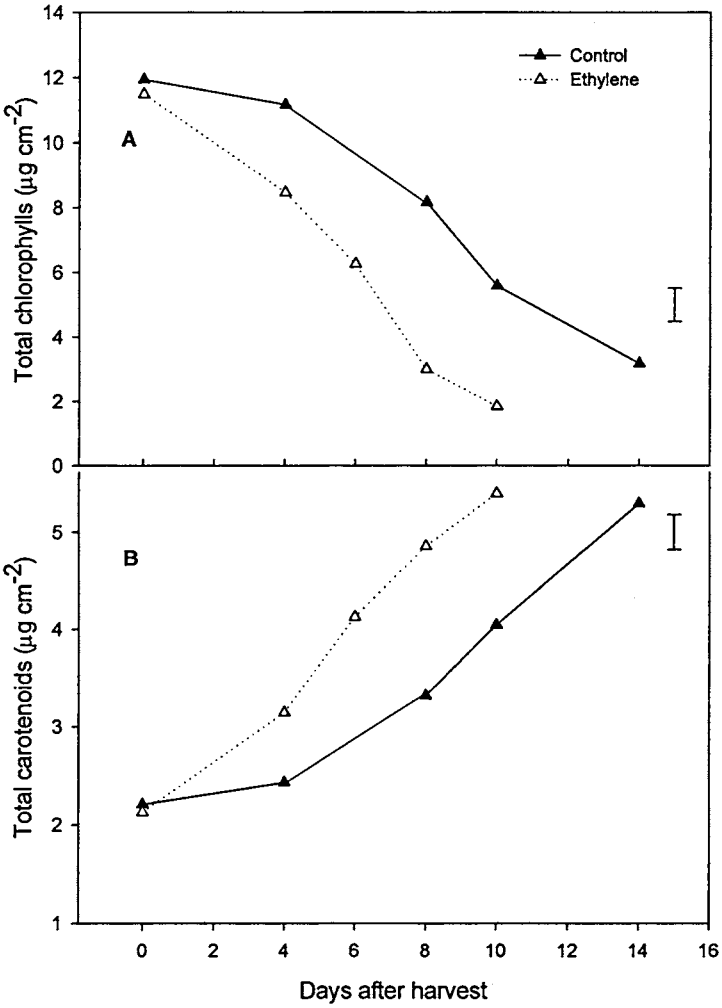


Figure 5.21 Experiment 4. Effect of ethylene application (10 µL L^{-1} for 2 days) on total skin chlorophylls (A) and total skin carotenoids (B) of 'Kensington Pride' mangoes during ripening at 20°C. Vertical bars represent LSDs ($P < 0.05$, $n = 9$).

Chlorophyllase activity was lower in the skin of the fruit 10 days after harvest compared with those assessed on day 4, in both the ethylene-treated and the control fruit (Figure 5.22). Ethylene-treated fruit had higher chlorophyllase activity 4 days after harvest (2 days after ethylene treatment) compared with control fruit, but not at day 10.

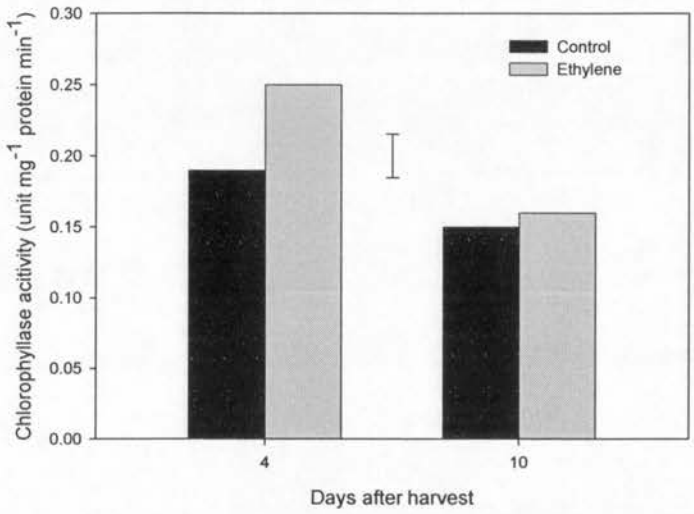


Figure 5.22 Experiment 4. Effect of ethylene application (10 μL L^{-1} for 2 days) on chlorophyllase activity in the skin of 'Kensington Pride' mangoes during ripening at 20°C. Vertical bar represents LSD ($P < 0.05$, $n = 9$).

5.4 Discussion

The results in Experiment 1 show that more green colour was retained on the skin of fruit ripened at 15°C than similar treatments at 20°C and 25°C. This is in agreement with previous reports that mangoes ripened at temperatures below 18°C can have less yellow colour on the skin (Thomas, 1975; O'Hare, 1995). The present results also suggest that treatment with higher ethylene concentration (100 μL L^{-1} and higher) at these low temperature (15°C) for a

long period (72 h) caused softening to occur more quickly than other ripening changes (loss of green skin colour and flesh acidity), so that the fruit reached the eating soft stage before flesh acidity and skin colour were acceptable.

In addition, treatment with ethylene (100 μL L^{-1} or 1000 μL L^{-1} for 24 h and 10 μL L^{-1} 100 μL L^{-1} for 72 h) at 20°C and (10 μL L^{-1} or above for 72 h) at 25°C resulted in less green colour on the skin of 'Kensington Pride' mangoes compared to no ethylene treatment. The loss of green colour of the skin during normal ripening is due primarily to chlorophyll loss (Chapter 4 and Experiment 4). Therefore, the above ethylene treatment conditions appear to accelerate chlorophyll degradation more quickly than softening, so that there is less green colour on the skin when the fruit are eating soft.

There appeared to be little benefit to de-greening by increasing ethylene concentration above 10 μL L^{-1} when treated for 24 h or 72 h at 20 or 25°C. This is similar to the findings on 'Tommy Atkins' with 10 μL L^{-1} ethylene significantly reduced green colour on ripe fruit, but with no additional benefit with ethylene concentrations of 100 μL L^{-1} or higher (Medlicott *et al.*, 1987). In addition, the results suggest that ethylene concentrations above 10 μL L^{-1} ethylene had only a small effect on DTR (shelf life).

Increasing ethylene concentrations and duration at high temperature (25°C) resulted in a less attractive skin colour due to more green colour, and a more uneven or blotchy or mottled colour, compared to those ripened at 20°C under the same conditions. A high ripening temperature of 30°C or above has been reported to cause poor colour development and abnormal softening in papaya (An and Paull, 1990) and at 35°C 'Cavendish' bananas fail to

de-green (Blackbourne and John, 1989). Mottled colour has been noted in Florida cultivars when ripened at 26.2-32.2°C (Hatton *et al.*, 1965) and 'Tommy Atkins' at 37°C (Medlicott *et al.*, 1986b). The failure of de-greening is thought to be a result of the thermal stability of the membrane–associated chloroplast enzymes determined by the changes in membrane lipid structure at high temperature (Raison, 1980).

The postharvest fungicide treatment may have contributed to the low anthracnose and stem-end rot severity, however there were still indications that ripening temperature could affect the severity of these diseases. Also, dendritic spot severity was very high particularly at low temperature. This result is different from the finding in avocado fruit where a lower temperature of 15°C reduced postharvest rots on ripe fruit (Hopkirk *et al.*, 1994). In our other trials on 'Kensington Pride' mango in the season 2000/2001 where fruit were ripened at 16, 19, 22 and 25°C (unpublished data), the severity of anthracnose and dendritic spot on ripe fruit ripened at 16°C was higher than on ripe fruit ripened at other temperatures. Higher severity of the disease occurred in fruit ripened at low temperature probably due to a longer time to reaching eating soft. The results suggest that occasionally ethylene may reduce disease. This shows that ethylene treatments that reduce DTR also generally reduce diseases. The relationship between storage duration and disease was investigated on avocado by Hopkirk *et al.* (1994) and they indicated that fruit had higher disease severity with longer storage duration.

The fact that low ripening temperature and high ethylene concentration for a long duration resulted in higher flesh acidity may be due to a similar mechanism to the green colour response in these fruit. The fruit softens at a faster rate than the reduction in flesh acidity

under these conditions. Similar results were obtained by Jobin-Décor (1988) and O'Hare (1995), again confirming the capacity of temperature and ethylene to differentially affect the some of the ripening processes in mango. There were very few treatment effects on TSS, suggesting that this is more closely linked to softening than colour and acidity changes. It is unclear whether these temperature effects are commercially significant, but the results of Jobin-Décor (1988) suggest that this area needs further investigation.

The results in Experiment 2 supported the finding of Experiment 1, confirming that in low ethylene concentration for a long period (72 h) was an effective treatment for de-greening. There were no significant differences in green colour between fruit treated with ethylene from 5 to 50 $\mu L\ L^{-1}$ for 72h. Despite anthracnose severity being lower in all fruit treated with ethylene for 72 h it is not clear whether this is an indirect result of ethylene reducing DTR. Generally, plants have the ability to defend themselves against pathogen attack by activating a series of both local and systemic defense responses (Norman-Setterblad *et al.*, 2000) and ethylene has been implicated in salicylic acid-independent defense responses. The levels of ethylene have been shown to increase upon wounding or infection by pathogens (Boller, 1991). Ethylene treatment induces several genes encoding pathogenesis-related proteins but mainly the basic vacuolar isoforms (Bol *et al.*, 1990).

The loss in % green colour and the increase of % yellow colour corresponded to the decreases in chlorophylls and increase in carotenoids concentration in the skin, as also reported by Medlicott *et al.* (1986a). The higher chlorophyllase activity in the skin of ethylene-treated fruit 4 days after harvest could indicate an ethylene stimulation of chlorophyllase activity (Trebitsh *et al.*, 1993 in citrus fruit), however, it may also be related

to the more rapid ripening (as suggested by firmness) of the ethylene fruit. The absence of a significant treatment effect at day 10 (8 days after ethylene treatment) may be because of a difference in ripening stages between fruit from the two treatments. In apple, chlorophyllase activity is thought to rise before commencement of the rise in respiration (Rhodes and Wooltorton, 1967). Further research is required in this area.

The chlorophyll and carotenoids results indicated that on day 14, total carotenoids in the skin of the control fruit reached similar concentrations to those in ethylene-treated fruit on day 10, while total chlorophyll concentration in the control fruit was higher than in the ethylene fruit on days 14 and 10, respectively. This suggests that ethylene application influences the total chlorophylls in the skin of ripe 'Kensington Pride' mangoes, more so than total carotenoids. Therefore, in relation to the de-greening process, the chlorophyll concentration in 'Kensington Pride' fruit skin is more important than the carotenoids concentration, as also suggested in Chapter 4.

Richmond and Biale (1966) showed that the respiratory climacteric may be a direct consequence of the increased concentrations of internal ethylene in climacteric fruits. In climacteric fruits such as mangoes, increased respiration rate is associated with an increase in the rate of enzyme synthesis which catalyses ripening reactions. In our trial, it is suggested that ethylene increased chlorophyllase activity which stimulates the de-greening process in the fruit skin of 'Kensington Pride' mangoes. However, there may be other enzymes which can also influence this process. For example, Mattoo and Modi (1969) reported that increased duration of ethylene treatments caused increased peroxidase activity in mango and

peroxidase activity is also thought to be related to chlorophyll degradation (Yamauchi and Watada, 1991; Ketsa *et al.*, 1999a).

Other researchers' work on CA storage have also found that CO_2 concentrations can result in retention of green colour on ripe mango fruit (McLauchlan and Barker, 1994 in 'Kensington Pride' cultivar; Bender *et al.*, 2000 in 'Tommy Atkins' cultivar). Our results indicate that 3 days exposure to high CO_2 concentration was enough to cause a slight increase in % green colour when ripe. This suggests that there is little ability to recover from CO_2 exposure at the start of ripening. The results also indicate that ethylene application was more effective in de-greening when concentrations of CO_2 are less than 2%. Therefore, it is important to reduce the concentration of CO_2 in the treatment room where ethylene treatment takes place. Furthermore, high CO_2 concentration (with or without ethylene) caused greater dullness on the skin of ripe fruit. Dull colour of 'Kensington Pride' fruit is also observed when fruit is ripened at low temperature, and is related to a reduction in carotenoids synthesis (O'Hare, 1995).

In conclusion, temperature was shown to be an important factor influencing the ripening process in 'Kensington Pride' mangoes. Fruit ripened at a low temperature (15°C) retained more green colour and had a higher flesh acidity, particularly in fruit treated with a high ethylene concentrations for 72 h. Ethylene was more effective at reducing the % green colour when using 20 and 25°C. Ethylene treatment at 10 $\mu L\ L^{-1}$ for 72 h at 20 or 25°C resulted in better colour in the ripe fruit. There was no benefit in relation to fruit colour with higher ethylene concentrations. However, at 25°C fruit were more sensitive to mottled colour. Therefore, low ethylene concentration (as low as 5 to 10 $\mu L\ L^{-1}$) for 72 h at 20°C is preferred

for 'Kensington Pride' mangoes to reduce the green, ripe problem on ripe fruit. The additional benefits were reduced diseases in the ripe fruit. In addition, attention needs to be given to the concentration of CO_2 in the ripening rooms.

CHAPTER SIX

SHORT TERM EXPOSURE TO HIGH OR LOW HOLDING TEMPERATURE DURING RIPENING

6.1 INTRODUCTION

The temperature during storage and ripening is important in relation to product quality. Its main effect is through the regulation of the metabolic rate of harvested products (Kays, 1991). Increasing the product temperature increases the metabolic reaction rates that accelerate ripening and deterioration. Fruit that ripen at temperatures above the optimum have a high respiration rate and a shorter shelf life. Temperature is the most critical single factor for the maintenance of quality in fruit and vegetables (Kays, 1991). If the temperature during ripening is too high, the product may suffer high temperature stress injury such as the inhibition of pigment synthesis or degradation, and the formation of surface burn (Blackbourn and John, 1989; An and Paull, 1990; Kays, 1991).

Refrigeration is used to reduce holding temperatures, and thereby reduce the respiration rate, delay the ripening process and extend the products life. However, many tropical and subtropical fruits are susceptible to low temperatures and may suffer chilling injury (Wang, 1982; Kader, 1992a). The severity of chilling injury depends on the temperature to which the fruit is exposed, the time of exposure, and cultivar sensitivity to the chilling temperature. Therefore, chilling injury severity increases with lower temperatures and longer exposure durations to temperatures below a certain threshold (Paull, 1990).

The optimum ripening temperatures for many mango cultivars is in the range of 18-24°C (Hatton *et al.*, 1965). The optimum ripening temperature for the Australian 'Kensington Pride' mango is 18-22°C (O'Hare, 1995). Our results (Chapter 5) indicate that ripening this cultivar with ethylene at 20°C will minimise the green, ripe fruit problem and other ripening disorders such as a mottled skin colour. In practice however, ripening temperature is not always in the optimum range during commercial handling and distribution, because of poor temperature control and delays in the marketing chain. Recent research has shown that, during the 2-3 day road transport of mango from North Queensland to southern markets such as Sydney and Melbourne, the fruit pulp temperature can reach 28°C, despite the truck refrigeration system being set within the optimum range of 18-22°C (Holmes and Kernot, 2002). These high pulp temperatures can be the result of fruit being inadequately pre-cooled before loading, or the fruit generating heat in the truck as they ripen. Therefore, it is important to determine whether these short term exposures to temperatures above the optimum can affect the skin colour and other quality attributes of ripe 'Kensington Pride' mangoes.

In addition, retailers may need to hold fruit that have been partly or fully ripened, because of short term over-supply. Low temperatures can delay mango ripening (O'Hare, 1995), but may also reduce the fruit quality by affecting skin colour (O'Hare, 1995) and inducing chilling injury (Veloz *et al.*, 1977; Medlicott *et al.*, 1990; Mohammed and Brecht, 2000). The stage of ripening can affect the sensitivity of fruit to low temperatures. For example, avocado (Kosiyachinda and Young, 1976) and papaya (Chen and Paull, 1986) at the pre-climacteric and mid-climacteric stage are more sensitive to chilling injury than fruit at the post-

climacteric stage. In 'Kensington Pride' mango, it is unclear what effect short exposures to low temperatures have on quality, and whether these responses depend on the stage of ripeness of the fruit.

The aim of the following two experiments was to investigate the effects of short exposures after harvest to temperatures outside the optimum ripening range, on skin colour and other quality attributes of ripe 'Kensington Pride' mangoes. In the first experiment, the effect of a short period of high temperature (24 or 28°C) was studied to simulate the effect of high temperatures during road transport. In the second experiment, the effect of a short period of low ripening temperatures (7, 10 and 13°C) was investigated to determine if the ripening of partly ripened fruit can be delayed.

6.2 Materials and Methods

6.2.1 Experiment 1: Short Term Exposure to High Temperature

Commercially harvested and packed 'Kensington Pride' mango fruit were collected from the packhouses of three orchards (as replicates) in the Bundaberg district on 10/01/02. The fruit were transported by car to the laboratory at Nambour within 8 h of harvest. Fruit were then exposed to 10 μL L^{-1} ethylene at 20°C for 2 days using a trickle method. After removal from ethylene, the fruit from each replication were randomly divided into 13 treatments containing 15 fruits from each orchard (total of 45 fruit per treatment). The fruit were held at 20°C until the mid-climacteric stage (about 6 h after removal from ethylene; a firmness rating of 2). The fruit were then exposed to either 24 or 28°C for either 1 or 3 days, then returned to 20°C,

or were held at 24 or 28°C, respectively, until ripe (Table 6.1). In addition, fruit at the post-climacteric stage (firmness rating of 3), were also held at 24 or 28°C for either 1 or 3 days and then returned to 20°C until ripe, or held at 24 or 28°C, respectively, until ripe (Table 6.1). Control fruit were held at 20°C until ripe.

Table 6.1 Treatments applied after all fruit had been exposed to 10 μL L^{-1} ethylene for 2 days at 20°C.

When transferred to holding temperature	Holding temperature	Ripening temperature
Held at 20 °C until ripe	Control treatment	
At mid-climacteric	24°C for 1 day	20°C until ripe
At mid-climacteric	24°C for 3 days	20°C until ripe
At mid-climacteric	24°C until ripe	24°C until ripe
At mid-climacteric	28°C for 1 day	20°C until ripe
At mid-climacteric	28°C for 3 days	20°C until ripe
At mid-climacteric	28°C until ripe	28°C until ripe
At post-climacteric	24°C for 1 day	20°C until ripe
At post-climacteric	24°C for 3 days	20°C until ripe
At post-climacteric	24°C until ripe	24°C until ripe
At post-climacteric	28°C for 1 day	20°C until ripe
At post-climacteric	28°C for 3 days	20°C until ripe
At post-climacteric	28°C until ripe	28°C until ripe

The number of days from harvest to ripe (DTR) was recorded. At the ripe stage (firmness rating of 5), fruit were assessed for the % green and % red blush area on the skin (% green and red blush colour), hue angle of the greenest and the most yellow part of the skin (hue angle of green and yellow skin), disease severity (anthracnose and SER), lenticel spotting,

flesh acidity and TSS (Section 3.3). The severity of blotchiness was determined on a rating scale of 0 to 4 (Section 5.2).

6.2.2 Experiment 2: Short Term Exposure to Low Temperature

Commercially harvested and packed 'Kensington Pride' mango fruit were obtained from the packhouses of three orchards (as replicates) in the Bundaberg district on 17/01/02. The fruit were taken directly to the laboratory at Nambour (arriving within 8 h of harvest) and exposed to 10 $\mu L\ L^{-1}$ ethylene at 20°C for 1.5 days. After removal from ethylene the fruit from each orchard were randomly divided into 19 treatment groups consisting of 20 fruit from each orchard. At the mid-climacteric stage (1 day after removal from ethylene), fruit were moved to 7, 10 or 13°C for 1, 3 or 7 days then returned to 20°C for ripening (Table 6.2). The remaining fruit were placed under the same treatment regime at the post-climacteric stage (2 days after removal from ethylene). Control fruit were ripened at 20°C until ripe.

At eating soft (firmness rating of 5) the DTR was recorded and the % green and red blush colour, hue angle of the green and yellow colour, disease severity (anthracnose and SER), lenticel spotting and the flesh acidity and TSS were assessed (Section 3.3). Skin chilling injury was measured based on the % of skin area affected using a scale of 0 to 4 (0 = no injury; 1 = 1-5%; 2 = 6-20%; 3 = 21-40%; and 4 = more than 40% area affected).

Table 6.2 Treatments applied after all fruit had been exposed to 10 μL L^{-1} ethylene for 1.5 days at 20°C.

When transferred to holding temperature	Holding temperature	Ripening temperature
Held at 20 °C until ripe	Control treatment	
At mid-climacteric	7°C for 1 day	20°C until ripe
At mid-climacteric	7°C for 3 days	20°C until ripe
At mid-climacteric	7°C for 7 days	20°C until ripe
At mid-climacteric	10°C for 1 day	20°C until ripe
At mid-climacteric	10°C for 3 days	20°C until ripe
At mid-climacteric	10°C for 7 days	20°C until ripe
At mid-climacteric	13°C for 1 day	20°C until ripe
At mid-climacteric	13°C for 3 days	20°C until ripe
At mid-climacteric	13°C for 7 days	20°C until ripe
At post-climacteric	7°C for 1 day	20°C until ripe
At post-climacteric	7°C for 3 days	20°C until ripe
At post-climacteric	7°C for 7 days	20°C until ripe
At post-climacteric	10°C for 1 day	20°C until ripe
At post-climacteric	10°C for 3 days	20°C until ripe
At post-climacteric	10°C for 7 days	20°C until ripe
At post-climacteric	13°C for 1 day	20°C until ripe
At post-climacteric	13°C for 3 days	20°C until ripe
At post-climacteric	13°C for 7 days	20°C until ripe

6.2.3 Statistical Analyses

Data were analysed with the Genstat 5® (Release 4.2) general analysis of variance model using a completely randomised design. There were three replications (orchards) per

treatment, and 15 and 20 fruits per replication for Experiment 1 and 2, respectively. The protected least significant difference (LSD) procedure at $P < 0.05$ was used to test for differences between treatment means. Only significant differences at $P < 0.05$ are discussed, unless otherwise stated. Skin colour, anthracnose and stem-end rot ratings with percentage data were angular transformed (in degrees) prior to analysis. Mean values and LSDs of these parameters are graphed on the transformed scales, with the corresponding non-transformed percentage values shown on the *y*-axis. In the tables, the back-transformed means are shown in brackets.

6.3 RESULTS

6.3.1 Experiment 1: Short Term Exposure to High Temperature

Skin colour: Mid-climacteric fruit held at 24°C until ripe, or at 28°C for 3 or more days had higher % green area on the skin (% green colour) of ripe fruit than those ripened continually at 20°C (control fruit) (Figure 6.1A). In addition, fruit ripened continually at 28°C had more % green colour than fruit held for only 1 day at 28°C. The treatment differences were not as strong in the post-climacteric treatment, since fruit held continually at 24°C had similar % green colour to the control, and there was no significant difference between 1 day at 28°C, and continual holding at 28°C.

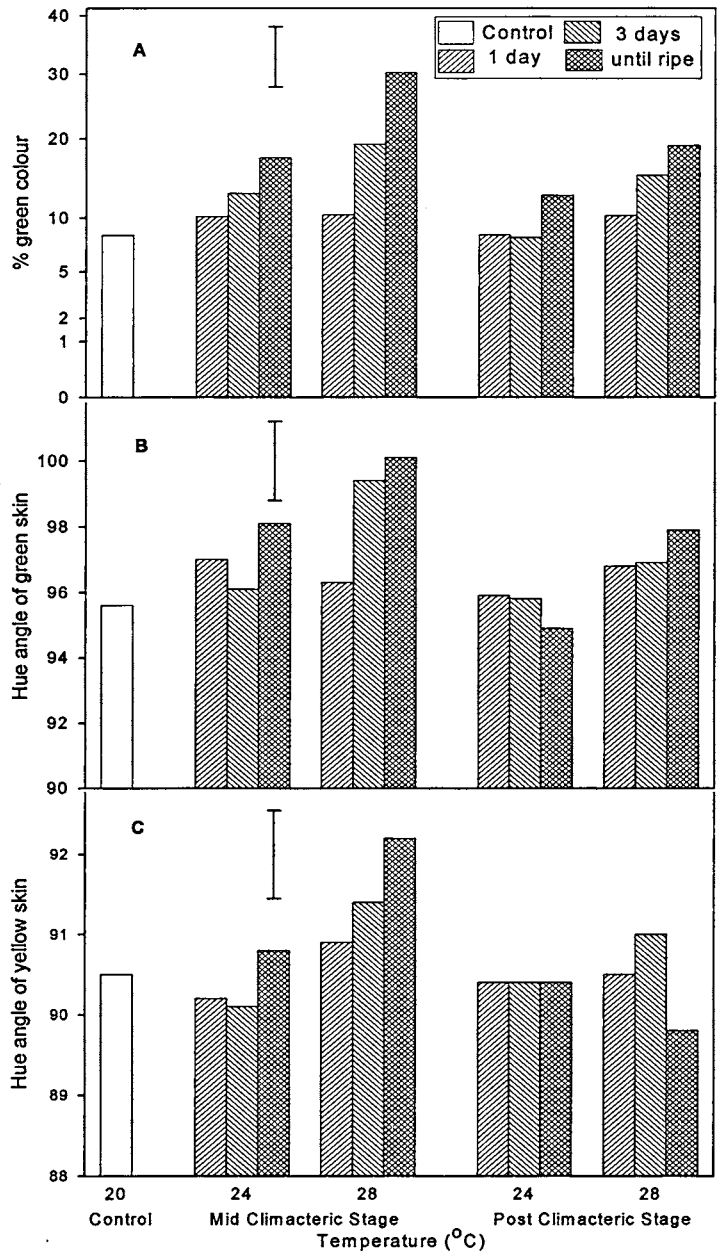


Figure 6.1 Experiment 1. Effect of short term exposure to high temperature on the % green colour (A), the hue angle of the greenest part of the skin (B), and hue angle of the most yellow part of the skin (C) of ripe 'Kensington Pride' mangoes. The y-axis scale of % green colour is angular transformed. Fruit were treated with ethylene for 2 days, then at the mid-climacteric and the post-climacteric stage were transferred to the higher temperatures then placed at 20°C or kept at the same temperatures until ripe. The control fruit were held continually at 20°C. Vertical bars represent LSDs ($P < 0.05$; $n = 45$).

Mid-climacteric fruit held at 24°C until ripe, or 28°C for 3 or more days also had a higher hue angle (more green) of the green skin compared to control fruit, but there were no significant treatment effects in the post-climacteric fruit (Figure 6.1B). Similarly, treatment at 28°C for 3 days or longer resulted in a higher hue angle of the yellow skin in mid-climacteric fruit compared to control fruit (Figure 6.1C), but not in the post-climacteric fruit.

The % red blush colour was not significantly affected by any temperature treatments with an average of 21% across all treatments (data not presented). However, temperatures of 24°C or above for 1 or more days increased the blotchy skin rating, compared to fruit ripened at 20°C (Figure 6.2). In addition, at both stages of ripeness, more blotchiness was noted in fruit held continually at either 24 or 28°C, compared to 1 day at the same temperatures. There was no significant treatment effect of the stage of ripeness.

The above results noted in ripe fruit were also obvious 7 days after harvest (Plates 6.1 and 6.2). At the mid-climacteric stage, fruit held at 28°C, particularly for 3 or more days, had more green colour remaining on the skin than control fruit or fruit held for a period of time (1 or 3 days or until ripe) at 24°C (Plate 6.1). Colour blotchiness is obvious in some fruit at 28°C. Similar treatment effects were observed on post-climacteric fruit 7 days after harvest (Plate 6.2). Generally, post-climacteric fruit had less green colour than mid-climacteric fruit.

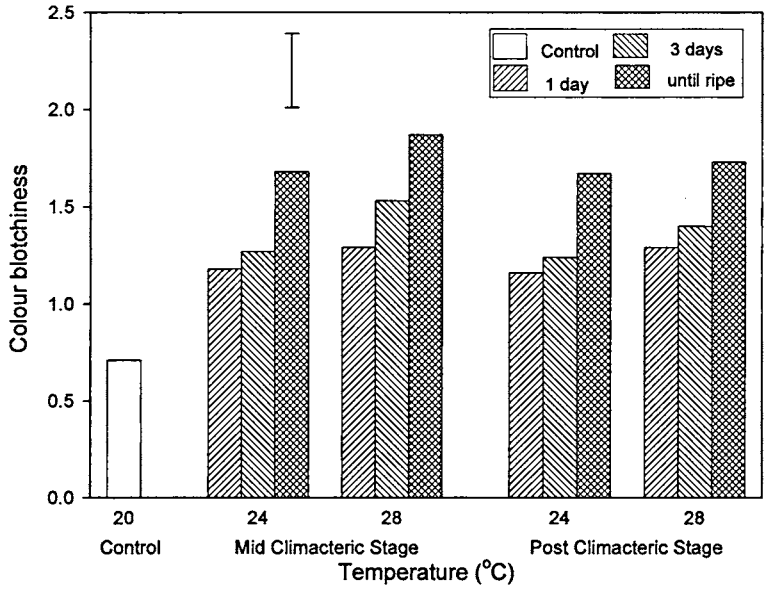


Figure 6.2 Experiment 1. Effect of short term exposure to high temperature on colour blotchiness (0 = no blotchiness to 4 = severe blotchiness) of ripe 'Kensington Pride' mangoes. Fruit were treated with ethylene for 2 days, then at the mid-climacteric and the post-climacteric stage were transferred to the higher temperatures then placed at 20°C or kept at the same temperatures until ripe. The control fruit were held continually at 20°C. Vertical bar represents LSD ($P < 0.05$; $n = 45$).

Days to ripe: Mid-climacteric fruit held at 24 or 28°C for 3 or more days generally had shorter DTR than control fruit, especially when held at these higher temperatures until ripe (Figure 6.3). There was no significant treatment effect when fruit were exposed to 24 or 28°C for 1 day at both ripening stages, compared to the controls. There was little difference between 24 and 28°C for the same duration, or between fruit transferred to the higher temperatures at the mid-climacteric or post-climacteric stage.

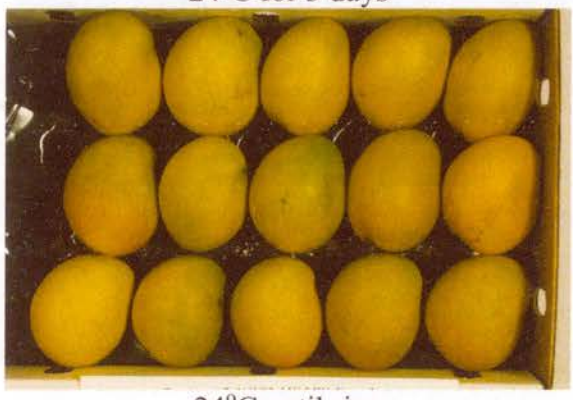
Control fruit (continually at 20°C)

24°C for 1 day

28°C for 1 day

24°C for 3 days

28°C for 3 days



24°C until ripe

28°C until ripe

Plate 6.1 Experiment 1. Effect of short term exposure to high temperatures at the mid-climacteric stage on skin colour of 'Kensington Pride' mangoes 7 days after harvest (with 10 $\mu L\ L^{-1}$ ethylene treated for 2 days).

Control fruit (continually at 20°C)

24°C for 1 day

28°C for 1 day

24°C for 3 days

28°C for 3 days

24°C until ripe

28°C for 3 days

Plate 6.2 Experiment 1. Effect of short term exposure to high temperatures at the post-climacteric stage on skin colour of 'Kensington Pride' mangoes 7 days after harvest (with 10 $\mu L\ L^{-1}$ ethylene treated for 2 days).

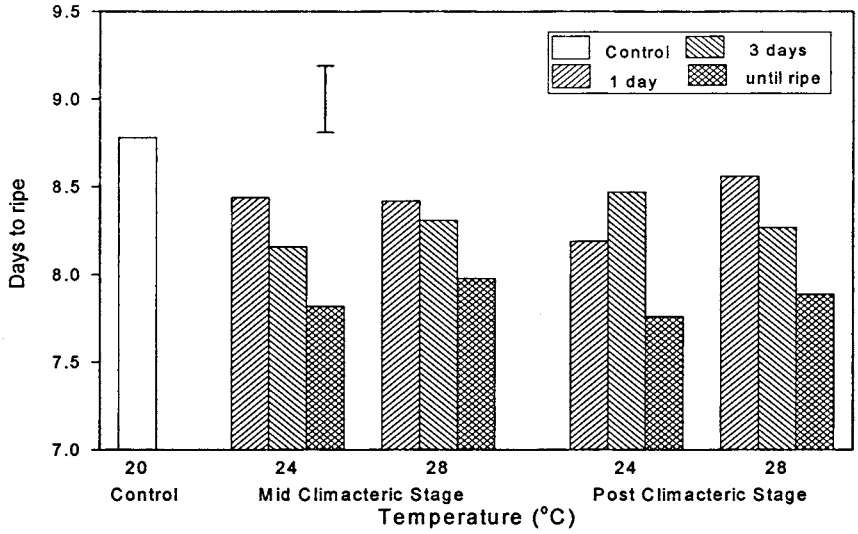


Figure 6.3 Experiment 1. Effect of short term exposure to high temperature on the days from harvest to ripe (DTR) of 'Kensington Pride' mangoes. Fruit were treated with ethylene for 2 days, then at the mid-climacteric and the post-climacteric stage were transferred to the higher temperatures then placed at 20°C or kept at the same temperatures until ripe. The control fruit were held continually at 20°C. Vertical bar represents LSD ($P < 0.05$; $n = 45$).

Diseases: Anthracnose severity was very low in all treatments. There were no significant treatment effects, with an average of 0.6% of the skin area affected across all treatments (data not presented). Fruit were free from SER in all treatments (data not presented).

Other quality attributes: The flesh hue angle of the mid-climacteric fruit was reduced (more yellow) by holding for 3 or more days at 24 or 28°C compared with the controls (Table 6.3). Similar results were noted in the post-climacteric treatments. There were few significant differences between mid-climacteric and post-climacteric treatments held under the same conditions.

Table 6.3 Experiment 1. Effect of short term exposure to high temperature on the flesh hue angle (H°), lenticel spotting (0 = no spotting to 4 = severe spotting), and the flesh TSS and acidity of ripe 'Kensington Pride' mangoes. Fruit were treated with ethylene for 2 days, then at the mid-climacteric and the post-climacteric stage were transferred to the higher temperatures then placed at 20°C until ripe. The control fruit were held continually at 20°C.

Treatment	Flesh H°	Lenticel spotting	TSS (°Brix)	Acidity (%)
Control	87.7 ^{e}	3.20 ^{ab}	14.4	0.29
Mid-climacteric stage				
24°C for 1 day	87.8 ^{e}	3.29 ^{abc}	14.8	0.29
24°C for 3 days	86.8 ^{cd}	3.02 ^{a}	14.7	0.25
24°C until ripe	86.5 ^{bcd}	3.54 ^{c}	14.4	0.21
28°C for 1 day	87.0 ^{de}	3.11 ^{a}	14.1	0.25
28°C for 3 days	86.2 ^{abcd}	3.24 ^{abc}	14.9	0.20
28°C until ripe	85.5 ^{a}	3.51 ^{bc}	14.6	0.18
Post-climacteric stage				
24°C for 1 day	86.8 ^{cd}	3.16 ^{a}	15.1	0.23
24°C for 3 days	86.3 ^{abcd}	3.20 ^{ab}	15.0	0.21
24°C until ripe	86.6 ^{cd}	3.11 ^{a}	14.7	0.21
28°C for 1 day	86.8 ^{cd}	3.04 ^{a}	14.9	0.23
28°C for 3 days	85.9 ^{abc}	3.56 ^{c}	15.4	0.22
28°C until ripe	85.6 ^{ab}	3.51 ^{bc}	14.3	0.20
LSD	0.89	0.34	n.s	n.s

Means followed by the same letter in each column for each parameter are not significantly different at $P < 0.05$ (n = 45). n.s = not significant.

Mid-climacteric fruit held continually at 24°C or 28°C until ripe and post-climacteric fruit held at 28°C for 3 or more days had more lenticel spotting than control fruit (Table 6.3). There were no significant differences in lenticel spotting between other treatments and the control. The flesh TSS and acidity were not influenced by treatment.

6.3.2 Experiment 2: Short Term Exposure to Low Temperature

Skin colour: With the mid-climacteric treatment, the % green colour on ripe fruit was higher than the controls when fruit were held at 7°C for 3 or more days or at 10°C for 7 days (Figure 6.4A). The fruit were less sensitive at the post-climacteric stage, with only 7°C for 7 days resulting in more % green area on the skin compared with the controls (ripened at 20°C). There were no significant treatment effects at 10 and 13 °C.

The hue angle of the green skin showed similar responses to % green colour (Figure 6.4B). Both mid-climacteric and post-climacteric fruit held at 7°C for 7 days had higher hue angle (more green intensity) compared to the control. There were no significant differences in hue angle of the yellow skin between control and treatment fruit (Figure 6.4C). However, post-climacteric fruit held at 7°C for 7 days had higher hue angle compared with those held for 1 day.

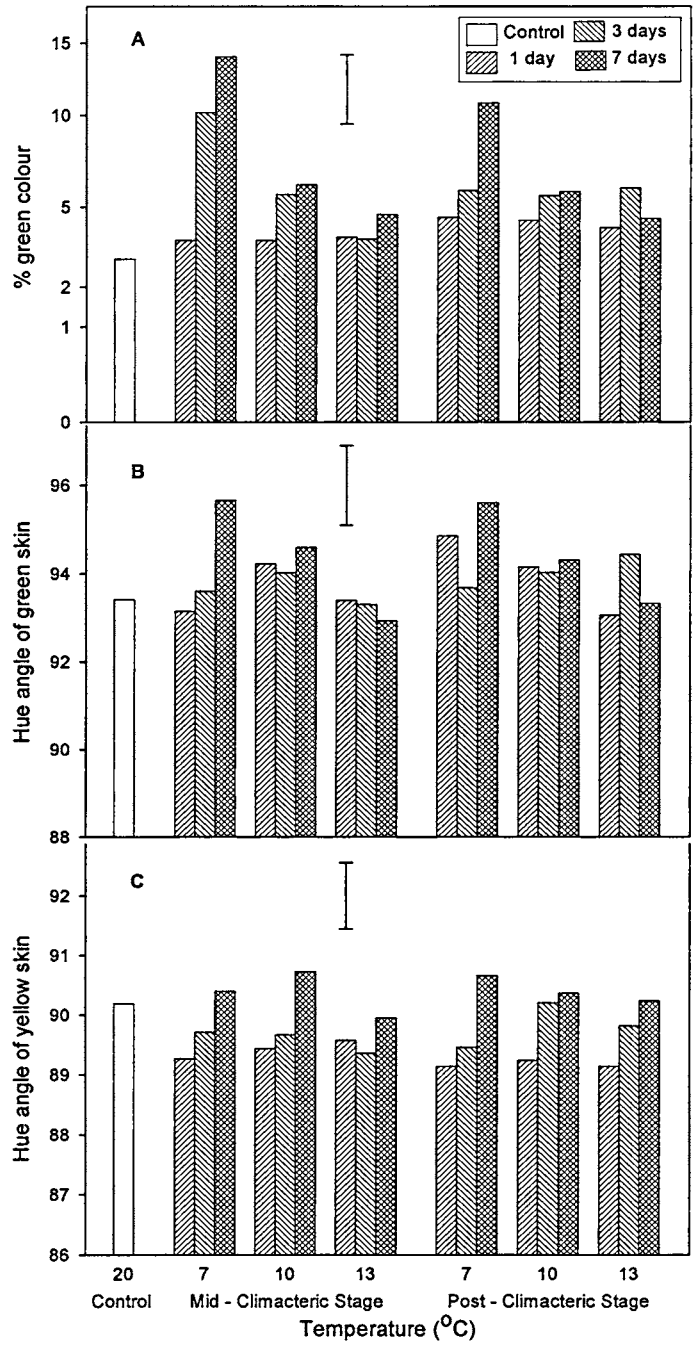


Figure 6.4 Experiment 2. Effect of short term exposure to low temperatures on the % green colour (A), the hue angle of the greenest part of the skin (B) and the hue angle of the most yellow part of the skin (C) of ripe 'Kensington Pride' mangoes. The y-axis scale of % green colour is angular transformed. Fruit were treated with ethylene for 1.5 days, then at the mid-climacteric and the post-climacteric stage were transferred to the lower temperatures then placed at 20°C until ripe. The control fruit were held continually at 20°C. Vertical bars represent LSDs ($P < 0.05$; $n = 60$).

Most of the low temperature treatments resulted in a duller colour than control fruit (Figure 6.5A). In particular, treatment at 7°C for 3 or more days at both the mid-climacteric and post-climacteric stage resulted in duller skin colour compared to the controls and most of the other treatments.

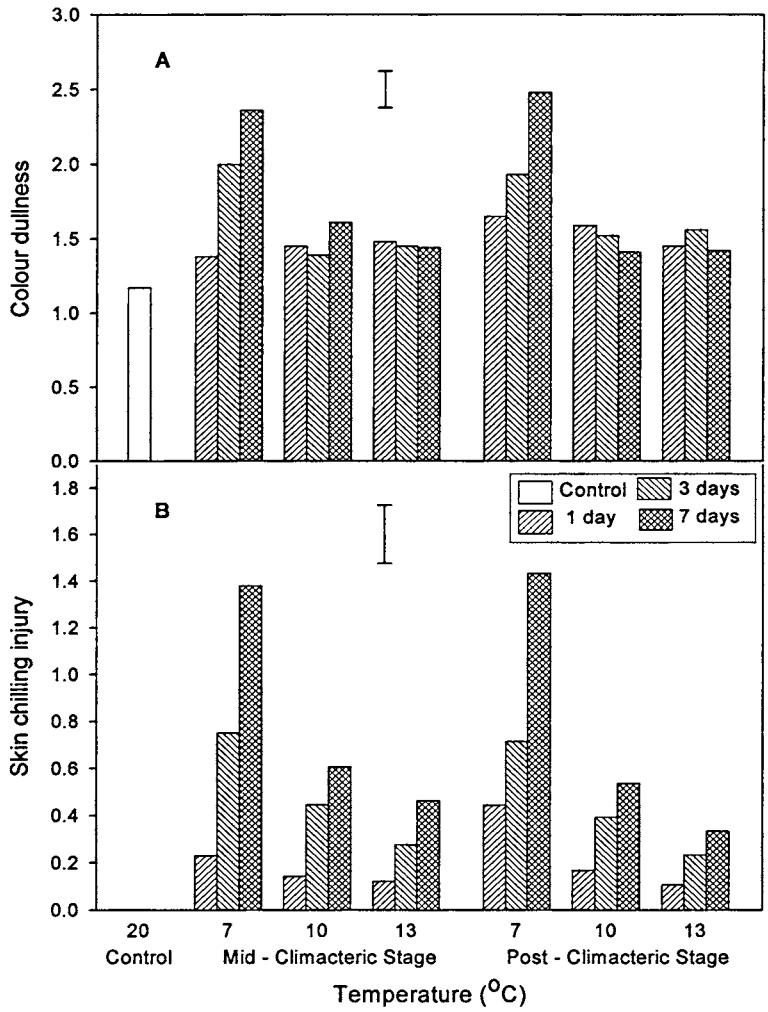


Figure 6.5 Experiment 2. Effect of short term exposure to low temperature on colour dullness (0 = no dullness to 4 = full dull; A) and skin chilling injury (0 = no injury to 4 = extreme injury; B) of ripe 'Kensington Pride' mangoes. Fruit were treated with ethylene for 1.5 days, then at the mid-climacteric and the post-climacteric stage were transferred to the lower temperatures then placed at 20°C until ripe. The control fruit were held continually at 20°C. No bar indicates nil chilling injury. Vertical bars represent LSDs ($P < 0.05$; $n = 60$).

There was no skin chilling injury on control fruit (ripened at 20°C) (Figure 6.5B). In both mid and post-climacteric fruit, skin chilling injury was higher at 7 or 10°C for 3 or more days, and at 13°C for 7 days, compared with the control. The highest skin chilling injury severity occurred at 7°C for 7 days in both the mid and post-climacteric fruit. The symptoms of skin chilling injury are shown in Plate 6.3.

Plates 6.4 and 6.5 show the skin colour of 'Kensington Pride' mangoes 7 days after harvest, when exposed to low temperatures for 1 or 3 days at different ripening stages. Fruit exposed to low temperatures at the mid-climacteric (Plate 6.4) or post-climacteric stage (Plate 6.5) had more green colour than the control fruit. In general, mid-climacteric fruit were greener than post-climacteric fruit at 7 days. Lower temperatures or longer holding times appeared to result in more green colour on the skin.

At 13 days from harvest, there appeared to be more green colour on fruit held at 7°C for 7 days at both the mid- and post-climacteric stage compared to fruit held at 10 and 13°C (Plate 6.6). In addition, pre-climacteric fruit held at 7°C for 7 days appeared to have more green colour than post-climacteric fruit held at 7°C for the same duration.

Days to ripe: There were no treatment effects on DTR with 1 day at 7-13°C, compared with the control (Figure 6.6). However, exposure for 3 or more days at 7 and 10°C or 7 days at 13°C reduced DTR. There was no treatment effect of the stage of ripeness at the same holding conditions on the DTR.

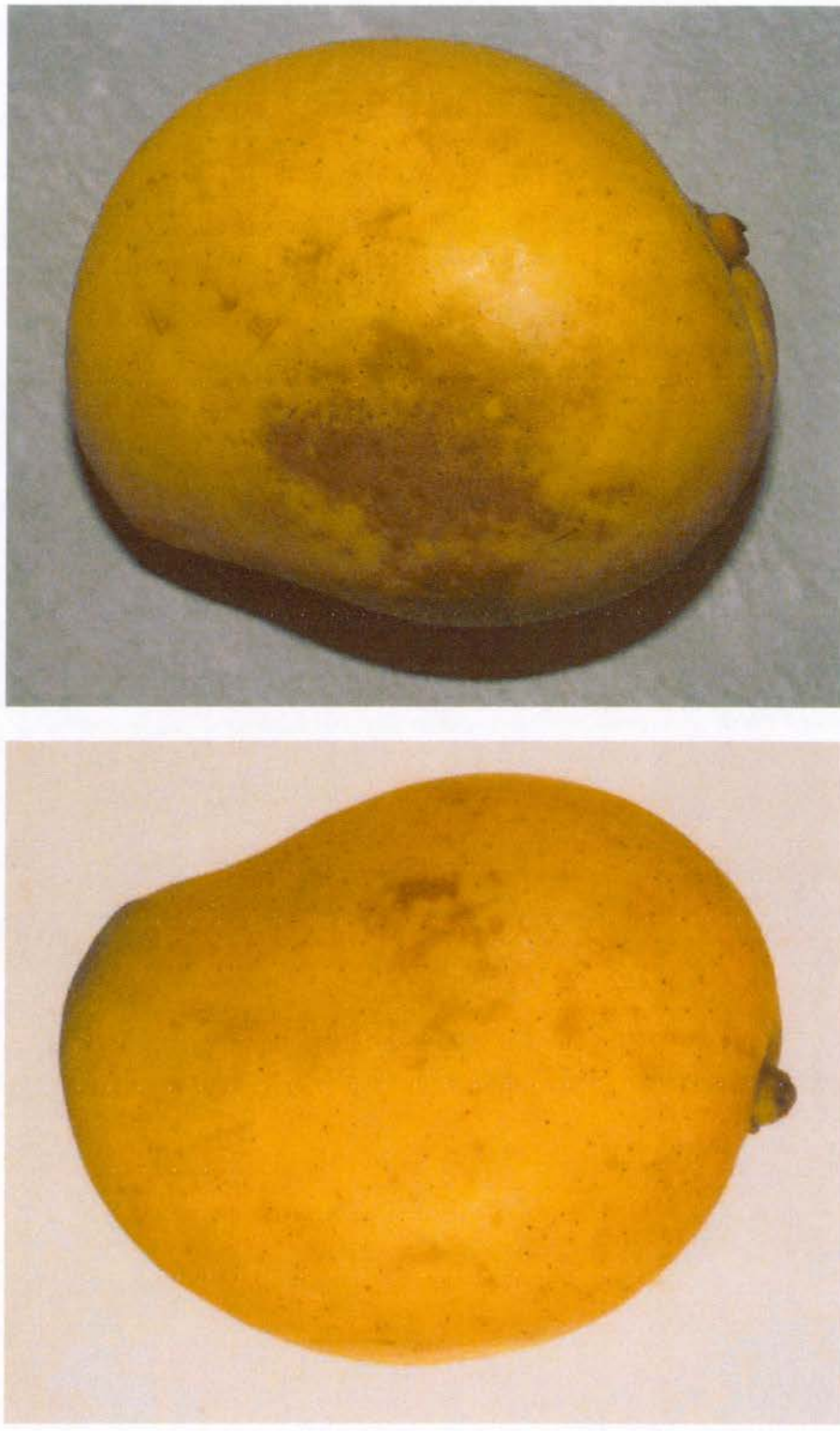


Plate 6.3 Experiment 2. The symptoms of the skin chilling injury of 'Kensington Pride' mangoes when short term exposed to low temperatures.

Control fruit (continually at 20°C)

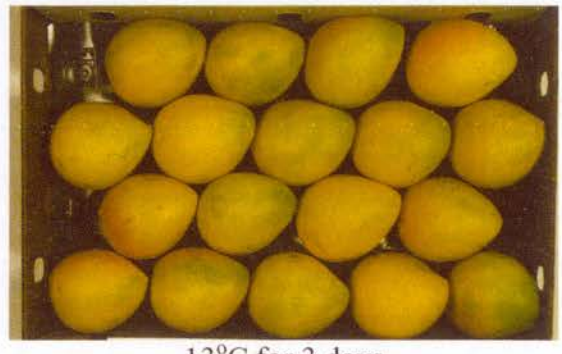
7°C for 1 day

7°C for 3 days

10°C for 1 day

10°C for 3 days

13°C for 1 day



13°C for 3 days

Plate 6.4 Experiment 2. Effect of short term exposure (1 or 3 days) to low temperatures at the mid-climacteric stage on skin colour of 'Kensington Pride' mangoes 7 days after harvest (with 10 μL L^{-1} ethylene treated for 1.5 days).

Control fruit (continually at 20°C)

7°C for 1 day

7°C for 3 days

10°C for 1 day

10°C for 3 days

13°C for 1 day

13°C for 3 days

Plate 6.5 Experiment 2. Effect of short term exposure (1 or 3 days) to low temperatures at the post-climacteric stage on skin colour of 'Kensington Pride' mangoes 7 days after harvest (with 10 μL L^{-1} ethylene treated for 1.5 days).

7°C for 7 days at MC

7°C for 7 days at PC

10°C for 7 days at MC

10°C for 7 days at PC

13°C for 7 days at MC

13°C for 7 days at PC

Plate 6.6 Experiment 2. Effect of short term exposure (7 days) to low temperatures at the mid-climacteric (MC) and post-climacteric (PC) stage on skin colour of 'Kensington Pride' mangoes 13 days after harvest (with 10 µL L^{-1} ethylene treated for 1.5 days).

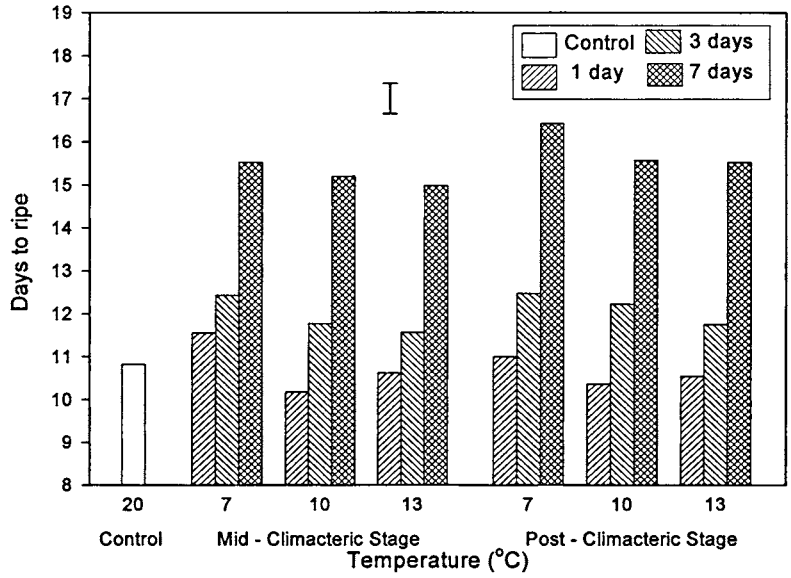


Figure 6.6 Experiment 2. Effect of short term exposure to low temperature on the days from harvest to ripe (DTR) of 'Kensington Pride' mangoes. Fruit were treated with ethylene for 1.5 days, then at the mid-climacteric and the post-climacteric stage were transferred to the lower temperatures then placed at 20°C until ripe. The control fruit were held continually at 20°C. Vertical bar represents LSD ($P < 0.05$; $n = 60$).

Diseases: Post-climacteric fruit held at 13°C for 7 days had more anthracnose infection compared with control fruit (Figure 6.7A). There were no significant differences in severity of anthracnose between other treatments and control fruit. However, holding fruit for 7 days resulted in higher anthracnose severity in mid-climacteric fruit held at 10°C and post-climacteric fruit held at 13°C compared with the control. SER severity was low (less than 1%) in all treatments (Figure 6.7B). There was no treatment effect in mid-climacteric fruit, while post-climacteric fruit had higher SER severity when held at 10 and 13°C for 7 days, compared to the control.

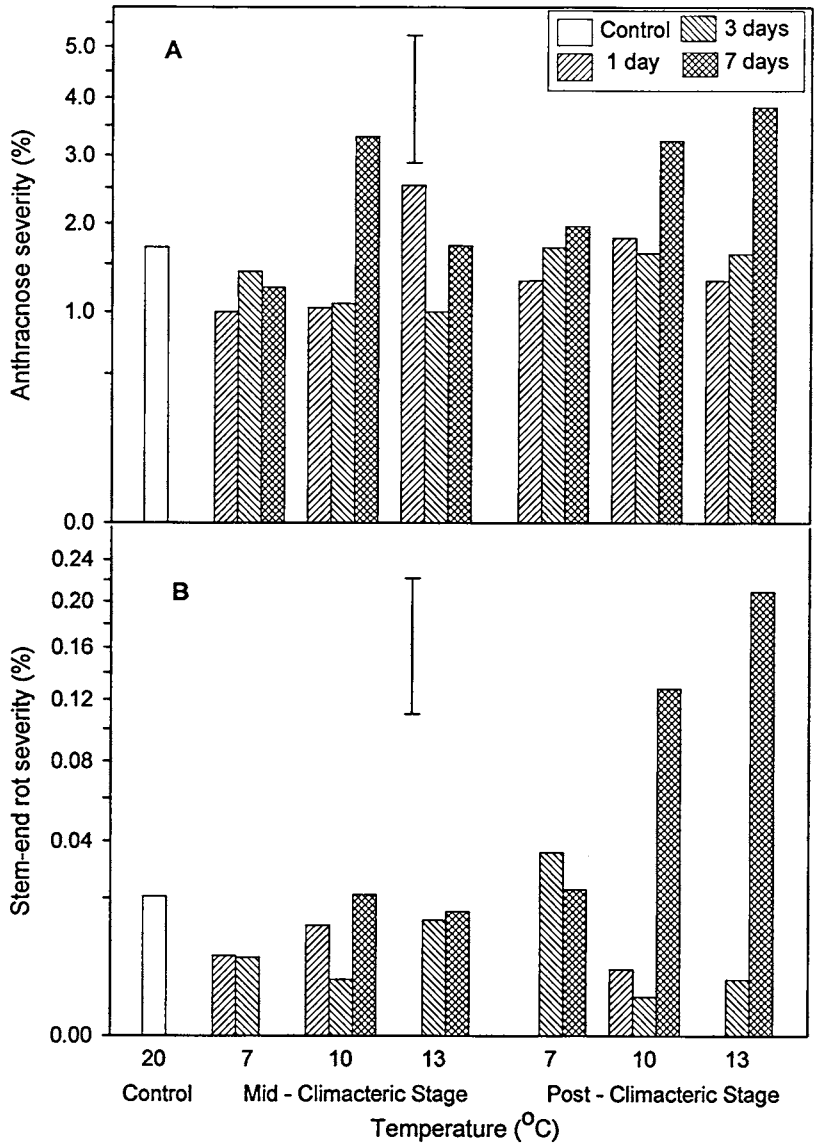


Figure 6.7 Experiment 2. Effect of short term exposure to low temperature on the severity of anthracnose (% skin area affected; A) and stem-end rots (% flesh volume affected; B) of ripe 'Kensington Pride' mangoes. Fruit were treated with ethylene for 1.5 days, then at the mid-climacteric and the post-climacteric stage were transferred to the lower temperatures then placed at 20°C until ripe. The control fruit were held continually at 20°C. The y-axis scales are angular transformed. Vertical bars represent LSDs ($P < 0.05$; $n = 60$).

Other quality attributes: The flesh hue angle was generally higher in the mid and post-climacteric fruit held for 7 days at 7-13°C, compared with the control and 1 day treatments (Table 6.4). In most cases, the 3 day duration resulted in a higher hue angle compared with the control fruit in both mid and post-climacteric treatments, but not with the 1 day duration. There were no significant differences in flesh hue angle between the 3 day treatments at both ripening stages.

Fruit in all treatments had high lenticel spotting (greater than 2 on the 0-4 scale). Lenticel spotting severity was significantly higher in fruit held at 7°C for 3 or more days, compared with the controls (Table 6.4). There was little consistent treatment effect on lenticel spotting in the 10 and 13°C treatments for both mid and post-climacteric fruit.

The flesh TSS and acidity were not affected by the treatments, with average means of 15.6 °Brix and 0.26% acidity (Table 6.4).

Table 6.4 Experiment 2. Effect of short term exposure to low temperature on the flesh hue angle (H°), lenticel spotting (0 = no spotting to 4 = severe spotting), and the flesh TSS and acidity of ripe 'Kensington Pride' mangoes. Fruit were treated with ethylene for 1.5 days, then at the mid-climacteric and the post-climacteric stage were transferred to the lower temperatures then placed at 20°C until ripe. The control fruit were held continually at 20°C.

Treatment	Flesh H°	Lenticel spotting	TSS (°Brix)	Acidity (%)
Control	86.6 ^{ab}	2.60 ^{abcd}	15.6	0.18
Mid-climacteric stage				
7°C for 1 day	87.0 ^{abc}	2.84 ^{cde}	15.7	0.21
7°C for 3 days	87.8 ^{cde}	3.43 ^{fg}	15.4	0.31
7°C for 7 days	89.2 ^{f}	3.28 ^{f}	15.3	0.46
10°C for 1 day	87.6 ^{bcd}	2.48 ^{ab}	15.7	0.26
10°C for 3 days	87.8 ^{cde}	2.91 ^{e}	15.3	0.26
10°C for 7 days	88.7 ^{ef}	2.52 ^{abc}	15.8	0.27
13°C for 1 day	87.0 ^{abc}	2.81 ^{cde}	15.7	0.21
13°C for 3 days	87.7 ^{cd}	2.97 ^{e}	15.8	0.30
13°C for 7 days	88.7 ^{ef}	2.85 ^{cde}	15.6	0.25
Post-climacteric stage				
7°C for 1 day	87.2 ^{abc}	2.82 ^{de}	15.2	0.22
7°C for 3 days	87.5 ^{bcd}	3.50 ^{fg}	15.9	0.31
7°C for 7 days	88.4 ^{cdef}	3.63 ^{g}	15.3	0.24
10°C for 1 day	86.2 ^{a}	2.76 ^{bcde}	15.9	0.26
10°C for 3 days	88.0 ^{cde}	2.61 ^{abcd}	15.7	0.28
10°C for 7 days	88.5 ^{def}	2.32 ^{a}	15.9	0.22
13°C for 1 day	86.6 ^{ab}	2.77 ^{bcde}	15.1	0.22
13°C for 3 days	87.6 ^{bcd}	2.48 ^{ab}	15.7	0.26
13°C for 7 days	87.9 ^{cde}	2.52 ^{ab}	15.5	0.25
LSD	1.0	0.29	n.s	n.s

Means followed by the same letter in each column for each parameter are not significantly different at $P < 0.05$ (n = 60). n.s = not significant.

6.4 DISCUSSION

The results suggest that the fruit quality of 'Kensington Pride' mangoes can be reduced by short term exposure to higher or lower temperatures than the optimum recommended ripening conditions (18-22°C). However, there is some tolerance to these sub-optimal conditions, suggesting that some of the conditions observed during commercial transport and ripening (Holmes and Kernot, 2002) may not have a detrimental effect on fruit quality. Therefore, exposures to 24°C for no more than 3 days and 28°C for no more than 1 day at the mid-climacteric stage generally will not cause any loss of quality. In addition, holding ripening fruit at 7°C for no more than 1 day, 10°C for no more than 3 days and 13°C for 7 days will delay the DTR with little negative effect on quality.

In experiment 1, the higher % of the skin area with green colour observed after 24 and 28°C holding is likely to be due to the retention of chlorophyll in the fruit skin (Kalra and Tandon, 1983; Medlicott *et al.*, 1986a). Pigment metabolism, which influences skin colour development, is temperature dependent in a number of fruits. The de-greening process is inhibited above 30°C in bananas (Yoshioka *et al.*, 1978; Seymour *et al.*, 1987; Blackbourn and John, 1989). Papaya held for 10 days at 32.5°C failed to develop an even yellow colour because of a retention of green colour, and a temperature range of 22.5-27.5°C was suggested for optimum skin colour development (An and Paull, 1990). In apple, the rate of chlorophyll loss in the fruit skin was greatest at the optimum ripening temperature of 20-24°C (Dixon and Hewett, 1998). The rate of chlorophyll loss was lower if the ripening temperature was higher or lower than the optimum range, resulting in greener coloured fruit. In tomato, the rate of

chlorophyll loss during ripening after short term exposure to high temperature was less than that observed at the optimum ripening temperature of 21°C, which resulted in more green colour on the ripe fruit (Sozzi *et al.*, 1996). Furthermore, lycopene accumulation was also reduced by exposure to temperatures of 36°C or above for 48 h (Sozzi *et al.*, 1996).

In mangoes, the response of the de-greening process in the skin to temperature can be influenced by cultivar. Florida mango cultivars developed a mottled green skin colour (uneven colour development or blotchiness, which is a symptom of inhibited de-greening) when ripened at 26.2-32.2°C (Hatton *et al.*, 1965), while this did not occur in 'Keitt' or 'Kent' mangoes at 26.7°C. Mottled colour was observed in 'Tommy Atkins' ripened at 37°C, but not in 'Kent' or 'Haden' mangoes (Medlicott *et al.*, 1986b).

In 'Kensington Pride' mango, the optimum ripening temperature is within the range of 18-22°C (O'Hare, 1995). In experiment 1, uneven colour development (blotchiness) with short term exposure to 24-28°C was also observed, suggesting that 'Kensington Pride' is susceptible to similar ripening disorders as the cultivars mentioned above. The uneven de-greening may be related to the partial inhibition of the chlorophyll degradative pathway. Enhanced breakdown of galactolipids can occur at high temperatures, which leads to a loss of membrane structure and disrupting the spatial arrangement between the degradative system and chlorophyll complexes (Blackbourn and John, 1989). Short period exposure to high temperature has reduced of both □- and □-galactosidase (□- and □-Gal) activities during tomato ripening, and □-Gal is a key factor in galactolipid degradation (Sozzi *et al.*, 1996). Furthermore, the failure of fruit to ripen normally at high temperatures can be related to the reduction of ethylene production and respiration rate at these temperatures (Eaks, 1978; Lee

and Young, 1984; Antunes and Sfakiotakis, 2000). High temperature stress inhibits 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) more than 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) (Yu *et al.*, 1980; Field, 1985), and ACC synthase is thought to recover faster than ACC oxidase activity after removal from high temperature stress (Biggs *et al.*, 1988).

Generally, holding mid-climacteric fruit at the higher temperatures resulted in greener fruit than those held at the post-climacteric stage. These results can be explained by the fact that chlorophyll concentration decreases in the mango skin during ripening (Medlicott *et al.*, 1986a; Chapter 5). At the mid-climacteric stage, the fruit are still firm and the chlorophyll concentration (and % green colour) in the skin is higher than in post-climacteric fruit. Therefore, at the post-climacteric stage there is less potential for treatment differences in relation to green skin colour to develop.

In addition to fruit skin colour, the results in Experiment 1 indicate that short term exposure to high temperatures, especially for 3 or more days could lead to shorter shelf life (DTR). Exposure to high temperature can result in an increased respiration rate and reduced storage time for many fruits (Biale and Young, 1981; Kays, 1991).

The absence of a treatment effect on diseases in this experiment may be related to the low disease severity in all fruit, due to a seasonal effect and/or good hot fungicide treatment at the packinghouse.

In Experiment 2, the higher % green area in fruit held at 7°C for 3 days or longer and 10°C for 7 days or longer was similar to the higher green colour noted in 'Kensington Pride' mango (O'Hare, 1995), and in apple (Dixon and Hewett, 1998) when these fruit were ripened at sub-optimal temperatures. This was attributed to a decrease in the rate of chlorophyll degradation and in carotenoids synthesis in mangoes. However, in their experiments fruit were continually ripened at low temperature, while fruit in our experiment were exposed for a short period. It suggests that the low temperature effect on % green colour can be permanent after only short durations.

Generally, holding fruit at low temperature can increase the shelf life. However, exposure to excessively low temperature or for too long can negatively affect quality. For example, in mango the ripe fruit can have more green colour on the skin (O'Hare, 1995) and higher chilling injury (Veloz *et al.*, 1977). Symptoms of chilling injury in mango stored below 10°C include a greyish scald-like discolouration of the skin and skin pitting, as well as uneven ripening, reduction in carotenoids, aroma and flavour (Hatton *et al.*, 1965; Abou-Aziz *et al.*, 1976; Thomas and Oke, 1983; Mohammed and Brecht, 2000). The greyish scald-like discolouration was also noted in experiment 2, but severity was low and limited only to the fruit skin. There was no evidence of off-flavour or eating quality (data not presented) of fruit held at 7, 10 or 13°C for 7 days at both mid- and post-climacteric stages. However, if fruit were held at 7°C for 2 weeks at either ripening stages, the symptoms of chilling injury were very severe with most fruit suffering high level of chilling injury (data not presented). This is different to the findings of McLauchlan and Wells (1994) with 'Kensington Pride' fruit where chilling injury was not evident in fruit held at 7°C for 2 weeks. This difference may be because in their trials, fruit were held at low temperature immediately after harvest when

fruit were still firm, while in our trial, fruit were treated with ethylene at 20°C and were already in mid- and post-climacteric stages, before storage. In addition, the dull colour, which is associated with low carotenoids production (O'Hare, 1995), was also observed in our experiments.

The higher disease severity in post-climacteric fruit held at 13°C for 7 days suggests that the fungi are still active at 13°C, and/or that fungal growth continues at a faster rate than the fruit softening during storage and ripening. This does not appear to be the case at 7 and 10°C. Similar results have been reported in guava, where temperatures below 10°C completely suppressed anthracnose disease development (Singh and Daulta, 1983). In addition, the higher anthracnose and SER severity in post-climacteric fruit held at 13°C for 7 days may be partly due to these fruit having a longer DTR than the control fruit. Similar observations have been made in avocado, where body rot and SER severity is higher in fruit that are cold stored for long periods (Hopkirk *et al.*, 1994).

CHAPTER SEVEN

GENERAL DISCUSSION AND CONCLUSIONS

The presence of green colour on the skin of ripe 'Kensington Pride' mango fruit is a significant commercial problem (Hofman *et al.*, 1997a), since it reduces the visual appeal, and potentially the market value. This problem is also evident in other mango cultivars such as 'Sensation', 'Keitt' and 'Kent' (McKenzie, 1994). The continued increase in Australian mango production has increased the need to expand existing markets and establish new ones, which often requires consistent and high quality fruit. Therefore, our experiments investigated how field pre-harvest N applications and postharvest practices (ripening temperature, ethylene and CO_2 concentration) influence the skin colour and other quality attributes of ripe 'Kensington Pride' mango fruit.

The survey (Chapter 4) confirmed that green, ripe fruit is a serious problem of 'Kensington Pride' mango in the Burdekin district, a major mango production area in Australia. Seventy percent of surveyed orchards with different soil types had more than 25% green colour remaining on ripe fruit, with one orchard showing more than 40%. The % green colour was highly related to the hue angle of the greenest part of the skin and total chlorophylls retained in the skin of the greenest part. This suggests that fruit with higher % green area on the skin also had a more intense green colour of the affected skin. The survey results also indicated that the retention of green colour may be related to the history of N fertilization since most of orchards with high N status had a high % green colour. The survey also indicated that trees on sandy loam soil were more likely to produce fruit with less green colour than those on higher clay soils. However, green, ripe fruit were produced on trees grown on both soil types,

suggesting that other factors, such as previous N history, are also important. It is likely that the clay soils retain more of the N applied, so that the effects on skin colour were more long lasting in trees on these soils, compared to those on sandy soils. There is a need for more research on this area to confirm the relation between soil type, N application and green, ripe fruit.

SER was also a quality issue for Burdekin mango fruit in the 99/00 season, with 30% of surveyed orchards having 5% or more of flesh volume affected when ripe. Soil type may also influence postharvest SER incidence since SER severity was very low in orchards on sandy soil. Fruit with high SER severity also had more green colour, but the relation between SER and skin colour requires further investigation.

Further investigations of the relationship between pre-harvest N application and mango quality (Chapter 4) confirmed that N application can cause greener fruit. This finding is similar to that found in apple (Neilsen *et al.*, 1984; Fallahi *et al.*, 1985a). However, the response of the % green on ripe fruit to N application rates was different in fruit from HG orchard compared with those from LG orchards. In the second experiment, the magnitude of the response to the same N applied to the soil was less for fruit from the HG orchard than from the LG orchards. The results suggest that the HG orchard already had higher N in soil and leaves that caused the higher % green colour, so that added N had less effect than in the LG, where presumably N was less. In contrast, foliar treatment in HG orchard had more affect on green colour than that in LG orchards. Foliar N treatment is believed to affect green colour in apple fruit more than soil N application (Meheriuk *et al.*, 1996).

As fruit ripen, the loss of green colour and the increase in yellow colour are associated with the loss of total chlorophylls and the gain of total carotenoids in the fruit skin. This is similar to the results on cv.'Tommy Atkin' by Medlicott *et al.* (1986a). While total chlorophylls were significantly higher in high N fruit when approaching eating soft, there were no differences in total carotenoids between high N and control fruit at all ripening stages. The higher retention of chlorophylls (green colour) in the skin of high N fruit may be explained by lower chlorophyllase activity. Chlorophyllase activity is reported to be lower in the skin of green mango cultivars in Thailand (Ketsa *et al.*, 1999a). In addition, the sudden appearance of red blush colour may be due to "unmasking" of the red pigments as a result of chlorophyll breakdown rather than anthocyanin synthesis, as was also suggested by Medlicott *et al.* (1986a) in 'Tommy Atkins' mango. These results suggest that the more green colour on ripe 'Kensington Pride' fruit from high N treated trees is due to the higher concentration of total chlorophylls in the skin, rather than lack of carotenoids synthesis.

In addition to affecting green colour on ripe fruit, high N rates were also associated with higher postharvest disease severity. This may be due to a reduction in the concentration of some phenolics, lignin and silicon in plant tissues which are important components of the defense mechanism against pathogens (Matsuyama amd Diamond, 1973; Menzies *et al.*, 1991; Stange and McDonald, 1999). In addition, high N (ammonium) can result in Ca deficiency in the fruit and reduce the resistance to disease (Sugar *et al.*, 1992; Sugar, 2002). The effects of N on disease susceptibility, including the possible interactions with Ca, and the yield/quality balance, require further investigation.

The present results show that fruit yield was not significantly improved if pre-harvest N application is increased in both categories of orchards at two different timings. However, reducing N eventually results in decreased yield in other species such as avocado (Lovatt, 2001). The reasons for no significant effect on yield in our experiments may be due to the large variation in fruit yield between trees. Therefore, there is a need for more research in this area.

The retention of green colour on the skin of ripe 'Kensington Pride' mangoes can also be influenced by postharvest practices (Chapter 5 and 6). During mango ripening, many changes occur, including colour, softening and chemical changes such as reduction in flesh acidity and increases in sugars. The present results indicate that these changes are influenced by postharvest factors such as ripening temperature, ethylene and CO_2 concentration, and that the relative rate of these processes can be affected by ripening conditions. This results in varying quality of ripe fruit.

The combination of ripening temperature, and ethylene concentration and duration all affected the % green colour on the ripe 'Kensington Pride' fruit (Chapter 5). High ethylene concentrations (up to 1000 $\mu L\ L^{-1}$) for a long period of 72 h at 15°C caused softening to occur more quickly than colour change, resulting in soft, green fruit. A similar effect occurred with the flesh acidity, since the fruit were eating soft when the flesh acidity was still high. Therefore, a combination of low temperature and high ethylene appeared to cause a "separation" of the ripening processes, with ethylene having more effect on the softening process than de-greening and acidity reduction. Therefore, we recommend avoiding the use of ethylene treatment at low temperature to reduce the green colour of ripe fruit.

At 20 and 25°C, most ethylene treatments reduced the % green area on ripe fruit. This may be related to chlorophyllase activity since chlorophyllase activity in the skin of ethylene-treated fruit was higher than control fruit 2 days after treatment. A similar result was reported in citrus by Trebitsh *et al.* (1993). At 20-25°C, the relative changes in colour and softening appeared to be closer compared with at 15°C. However, even at 20-25°C, the coordination of these processes is still dependent on ethylene concentration and duration, since 10 μL L^{-1} ethylene for 72 h resulted in less % green compared to untreated fruit. Using low ethylene concentration over a long period indicates that ethylene may play a continuing role in regulating the de-greening process in mango rather than simply being a trigger.

Treatment with high ethylene concentration (100 μL L^{-1} or 1000 μL L^{-1}) for 72 h at 25°C resulted in blotchy skin. This "abnormal" colour development is associated with a suppression of chlorophyll breakdown due to high temperature or combination of high ethylene concentration and high temperature and is not related to carotenoids synthesis (Seymour *et al.*, 1987a; Seymour *et al.*, 1987b). This supports the argument (Chapter 4) that chlorophylls degradation is more important than carotenoids synthesis in mango de-greening during ripening.

At 20°C, fruit had less green colour than at 15°C, and less blotchiness severity than at 25°C. Therefore, of the temperatures tested, 20°C resulted in the least green colour, and lowest blotchiness, which agrees with findings by other researchers that 18-22°C was the optimum range for ripening of 'Kensington Pride' mango (Jobin-Décor, 1988, O'Hare, 1995).

In addition to ethylene and temperature, increased CO_2 concentrations in the ripening room can increase the % green area of ripe fruit (Experiment 3), possibly through the same mechanisms as temperature and ethylene (allowing softening to occur at a relatively faster rate than colour change). The retention of green colour under high CO_2 concentrations is also associated with inhibited chlorophylls degradation. In addition high CO_2 concentrations are thought to inhibit ethylene production by inhibiting ACC oxidase (Bender *et al.*, 2000), which may further "separate" the softening and colour development processes. In addition, the colour dullness observed at high CO_2 concentrations may be due to both low chlorophylls degradation and low carotenoids synthesis as noted under low ripening temperatures (O'Hare, 1995).

The present results indicate that, although there was some tolerance to variable temperatures during storage and transportation, adverse temperatures can influence 'Kensington Pride' mango fruit quality (Chapter 6). Surveys of commercial mango consignments in Australia showed that temperatures greater than 24°C are not unusual (Holmes and Kernot, 2002) and the results indicate that these conditions could contribute to the poor mango skin colour often seen at the markets. Fruit at the mid-climacteric stage of ripening were more sensitive to temperatures above the optimal range than those at the post-climacteric stage. This may be because mid-climacteric fruit are more metabolically active than post-climacteric fruit. Fruit exposed to higher or lower temperatures than the optimal for short durations can have more green colour on the ripe fruit due to lower chlorophylls degradation rate, compared to continually holding at the optimum temperature. Similar results have been reported in apple (Dixon and Hewett, 1998). In addition to more green colour, other disorders such as colour blotchiness, dullness and skin chilling injury were observed when fruit were held outside the

optimal range. This suggests that some fruit quality attributes were permanently affected by a short term exposure to these temperatures.

In conclusion, the results clearly demonstrate that increased field N application can increase the green, ripe fruit problem in 'Kensington Pride' mangoes. In addition, high N can increase fruit diseases. The negligible effect on other quality attributes suggests that evaluation of N effects within the ranges tested need only consider skin colour and diseases. The absence of a significant effect on yield needs to be studied further with a view to determining the optimal yield/quality balance for each growing environment and market situation. For example, other factors such as N run-off into the environment need to be considered. 'Kensington Pride' mango quality can also be manipulated through postharvest factors such as ripening temperature, ethylene treatment and atmospheric composition. A ripening temperature of about 20°C, with an ethylene concentration of about 10 $\mu L\ L^{-1}$ for as long as 3 days, will reduce the green colour and can counteract the negative effects of growing conditions. Increasing the ethylene concentration to 100 $\mu L\ L^{-1}$ or higher can increase the rate of ripening, which is sometimes a desirable commercial outcome. However, this is best achieved by increasing the duration of exposure rather than increasing the ethylene concentration, since the latter can increase the % green skin and disease severity. Conversely, delayed ripening should be achieved by holding the fruit at temperatures below 13°C, then ripening them at about 20°C with ethylene, rather than ripening at 15°C. In addition, regular ventilation is needed in the ripening room to keep the CO_2 concentration below 4%. Temperatures above 24°C for 3 days or more and temperatures bellow 10°C for 7 days or more, especially with fruit in the pre-climacteric stage, should be avoided.

Further investigations on the effects of cultural practices on skin colour of ripe fruit should focus on obtaining the balance between yield and quality. This may be required for each production area or orchard. In addition, the influence of other macro and micro elements such as K and P need to be considered. There is also a need to understand the relationship between the N and Ca supply to mango fruit, to maximise the benefits of N on yield, and maximise the potential for Ca to improve fruit quality, including disease.

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Appendix 1. Conferences attended and publications from Thesis

1. Conferences Attended and Presented at during PhD:

29 September – 2 October 2002: Australian Society of Horticultural Science Conference, Sydney, Australia.

23-27 September 2001: Australian Postharvest Conference, Adelaide, Australia.

26 November – 1 December 2000: International Symposium on Tropical and Subtropical Fruits, Cairns, Australia.

9-12 November 1999: ASEAN and APEC Seminar on Postharvest Technology, Hochiminh City, Vietnam.

2. Publications from Thesis:

Nguyen, H., P. Hofman, R. Holmes, B. Stubbings, I. Bally and R. McConchie (2002). Effect of nitrogen on the skin colour and other quality attributes of ripe 'Kensington Pride' mango (*Mangifera indica* L.) fruit. *Journal of Horticultural Science and Biotechnology*, **submitted.**

Nguyen, H., P. Hofman, B. Stubbings, S. Ledger and R. McConchie (2002). Effect of a period change of temperature during storage and transportation on quality of 'Kensington Pride' mangoes. *Proceedings of Australian Society of Horticultural Science Conference*, 29 Sep. – 2 Oct., Sydney, Australia.

Hofman, P., R. Marques, L. Coates, H. Nguyen, S. Ledger, B. Stubbings and R. McConchie (2002). An integrated approach to product quality – case studies with avocado and mango.

Proceedings of Australian Society of Horticultural Science Conference, 29 Sep. – 2 Oct., Sydney, Australia.

Nguyen, H., R. McConchie, P. Hofman, L. Smith, B. Stubings and M. Adkins (2002). Effect of ethylene and ripening temperatures on the skin colour and flesh characteristics of ripe 'Kensington' mango fruit. *Acta Horticulturae*, **575**, 635-642.

Nguyen, H., P. Hofman, B. Stubbings, M. Adkins, I. Bally and R. McConchie (2001). Effect of nitrogen fertilizer on skin colour of ripe 'Kensington' mangoes. *Proceedings of Australian Postharvest Conference*, 23-27 Sep., Adelaide, Australia.

Appendix 2. Analysis of variance and data for graphs in Chapter 4.

Appendix 2.1. Analysis of variance for Experiment 1 - Chapter 4.

% Green colour

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	1731.0	346.2	2.15	
Tree.*Units* stratum					
Orchard	9	37672.2	4185.8	25.95	<.001
Residual	555(30)	89517.1	161.3		
Total	569(30)	127749.8			

Hue angle of green skin

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	414.90	82.98	1.70	
Tree.*Units* stratum					
Orchard	9	5221.18	580.13	11.88	<.001
Residual	554(31)	27050.48	48.83		
Total	568(31)	32526.03			

Total chlorophylls

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	5.911	1.182	0.46	
Tree.*Units* stratum					
Orchard	9	153.093	17.010	6.66	<.001
Residual	45	114.946	2.554		
Total	59	273.950			

Total carotenoids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.8128	0.1626	0.62	
Tree.*Units* stratum					
Orchard	9	21.9935	2.4437	9.25	<.001
Residual	45	11.8923	0.2643		
Total	59	34.6986			

DTR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	40.560	8.112	1.05	
Tree.*Units* stratum					
Orchard	9	755.973	83.997	10.88	<.001
Residual	585	4515.707	7.719		
Total	599	5312.240			

Anthracnose

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	154.23	30.85	2.19	
Tree.*Units* stratum					
Orchard	9	619.20	68.80	4.90	<.001
Residual	555(30)	7799.62	14.05		
Total	569(30)	8493.22			

SER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	3205.0	641.0	1.63	
Tree.*Units* stratum					
Orchard	9	54922.4	6102.5	15.50	<.001

Residual	585	230339.2	393.7		
Total	599	288466.5			

Hue angle of flesh colour

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	58.503	11.701	3.35	
Tree.*Units* stratum					
Orchard	9	339.278	37.698	10.81	<.001
Residual	555(30)	1935.858	3.488		
Total	569(30)	2304.074			

% Red Blush colour

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	686.0	137.2	1.01	
Tree.*Units* stratum					
Orchard	9	36852.3	4094.7	30.27	<.001
Residual	555(30)	75069.3	135.3		
Total	569(30)	111284.5			

Lenticel spotting

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	9.0589	1.8118	2.42	
Tree.*Units* stratum					
Orchard	9	123.8800	13.7644	18.38	<.001
Residual	554(31)	414.7891	0.7487		
Total	568(31)	543.7856			

TSS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.4615	0.0923	0.19	
Tree.*Units* stratum					
Orchard	9	35.9135	3.9904	8.11	<.001
Residual	45	22.1535	0.4923		
Total	59	58.5285			

Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.0009315	0.0001863	1.26	
Tree.*Units* stratum					
Orchard	9	0.0070693	0.0007855	5.32	<.001
Residual	45	0.0066425	0.0001476		
Total	59	0.0146433			

APPENDIX 2.2. Table of data for graphs in Experiment 1 - Chapter 4.

Orchard	Green colour (%)		Green H°	Chl ((µg cm^{-2})	Car (µg cm^{-2})	DTR (days)	Anthracnose (%)		SER (%)	
1	12.7	(4.8)	81.7	4.2	3.4	17.2	0.00	(0.00)	2.81	(0.24)
2	42.1	(44.9)	90.1	9.2	5.0	18.6	0.50	(0.01)	4.66	(0.68)
3	32.2	(28.4)	89.0	7.6	4.0	19.0	0.17	(0.00)	15.76	(7.40)
4	32.4	(28.7)	89.1	7.9	4.6	18.9	0.76	(0.02)	12.76	(4.90)
5	36.1	(34.7)	88.5	7.6	5.2	19.4	3.21	(0.32)	35.92	(34.4)
6	31.8	(27.8)	87.5	6.5	5.0	16.4	1.37	(0.06)	9.73	(2.85)
7	29.9	(24.8)	86.6	6.7	4.5	16.5	0.79	(0.02)	7.09	(1.54)
8	29.9	(24.8)	87.2	7.3	5.0	19.5	2.31	(0.16)	20.99	(12.8)
9	26.2	(19.7)	84.3	4.9	3.7	19.1	2.15	(0.13)	11.29	(3.85)
10	18.8	(10.4)	81.2	4.1	3.9	18.9	0.00	(0.00)	2.98	(0.27)
LSD	4.6		2.5	1.5	0.5	1.0	1.35		7.13	

Chl = total chlorophylls; Car = total carotenoids

Appendix 2.3. Analysis of variance for Experiment 2-HG Orchard-Chapter 4.

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	1401.8	280.4	1.71	
Tree.*Units* stratum					
Treatment	4	12907.5	3226.9	19.65	<.001
Residual	350	57468.4	164.2		
Total	359	71777.8			

Hue angle of green skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	126.14	25.23	1.01	
Tree.*Units* stratum					
Treatment	4	1287.53	321.88	12.87	<.001
Residual	350	8751.24	25.00		
Total	359	10164.91			

DTR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	130.492	26.098	2.98	
Tree.*Units* stratum					
Treatment	4	49.594	12.399	1.42	0.228
Residual	350	3060.689	8.745		
Total	359	3240.775			

Total chlorophylls

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	4.989	2.495	2.22	
Tree.*Units* stratum					
Treatment	4	43.819	10.955	9.75	0.004
Residual	8	8.986	1.123		
Total	14	57.795			

Total carotenoids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	0.8531	0.4266	2.44	
Tree.*Units* stratum					
Treatment	4	0.5226	0.1307	0.75	0.587
Residual	8	1.3998	0.1750		
Total	14	2.7755			

Anthracnose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	409.3	81.9	0.63	
Tree.*Units* stratum					
Treatment	4	3181.3	795.3	6.13	<.001
Residual	350	45392.7	129.7		
Total	359	48983.2			

SER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	4936.2	987.2	4.79	
Tree.*Units* stratum					
Treatment	4	1399.8	350.0	1.70	0.150
Residual	350	72186.9	206.2		
Total	359	78522.9			

Hue angle of flesh colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	73.148	14.630	3.61	
Tree.*Units* stratum					
Treatment	4	57.962	14.491	3.58	0.007
Residual	350	1416.513	4.047		
Total	359	1547.623			

% Red Blush colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	413.5	82.7	0.53	
Tree.*Units* stratum					
Treatment	4	1153.3	288.3	1.84	0.121
Residual	350	54868.5	156.8		
Total	359	56435.4			

Lenticel spotting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	2.4250	0.4850	1.21	
Tree.*Units* stratum					
Treatment	4	3.5111	0.8778	2.20	0.069
Residual	350	139.8389	0.3995		
Total	359	145.7750			

TSS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	4.2977	0.8595	1.24	
Tree.*Units* stratum					
Treatment	4	0.8900	0.2225	0.32	0.861
Residual	20	13.8940	0.6947		
Total	29	19.0817			

Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.0031132	0.0006226	2.46	
Tree.*Units* stratum					
Treatment	4	0.0005815	0.0001454	0.58	0.684
Residual	20	0.0050525	0.0002526		
Total	29	0.0087472			

Leaf N at panicle emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.13291	0.02658	2.40	
Tree.*Units* stratum					
Treatment	4	0.00737	0.00184	0.17	0.953
Residual	20	0.22145	0.01107		
Total	29	0.36172			

Leaf N at harvest

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	0.003515	0.001758	0.33	
Tree-No.*Units* stratum					
Treatment	4	0.041077	0.010269	1.95	0.196
Residual	8	0.042235	0.005279		
Total	14	0.086828			

Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	3385.2	677.0	1.72	
Tree.*Units* stratum					
Treatment	4	1059.9	265.0	0.67	0.619
Residual	20	7881.2	394.1		
Total	29	12326.3			

Appendix 2.4. Analysis of variance for Experiment 2-LG1 Orchard-Chapter 4.

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	1109.7	221.9	1.80	
Tree.*Units* stratum					
Treatment	4	23384.4	5846.1	47.36	<.001
Residual	349	43079.1	123.4		
Total	358	67573.2			

Hue angle of green skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	150.78	30.16	1.77	
Tree.*Units* stratum					
Treatment	4	977.59	244.40	14.31	<.001
Residual	349	5960.20	17.08		
Total	358	7088.58			

DTR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	234.05	46.81	3.13	
Tree.*Units* stratum					
Treatment	4	188.33	47.08	3.15	0.014
Residual	349	5214.42	14.94		
Total	358	5636.81			

Anthracnose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	1800.9	360.2	2.90	
Tree.*Units* stratum					
Treatment	4	640.5	160.1	1.29	0.274
Residual	349	43334.3	124.2		
Total	358	45775.6			

SER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	1672.0	334.4	3.17	
Tree.*Units* stratum					
Treatment	4	473.8	118.5	1.12	0.346
Residual	349	36855.9	105.6		
Total	358	39001.7			

Hue angle of flesh colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	183.895	36.779	9.09	
Tree.*Units* stratum					
Treatment	4	57.001	14.250	3.52	0.008
Residual	349	1411.959	4.046		
Total	358	1652.855			

% Red Blush colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	4950.0	990.0	5.20	
Tree.*Units* stratum					
Treatment	4	922.9	230.7	1.21	0.306
Residual	349	66463.0	190.4		
Total	358	72335.8			

Lenticel spotting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	28.8299	5.7660	12.85	
Tree.*Units* stratum					
Treatment	4	5.6303	1.4076	3.14	0.015
Residual	349	156.6595	0.4489		
Total	358	191.1198			

TSS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	1.462	0.292	0.29	
Tree.*Units* stratum					
Treatment	4	5.119	1.280	1.27	0.315
Residual	20	20.153	1.008		
Total	29	26.734			

Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.0023307	0.0004661	1.37	
Replicate.*Units* stratum					
Treatment	4	0.0030899	0.0007725	2.26	0.098
Residual	20	0.0068293	0.0003415		
Total	29	0.0122499			

Leaf N at panicle emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.018292	0.003658	0.50	
Tree.*Units* stratum					
Treatment	4	0.042233	0.010558	1.44	0.258
Residual	20	0.146775	0.007339		
Total	29	0.207299			

Leaf N at harvest

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	0.006973	0.003487	2.59	
Tree.*Units* stratum					
Treatment	4	0.004521	0.001130	0.84	0.538
Residual	8	0.010787	0.001348		
Total	14	0.022281			

Appendix 2.5. Analysis of variance for Experiment 2-LG2 Orchard-Chapter 4.

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	22126.0	5531.5	34.05	<.001
Residual	324	52639.0	162.5		
Total	333	77773.3			

Hue angle of green skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	999.94	249.98	15.11	<.001
Residual	324	5359.68	16.54		
Total	333	6492.51			

DTR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	307.596	76.899	15.41	<.001
Residual	324	1617.135	4.991		
Total	333	1959.030			

Anthracnose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	611.1	152.8	1.35	0.252
Residual	324	36704.3	113.3		
Total	333	37663.0			

SER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	600.62	150.15	1.55	0.188
Residual	324	31416.71	96.97		
Total	333	34413.63			

Hue angle of flesh colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	181.908	45.477	8.05	<.001
Residual	324	1831.183	5.652		
Total	333	2215.133			

%Red Blush colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	590.8	147.7	0.82	0.514
Residual	324	58482.4	180.5		
Total	333	64079.9			

TSS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.6763	0.1353	0.88	
Tree.*Units* stratum					
Treatment	4	2.3539	0.5885	3.85	0.018
Residual	20	3.0610	0.1530		
Total	29	6.0912			

Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.0027459	0.0005492	1.84	
Tree.*Units* stratum					
Treatment	4	0.0007245	0.0001811	0.61	0.663

Residual	20	0.0059783	0.0002989		
Total	29	0.0094487			

Leaf N at panicle emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.014469	0.002894	0.94	
Tree.*Units* stratum					
Treatment	4	0.034350	0.008588	2.77	0.055
Residual	20	0.061896	0.003095		
Total	29	0.110715			

Leaf N at harvest

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	0.018688	0.009344	1.29	
Tree.*Units* stratum					
Treatment	4	0.050285	0.012571	1.73	0.235
Residual	8	0.057986	0.007248		
Total	14	0.126959			

Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	6382.6	1276.5	1.86	
Tree.*Units* stratum					
Treatment	4	2152.3	538.1	0.79	0.548
Residual	20	13708.1	685.4		
Total	29	22243.1			

APPENDIX 2.6. Table of data for graphs in Experiment 2 - Chapter 4.

Treatment (g N/tree)	Green colour (%)		Green H°	Chl (µg cm^{-2})	Car (µg cm^{-2})	DTR (days)	Anthracnose (%)		SER (%)		Yield (kg/tree)
HG orchard											
0	15.8	(7.4)	89.7	6.8	4.9	21.6	11.7	(4.1)	8.2	(2.1)	72.0
75	18.1	(9.7)	89.2	7.5	4.8	21.9	13.2	(5.2)	9.8	(2.9)	82.5
150	25.8	(18.8)	91.6	9.7	4.9	21.0	13.1	(5.1)	7.7	(1.8)	77.2
300	26.9	(20.5)	91.8	10.8	5.3	21.1	15.5	(6.9)	9.7	(2.8)	85.6
Foliar	32.1	(28.3)	94.6	11.0	5.2	20.9	20.2	(11.9)	13.3	(5.3)	70.1
LSD	4.2		1.6	1.5	n.s	n.s	3.7		n.s		n.s
LG1 Orchard											
0	4.5	(0.7)	86.7	-	-	23.0	11.1	(3.7)	3.1	(0.3)	-
150	8.5	(2.2)	86.8	-	-	24.1	14.2	(6.0)	3.4	(0.4)	-
300	21.7	(13.7)	89.0	-	-	24.5	14.8	(6.5)	5.5	(0.9)	-
450	26.0	(19.2)	89.9	-	-	22.7	12.2	(4.5)	3.5	(0.4)	-
Foliar	12.4	(4.6)	90.8	-	-	22.8	13.2	(5.2)	5.8	(1.0)	-
LSD	3.7		1.4			1.3	n.s		n.s		
LG2 Orchard											
0	7.1	(1.6)	87.0	-	-	22.0	24.0	(16.6)	6.8	(1.4)	39.7
150	12.2	(4.5)	87.1	-	-	21.6	25.2	(18.1)	2.7	(0.2)	35.9
300	22.8	(15.0)	89.1	-	-	21.4	22.7	(14.9)	2.8	(0.3)	49.4
450	30.7	(26.0)	91.9	-	-	20.4	26.0	(19.2)	2.7	(0.2)	40.7
Foliar	17.8	(9.3)	89.2	-	-	19.4	22.5	(14.7)	4.3	(0.6)	23.3
LSD	4.5		1.4			0.8	n.s		n.s		17.1

Chl = total chlorophylls; Car = total carotenoids

Appendix 2.7. Analysis of variance for Experiment 3-HG* Orchard-Chapter 4.

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	1685.5	337.1	2.20	
Tree.*Units* stratum					
Treatment	7	13803.0	1971.9	12.86	<.001
Residual	707	108419.6	153.4		
Total	719	123908.1			

Hue angle of green skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	370.67	74.13	3.25	
Tree.*Units* stratum					
Treatment	7	2237.36	319.62	14.03	<.001
Residual	707	16110.49	22.79		
Total	719	18718.52			

DTR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	122.946	24.589	3.21	
Tree.*Units* stratum					
Treatment	7	274.888	39.270	5.12	<.001
Residual	707	5421.354	7.668		
Total	719	5819.188			

Anthracnose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	119.54	23.91	1.19	
Tree.*Units* stratum					
Treatment	7	466.78	66.68	3.32	0.002
Residual	707	14201.71	20.09		
Total	719	14788.03			

SER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	68.02	13.60	0.81	
Tree.*Units* stratum					
Treatment	7	129.03	18.43	1.10	0.361
Residual	707	11843.86	16.75		
Total	719	12040.91			

Hue angle of flesh colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	25.511	5.102	1.15	
Tree.*Units* stratum					
Treatment	7	195.809	27.973	6.32	<.001
Residual	707	3129.322	4.426		
Total	719	3350.642			

% Red Blush colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	4074.0	814.8	6.11	
Tree.*Units* stratum					
Treatment	7	2012.5	287.5	2.16	0.036
Residual	707	94299.4	133.4		
Total	719	100385.9			

Lenticel spotting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	6.3569	1.2714	2.79	
Tree.*Units* stratum					
Treatment	7	7.7208	1.1030	2.42	0.019
Residual	707	322.2542	0.4558		
Total	719	336.3319			

TSS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	1.7760	0.3552	0.42	
Tree.*Units* stratum					
Treatment	7	4.5798	0.6543	0.77	0.615
Residual	35	29.6990	0.8485		
Total	47	36.0548			

Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.0016627	0.0003325	1.49	
Tree.*Units* stratum					
Treatment	7	0.0011089	0.0001584	0.71	0.665
Residual	35	0.0078323	0.0002238		
Total	47	0.0106039			

Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	1478.4	295.7	1.00	
Tree.*Units* stratum					
Treatment	7	3999.2	571.3	1.93	0.093
Residual	35	10338.2	295.4		
Total	47	15815.8			

Appendix 2.8. Analysis of variance for Experiment 3-LG* Orchard-Chapter 4.

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	15930.9	2275.8	16.81	<.001
Residual	711	96239.8	135.4		
Total	718	112170.7			

Hue angle of green skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	2337.02	333.86	14.11	<.001
Residual	711	16821.91	23.66		
Total	718	19158.93			

DTR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	336.53	48.08	3.77	<.001
Residual	711	9059.15	12.74		
Total	718	9395.67			

Anthracnose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	305.63	43.66	1.62	0.127
Residual	711	19200.76	27.01		
Total	718	19506.39			

SER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	79.60	11.37	0.38	0.914
Residual	711	21255.79	29.90		
Total	718	21335.38			

Hue angle of flesh colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	206.547	29.507	3.76	<.001
Residual	711	5576.921	7.844		
Total	718	5783.469			

% Red Blush colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	1090.0	155.7	1.23	0.281
Residual	711	89718.2	126.2		
Total	718	90808.2			

Lenticel spotting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	15.0849	2.1550	2.46	0.017
Residual	711	623.5355	0.8770		
Total	718	638.6203			

TSS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	8.9567	1.7913	2.20	
Tree.*Units* stratum					
Treatment	7	10.1200	1.4457	1.78	0.123
Residual	35	28.4700	0.8134		
Total	47	47.5467			

Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	0.0011697	0.0002339	1.75	
Tree.*Units* stratum					
Treatment	7	0.0007420	0.0001060	0.79	0.600
Residual	35	0.0046890	0.0001340		
Total	47	0.0066007			

Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	5	692.5	138.5	0.48	
Tree.*Units* stratum					
Treatment	7	1546.8	221.0	0.76	0.620
Residual	35	10110.6	288.9		
Total	47	12349.8			

APPENDIX 2.9. Table of data for graphs in Experiment 3A - Chapter 4.

Treatment (g N/tree)	Green colour (%)		Green H°	DTR (days)	Anthracnose (%)		SER (%)		Yield (kg/tree)
HG orchard*									
0	23.3	(15.7)	92.6	15.7	1.67	(0.08)	0.00	(0.00)	60.4
75	27.1	(20.8)	95.5	15.5	3.25	(0.32)	0.14	(0.00)	55.6
175	26.0	(19.3)	94.6	15.5	3.13	(0.30)	0.35	(0.00)	48.2
275	30.5	(25.8)	95.2	15.6	3.57	(0.40)	1.23	(0.05)	62.2
375	37.1	(36.5)	98.4	15.1	3.27	(0.33)	0.30	(0.00)	73.7
575	33.2	(28.5)	97.2	16.6	4.47	(0.61)	0.94	(0.03)	52.1
375B	31.3	(27.0)	97.3	15.3	3.37	(0.35)	0.96	(0.03)	74.9
275K	34.8	(32.6)	97.3	14.2	2.13	(0.35)	0.25	(0.00)	54.4
LSD	3.6		1.4	0.8	1.31		n.s		n.s
LG Orchard*									
0	9.0	(2.5)	92.6	19.6	3.31	(0.34)	0.58	(0.01)	84.9
75	9.9	(2.9)	92.0	19.0	3.92	(0.47)	0.40	(0.01)	90.5
175	11.0	(3.6)	91.0	18.2	4.10	(0.53)	0.40	(0.01)	96.9
275	18.1	(9.7)	94.9	18.7	4.71	(0.66)	0.64	(0.01)	97.3
375	18.6	(10.2)	95.0	18.1	5.14	(0.80)	1.00	(0.03)	97.8
575	22.6	(14.7)	96.1	17.3	5.49	(0.92)	1.37	(0.06)	91.1
375B	17.9	(9.4)	95.4	19.0	4.17	(0.53)	0.67	(0.01)	94.1
275K	19.0	(10.6)	95.9	18.1	4.31	(0.57)	1.15	(0.04)	105.2
LSD	3.4		1.4	1.0	n.s		n.s		n.s

APPENDIX 2.10. Analysis of variance for Experiment 3B - Chapter 4.

Firmness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	0.6741	0.3370	1.26	
Tree.*Units* stratum					
Treatment	9	36.9481	4.1053	15.37	<.001
Residual	18	4.8074	0.2671		
Total	29	42.4296			

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	297.4	148.7	0.66	
Tree.*Units* stratum					
Treatment	9	28632.2	3181.4	14.12	<.001
Residual	18	4055.5	225.3		
Total	29	32985.1			

% Yellow colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	344.6	172.3	1.04	
Tree.*Units* stratum					
Treatment	9	16170.7	1796.7	10.88	<.001
Residual	18	2972.0	165.1		
Total	29	19487.4			

% Red Blush colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	25.40	12.70	0.93	
Tree.*Units* stratum					
Treatment	9	2765.99	307.33	22.62	<.001
Residual	18	244.53	13.58		
Total	29	3035.91			

Hue angle (H°)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	6.37	3.18	0.16	
Tree.*Units* stratum					
Treatment	9	2605.83	289.54	14.69	<.001
Residual	18	354.88	19.72		
Total	29	2967.07			

Chroma (C)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	9.705	4.852	4.04	
Tree.*Units* stratum					
Treatment	9	458.111	50.901	42.33	<.001
Residual	18	21.646	1.203		
Total	29	489.463			

L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	1.099	0.549	0.22	
Tree.*Units* stratum					
Treatment	9	507.481	56.387	22.56	<.001
Residual	18	44.995	2.500		
Total	29	553.575			

Total chlorophylls

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	2.8217	1.4108	3.41	
Tree.*Units* stratum					
Treatment	9	436.8067	48.5341	117.37	<.001
Residual	18	7.4432	0.4135		
Total	29	447.0715			

Total carotenoids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tree stratum	2	0.06466	0.03233	1.32	
Tree.*Units* stratum					
Treatment	9	20.60877	2.28986	93.55	<.001
Residual	18	0.44059	0.02448		
Total	29	21.11402			

APPENDIX 2.11. Table of data for graphs in Experiment 3B – Chapter 4.

Treatment (N, day)	Firmness	Green Colour (%)		Yellow colour (%)		Blush colour (%)		H°	C	L	Chl ($\mu g\ cm^{-2}$)	Car ($\mu g\ cm^{-2}$)
0, 1	1.00	90.0	(100.0)	0.0	(0.0)	0.0	(0.0)	116.0	64.4	44.4	11.49	2.13
0, 5	1.89	69.6	(87.9)	20.4	(11.8)	0.0	(0.0)	112.1	67.4	45.4	8.29	2.93
0, 9	3.00	48.3	(55.8)	39.5	(40.5)	6.8	(1.4)	103.3	70.4	49.3	2.58	3.33
0, 12	3.89	21.9	(13.9)	53.7	(65.0)	23.8	(16.3)	94.0	73.0	52.4	1.53	3.93
0, 18	4.20	13.8	(5.7)	59.4	(74.1)	22.6	(14.8)	90.7	73.5	55.4	1.50	4.46
N, 1	1.00	90.0	(100.0)	0.0	(0.0)	0.0	(0.0)	120.1	60.1	42.2	11.94	2.28
N, 5	2.00	71.6	(90.0)	18.4	(10.0)	0.0	(0.0)	113.9	64.5	44.4	9.49	2.85
N, 9	2.67	57.6	(71.3)	30.9	(26.4)	5.7	(1.0)	105.5	67.6	47.2	7.41	3.47
N, 12	3.56	42.3	(45.3)	32.5	(28.4)	28.7	(23.0)	100.2	69.3	50.7	4.06	4.15
N, 18	3.80	38.1	(38.1)	39.4	(40.3)	24.9	(17.8)	98.7	69.8	52.5	3.25	4.63
LSD	0.64	13.2		12.6		6.9		2.5	1.7	3.5	1.22	0.27

N = 575 g N/tree; 0 = 0 g N/tree; day = days after harvest
H° = hue angle; C = Chroma; L = Light; Chl = total chlorophylls; Car = total carotenoids

APPENDIX 3. Analysis of variance and data for graphs in Chapter 5.

APPENDIX 3.1. Analysis of variance for Experiment 1 - Chapter 5.

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Container stratum	2	1359.5	679.7	4.85	
Container.*Units* stratum					
Temp	2	48347.7	24173.9	172.64	<.001
Ethylene	3	2082.2	694.1	4.96	0.002
Time	1	557.1	557.1	3.98	0.046
Temp.Ethylene	6	7949.2	1324.9	9.46	<.001
Temp.Time	2	1400.9	700.4	5.00	0.007
Ethylene.Time	3	3534.3	1178.1	8.41	<.001
Temp.Ethylene.Time	6	2449.3	408.2	2.92	0.008
Residual	982	137504.1	140.0		
Total	1007	205184.2			

(Temp = temperature; Ethylene = ethylene concentration; Time = treatment duration)

Hue angle of green skin

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Container stratum	2	483.63	241.81	8.59	
Container.*Units* stratum					
Temp	2	3193.37	1596.69	56.72	<.001
Ethylene	3	202.27	67.42	2.39	0.067
Time	1	742.63	742.63	26.38	<.001
Temp.Ethylene	6	271.31	45.22	1.61	0.142
Temp.Time	2	429.32	214.66	7.62	<.001
Ethylene.Time	3	419.71	139.90	4.97	0.002
Temp.Ethylene.Time	6	476.69	79.45	2.82	0.010
Residual	978(4)	27533.33	28.15		
Total	1003(4)	33736.04			

Hue angle of yellow skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Container stratum	2	2.63	1.32	0.10	
Container.*Units* stratum					
Temp	2	3934.16	1967.08	152.87	<.001
Ethylene	3	233.29	77.76	6.04	<.001
Time	1	0.24	0.24	0.02	0.891
Temp.Ethylene	6	336.93	56.16	4.36	<.001
Temp.Time	2	1257.38	628.69	48.86	<.001
Ethylene.Time	3	41.53	13.84	1.08	0.358
Temp.Ethylene.Time	6	404.82	67.47	5.24	<.001
Residual	982	12636.29	12.87		
Total	1007	18847.28			

% Red Blush colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Container stratum	2	2028.1	1014.1	5.50	
Container.*Units* stratum					
Temp	2	906.4	453.2	2.46	0.086
Ethylene	3	684.8	228.3	1.24	0.295
Time	1	12.1	12.1	0.07	0.798

Temp.Ethylene	6	666.2	111.0	0.60	0.729
Temp.Time	2	331.3	165.6	0.90	0.408
Ethylene.Time	3	255.5	85.2	0.46	0.709
Temp.Ethylene.Time	6	494.3	82.4	0.45	0.848
Residual	982	181211.1	184.5		
Total	1007	186589.9			

Blotchiness

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Container stratum	2	5.6471	2.8236	6.33	
Container.*Units* stratum					
Temp	2	68.5666	34.2833	76.80	<.001
Ethylene	3	31.0306	10.3435	23.17	<.001
Time	1	4.6560	4.6560	10.43	0.001
Temp.Ethylene	6	9.9270	1.6545	3.71	0.001
Temp.Time	2	0.3484	0.1742	0.39	0.677
Ethylene.Time	3	13.1783	4.3928	9.84	<.001
Temp.Ethylene.Time	6	2.4748	0.4125	0.92	0.477
Residual	981(1)	437.9105	0.4464		
Total	1006(1)	573.7200			

DTR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Container stratum	2	51.056	25.528	6.79	
Container.*Units* stratum					
Temp	2	10452.454	5226.227	1389.12	<.001
Ethylene	3	2561.885	853.962	226.98	<.001
Time	1	279.212	279.212	74.21	<.001
Temp.Ethylene	6	22.440	3.740	0.99	0.428
Temp.Time	2	96.623	48.312	12.84	<.001
Ethylene.Time	3	246.694	82.231	21.86	<.001
Temp.Ethylene.Time	6	35.768	5.961	1.58	0.148
Residual	982	3694.530	3.762		
Total	1007	17440.663			

Anthracnose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Container stratum	2	0.187	0.094	0.03	
Container.*Units* stratum					
Temp	2	125.719	62.860	17.00	<.001
Ethylene	3	36.397	12.132	3.28	0.020
Time	1	11.581	11.581	3.13	0.077
Temp.Ethylene	6	45.654	7.609	2.06	0.056
Temp.Time	2	9.623	4.812	1.30	0.273
Ethylene.Time	3	5.991	1.997	0.54	0.655
Temp.Ethylene.Time	6	9.883	1.647	0.45	0.848
Residual	982	3630.722	3.697		
Total	1007	3875.759			

SER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Container stratum	2	5.207	2.604	0.31	
Container.*Units* stratum					
Temp	2	159.527	79.764	9.45	<.001
Ethylene	3	52.982	17.661	2.09	0.100
Time	1	2.141	2.141	0.25	0.615
Temp.Ethylene	6	68.159	11.360	1.35	0.234
Temp.Time	2	10.098	5.049	0.60	0.550

Ethylene.Time	3	5.174	1.725	0.20	0.893
Temp.Ethylene.Time	6	32.894	5.482	0.65	0.691
Residual	982	8291.172	8.443		
Total	1007	8627.355			

Dendritic rot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Container stratum	2	24.579	12.290	8.26	
Container.*Units* stratum					
Temp	2	648.014	324.007	217.77	<.001
Ethylene	3	16.214	5.405	3.63	0.013
Time	1	9.921	9.921	6.67	0.010
Temp.Ethylene	6	37.742	6.290	4.23	<.001
Temp.Time	2	22.516	11.258	7.57	<.001
Ethylene.Time	3	11.911	3.970	2.67	0.046
Temp.Ethylene.Time	6	10.423	1.737	1.17	0.321
Residual	982	1461.092	1.488		
Total	1007	2242.413			

Lenticel spotting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Container stratum	2	1.1488	0.5744	1.20	
Container.*Units* stratum					
Temp	2	5.5417	2.7708	5.77	0.003
Ethylene	3	9.8204	3.2735	6.82	<.001
Time	1	2.4971	2.4971	5.20	0.023
Temp.Ethylene	6	4.2067	0.7011	1.46	0.189
Temp.Time	2	5.1891	2.5945	5.41	0.005
Ethylene.Time	3	2.8644	0.9548	1.99	0.114
Temp.Ethylene.Time	6	2.3525	0.3921	0.82	0.557
Residual	982	471.2989	0.4799		
Total	1007	504.9196			

Hue angle of flesh colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Container stratum	2	43.435	21.717	4.58	
Container.*Units* stratum					
Temp	2	2907.458	1453.729	306.66	<.001
Ethylene	3	1245.664	415.221	87.59	<.001
Time	1	0.628	0.628	0.13	0.716
Temp.Ethylene	6	465.209	77.535	16.36	<.001
Temp.Time	2	0.696	0.348	0.07	0.929
Ethylene.Time	3	9.189	3.063	0.65	0.585
Temp.Ethylene.Time	6	26.773	4.462	0.94	0.464
Residual	982	4655.238	4.741		
Total	1007	9354.290			

Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Container stratum					
Temp	2	4.13372	2.06686	89.76	<.001
Ethylene	3	4.12806	1.37602	59.76	<.001
Time	1	3.38620	3.38620	147.06	<.001
Temp.Ethylene	6	0.38795	0.06466	2.81	0.020
Temp.Time	2	2.81386	1.40693	61.10	<.001
Ethylene.Time	3	2.21257	0.73752	32.03	<.001
Temp.Ethylene.Time	6	2.55942	0.42657	18.53	<.001
Residual	48	1.10525	0.02303	1.17	

Container.*Units* stratum			
	431	8.45523	0.01962
Total	502	29.18226	

TSS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Container stratum					
Temp	2	0.2462	0.1231	0.19	0.825
Ethylene	3	6.0362	2.0121	3.16	0.033
Time	1	0.0001	0.0001	0.00	0.991
Temp.Ethylene	6	14.2248	2.3708	3.73	0.004
Temp.Time	2	2.6677	1.3339	2.10	0.134
Ethylene.Time	3	6.1812	2.0604	3.24	0.030
Temp.Ethylene.Time	6	3.3519	0.5586	0.88	0.518
Residual	48	30.5263	0.6360	0.93	
Container.*Units* stratum					
	431	293.3455	0.6806		
Total	502	356.5800			

APPENDIX 3.2. Tables of data for graphs in Experiment 1 - Chapter 5.

Treatment (T, EthC, t)	Green colour (%)		Green H°	Yellow H°	Blotchiness	DTR (days)	Anthracnose (%)	
15, 0, 3	33.0	(29.6)	98.2	88.3	0.17	18.3	0.47	(0.01)
15, 0, 1	36.2	(34.9)	99.7	88.7	0.24	17.8	1.22	(0.05)
15, 10, 3	39.1	(39.8)	98.3	90.8	0.21	14.2	0.95	(0.03)
15, 10, 1	38.4	(38.5)	99.3	88.7	0.19	16.2	1.61	(0.08)
15, 100, 3	44.5	(49.2)	99.3	92.2	0.33	13.2	0.14	(0.00)
15, 100, 1	37.1	(36.4)	99.3	88.7	0.17	15.8	0.00	(0.00)
15, 1000, 3	50.2	(59.0)	100.6	93.6	0.43	12.0	0.85	(0.02)
15, 1000, 1	37.7	(37.4)	99.3	89.1	0.21	15.4	1.53	(0.07)
20, 0, 3	30.7	(26.0)	97.3	86.2	0.17	11.6	0.27	(0.00)
20, 0, 1	27.8	(21.7)	96.5	85.2	0.29	10.9	0.27	(0.00)
20, 10, 3	18.6	(10.2)	91.9	85.3	0.19	8.1	0.00	(0.00)
20, 10, 1	23.5	(15.9)	96.7	85.7	0.24	9.4	0.33	(0.00)
20, 100, 3	21.6	(13.5)	95.0	86.8	0.62	7.1	0.00	(0.00)
20, 100, 1	21.4	(13.3)	96.9	86.5	0.30	8.7	0.00	(0.00)
20, 1000, 3	27.2	(20.8)	95.0	87.5	0.95	6.8	0.00	(0.00)
20, 1000, 1	20.2	(11.9)	95.1	86.4	0.41	8.2	0.00	(0.00)
25, 0, 3	32.2	(28.4)	95.5	85.9	0.57	11.6	0.00	(0.00)
25, 0, 1	29.3	(24.0)	95.2	86.6	0.62	10.8	0.33	(0.00)
25, 10, 3	19.9	(11.6)	92.4	83.5	0.64	7.5	0.19	(0.00)
25, 10, 1	28.0	(22.0)	97.2	86.8	0.67	8.3	0.00	(0.00)
25, 100, 3	24.7	(17.4)	92.6	83.1	1.10	6.9	0.00	(0.00)
25, 100, 1	24.6	(17.3)	98.0	87.0	0.81	7.4	0.00	(0.00)
25, 1000, 3	24.4	(17.1)	94.3	83.7	1.60	6.5	0.00	(0.00)
25, 1000, 1	24.9	(17.5)	98.3	87.5	0.98	7.5	0.14	(0.00)
LSD	5.2		2.3	1.6	0.29	0.9	0.83	

T = Temperature (°C); EthC = ethylene concentration (µL L^{-1}); t = treatment duration (days)

APPENDIX 3.2. Tables of data for graphs in Experiment 1 - Chapter 5.
(continue)

Treatment (T, EthC, t)	SER (%)		Dendritic rot	Lenticel spot	Flesh H°	Acidity (%)	TSS (%)
15, 0, 3	0.94	(0.03)	2.69	2.69	86.4	0.28	12.4
15, 0, 1	1.19	(0.04)	2.88	2.81	86.7	0.34	12.0
15, 10, 3	1.31	(0.05)	2.33	2.57	86.3	0.47	11.9
15, 10, 1	1.94	(0.12)	2.57	2.79	87.0	0.29	11.5
15, 100, 3	0.00	(0.00)	1.88	2.76	87.5	0.76	11.8
15, 100, 1	0.44	(0.01)	2.76	2.88	87.2	0.32	12.1
15, 1000, 3	1.25	(0.05)	1.52	2.81	87.5	0.94	11.9
15, 1000, 1	0.84	(0.02)	2.69	2.76	87.2	0.14	11.8
20, 0, 3	0.58	(0.01)	1.14	2.38	82.3	0.18	11.8
20, 0, 1	0.00	(0.00)	1.14	2.36	82.2	0.17	11.8
20, 10, 3	0.19	(0.00)	1.45	2.69	83.7	0.39	12.0
20, 10, 1	0.00	(0.00)	1.36	2.50	84.4	0.27	12.0
20, 100, 3	0.00	(0.00)	0.60	2.67	85.2	0.55	11.4
20, 100, 1	0.00	(0.00)	0.85	2.53	84.7	0.27	12.0
20, 1000, 3	0.00	(0.00)	0.76	2.95	84.6	0.61	12.2
20, 1000, 1	0.00	(0.00)	0.76	2.62	84.7	0.34	12.4
25, 0, 3	0.19	(0.00)	0.29	2.43	79.9	0.16	12.0
25, 0, 1	1.28	(0.05)	0.29	2.55	79.8	0.14	12.2
25, 10, 3	0.00	(0.00)	0.71	2.74	83.0	0.16	12.1
25, 10, 1	0.00	(0.00)	0.41	2.36	82.7	0.18	11.8
25, 100, 3	0.14	(0.00)	0.81	2.83	84.4	0.24	12.1
25, 100, 1	0.00	(0.00)	0.52	2.48	84.6	0.27	11.8
25, 1000, 3	0.00	(0.00)	0.41	2.98	84.6	0.28	12.1
25, 1000, 1	0.00	(0.00)	0.79	2.69	85.0	0.33	11.7
LSD	1.26		0.53	0.30	1.0	0.09	0.5

APPENDIX 3.3. Analysis of variance for Experiment 2 - Chapter 5

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	37768.3	18884.1	185.91	
Orchard.*Units* stratum					
Ethylene	4	2404.7	601.2	5.92	<.001
Time	1	3257.8	3257.8	32.07	<.001
Ethylene.Time	4	1553.4	388.3	3.82	0.005
Residual	438	44489.9	101.6		
Total	449	89474.1			

Hue angle of green skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	2944.58	1472.29	96.74	
Orchard.*Units* stratum					
Ethylene	4	216.57	54.14	3.56	0.007
Time	1	843.78	843.78	55.44	<.001
Ethylene.Time	4	124.08	31.02	2.04	0.088
Residual	438	6666.23	15.22		
Total	449	10795.24			

Hue angle of yellow skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	20.770	10.385	2.58	
Orchard.*Units* stratum					
Ethylene	4	212.461	53.115	13.21	<.001
Time	1	104.064	104.064	25.88	<.001
Ethylene.Time	4	16.323	4.081	1.01	0.399
Residual	438	1761.489	4.022		
Total	449	2115.107			

% Red Blush colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	9259.2	4629.6	27.22	
Orchard.*Units* stratum					
Ethylene	4	461.1	115.3	0.68	0.608
Time	1	77.5	77.5	0.46	0.500
Ethylene.Time	4	362.4	90.6	0.53	0.712
Residual	438	74485.2	170.1		
Total	449	84645.4			

DTR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	331.151	165.576	70.09	
Orchard.*Units* stratum					
Ethylene	4	1539.476	384.869	162.92	<.001
Time	1	246.420	246.420	104.32	<.001
Ethylene.Time	4	169.147	42.287	17.90	<.001
Residual	438	1034.671	2.362		
Total	449	3320.864			

Anthracnose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	3000.12	1500.06	35.43	
Orchard.*Units* stratum					
Ethylene	4	300.93	75.23	1.78	0.132

Time	1	269.78	269.78	6.37	0.012
Ethylene.Time	4	169.41	42.35	1.00	0.407
Residual	438	18545.27	42.34		
Total	449	22285.51			

SER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	47.23	23.62	2.19	
Orchard.*Units* stratum					
Ethylene	4	64.51	16.13	1.49	0.203
Time	1	5.34	5.34	0.49	0.482
Ethylene.Time	4	34.82	8.70	0.81	0.522
Residual	438	4731.11	10.80		
Total	449	4883.01			

Dendritic rot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	110.2533	55.1267	82.79	
Orchard.*Units* stratum					
Ethylene	4	2.3867	0.5967	0.90	0.466
Time	1	0.1422	0.1422	0.21	0.644
Ethylene.Time	4	0.2800	0.0700	0.11	0.981
Residual	438	291.6578	0.6659		
Total	449	404.7200			

Hue angle of flesh colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	368.864	184.432	72.68	
Orchard.*Units* stratum					
Ethylene	4	156.368	39.092	15.41	<.001
Time	1	189.605	189.605	74.72	<.001
Ethylene.Time	4	60.691	15.173	5.98	<.001
Residual	438	1111.436	2.538		
Total	449	1886.965			

Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	0.004086	0.002043	1.64	
Orchard.*Units* stratum					
Ethylene	4	0.018249	0.004562	3.65	0.024
Time	1	0.008875	0.008875	7.11	0.016
Ethylene.Time	4	0.006493	0.001623	1.30	0.307
Residual	18	0.022468	0.001248		
Total	29	0.060171			

TSS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	9.6647	4.8323	16.06	
Orchard.*Units* stratum					
Ethylene	4	0.5787	0.1447	0.48	0.749
Time	1	0.1080	0.1080	0.36	0.557
Ethylene.Time	4	0.7920	0.1980	0.66	0.629
Residual	18	5.4153	0.3009		
Total	29	16.5587			

APPENDIX 3.4. Tables of data for graphs in Experiment 2 - Chapter 5.

Treatment (EthC, t)	Green colour (%)		Green H°	Yellow H°	DTR (days)	Anthracnose (%)		Lenticels	Flesh H°	Acidity (%)
0, 1	24.0	(16.5)	93.7	87.2	17.5	7.02	(1.50)	2.36	86.5	0.16
0, 3	24.7	(17.4)	92.5	86.8	18.2	7.23	(1.59)	2.36	86.6	0.15
5, 1	22.6	(14.7)	92.5	85.6	14.7	8.06	(1.96)	1.87	85.9	0.15
5, 3	17.0	(8.6)	90.3	84.7	13.0	4.81	(0.71)	2.18	84.0	0.17
10, 1	20.9	(12.7)	93.2	85.9	14.6	5.97	(1.08)	1.93	86.1	0.16
10, 3	15.9	(7.5)	90.6	84.6	12.5	4.97	(0.75)	2.07	84.3	0.23
20, 1	23.5	(15.9)	93.3	86.7	14.1	5.70	(0.98)	1.91	86.3	0.21
20, 3	12.5	(4.7)	89.0	85.4	11.9	4.53	(0.64)	2.09	84.7	0.23
50, 1	21.9	(13.9)	93.1	86.5	14.2	6.34	(1.22)	1.80	86.7	0.17
50, 3	15.9	(7.5)	89.7	85.7	11.9	3.76	(0.43)	2.29	85.4	0.24
LSD	5.7		1.9	0.83	0.73	2.89		0.38	0.76	0.06

EthC = ethylene concentration (μL L^{-1}); t = treatment duration (days)

APPENDIX 3.5. Analysis of variance for Experiment 3 - Chapter 5

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	4598.8	2299.4	17.18	
Orchard.*Units* stratum					
CO2	4	1450.6	362.6	2.71	0.030
Ethylene	1	99.9	99.9	0.75	0.388
CO2.Ethylene	4	3015.8	754.0	5.63	<.001
Residual	438	58627.3	133.9		
Total	449	67792.5			

(CO2 = CO2 concentration; Ethylene = ethylene concentration)

Hue angle of green skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	1029.88	514.94	24.23	
Orchard.*Units* stratum					
CO2	4	107.04	26.76	1.26	0.028
Ethylene	1	38.54	38.54	1.81	0.179
CO2.Ethylene	4	94.72	23.68	1.11	0.349
Residual	438	9308.89	21.25		
Total	449	10579.08			

Hue angle of yellow skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	201.438	100.719	17.27	
Orchard.*Units* stratum					
CO2	4	53.138	13.285	2.28	0.060
Ethylene	1	1.323	1.323	0.23	0.634
CO2.Ethylene	4	9.282	2.321	0.40	0.810
Residual	438	2554.198	5.832		
Total	449	2819.379			

% Red Blush colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	24489.2	12244.6	67.86	
Orchard.*Units* stratum					
CO2	4	1233.2	308.3	1.71	0.147
Ethylene	1	144.2	144.2	0.80	0.372
CO2.Ethylene	4	377.0	94.3	0.52	0.719
Residual	438	79030.7	180.4		
Total	449	105274.3			

Colour Dullness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	4.173	2.087	1.84	
Orchard.*Units* stratum					
CO2	4	13.444	3.361	2.96	0.020
Ethylene	1	2.136	2.136	1.88	0.171
CO2.Ethylene	4	36.120	9.030	7.96	<.001
Residual	438	496.627	1.134		
Total	449	552.500			

DTR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	638.458	319.229	294.58	

Orchard.*Units* stratum					
CO2	4	1705.858	426.464	393.53	<.001
Ethylene	1	81.069	81.069	74.81	<.001
CO2.Ethylene	4	373.653	93.413	86.20	<.001
Residual	438	474.653	1.084		
Total	449	3273.691			

Anthracnose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	221.43	110.71	3.57	
Orchard.*Units* stratum					
CO2	4	500.56	125.14	4.03	0.003
Ethylene	1	7.84	7.84	0.25	0.616
CO2.Ethylene	4	390.75	97.69	3.15	0.014
Residual	438	13597.83	31.05		
Total	449	14718.40			

SER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	11.208	5.604	1.33	
Orchard.*Units* stratum					
CO2	4	23.962	5.990	1.42	0.227
Ethylene	1	5.038	5.038	1.19	0.275
CO2.Ethylene	4	13.020	3.255	0.77	0.544
Residual	438	1849.112	4.222		
Total	449	1902.341			

Dendritic rot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	0.8711	0.4356	0.66	
Orchard.*Units* stratum					
CO2	4	4.2667	1.0667	1.62	0.169
Ethylene	1	0.1089	0.1089	0.17	0.685
CO2.Ethylene	4	4.1244	1.0311	1.56	0.183
Residual	438	288.9067	0.6596		
Total	449	298.2778			

Lenticel spotting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	10.4044	5.2022	9.04	
Orchard.*Units* stratum					
CO2	4	16.0000	4.0000	6.95	<.001
Ethylene	1	0.4356	0.4356	0.76	0.385
CO2.Ethylene	4	7.4311	1.8578	3.23	0.013
Residual	438	252.1733	0.5757		
Total	449	286.4444			

Hue angle of flesh colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	115.091	57.545	13.40	
Orchard.*Units* stratum					
CO2	4	81.954	20.489	4.77	<.001
Ethylene	1	0.728	0.728	0.17	0.681
CO2.Ethylene	4	50.862	12.716	2.96	0.020
Residual	438	1880.552	4.293		
Total	449	2129.188			

Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	0.0092425	0.0046212	12.42	
Orchard.*Units* stratum					
CO2	4	0.0056058	0.0014015	3.77	0.210
Ethylene	1	0.0009075	0.0009075	2.44	0.136
CO2.Ethylene	4	0.0009357	0.0002339	0.63	0.648
Residual	18	0.0066995	0.0003722		
Total	29	0.0233910			

TSS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	31.0287	15.5143	86.96	
Orchard.*Units* stratum					
CO2	4	2.2987	0.5747	3.22	0.067
Ethylene	1	0.3413	0.3413	1.91	0.184
CO2.Ethylene	4	0.3387	0.0847	0.47	0.754
Residual	18	3.2113	0.1784		
Total	29	37.2187			

APPENDIX 3.6. Tables of data for graphs in Experiment 3 - Chapter 5.

Treatment (%CO_2, EthC)	Green colour (%)		Green H°	Dullness	DTR (days)	Anthracnose (%)		Lenticels	Flesh H°
0, 0	22.2	(14.3)	90.6	2.44	14.4	5.85	(1.04)	2.47	84.6
1, 0	22.0	(14.0)	90.9	2.29	14.4	5.26	(0.84)	2.36	84.2
2, 0	22.4	(14.5)	90.9	2.56	14.7	5.28	(0.85)	2.49	84.1
4, 0	24.5	(17.2)	89.8	2.47	14.6	5.61	(0.96)	2.60	84.2
6, 0	27.6	(21.5)	90.8	2.93	15.0	6.13	(1.14)	2.49	83.2
0, 10	16.5	(8.1)	88.8	1.78	10.2	2.38	(0.17)	3.02	84.6
1, 10	16.5	(8.1)	89.1	1.93	10.1	2.15	(0.14)	3.04	85.2
2, 10	19.7	(11.4)	89.5	2.49	10.1	3.40	(0.35)	2.84	84.9
4, 10	21.2	(13.1)	90.6	2.64	10.7	2.90	(0.25)	2.80	85.0
6, 10	22.5	(14.6)	90.1	2.80	11.3	3.49	(0.37)	2.78	84.7
LSD	5.0		1.3	0.44	0.7	2.32		0.32	0.9

%CO_2 = CO_2 concentration (%); EthC = ethylene concentration (µL L^{-1})

APPENDIX 3.7. Analysis of variance for Experiment 4 - Chapter 5.

Firmness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	0.01067	0.00533	0.17	
Orchard.*Units* stratum					
Treatment	9	63.31200	7.03467	219.83	<.001
Residual	18	0.57600	0.03200		
Total	29	63.89867			

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	288.57	144.29	3.97	
Orchard.*Units* stratum					
Treatment	9	25212.04	2801.34	77.15	<.001
Residual	18	653.60	36.31		
Total	29	26154.21			

% Yellow colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	674.17	337.08	7.79	
Orchard.*Units* stratum					
Treatment	9	16889.54	1876.62	43.38	<.001
Residual	18	778.74	43.26		
Total	29	18342.45			

% Red Blush colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	448.06	224.03	4.59	
Orchard.*Units* stratum					
Treatment	9	2327.74	258.64	5.30	0.001
Residual	18	878.05	48.78		
Total	29	3653.84			

Hue angle of green skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	20.747	10.373	1.34	
Orchard.*Units* stratum					
Treatment	9	3027.630	336.403	43.51	<.001
Residual	18	139.176	7.732		
Total	29	3187.553			

Hue angle of flesh colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	21.220	10.610	5.30	
Orchard.*Units* stratum					
Treatment	9	1294.713	143.857	71.92	<.001
Residual	18	36.002	2.000		
Total	29	1351.935			

Total chlorophylls

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	0.0769	0.0384	0.09	
Orchard.*Units* stratum					
Treatment	9	375.1740	41.6860	101.89	<.001
Residual	18	7.3643	0.4091		
Total	29	382.6152			

Total carotenoids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	0.06917	0.03459	0.73	
Orchard.*Units* stratum					
Treatment	9	41.50569	4.61174	97.14	<.001
Residual	18	0.85453	0.04747		
Total	29	42.42939			

APPENDIX 3.8. Table of data for graphs in Experiment 4 - Chapter 5.

Treatment (E, day)	Firmness	Green Colour (%)		Yellow colour (%)		Blush colour (%)		Green H°	Flesh H°	Chl ($\mu g\ cm^{-2}$)	Car ($\mu g\ cm^{-2}$)
0, 0	1.00	90.0	(100.0)	0.0	(0.0)	0.0	(0.0)	117.8	102.8	11.94	2.21
0, 4	1.20	73.9	92.3	4.2	(0.6)	14.5	(6.3)	117.5	100.6	11.17	2.43
0, 8	2.47	58.3	(72.4)	19.0	(10.6)	17.0	(8.5)	109.8	91.2	8.17	3.32
0, 10	3.67	36.5	(35.3)	42.3	(45.3)	19.4	(11.0)	99.0	88.8	5.58	4.04
0, 14	4.48	14.6	(6.4)	57.4	(71.0)	22.1	(14.2)	92.7	86.4	3.18	5.30
E, 0	1.00	90.0	(100.0)	0.0	(0.0)	0.0	(0.0)	116.6	103.5	11.49	2.13
E, 4	2.13	41.5	(43.9)	37.0	(36.3)	21.7	(13.7)	105.0	95.5	8.47	3.14
E, 6	3.73	25.3	(18.3)	49.4	(57.7)	24.5	(17.2)	98.9	90.9	6.26	4.12
E, 8	4.67	14.3	(6.1)	57.8	(71.6)	25.9	(19.1)	94.4	87.9	3.00	4.86
E, 10	4.73	13.3	(5.3)	62.0	(78.0)	19.3	(10.9)	92.4	85.5	1.85	5.40
LSD	0.32	8.5		8.6		9.0		3.9	2.1	1.02	0.36

E = 10 $\mu L\ L^{-1}$; 0 = 0 $\mu L\ L^{-1}$; day = days after harvest
Chl = total chlorophylls; Car = total carotenoids

Appendix 4. Analysis of variance and data for graphs in Chapter 6.

Appendix 4. Analysis of variance for Experiment 1 - Chapter 6.

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	37785.1	18892.5	119.76	
Orchard.*Units* stratum					
Treatment	12	12877.9	1073.2	6.80	<.001
Residual	570	89915.9	157.7		
Total	584	140578.8			

Hue angle of green skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	6264.12	3132.06	135.53	
Orchard.*Units* stratum					
Treatment	12	1368.58	114.05	4.94	<.001
Residual	570	13172.65	23.11		
Total	584	20805.35			

Hue angle of yellow skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	913.362	456.681	79.03	
Orchard.*Units* stratum					
Treatment	12	207.947	17.329	3.00	<.001
Residual	570	3293.670	5.778		
Total	584	4414.978			

% Red Blush colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	10388.8	5194.4	23.77	
Orchard.*Units* stratum					
Treatment	12	2332.2	194.4	0.89	0.558
Residual	570	124549.8	218.5		
Total	584	137270.8			

Blotchiness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	48.1778	24.0889	33.29	
Orchard.*Units* stratum					
Treatment	12	52.3385	4.3615	6.03	<.001
Residual	570	412.4000	0.7235		
Total	584	512.9162			

DTR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	120.1778	60.0889	96.26	
Orchard.*Units* stratum					
Treatment	12	54.5573	4.5464	7.28	<.001
Residual	570	355.8222	0.6242		
Total	584	530.5573			

Anthracnose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	17.12	8.56	0.59	
Orchard.*Units* stratum					
Treatment	12	222.75	18.56	1.29	0.223

Residual	570	8232.12	14.44		
Total	584	8471.99			

SER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	1.1619	0.5809	1.00	
Orchard.*Units* stratum					
Treatment	12	6.9712	0.5809	1.00	0.447
Residual	570	331.1333	0.5809		
Total	584	339.2664			

Hue angle of flesh colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	311.204	155.602	38.47	
Orchard.*Units* stratum					
Treatment	12	258.270	21.523	5.32	<.001
Residual	570	2305.262	4.044		
Total	584	2874.736			

Lenticel spotting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Orchard stratum	2	1.9521	0.9761	1.52	
Orchard.*Units* stratum					
Treatment	12	20.9709	1.7476	2.72	0.001
Residual	570	366.4034	0.6428		
Total	584	389.3265			

TSS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Farm stratum	2	37.7890	18.8945	63.11	
Farm.*Units* stratum					
Treatment	13	4.9314	0.3793	1.27	0.293
Residual	26	7.7843	0.2994		
Total	41	50.5048			

Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Farm stratum	2	0.106181	0.053091	28.12	
Farm.*Units* stratum					
Treatment	13	0.046706	0.003593	1.90	0.079
Residual	26	0.049084	0.001888		
Total	41	0.201971			

APPENDIX 4.2. Table of data for graphs in Experiment 1 - Chapter 6.

Treatment (stage, T, t)	Green colour		Green H°	Yellow H°	Blotchiness	DTR (days)
Control	16.7	(8.3)	95.6	90.5	0.71	8.8
M, 24, 1	18.6	(10.2)	97.0	90.2	1.18	8.4
M, 24, 3	20.9	(12.7)	96.1	90.1	1.27	8.1
M, 28, 1	18.7	(10.3)	96.3	90.9	1.29	8.4
M, 28, 3	26.0	(19.3)	99.4	91.4	1.53	8.3
M, 24, UR	24.6	(17.3)	98.1	90.8	1.68	7.8
M, 28, UR	33.4	(30.3)	100.1	92.2	1.87	8.0
P, 24, 1	16.7	(8.3)	95.9	90.4	1.16	8.1
P, 24, 3	16.4	(8.0)	95.8	90.4	1.24	8.5
P, 28, 1	18.7	(10.3)	96.8	90.5	1.29	8.6
P, 28, 3	22.8	(15.0)	96.9	91.0	1.40	8.3
P, 24, UR	20.7	(12.5)	94.9	90.4	1.67	7.8
P, 28, UR	25.9	(19.1)	97.9	89.8	1.73	7.9
LSD	6.2		2.4	1.1	0.38	0.4

Stage of ripening: M = mid-climacteric; P = post-climacteric
T = temperature; t = exposure time; UR = until ripe
Control = ripened at 20°C all the time

Appendix 4.3. Analysis of variance for Experiment 2 – Chapter 6.

% Green colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	18	10377.4	576.5	4.48	<.001
Residual	1060	136327.2	128.6		
Total	1078	146704.6			

Hue angle of green skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	18	660.09	36.67	1.42	0.014
Residual	1060	27410.32	25.86		
Total	1078	28070.41			

Hue angle of yellow skin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	18	603.27	33.51	3.27	<.001
Residual	1060	10870.25	10.25		
Total	1078	11473.52			

CI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	18	161.2598	8.9589	18.95	<.001
Residual	1060	501.1165	0.4728		
Total	1078	662.3763			

DTR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	18	4657.196	258.733	72.72	<.001
Residual	1060	3771.494	3.558		
Total	1078	8428.690			

Anthracnose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	18	2839.26	157.74	1.78	0.023
Residual	1060	93959.49	88.64		
Total	1078	96798.75			

SER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	18	554.60	30.81	1.70	0.034
Residual	1060	19245.49	18.16		
Total	1078	19800.09			

Hue angle of flesh colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	18	642.455	35.692	4.64	<.001
Residual	1060	8158.723	7.697		
Total	1078	8801.178			

Lenticel spotting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	18	142.1874	7.8993	12.42	<.001
Residual	1060	674.1889	0.6360		
Total	1078	816.3763			

TSS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	18	3.584	0.199	0.11	1.000
Residual	38	68.993	1.816		
Total	56	72.577			

Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	18	0.208214	0.011567	1.50	0.143
Residual	38	0.292571	0.007699		
Total	56	0.500785			

APPENDIX 4.4. Table of data for graphs in Experiment 2 - Chapter 6.

Treatment (stage, T, t)	Green colour (%)		Green H°	Yellow H°	Dullness	CI	DTR (days)	Anthracnose (%)		SER (%)	
Control	9.8	(2.9)	93.4	90.2	1.17	0.00	10.8	7.49	(1.70)	0.82	(0.02)
M, 7, 1	10.9	(3.6)	93.2	89.3	1.38	0.23	11.6	5.74	(1.00)	0.47	(0.01)
M, 7, 3	18.6	(10.2)	93.6	89.7	2.00	0.75	12.4	6.83	(1.41)	0.46	(0.01)
M, 7, 7	22.0	(14.1)	95.7	90.4	2.36	1.38	15.5	6.40	(1.25)	0.00	(0.00)
M, 10, 1	10.9	(3.6)	94.2	89.4	1.45	0.14	10.2	5.85	(1.04)	0.65	(0.01)
M, 10, 3	13.7	(5.6)	94.0	89.7	1.39	0.44	11.8	5.96	(1.08)	0.33	(0.00)
M, 10, 7	14.3	(6.1)	94.6	90.7	1.61	0.61	15.2	10.48	(3.31)	0.83	(0.02)
M, 13, 1	11.1	(3.7)	93.4	89.6	1.48	0.12	10.6	9.16	(2.52)	0.00	(0.00)
M, 13, 3	11.0	(3.6)	93.3	89.4	1.45	0.28	11.6	5.74	(1.00)	0.68	(0.01)
M, 13, 7	12.5	(4.7)	92.9	90.0	1.44	0.46	15.0	7.53	(1.72)	0.73	(0.02)
P, 7, 1	12.3	(4.5)	94.9	89.1	1.65	0.44	11.0	6.58	(1.31)	0.00	(0.00)
P, 7, 3	13.9	(5.8)	93.7	89.5	1.93	0.71	12.5	7.48	(1.69)	1.08	(0.04)
P, 7, 7	19.2	(10.8)	95.6	90.7	2.48	1.43	16.4	8.06	(1.97)	0.86	(0.02)
P, 10, 1	12.2	(4.5)	94.2	89.2	1.59	0.17	10.4	7.74	(1.81)	0.39	(0.01)
P, 10, 3	13.6	(5.5)	94.0	90.2	1.52	0.39	12.2	7.33	(1.63)	0.23	(0.00)
P, 10, 7	13.9	(5.8)	94.3	90.4	1.41	0.54	15.6	10.38	(3.25)	2.05	(0.13)
P, 13, 1	11.7	(4.1)	93.1	89.1	1.45	0.11	10.6	6.58	(1.31)	0.00	(0.00)
P, 13, 3	14.1	(5.9)	94.4	89.8	1.56	0.23	11.8	7.31	(1.62)	0.33	(0.00)
P, 13, 7	12.3	(4.5)	93.3	90.2	1.41	0.33	15.5	11.28	(3.83)	2.62	(0.21)
LSD	4.8		1.8	1.1	0.25	0.25	0.9	3.46		0.80	

Stage of ripening: M = mid-climacteric; P = post-climacteric
T = temperature; t = exposure time; Control = ripened at 20°C all the time