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Effects of 1-methylcyclopropene (1-MCP) on shelf life and quality of Cavendish bananas

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Summary

Banana quality and shelf life are variable and often insufficient to meet the needs of marketers and consumers. Hence, postharvest treatment by 1-methylcyclopropene (1-MCP) may be variable. This study examined how the efficacy of 1-MCP varies throughout the year; the effect of concentration, duration and timing of ethylene application; the effect of hand position on the bunch; the timing of 1-MCP application in relation to ripening and harvest time; and the effect of ripening storage temperatures and chilling storage. Additionally, impact of 1-MCP treatment on ethylene synthesis was studied. All studies used Cavendish cv. Williams bananas grown on a single property in Innisfail, north Queensland, harvested at 75 to 80% maturity (when they were hard and green) and then transported at 14 °C to Adelaide.

When examining the effect of 24 h application of different concentrations of 1-MCP (0 to 10000 nL L^{-1}) prior to storage at 22 °C to partially ripened bananas throughout the year, 1-MCP at a concentration of 300 nL L^{-1} increased shelf life significantly in fruit harvested in different months across the year except March where 3000 nL L^{-1} was required. Fruit harvested in May was significantly more responsive with a greater than two-fold increase in shelf life. Firmness of 1-MCP treated fruit was up to 19% greater than the control across the year. Lower levels of weight loss and discolouration were observed in 1-

MCP-treated fruit regardless of harvest time while 1-MCP had no effect on total soluble solids (TSS).

Early-climacteric 1-MCP treatment significantly increased shelf life to a greater extent in fruit from the top of the bunch compared with fruit from the bottom of the bunch. While 1-MCP was effective on fruit from the top of the bunch regardless of the time of year fruit was harvested, it was only effective on fruit harvested from the bottom of the bunch in October and April.

Concentration and the duration of ethylene exposure had impact on efficacy of 1-MCP. 1-MCP was most effective at increasing shelf life and firmness when fruit were treated with 100 $\mu L\ L^{-1}$ ethylene for the first day and 2 $\mu L\ L^{-1}$ for the second day. Winter-harvested bananas that were exposed to 100 $\mu L\ L^{-1}$ ethylene for 50 h had a longer shelf life compared to bananas treated for 40 h. 1-MCP was only more effective in summer-harvested fruit when they were exposed to ethylene for 40 h with an increase in firmness. 1-MCP did not affect weight loss or discolouration when fruit were treated with ethylene at different concentrations or duration.

Simultaneous application of 1-MCP (30 $nL\ L^{-1}$) with ethylene (100 $\mu L\ L^{-1}$) in the second day and reapplication of 1-MCP (300 $nL\ L^{-1}$) alone in the third day more than doubled banana shelf life. When bananas were treated with 100 $\mu L\ L^{-1}$ ethylene for two days followed by 1-MCP for 24 h and ripened at 16 and 19 °C shelf life was extended significantly but not at 22 or 25 °C. Pre-

ripening chilling temperature (5 °C) also decreased shelf life to a greater extent than when fruit were stored at 15 °C.

Measuring activity of ethylene biosynthesis enzymes during ripening showed that pulp and the peel of bananas respond differently to ethylene and 1-MCP treatment with a greater and quicker impact on peel than the pulp. The findings of this study allow 1-MCP to be used in a more commercially reliable manner.

Declaration

I, FARID MORADINEZHAD, certify that this thesis does not incorporate without acknowledgement any material submitted for the award of any degree or diploma in any university or other tertiary institution, and that to the best of my knowledge, contains no material previously published or written by any other person, except where due reference is made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Farid Moradinezhad

Date 30.10.2006

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Abbreviations

1-MCP	1- methylcyclopropene
a.i.	active ingredient
ACC	1-aminocyclopropene-1-carboxylic acid
ACO	ACC oxidase
ACS	ACC synthase
ANOVA	analysis of variance
CA	controlled atmosphere
C_2H_4	Ethylene
$Ca(OH)_2$	calcium hydroxide
conc.	concentration
cm	centimetre
CO_2	carbon dioxide
CSIRO	Commonwealth Scientific and Industrial Research Organisation
cv.	cultivar
°C	degrees Celsius
dd	degree-days
e.g.	for example
et al.	et alia (Latin)
FAO	Food and Agriculture Organisation
FID-GC	flame ionisation detector- gas chromatography
FW	fruit weight
g	gram
h	hour
$HgCl_2$	Mercuric Chloride
i.d.	internal diameter
kg	kilograms
K_m	binding constant
KNO_3	potassium nitrate
kPa	kilo pascals

L	litres
LSD	least significant differences
Ltd.	limited
m	metre
M	molar
MA	modified atmosphere
min	minute
mg	milligram
mL	millilitre
mm	millimetre
mM	millimolar
mmol	millimole
MT	metric tons
N_2	nitrogen
NaOCl	Sodium Hypochlorite
nL	nanolitres
nmoles	nanomoles
n (no)	number
NSW	New South Wales
O_2	oxygen
P	probability
pH	potential of Hydrogen
ppb	parts per billion
ppm	parts per million
P>0.05	non-significance level of over 5%
P<0.05	significance level of 5%
QLD	Queensland
®	Registered trademark
r	coefficient of correlation
RH	relative humidity
sec	second
TSS	Total soluble solids

UK	United Kingdom
USA	United States of America
var.	variety
v/v	volume to volume
µL	microlitres
µM	micromolar
%	percent

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Chapter 1

Literature review

1.1 Introduction

1.1.1 Background

Bananas are a common everyday fruit in most countries around the world. They are the staple food for over 400 million people in the developing world (Lopez-Gomez et al., 1997). When bananas are harvested at a mature stage that can withstand the rigors and duration of transport and then treated with ethylene to promote ripening before sale, their quality is increased (Saltveit, 2004). However, autocatalytic ethylene production causes unwanted changes such as over-ripening of fruit. Hence, there is a requirement to control the action of ethylene through methods such as inhibition of ethylene perception.

In recent years effective agents for blocking the ethylene receptor have been discovered and hold the promise of being a new way of controlling ripening, senescence and other ethylene responses (Sisler and Serek, 1999). 1-methylcyclopropene (1-MCP) is the best example of synthetic cyclopropenes which block ethylene receptors and prevent ethylene effects in plant tissues for extended periods (Sisler et al., 1996a; Sisler and Serek, 1997). This chemical provides a valuable tool to investigate ethylene metabolism and has the

potential to extend the storage life of horticultural products (Watkins et al., 2000).

The ability of 1-methylcyclopropene (1-MCP) to delay ripening of pre-climacteric bananas has been demonstrated (Golding et al., 1998; Jiang et al., 1999a; Sisler and Serek, 1997) as well as the time-concentration-temperature dependence of this response (Jiang et al., 1999a; Jiang et al., 1999b; Macnish et al., 2000b). However, the reported efficacy of 1-MCP in these studies was quite variable. A variety of factors may need to be considered when using 1-MCP on bananas, including concentration, timing, developmental stage, ethylene, temperature and harvesting season. All of these factors are significant as the final quality of fruit can be determined by them. Hence, the goal of postharvest physiologists is to maximise the postharvest quality of horticultural commodities through not only postharvest treatment but also potential consideration of preharvest elements.

1.1.2 Outcomes for industry

The overall aims of this work were to evaluate the efficacy of 1-MCP on extending the shelf life of bananas; to develop management strategies for commercial 1-MCP application; and to find out how 1-MCP affects the ripening process. This investigation also aimed to examine the external and internal quality of harvested bananas caused by exposure to 1-MCP and exogenous ethylene. Contribution to this knowledge will help to improve the

postharvest quality and shelf life of bananas for industry and consumer satisfaction.

1.2 Banana

1.2.1 Classification, morphology and human nutrition

Bananas are monocotyledonous and classified in the family Musaceae of the order Zingiberales. The Musaceae family has two genera: *Musa* and *Ensete*. All edible banana cultivars are classified into *Musa* (Hulme, 1971). Banana is a tropical tree-like perennial herbaceous plant, two to nine metres tall, consisting of an underground corm and a trunk comprised of concentric layers of leaf sheaths. At 10 to 15 months after the emergence of a new plant, its true stem rapidly grows up through the centre and emerges as a terminal inflorescence, which bears fruit. Banana varieties, which produce fruit of commercial use, are parthenocarpic, triploids (2n = 33) and derived from the wild diploid species *Musa acuminate* (2n = 22) or by hybridisation between this species and the wild diploid *Musa balbisiana* (Seymour, 1993). Cultivars belonging to *Musa* AAA include the Cavendish bananas, which form the basis of international trade (Seymour, 1993). The banana fruit is classified as a berry, which is an edible seedless pulp formed in the absence of pollination.

Nutritionally, the fruit of bananas contain significant amounts of calories, vitamins, proteins and carbohydrates; are low fat and are also rich in useful essential elements such as calcium, phosphorous, iron and potassium (Table

1.1). Although an important source of nutrition, the fact that per capita consumption worldwide is one to two per week (Economic Research Service, 2005; Hulme, 1971) might suggest on appreciation of its flavour and sweetness. It is also a source of vitamins A, B2, B3 and C (Gebhardt and Thomas, 2003).

Table 1.1: Typical composition of unripe and ripe banana fruit (g/100g edible portion of macronutrients and mg/100g of vitamins and minerals).[z]

Composition	Unripe	Ripe
Water	71.9	75.2
Protein	1.9	1.7
Fat	0.1	0.1
Sugar	1.3	17.3
Starch	21.2	3.1
Dietary fibre	3.2	2.8
Vitamin C	18	12
ß Carotene	0.2	0.1
Potassium	320	350
Calcium	5	5

[z]Adapted from Wills et al., (1998).

1.2.2 World production

Since 1981, the Food and Agriculture Organisation of the United Nations (FAO) estimates that total world banana production has increased from almost 40 million metric tons (MT) (Stover and Simmonds, 1987) to 72.4 million MT in 2005 (FAO, 2005). Currently, bananas rank first place in world fruit volume, followed by grapes (66.5 million MT), apples (63.4 million MT) and oranges (59.8 million MT) (FAO, 2005).

In 2004, a total of 130 countries produced bananas. However, production, as well as exports and imports of bananas, are highly concentrated in a few countries. The 10 major banana producing countries accounted for about 75% of total banana production in 2004 (FAO, 2005). India, Brazil, Ecuador, China, the Philippines and Indonesia alone produced about two-thirds in the 2000-2004 period (FAO, 2005) (Figure 1.1). Banana production in Australia was approximately 250,000 MT in 2005 (FAO, 2005). About 25% of world production is exported from the tropics to temperate climates. Before the 1940s the cultivar 'Gros Michel' dominated the international banana trade, until it succumbed to Fusarium wilt (Panama disease). Since the 1940s the trade has adopted cultivars of the Cavendish subgroup, because they are resistant to this disease (Hulme, 1971). Although Cavendish cultivars are resistant to Race 1 (FOC-1) and Race 2 (FOC-2), the Race 4 of the pathogen (FOC-4) damages these cultivars in subtropical banana-growing regions (Fernandez-Falcon and Borges, 2003) including Australia (Taylor et al., 2005). However, the

Cavendish banana dominates the market because of its sweet taste and its attractive peel colour.

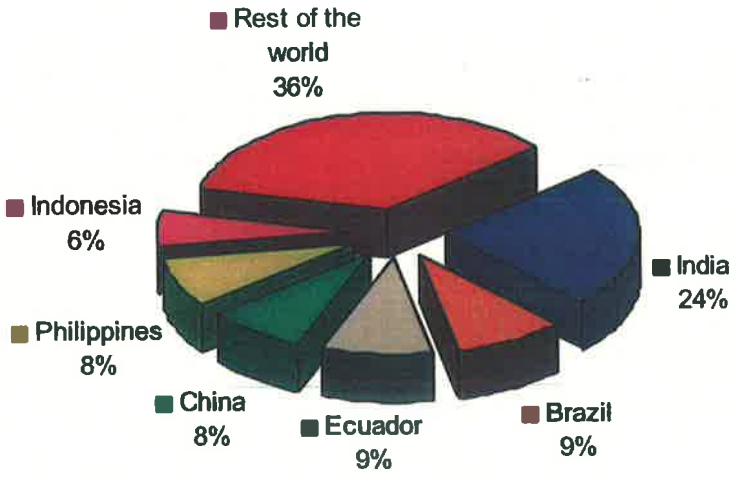


Figure 1.1: Distribution of world banana production in the 2000-2004 period (FAO, 2005).

1.2.3 Physiology and ripening of bananas

1.2.3.1 Physiology

1.2.3.1.1 Climacteric fruit

Fruit are typically classified into two groups based on their trends in respiratory behaviour during the final stages of ontogeny of the organ. Fruit that are able to produce ethylene autocatalytically after harvest and exhibit a marked upsurge in respiratory activity at the end of the maturation phase are

called climacteric, whereas nonclimacteric fruits do not exhibit a sizeable increase in their respiration rate (Kader et al., 1992). However, the respiration rate in bananas only increases after endogenous ethylene production increases from 0.05 to 3 μL ethylene kg^{-1} h^{-1} (Mitra, 1997).

Bananas are reported to be a typical climacteric fruit that shows a rise in ethylene production and respiration rate during ripening, followed a few days later by the production of a range of aromatic volatiles (Golding et al., 1999). Thus, only climacteric fruit produce ethylene autocatalytically, and this is triggered commercially by exogenous application of ethylene to ensure that all fruit ripen uniformly when ripened. Harvested mature bananas left to ripen naturally will eventually soften, but the change in colour and appearance of the peel will be unattractive (Kerbel, 2003).

1.2.3.1.2 Ethylene

Ethylene gas is a natural plant hormone produced by many horticultural commodities (Reid, 1992). Ethylene is a simple gaseous hydrocarbon (C_2H_4) that can diffuse into and out of plant tissues readily, from both endogenous and exogenous sources (Watkins, 2002). Ethylene is physiologically active at very low concentrations, such as 0.1 μL L^{-1} (Peacock, 1972; Wills et al., 2001). Pre-climacteric bananas have a constant endogenous ethylene production level (0.05 μL kg^{-1} h^{-1}) (Seymour, 1993). During ripening, ethylene production then increases and this is followed by a rise in the rate of respiration (Burg and Burg, 1965). At 15 °C, the typical respiration rate of green banana fruit is 45

mL CO_2 kg^{-1} h^{-1}, rising to 200 mL CO_2 kg^{-1} h^{-1} in ripening fruits (Wills et al., 1998). When the climacteric has peaked, ethylene production drops rapidly and respiration reaches its maximum (Seymour, 1993).

1.2.3.2 The ripening process of banana

Fruit ripening is a sequence of biochemical events resulting in loss of chlorophyll, formation of pigments, flavours and aromas, softening of the flesh, and eventual abscission of the fruit (Knee, 2002). The rate at which this ripening occurs is often controlled to prevent spoilage and to ensure uniformity and high quality, especially in climacteric perishable fruit like bananas.

1.2.3.2.1 Commercial ripening

It has been reported that very low concentrations of ethylene (10 to 50 μL L^{-1}) are sufficient to ripen the fruit (Kerbel, 2003). In commercial practice, however, the green bananas are placed in special ripening rooms and ripening is initiated by treatment with 1000 μL L^{-1} of ethylene for 24 to 48 h at 14.5 to 21 °C and at high relative humidity (90 to 95%) to ensure uniform ripening (Kerbel, 2003). Room ventilation after gassing with ethylene is also essential to keep CO_2 below 1% and avoid its impact of delaying ethylene action (Kays, 1991).

The most identifiable difference that consumers associate with unripe and ripe fruit is the peel colour change that occurs during banana ripening. The

Commonwealth Scientific and Industrial Research Organisation (CSIRO) banana ripening chart (1972) used by most commercial businesses (Figure 1.2), shows fruit colour converting from green into yellow with brown areas, in a total of eight ripening stages (Table 1.2). Bananas between ripening stage 4 (more yellow than green) to stage 7 (yellow with some brown flecks) are preferred by most consumers. Consumers also prefer fruit with sugar above 18% (Table 1.2) in fully ripe Cavendish bananas (CSIRO, 1972).

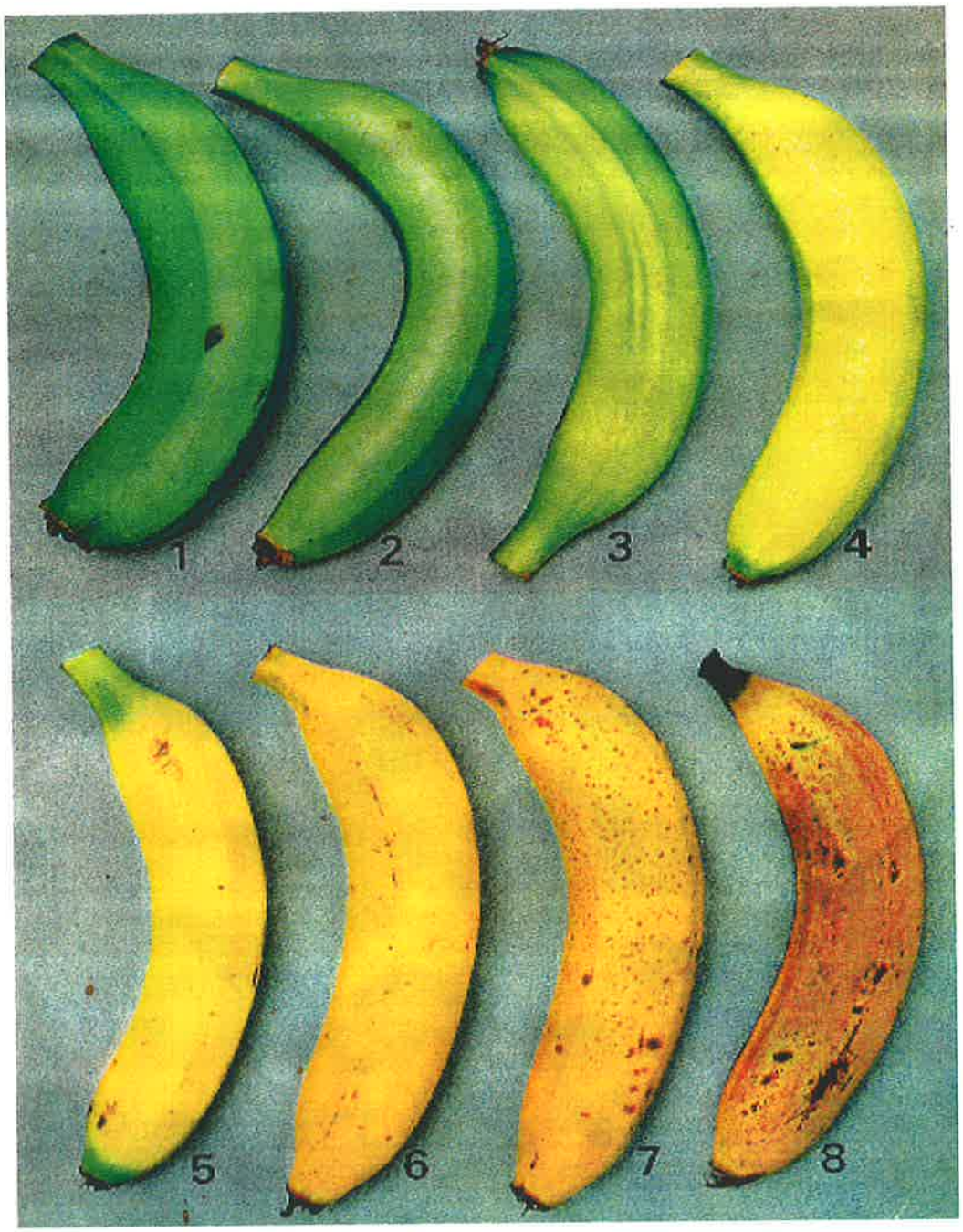


Figure 1.2: Banana ripening chart (CSIRO, 1972)

Table 1.2: Peel colour and carbohydrate correlation from the Australian Cavendish colour chart (CSIRO, 1972).

Stage	Peel colour	Sugar (%)	Starch (%)	Observations
1	Green	0.5	20	Hard, rigid, no ripening
Sprung	Green	1.0	19.5	Bends slightly, ripening started
2	Green, trace of yellow	2.5	18	
3	More green than yellow	4.5	16	
4	More yellow than green	7.5	13	
5	Yellow, green tip	13.5	7	
6	Full yellow	18	2.5	Peels readily; firm ripe
7	Yellow, lightly flecked with brown	19	1.5	Fully ripe; aromatic
8	Yellow with increasing brown flecks	19	1.0	Over-ripe; pulp very soft and darkening, highly aromatic

1.2.3.2.2 Ethylene biosynthesis

Ethylene is synthesised in plants from methionine via S-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC). SAM is the precursor for ethylene biosynthesis (Yang and Hoffman, 1984) (Figure 1.3). The two key enzymes of the pathway are ACC synthase and ACC oxidase. The enzyme involved in the conversion of SAM to ACC is ACC synthase. Conversion of ACC to ethylene is by ACC oxidase. In climacteric fruits, increasing ethylene production and increasing respiration are strongly related.

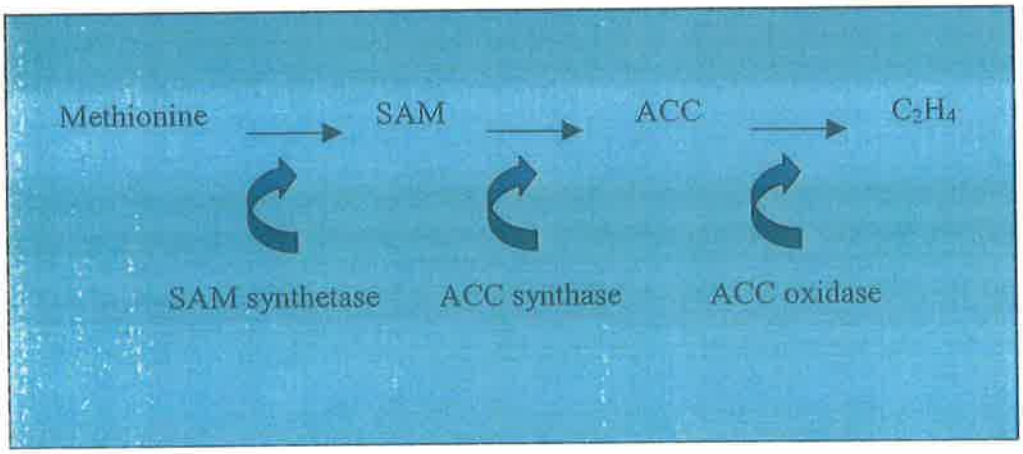


Figure 1.3: Ethylene biosynthesis pathway (adapted from, Yang and Hoffman, 1984). The formation of SAM is catalysed by SAM synthetase from the methionine. On the basis of Yang cycle, the first committed step of ethylene biosynthesis is the conversion of SAM to ACC by ACC synthase. ACC is the immediate precursor of ethylene. ACC is oxidised by ACC oxidase to form ethylene.

In banana, the rise in ethylene production is associated with an increase in the activity of the enzymes involved in ethylene synthesis (Lopez-Gomez et al., 1997). Ethylene is naturally produced during fruit ripening and all of the strategies in use nowadays to extend the shelf life of banana are based on the control of ethylene action and production.

1.2.3.2.3 Involvement of ethylene in fruit ripening

The ripening of climacteric fruit is divided into a preclimacteric and climacteric stage. The effects of exogenously applied ethylene and the pattern of ethylene production can be used to classify these stages. Two systems of ethylene production can be distinguished (McMurchie et al., 1972 cited in Oetiker and Yang, 1995). In *System 1* a low level of ethylene is present in early preclimacteric fruit before the onset of ripening while in *System 2*, the autocatalytic burst of ethylene production is high and accompanies the ripening process (Oetiker and Yang, 1995).

Oetiker and Yang (1995) stated that preclimacteric fruit are responsive to ethylene and their response is slow and not immediate. They also noted that the duration and passage of the preclimacteric stage is controlled by S*ystem 1* ethylene. Reduction of ethylene prolongs the preclimacteric life while an increase of ethylene shortens it. However, the onset of climacteric fruit ripening is reflected by a dramatic increase in ethylene production, enhanced ACC synthase production and an increase in respiration. This rapid developmental change from preclimacterium to climacterium, caused by the

transition from *System 1* to *System 2* ethylene production has been the target of numerous developmental studies in terms of ethylene (Oetiker and Yang, 1995). A recent study suggested that ethylene induced ripening of banana is characteristically different from that of other climacteric fruits, and that during ripening, ethylene biosynthesis may have more than one mechanism which are tightly controlled at various levels (Pathak et al., 2003).

Although the physiological aspect of fruit ripening has been well documented (Pua and Lee, 2003), its molecular basis is still being determined. Ethylene is a plant hormone regulating fruit ripening by coordinating the expression of genes (Jiang and Joyce, 2000). Components of the ethylene signal transduction pathway have been identified by characterisation of ethylene-response mutants in *Arabidopsis thaliana* (Bleecker, 2000). Perception of ethylene occurs through a chain of events involving proteins that were first identified in *Arabidopsis*. The essential elements of this pathway appear to be conserved among plants where it has been examined, suggesting its importance to plant development and survival. There are five known ethylene receptor proteins in *Arabidopsis:* ETR1, ETR2, EIN4, ERS1 and ERS2 (Ethylene Receptor 1, Ethylene Receptor 2, Ethylene Insensitive 4, Ethylene Response Sensor 1 and Ethylene Response Sensor 2, respectively) (Stearns and Glick, 2003). Ethylene binds to these receptors with the help of a copper cofactor that is bound in the receptors' transmembrane domain. Further molecular genetic studies in *Arabidopsis thaliana* have led to the development

of a model for ethylene signal transduction in plants (Guo and Ecker, 2004). Regulation of the five receptors is quite complex and interdependent.

1.2.3.2.4 Compositional changes in the ripening banana

The most abundant constituent of banana fruit is water. The peel of green bananas has 90% water, while it is about 73% in the pulp. Ripening increases the water concentration in the pulp from 73 to 82%. As the fruit ripens water is lost from the peel because of a threefold increase in evaporation (Turner, 1997). This implies the importance of the high humidity (90 to 95%) recommended by Blankenship and Herdeman (1995) for the ripening period after ethylene gassing to ensure bananas maintain high quality. It has also been reported that moisture loss from preclimacteric fruits including banana fruit hastens ripening (Littmann, 1972).

Some other important compositional changes happen during banana ripening such as the hydrolysis of starch and the accumulation of sugars. Starch is almost completely hydrolysed when ripening is completed. The conversion of starch to sugars in the fruit is an important component of the ripening process in banana, giving the fruit its distinctive sweet flavour as well as precursors for many of the aromatic flavour compounds (Kays, 1991). Sucrose, glucose and fructose are the major sugars in banana pulp (Hulme, 1971). A number of different hydrolase enzymes break down the carbohydrate polymers (e.g. pectins and celluloses) responsible for the structural integrity of cell walls. Among these enzymes is the enzyme polygalacturonase (PG), which

hydrolyses the α (1-4) linkage between galacturonic acid residues in pectins, and this could be one of the enzymes responsible for fruit softening during ripening (Pathak et al., 2000). Pectin-methyl-esterase (PME) shows activity throughout fruit development and may increase accessibility of PG to its pectin substrate (Giovannoni, 2001). PME seems not to affect softening during ripening; however, in tomato, endo-PG (Orfila et al., 2002) and expansins (Giovannoni, 2001) may play a role.

The change in colour of the peel from green to yellow is the most obvious change which occurs during ripening, and so peel colour is used as an indicator of ripening. Most colour changes in fruit are associated with a decrease in the concentration of chlorophyll in the chloroplasts, so pigmentation provides quality information, such as the degree of ripeness of banana fruit (Kays, 1991). The loss in chlorophyll is mediated through an increase in the activity of the enzyme chlorophyllase.

During ripening 350 volatile compounds are produced by bananas (Stover and Simmonds, 1987); the volatiles are mainly a complex mixture of esters, but alcohols, aldehydes, ketones and aromatic compounds are also present. In banana, amyl esters and isoamyl acetate are two character impact compounds responsible for the characteristic aroma (Kays, 1991). The pH of pulp falls during ripening from about 5.4 to 4.5 and malic acid increases between 2 and 6.5 fold (Hulme, 1971). Several researchers have studied the relationship between banana ripening and other compounds such as proteins

(Stratton and Loesecke, 1930; Steward et al., 1960a; Sacher, 1967; Brady et al., 1970a, in Hulme, 1971), vitamins (Thornton, 1943, in Hulme, 1971) and lipids (Golstein and Wick, 1970, in Hulme, 1971); however, no significant changes in these compounds were found during ripening. A recent study noted that no differences in the total amount of aroma compounds of bananas between control and 1-MCP-treated fruit were observed at 20 °C (Pelayo et al., 2003).

1.2.4 Fruit quality

1.2.4.1 Introduction

Quality, that is the degree of excellence or superiority, of fresh fruits and their products is determined by a combination of attributes, properties or characteristics that give each commodity value in terms of human food. The relative importance of each quality component depends upon the commodity, the perception of different individuals in the handling chain and its intended use. To producers a given commodity must have good appearance and high yield as well as be easy to harvest and able to withstand long-distance shipping to markets. Firmness, appearance quality and shelf life are important for wholesale and retail marketers, whereas consumers judge quality on the basis of firmness and appearance and subsequently flavour, nutritional quality (Kader, 1999) and shelf life of the product.

1.2.4.2 Appearance (visual) quality factors

Product appearance is characterised by shape, size, colour and also freedom from defects (Kader, 2000). Colour is one of the most important quality criteria for banana fruits (Medlicott et al., 1992) especially during ripening. Defects can originate before harvest as a result of damage by diseases, insects birds and so on; chemical injuries; and various blemishes such as russeting or scars. However, they become visible on ripened banana fruit before or after harvest. Many defects such as bruising, malformation, cuts, neck injury, split peel, crown end rot and maturity bronzing can occur when the conditions are not optimal (Chiquita, 1997). Banana shelf life (number of days between stage 4 to 7) is also significant, as consumers prefer fruit with a good appearance too.

1.2.4.3 Texture (feel) quality factors

These may include firmness, juiciness, mealiness and toughness depending on the commodity. The importance of textural quality is not only for their eating quality but also for their shipping ability. The firmness of many ripening fruit decreases with ethylene treatment. This is usually beneficial when associated with ripening, but if applied for too long, ripening can progress into senescence and the flesh can become too soft leaving a product that is not desirable for shipping (Saltveit, 1999).

1.2.4.4 Flavour (eating) quality factors

These include sweetness, acidity, bitterness and aroma. Sensory evaluation is an important aspect of fruit quality. In general, ethylene enhances taste and flavour by stimulating fruit ripening. Exogenous ethylene not only caused the peel and flesh to ripen out of phase, with the flesh ripening faster than the peel, but also the ethylene-treated fruit were more fruity and softer than fruit ripened without ethylene applied (Scriven et al., 1989). Soluble solids can be easily measured by refractometry.

1.2.5 Pre and postharvest factors influencing postharvest life of bananas

1.2.5.1 Time of harvest and maturity

In the commercial production of bananas the fruit is harvested while green and transported to market where, in some countries, it is ripened under controlled conditions. There is almost always a need to transport the fruit in a green state, as banana is a perishable fruit. Harvest time therefore represents a compromise between leaving the fruit on the plant long enough to maximise yield, but harvesting it soon enough so that sufficient green life remains to market the fruit in an acceptable manner. Despite the importance of these two components of fruit quality they are rarely measured in experiments (Turner, 1997) even though it has been established that green life declined exponentially with time while fruit size increases exponentially (Turner, 1997).

In this case, maturity will be a function of fruit growth rate, bunch emergence to harvest interval, market needs for fruit size and environmental conditions during postharvest as well as the developmental lag within a banana bunch. The fruit mature over the length of the bunch, with basal fruit being more mature than distal fruit. Singh et al. (1976) showed that it took 10 to 11 days from the emergence of the bunch until the appearance of the last hand of fruit. Some of the important strategies that are used in practice to estimate harvest maturity are bunch emergence to harvest interval, thermal time and fruit size.

The bunch emergence to harvest interval varies from an average of 80 days in the summer months to 120 days in winter (Stover and Simmonds, 1987). Given that north Queensland (the primary banana production area in Australia) is subject to temperature fluctuations and also temperature variations between seasons (Palmer, 1971), the thermal units that fruit experience during different seasons of year and subsequent quality may vary to some extent. Time of year at harvest and fruit maturity therefore may affect the quality and ripening of 1-MCP-treated fruit as bananas are harvested in both sub-tropical and tropical climates throughout the year. Previous studies have indicated that seasonal effects influence postharvest life and ripening of bananas (Bagnato et al., 2002; Palmer, 1971).

1.2.5.2 Ethylene application

From a postharvest perspective, for banana and other climacteric fruit, role of ethylene as a principal regulator of fruit ripening is the most important. The response to endogenously produced and exogenously applied ethylene are numerous and varied, and can be beneficial or detrimental (Table 1.3).

In climacteric fruits, ethylene is produced in relatively large amounts. The autocatalytic biosynthesis of ethylene that occurs in pre-climacteric banana can be initiated by exogenous ethylene application. Once triggered, the fruit ripen, even if the exogenous ethylene source is removed. This is due to the ability of ripening bananas to produce endogenous ethylene. Peak ethylene production by Cavendish bananas was reported to be around 3 $\mu L\ kg^{-1}\ h^{-1}$ (Seymour, 1993). Peacock (1972) demonstrated that even very low concentrations of ethylene (0.3 to 0.4 $\mu L\ L^{-1}$) can initiate ripening of bananas. He concluded that ethylene was physiologically active during the preclimacteric life of the banana. The three factors affecting the response of the fruit to green life were the time since harvest that exposure to ethylene commenced, the length of the exposure time and the fruit maturity (Peacock, 1972).

Table 1.3: Some of the beneficial and detrimental effects of ethylene on the quality of fruits[z]

Beneficial effects	Detrimental effects
Promotes colour development in fruit	Accelerates senescence
Stimulates ripening of climacteric fruit	Stimulates chlorophyll loss (e.g. yellowing)
Promotes de-greening of citrus	Enhances excessive softening of fruits
Stimulates dehiscence in nuts	Promotes discolouration (e.g. browning)

[z]Adapted from Saltveit (1999)

Ethylene at concentrations of 100 to 1000 μL L^{-1} for 24 to 48 hours, applied as either a single shot of ethylene gas or in a trickle mechanism, are used for initiating ripening in commercial situations, even though levels as low as 0.1 or 1 μL L^{-1} for 24 hours are equally as effective in initiating ripening (Wills et al., 2001). Despite this, it is not known what the optimum amount of exogenous ethylene gas is to initiate ripening while resulting in the best quality produce with an extended shelf life, when followed by 1-MCP application. Bagnato (2002) stated that no significant differences were found between quality and shelf life of bananas that were ripened with ethylene at different concentrations (50, 300 or 1000 μL L^{-1}). Further studies indicated that time to ripen of all climacteric fruits increased linearly with a logarithmic decrease in ethylene concentration over the whole concentration range (0.005 to 10 μL L^{-1}) (Wills et al., 2001). However, the quality and shelf life of bananas exposed to

lower (<50 μL L^{-1}) ethylene levels or for different durations (30 to 50 h) were not compared to those exposed to higher (>50 μL L^{-1}) ethylene levels, as well as when 1-MCP is applied to the partially ripened ethylene initiated bananas. This may be a useful evaluation to observe whether quality and shelf life extension differences occur.

1.2.5.3 Temperature

Temperature management is the most important tool to extend shelf life and maintain quality of fresh fruits. Temperature control of banana fruit should be considered while in the field, at harvest, as well as in postharvest storage and during the ripening period. Temperature control of fruit while in the field is difficult and therefore differences in field temperatures between winter and summer season occur. Temperatures close to harvest influence fruit development. High temperatures will hasten fruit ripening and lead to early harvest (Lurie, 2002).

Unlike in the field, temperatures of fruit after harvest can be controlled. Regulating temperature is the principal way to control respiration rate, ripening and senescence. The major reason that postharvest life of a fruit is extended by cooling is that metabolism is slowed by low temperatures. Each 10 °C decrease in temperature will reduce respiratory activity by a factor of two to four (Lurie, 2002). Another benefit of lowering temperature is that ethylene production is reduced. The ethylene synthesising enzymes are sensitive to low temperatures and, as the temperature is lowered, less ethylene will be produced

(Larrigaudiere et al., 1997). In addition, lowering of storage temperatures decreases fungal growth, tissue softening and water loss (Mitra, 1997; Thompson, 2003).

Recommended ripening temperatures for bananas are 20 or 21 °C in summer and 19 °C in winter (Young et al. 1932, in Bagnato et al., 2002). Ripening bananas at 14 and 16 °C extended shelf life by up to 50 and 32%, respectively, but ripening at 18 or 20 °C throughout the year results in a better visual appearance of the fruit, which is essential for consumers (Bagnato et al., 2002). At lower temperatures, ripening processes and decay are slowed but not stopped and storage time is limited. However, subtropical and tropical fruits do react adversely to temperatures between 0 and 13 °C (Wills et al., 1998). Bananas particularly show the negative effects of low temperature at 12 °C and below (Wills et al., 1998) as the threshold for chilling injury of banana is in the 12 to 13 °C range. This temperature stress causes the physiological disorder chilling injury, which is not only a postharvest problem, but can also occur in plants throughout their development including in the field, during transport, as well as in storage (Kays, 1991; Kays, 1999). Unlike freezing injury, chilling injury generally requires an extended exposure. Lower temperatures for short periods therefore, could possibly be used to store ripe bananas to extend their shelf life. It has been reported that chilling treatment also influences ripening of climacteric fruits like pears through accelerating ethylene synthesis (Wang et al., 1972 cited in Lelievre et al., 1997). Recently, it has been reported that

chilling injury in banana reduced the sensitivity of fruit to ethylene and therefore affected the ripening process (Jiang et al., 2004). Thus, low temperatures may accelerate or delay ethylene synthesis and subsequently alter the speed of ripening. This may in turn have an effect on the inhibitory action of 1-MCP on banana ripening. Chilling temperatures that may occur during the postharvest chain or during the preharvest stage may also influence the efficacy of 1-MCP on partially ripened bananas.

Despite this, generally almost two-thirds of the ripening period after ethylene gassing occurs in supermarkets, where they are stored at a common temperature (approximately between 20 to 24 °C), hence, the ripening temperature for most of the experiments in this project are set at 22 °C to reflect market temperature in order to properly evaluate the 1-MCP efficacy in extending the banana shelf life and improving fruit quality in a similar situation like handling and marketing.

1.2.6 Post-ripening storage

Bananas are delivered to market in a firm green condition and as free of blemishes as possible (Stover and Simmonds, 1987). They are then ripened by releasing ethylene into a room with controlled temperature and humidity (Kerbel, 2003). However, high ethylene levels as a result of exogenous ethylene application and high respiratory rate in bananas hastens the ripening process and reduces storage life (Paull, 1993). Banana shelf life often can be as short as three days, when stored at supermarket temperatures of 23 °C,

compared to a postharvest life of seven to 28 days when stored at 14 °C (Paull, 1993). As this continual temperature regulation and high air humidity (95%) within the supermarket is unlikely, it is difficult to speed up the ripening process while extending the storage life and maintain the high quality fruit, to ensure consumer satisfaction and maximum profit. As most of the consumers prefer to eat bananas at ripening stages 5 to 6, post ripening treatments need to be used to extend banana shelf life (stages 4 to 7) for as long as possible. One approach to control ripening is to vary storage environmental conditions such as temperature, humidity and gasses including O_2, CO_2 and ethylene.

1.2.6.1 Controlled atmosphere (CA) and modified atmosphere (MA) storage

Optimum storage conditions for bananas are about 13 to 14 °C with a relative humidity of 85 to 90% (Sommer and Arpaia, 1992). Today, however, improved control atmosphere (CA) or modified atmosphere (MA) systems can also be used. Decreased O_2 and / or elevated CO_2 levels during storage conditions generally increase the storability of most horticultural crops (Watkins, 2002; Yahia, 1998).

CA storage is a technique for maintaining the quality of produce in atmospheres that differ from air with respect to the proportion of O_2 and / or CO_2. Respiration and ethylene production rates of bananas are decreased in a CA store of 2-5% O_2 and 2-5% CO_2 (Reid, 1992). Postharvest life potential of

mature-green bananas at 14 °C is 2 to 4 weeks in air and 4 to 6 weeks in CA (Kader et al., 1992).

MA storage is similar to CA storage except that atmospheric composition is obtained through the combined effect of respiration and the use of sealed semi-permeable enclosures (e.g. polyethylene bags) (Abdullah et al., 1990). Increase in CO_2 concentration within the container suppresses the activity of many enzymes that normally increase during ripening (Abdullah et al., 1990). However, in MA storage, ethylene accumulation in polyethylene bags can cause green ripe banana fruit when the storage period is too long. Removal of ethylene from the storage atmosphere can increase the green life of banana fruit (Saltveit, 1999). Green mature Cavendish bananas can be stored in low density polyethylene bags (0.05 mm thickness) for up to 30 days at 8 to 14 °C; such that in-package atmosphere was 3 to 11% O_2 and 3 to 5% CO_2 (Hewage et al., 1993). A disadvantage of MA storage is the need for the packaging to stay intact throughout storage to maintain the internal atmosphere. This can be costly due to the high labour intensity required to repackage bananas in damaged MA bags during the ripening period. Thus, using ethylene antagonist compounds such as aminoethyoxyvinylglycine (AVG); 2,5-norbornadiene (2,5-NBD) and diazocyclopentadiene (DACP) (Blankenship and Dole, 2003) seem to be a cheaper and easier method of improving quality and postharvest life of horticultural crops (including banana) than CA or MA packaging.

1.2.6.2 Ethylene antagonists

Ethylene biosynthesis in potted flowers was effectively prevented by treatment with aminoethyoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis (Serek and Sisler, 2001), but this treatment was not effective in the presence of exogenous ethylene. Quality improvement has been achieved by spraying plants with an inhibitor of the ethylene receptor, the anionic silver thiosulfate (STS) complex (Blankenship and Dole, 2003) and has become widely used commercially (Cameron and Reid, 2001). However, silver is a heavy metal pollutant, and there has been rising concerns about ground water pollution, thus its use has been restricted to ornamentals (Cameron and Reid, 2001). 2,5-norbornadiene (2,5-NBD) has the disadvantages of requiring continuous exposure, a high concentration and possessing a strong odour reviewed by Watkins (2002). Diazocyclopentadiene (DACP), a known ethylene inhibitor, also increased the display life of plants not treated with exogenous ethylene. It is also explosive at high concentrations (Watkins, 2002). Each of those presumed inhibitors of ethylene perception, therefore, has limitations that affect commercial acceptance.

Relatively recently, the discovery of 1-methylcyclopropene (1-MCP), an organic molecule that blocks the ethylene receptor preventing ethylene production in plants (Sisler and Serek, 1997), has been identified as an ethylene antagonist with potential commercial use on ornamental plants and recently on edible crops in the USA (Environmental Protection Agency, 2006).

1.3 1-methylcyclopropene (1-MCP)

1.3.1 Introduction

1-MCP is an organic compound which blocks ethylene receptors and prevents ethylene effects (an ethylene antagonist). The background work for the discovery of 1-MCP as an ethylene inhibitor came out of the laboratories of Edward Sisler and Sylvia Blankenship, North Carolina State University. Although there are other compounds which are or have been used extensively in scientific investigations to reduce ethylene effects, such as 2,5-NBD (2,5-bicyclohepta-2, 5-diene), trans-cyclooctene and DACP (diazocyclopentadiene); 1-MCP represents the best example of a group of active cyclopropene compounds when evaluated based on concentration. For example, the effective concentration of compound needed to protect plants like banana and carnation against ethylene is very low for 1-MCP, being 0.7 and 0.5 nL L^{-1}, respectively, compared with other compounds like 2,5-NBD which requires 55,000 nL L^{-1} on banana, and DACP which requires 700,000 nL L^{-1} on carnation (Sisler and Serek, 1999).

1.3.2 Commercialisation

1-MCP is a gas with a molecular weight of 54.09 at standard temperature and pressure and a formula of C_4H_6. EthylBloc®, the first product containing 1-MCP, was registered on April 22, 1999 in the USA. To release the active ingredient (1-MCP gas), a powder (EthylBloc® or SmartFresh®) has to be mixed with water or KOH buffer. EthylBloc® has been registered for use on cut

flowers, potted flowers and nursery plants while commercial application of 1-MCP to edible crops was undertaken by AgroFresh Inc, under the trade name SmartFresh® (Blankenship and Dole, 2003). 1-MCP is registered as EthylBloc® and SmartFresh® for use on ornamental and fruit in several countries including USA, New Zealand and Colombia (Macnish et al., 2004). The safety, toxicity and environment profiles of 1-MCP in regard to humans, animals and the environment are extremely favourable (Environmental Protection Agency, 2006). The United States Environmental Protection Agency (USEPA) accepted SmartFresh® for apples, pears, avocados, tomatoes, melons and some other fruits on July 17, 2002, but have not yet for bananas. Recently, Health Canada's Pest Management Regulatory Agency (PMRA) has issued temporary registration for 1-methylcyclopropene and the associated end-use product, SmartFresh™ Technology, for postharvest use on apples to delay fruit maturation and maintain fruit firmness (Pest Management Regulatory Agency, 2006). In addition, 1-MCP was authorised in Australia in 2004 for use on some fruits such as apples, kiwifruit, melons, tomatoes, avocados and pears (Donnelly, 2006) but not yet for bananas. For this reason, there is a need for more investigation of its effect on banana before registration for use on bananas.

1.3.3 1-methylcyclopropene (1-MCP) and horticultural crops

Watkins and Miller (2004) summarised the physiological processes or disorders in 68 species including fruits, vegetables and ornamental products

that have been affected by application of 1-MCP. 1-MCP has been reported to delay or reduce ethylene-induced effects of senescence in a variety of potted flowering plants and cut flowers (Heyes and Johnson, 1998; Porat et al., 1995; Serek and Sisler, 2001; Serek et al., 1994; Serek et al., 1995; Sisler et al., 1996a). Effects of 1-MCP on vegetables include inhibiting the ripening of tomatoes (Colelli et al., 2003; Mostofi et al., 2003; Nakatsuka et al., 1998; Serek et al., 1995; Sisler et al., 1996b; Sisler et al., 1999; Wills and Ku, 2002), and the senescence of broccoli (Ku and Wills, 1999), and delaying fruit ripening and improving storage quality of climacteric fruits including kiwifruit (Kim et al., 2001), plums (Abdi et al., 1998; Dong et al., 2002), apple (Fan et al., 1999; Watkins et al., 2000), apricots (Dong et al., 2002; Fan et al., 2000) and avocado (Feng et al., 2000; Jeong et al., 2002). 1-MCP, therefore, has proved a valuable tool to investigate ethylene action during ripening of climacteric fruit (Nakatsuka et al., 1997) and has the potential to extend the commercial storage life of horticultural products. A variety of factors have been investigated in relation to 1-MCP usage, such as temperature, active concentration, treatment duration, time from harvest to treatment and developmental stage. Watkins et al. (2000) stated that concentration, time, stage of ripening and type of fruit are the important factors affecting the efficacy of 1-MCP, whereas DeEll et al., (2002) reported temperature and treatment duration as two important factors governing efficacy. In most studies 1-MCP has been applied at temperatures of about 20 °C (Blankenship and Dole, 2003). Lower temperatures have been used, but a relationship exists

between concentration, temperature and treatment durations, and applications at low temperature are not effective on some crops such as penstemon (Serek et al., 1995) and broccoli (Ku and Wills, 1999). Additionally, the minimum concentration of 1-MCP for an effect varies between crops (Blankenship and Dole, 2003) while low concentrations of 1-MCP may be as effective as higher concentrations if applied over longer durations. Treatment duration has ranged from 12 to 24 h, which was sufficient to achieve a full response. 1-MCP efficacy will also vary between produce at different stages of ripeness and time from harvest to 1-MCP treatment. Generally the more perishable the crop, the more quickly after harvest 1-MCP should be applied (Blankenship and Dole, 2003).

For ethylene to have an effect, it must first be bound on the surface of the cells to its receptors. 1-MCP can block this ethylene binding and prevent or seriously interfere with ethylene induced fruit ripening and its effects on fruit quality (Weis and Bramlage, 2002). The findings that exogenous ethylene treatment cannot induce 1-MCP-treated fruit ripening for some time suggests that the ripening process depends on the synthesis of new ethylene receptors (Jiang, 2000). The most recent theory on how 1-MCP may act to block ethylene action, is based on extensive research using *Arabidopsis* (Prange and DeLong, 2003). The model suggests that 1-MCP suppresses the ethylene response pathway by permanently keeping ethylene receptors and particularly ETR1 activated. This blocks the CTR1 protein, which normally keeps the

EIN2 protein and subsequent ethylene responses deactivated. Hence, when 1-MCP is bound the CTR1 protein can not be deactivated and ethylene response does not occur.

1.3.4 1-methylcyclopropene (1-MCP) and banana

In most studies in bananas, 1-MCP has been applied at concentrations from 0.7 nL L^{-1} (Sisler et al., 2000) to 450 µL L^{-1} (Golding et al., 1998); and treatment durations have ranged from 1 to 72 h. Banana fruit softening was less at higher 1-MCP concentrations and generally less for shorter storage durations (Harris et al., 2000). In most studies on bananas 1-MCP treatment duration ranged from 12 to 24 h, which was sufficient to achieve a full response; however, in a few studies treatment durations between 1 and 12 h were effective. Pelayo et al. (2003) found exposure to 1-MCP delayed changes in skin colour and flesh softening of bananas and the magnitude of this effect was dependent on concentration, but not on duration of exposure. Moreover, fruit exposed to 1000 nL L^{-1} 1-MCP for 6 or 24 h did not show differences in skin colour. Similarly, Bagnato et al. (2003) reported that exposure periods from 24 to 72 h did not affect the efficacy of 1-MCP on banana fruits.

1.3.4.1 Effect of 1-MCP on green life

In some studies effects on the green life (time to ripen) of bananas have been considered for 1-MCP before ethylene treatment. It has been reported that 6 h fumigation with 450 µL L^{-1} 1-MCP increased the green life of bananas held

in air from 20 to 30 days (Golding et al., 1998). Exposure for 24 h to 500 or 1000 nL L^{-1} 1-MCP at 20 °C extended the green life of 'Cavendish' bananas from 16 to 31 days in the absence of ethylene compared with untreated controls (Harris et al., 2000). To inhibit ripening, an extremely low concentration (9 nL L^{-1}) of 1-MCP needed to be applied before the pre-climacteric stage (Roh et al., 2000). The green life of banana fruit that were harvested at different maturities, 173, 156 and 71 days from bunch emergence, and then treated with 500 nL L^{-1} 1-MCP varied with fruit maturity. In the two most mature bunches it was about 28 days, four-fold longer than control (0 nL L^{-1} 1-MCP), and in the least mature bunch, green life was about 40 days, 1.5-fold longer than control, which was about 26 days (Harris et al., 2000). Treatment with 500 nL L^{-1} 1-MCP led to an unacceptably uneven skin colouration (Harris et al., 2000). In a separate study, the ripening of banana fruits was significantly retarded by 100 or 300 nL L^{-1} 1-MCP at the green mature stage (Wu et al., 2001).

1.3.4.2 Effect of 1-MCP on shelf life

While 1-MCP has been shown to delay ripening in ethylene-treated fruit (Jiang et al., 1999a; Jiang et al., 1999b; Sisler and Serek, 1999), the effect of 1-MCP on shelf life (number of days after ethylene treatment) is quite variable among studies. It has been found that 24 h exposure with 10 nL L^{-1} 1-MCP was sufficient to protect fruit for 11 to 12 days at 25 °C against ripening induced by 18 h exposure to 1000 µL L^{-1} ethylene (Sisler et al., 1996b). One hour exposure at 20 °C to 1000 nL L^{-1} 1-MCP or 12 h at 20 °C to 50 nL L^{-1} 1-MCP essentially

eliminated ethylene stimulated ripening effects (Jiang et al., 1999b). Also, authors have suggested the degreening response of banana fruit to 1-MCP treatment depended largely on the interaction between concentration and time. 1-MCP treated banana fruit had extended shelf life, as judged by inhibition of fruit softening, in comparison with untreated fruit. 1-MCP associated shelf life extension could only be achieved when it was applied during the earliest phase of fruit ripening (Jiang et al., 1999b). Bananas treated with 300 nL L^{-1} 1-MCP had a shelf life of 6 days compared with 3 days for non-treated fruit and 4 days for fruit treated with 3 nL L^{-1} 1-MCP, but fruit treated with 30 µL L^{-1} (high concentration) were externally and internally commercially unacceptable, as fruit developed crown rot prior to ripening (Bagnato et al., 2003). Application of 1-MCP in combination with the use of polyethylene bags could greatly extend the postharvest life of banana fruit (Jiang et al., 1999a). Similar concentrations and durations had different effects on bananas in different replications of their experiments (Pelayo et al., 2003).

Some factors have been identified that may affect the efficacy of 1-MCP on shelf life. In one study, 1-MCP treatment only slowed ripening of ethylene-treated fruits when applied one day after ethylene treatment and was ineffective when applied three or five days after ethylene treatment, that is, the ripening response of fruits varied with time interval between 1-MCP and ethylene treatments (Jiang et al., 1999b).

As the review of literature shows, many studies have been dedicated to better understanding the efficacy of 1-MCP on quality and shelf life of horticultural products including bananas. Factors studied include concentration of 1-MCP, treatment duration and temperature, but there is very little information about the effect of preharvest and postharvest factors that could influence the effect of 1-MCP. For example, different harvesting seasons may be relevant, particularly in Australia, where other ripening parameters such as optimum ripening temperature was different for summer and winter-harvested fruit (Bagnato et al., 2002).

1.4 Summary

The fact that 1-methylcyclopropene (1-MCP) has potential for the commercial control of the ripening and senescence of horticultural crops including climacteric fruits like bananas has been established by previous research, but many questions remain regarding the effect of 1-MCP on banana fruit. Firstly, the effective concentration range has been reported as between 0.1 nL L^{-1} and 450 µL L^{-1} 1-MCP. Little information is available about the optimum concentration for improved quality and shelf life of 'Cavendish' banana worldwide, let alone in Australia. Secondly, despite over 100 studies to examine the effect of 1-MCP on different plant species over the past few years, it is not yet known why the results have shown a lack of consistency. For example, Harris et al. (2000) found that 500 nL L^{-1} 1-MCP was needed to delay ripening while Sisler et al. (1996a) reported that as little as 0.7 nL L^{-1} was

effective. Similarly, Pelayo et al. (2003) observed that similar concentrations and durations had different effects in different replicates of their experiments. These might have been the result of different growing conditions and seasons (preharvest factors), different fruit maturities (at harvest), or different ethylene and 1-MCP concentrations. Thirdly, application of 1-MCP (SmartFresh®) has recently been accepted for edible crops (2004) in Australia, and more recently for use on bananas (Sprague, 2005), but not yet in Australia. More evidence is therefore required about optimum treatments for bananas before this product can be suggested for commercial use, because sometimes unexpected results have been reported regarding 1-MCP application. Fourthly, little information is available about the activities of ethylene biosynthesis enzymes when 1-MCP is applied to the partially ripened bananas. Finally, no studies have investigated the effect of 1-MCP on shelf life and quality of 'Cavendish' banana, in relation to 1-MCP concentration and the effect of harvesting season, hand position on the bunch, ethylene concentration and duration; and also little work has been done on the timing of 1-MCP application, and the effect of ripening storage or chilling temperatures on 1-MCP-treated bananas.

The objective of this study therefore was to evaluate the efficacy of 1-MCP treatment in enhancing the quality and shelf life of bananas in relation to not only various preharvest conditions but also with attention to postharvest treatment. This study determined whether 1-MCP treatments can be of

commercial use, and through enzymatic studies led to a greater understanding of the effect of 1-MCP on the ripening processes of bananas.

Chapter 2

General materials and methods

2.1 Plant material

Preclimacteric Cavendish bananas (*Musa acuminate*) cv. Williams, were used for all experimentation. They were harvested between 2003 and 2005 from a commercial farm (Tully property) in Innisfail (17° 31' S, 146° 01' E), north Queensland. They were selected from the middle of the banana bunch to ensure a uniform size and shape (Figure 2.1) unless indicated otherwise. They were harvested at 80% maturity as this is considered to be optimum (Chang and Hwang, 1989). Immediately after harvest, mature and green bananas at ripening stage 1 (Figure 1.2) were packed in cartons and transported within three days in refrigerated trucks at 14 °C, to the Adelaide Produce Markets (Pooraka) before delivery to the Plant Research Centre at the University of Adelaide, Waite Campus where the experiments were performed at 22 °C unless indicated otherwise.

2.2 Preparation of fruit for experimentation

On arrival, about half of the acquired bunches were selected based on their uniformity in hand size, shape, colour, weight and also freedom from

defects. Thereafter, all hands from three selected bunches were separated, deflowered and crowns cleanly cut off using a clean sharp knife. All fruit were therefore of a closely related maturity, as not only were they selected from three hands of the middle section of the three similar banana bunches but also fingers (12 to 14) from the middle section of each hand were chosen to ensure uniformity of ripening (Liu, 1976) (Figure 2.1). Any damaged fingers were also removed from each hand and not used. Individual bananas were then dipped for 1 min in 1 mL L^{-1} Sportak® fungicide (active ingredient prochloraz) (Hoechst Schering AgrEvo, Glen Iris, Victoria) to control postharvest disease (Wade et al., 1993) and then air-dried for 1 hour.

Individual fruit were then marked with a number, weighed and randomly placed into 10 L plastic containers onto metal mesh (for better ventilation) covered with heavy-duty towel. Lids were then sealed using grease vacuum Molykote 3®. The containers also contained 100 g Ca $(OH)_2$ to scrub CO_2 and a 20 mL saturated KNO_3 (to maintain relative humidity at 90%). There were six fruit per container with three replicates per treatment.

2.3 Treatment application

Each container was either treated with ethylene or 1-MCP or both as detailed in individual Chapters.

2.3.1 Ethylene application

Banana ripening was initiated by withdrawing a pre-determined volume from a compressed cylinder of ethylene gas (99.9 % purity, BOC gases Australia Limited, NSW) and injecting into the sealed 10 L plastic containers (containing fruit) via the sampling septum fitted in the lid. The concentrations of ethylene used were based on empty container volume calculations (for example, 1 mL of ethylene gas was injected into the 10 L plastic container to give 100 μL L^{-1} ethylene).

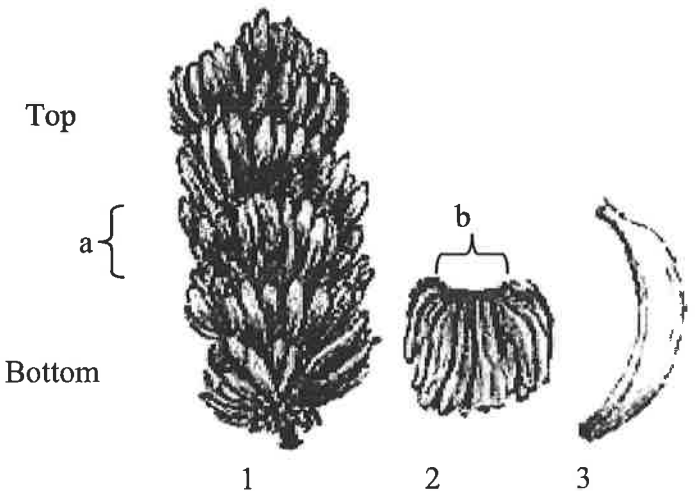


Figure 2.1: The banana bunch and its components. 1. bunch: a- middle section, 2. hand: b- 12 to 14 fingers of middle section 3. finger: individual fruit

2.3.2 1-Methylcyclopropene (1-MCP) application

1-Methylcyclopropene (1-MCP) atmosphere was created from SmartFresh® powder (3.3% active ingredient 1-MCP, Rohm and Haas, Philadelphia, USA) by dissolving a measured amount of SmartFresh® in 2%

potassium hydroxide (KOH) buffer in an airtight glass container (750 mL) in a ratio of 1 g SmartFresh® to 20 mL 2% KOH (Able et al., 2002) at 20 °C. The glass container was sealed with a stopper, Suba-seal no. 37. After 1 hour the appropriate quantity of 1-MCP was withdrawn from the stock 1-MCP atmosphere and then injected into the sealed plastic containers (10 L) containing the plant material resulting in the final 1-MCP concentration required. The quantity of 1-MCP used were based on empty container volume calculations. After 1-MCP treatment, fruit were ventilated, placed into unsealed clear polyethylene bags (255mm × 355mm × 38μm), placed onto shelves at 22 °C and assessed daily until the end of the experiment. Relative humidity in the plastic bag was approximately 85% as monitored using a digital humidity and temperature meter (Vaisala Sensor System, Vaisala Oy, HM 34, Finland).

2.4 Quantification of ethylene and 1-MCP

2.4.1 Monitoring ethylene levels

Ethylene levels of all treatments were monitored using a gas chromatograph (Varian 3400, Varian Associates Inc., Mulgrave, Victoria) connected to a flame ionisation detector (GC-FID) (Figure 2.2). A stainless steel column (60 cm x 3.175 mm i.d.) packed with Porapak Q with 80/100 mesh was used. Temperature conditions were set at 50 °C for the column, 135 °C for the injector and 150 °C for the detector. Flow rates of the carrier gases (Krajayklang et al., 2000) compressed air, nitrogen and hydrogen were 420, 50 and 40 mL/min, respectively. A 1.9 ± 0.1 μL L^{-1} ethylene gas mixture

(ethylene in nitrogen, β-standard, BOC gases Australia Limited, NSW) was used as a standard gas. For calibration of the GC-FID, two injections of the standard gas using a 1 mL syringe (fitted with a 25mm needle) were used. Ethylene concentrations were linearly calculated relative to the certified standard gas.

Figure 2.2: Gas chromatograph (Varian 3400)

2.4.2 Monitoring 1-MCP levels

1-MCP levels were monitored using a gas chromatograph (Varian 3400, Varian Associates Inc., Mulgrave, Victoria) fitted with a flame ionisation detector (GC-FID) and a stainless steel column (60 cm x 3.175 mm i.d.) packed with Porapak Q with 80/100 mesh was used. Temperatures were 90 °C for the column, 135 °C for injection and 150 °C for the detector. Flow rates of the carrier gases (Krajayklang et al., 2000) compressed air, nitrogen and hydrogen

were 600, 50 and 40 mL/min, respectively. Iso-butylene (103 ± 2 μL L^{-1}, iso-butylene in nitrogen, β-standard, BOC gases Australia Limited, NSW) was used as the standard gas to prepare the calibration curve and measure 1-MCP concentration (Pelayo et al., 2003). The retention times of iso-butylene and 1-MCP were ≈ 1.3 and 1.5 min, respectively, similar to Mir et al. (2001) who had used 1-butene as a standard gas. GC-FID calibration and sample injections were done as mentioned for ethylene (Section 2.4.1).

2.5 Quality assessments

Quality assessments of bananas were external and internal and included shelf life, discolouration index, weight loss, pulp firmness and total soluble solids (TSS). Nine fruit (three fruit per replicate) from each treatment were used to measure external parameters, and the remaining nine fruit (three fruit per replicate) allocated to each treatment were used to assess internal quality characteristics at colour stage 6 (full yellow; CSIRO, 1972). All assessments were performed at 22 °C.

2.5.1 Shelf life

The shelf life of bananas was evaluated by examining the surface colour of fruit peels twice daily and determining the number of days required for bananas to ripen from ripening stage 4 (where peel is more yellow than green) to ripening stage 7 (where peel is yellow light, with brown flecks) (Figure 1.2, CSIRO, 1972).

2.5.2 Discolouration index (DI)

Peel discolouration was observed visually at ripening stage 6 (full yellow, CSIRO, 1972) as follows: 0, no discolouration; 1, slight discolouration; 2, mild discolouration; 3, severe discolouration (Figure 2.3). Fruit scores (0, 1, 2, 3) were averaged into a discolouration index (DI), using the following formula (Bagnato et al., 2002). The formula uses the number of fruit with no discolouration (*a*), slight (*b*), mild (*c*), or severe discolouration (*d*) compared with the total number of fruit in each treatment (*n*).

$$\text{Discolouration index (DI)} = [(a \times 0) + (b \times 1) + (c \times 2) + (d \times 3)] / n \qquad (2.1)$$

2.5.3 Weight loss

Weight loss was measured by weighing individually marked fruit before ripening was initiated in ripening stage 1 as well as at ripening stage 6. Weight loss during this period was determined using the following formula.

$$\text{Weight loss (\%)} = [(FW_{S1} - FW_{S6}) / FW_{S1}] \times 100 \qquad (2.2)$$

Where,

FW_{S1} = Fruit Weight in stage 1 (before initiation of ripening)

FW_{S6} = Fruit Weight in stage 6

Figure 2.3: Discolouration score index (Bagnato, 2002)

Peel score and appearance			
0	1	2	3
			
Visual symptom			
yellow, no defects	dull grey/yellow coloured fruit	red/brown scarring along vascular tissue	red/dark brown marks all over banana
Assessment			
Healthy fruit	slightly discoloured	mild discolouration	severe discolouration

2.5.4 Pulp firmness

Pulp firmness was measured manually at ripening stage 6 (full yellow) using a twist tester (Department of Agricultural Engineering, Massey University, NZ) (Figure 2.4). Each banana was prepared for the twist test by cutting transversely. One half of the fruit was pushed (exposed pulp) onto the fixed blade using a firm pressure so that the blade penetrated a few millimeters into the fruit. Fruit were then slowly rotated in an anticlockwise direction until the pulp tissue failed and the maximum angle was recorded. This procedure was replicated using the second half of the banana. The two fruit scores were averaged and used in a twist tester formula as follows to convert all data into a crushing strength value.

$$\sigma = \frac{M \sin \theta}{a^2 b} \quad (2.3)$$

Where,

σ = crushing strength (Pa)

θ = maximum angle recorded (°)

M = Maximum moment produced by the arm when θ = 90 °, measured in Newton-meters (N.m)

a = radius of the blade (m)

b = width of the blade (m)

Figure 2.4: Twist tester (Massey University, NZ)

2.5.5 Total soluble solids (TSS)

Total soluble solids (TSS) were measured using a hand held refractometer (0 to 30% sugar w/w, Bellingham and Stanley Ltd., Tunbridge Wells, UK). Pulp from the middle section (a cross-section, approximately 4 mm in thickness) was squeezed through miracloth (Stretcher sheets no. 2055, Medical Concepts, Australia) onto the glass surface of the refractometer.

2.6 Fruit diameter

One of the important pomological characteristics of the finger is grade (diameter). In commercial practice using middle fruit in the outer whorl of the second hand on the bunch, the grade is measured with calipers. A grade of 34 mm is considered the optimum fruit diameter at harvest in banana bunch (Stover and Simmonds, 1987). To determine harvest grade the middle finger in

the outer whorl was calipered with a plastic digital calipers (Digimax®, Swiss) at the thickest part of the fruit as shown in Figure 2.5. Fruit diameter was recorded for all fingers used in experimentation.

Figure 2.5: Method of measuring finger grade using digital calipers (Digimax®, Swiss).

2.7 Statistics

A randomised block design or a split-plot design was used in all experiments, with three replicates. Each treatment within an experiment contained 18 bananas. Each experiment was usually repeated two or three times as detailed in individual Chapters. Data were analysed with the Genstat 6 program (Release 6.2, 6th edition, 2002, Lawes Agricultural Trust, VSN International Ltd) using one-way or two-way analysis of variance (ANOVA). A least significant difference test (LSD) at the 5% level was used to determine significant differences between means. Total weight loss data were expressed

as a percentage of the weight of fruit before ripening was initiated (ripening stage 1). Correlations between shelf life and quality assessments were also calculated.

2.8 Photographics

Images were taken using a digital camera (Kodak, EasyShare CX 4200, 2.0 Mega Pixels, Kodak, China), and a scanner (Epson Perfection 4180 Photo, Seiko Epson Corp., Indonesia), and processed using a photo editor (Release 3.01, 1998, Based upon HALO Desktop Imager, Media Cybernetics, L. P.).

Chapter 3

Effect of preharvest conditions and maturity on responses of bananas to 1-MCP

3.1 Introduction

Banana is a climacteric fruit, which is harvested throughout the year in subtropical and tropical climates. Environmental conditions (such as climate and soil) and physiological factors (such as maturity) are well documented as preharvest factors that influence fruit quality and maturation (Arpaia, 1994). Given that north Queensland (the primary production area for Australia) is subject to summer cyclones, temperature fluctuations and occasional chilling conditions, the quality of bananas can vary quite significantly.

This environmental variability also makes the interval between bunch emergence and harvest (Seberry and Harris, 1993) and ripening of harvested bananas (Palmer, 1971) difficult to predict. The bunch emergence-harvest interval varies from an average of 80 days in the summer months to 120 days in winter provided adequate moisture is available (Stover and Simmonds, 1987). In addition, the physiological maturity of banana fruit is not always the same, even in fruit of the same age (Marin et al., 1996). Physiological maturity

may be influenced by different factors, such as time of the year or other environmental conditions (Liu, 1976; Marin et al., 1996). Maturity at harvest significantly impacts the time before green bananas will initiate to ripening (Thompson, 2003).

Moreover, within a ripening bunch of bananas there is an unavoidable variation in maturity, that is, a bunch developmental lag between hands depending on their position on the bunch. The fruit mature over the length of the bunch, with fruit at the top (basal) being more mature than those at the bottom (distal) (Ahmad et al., 2001; Jullien et al., 2001). The youngest fruit (distal end) on the most immature bunches take longer to ripen than the youngest fruit on the more mature bunches (Ahmad et al., 2001) as well as the fruit at the top of the same bunch (Ahmad et al., 2001; Jullien et al., 2001).

To date, seasonal effects and those of the banana's physiological age have not been considered in studies investigating the application of 1-MCP to ethylene-initiated bananas (Bagnato et al., 2003; Golding et al., 1999; Jiang et al., 1999a; Jiang et al., 1999b; Macnish et al., 2000; Macnish et al., 1997; Pelayo et al., 2003). Given that seasonal differences influence shelf life and ripening of bananas grown in Australia (Bagnato et al., 2002; Palmer, 1971; Seberry and Harris, 1993), it is likely that preharvest factors may be a reason that bananas are variable in their response to the inhibitory effect of 1-MCP.

Although, to date, publications showing the impact of maturity on the response of bananas to 1-MCP have been limited, 1-MCP efficacy has been shown to decline slightly as harvest maturity increases in Red Delicious apples (Mattheis et al., 2005) and in Williams pears (Calvo, 2002) and much more dramatically in some faster ripening cultivars (Watkins, 2000). Previous studies on bananas (Harris et al., 2000; Wu et al., 2001) also noted that effectiveness of 1-MCP in extending postharvest life of bananas varied significantly according to the maturity of fruit at harvest. Although treatment with 1-MCP in the pre-climacteric stage at 22 °C for 24 h was effective in delaying ripening, as maturity progressed from 71 to between 156 or 173 days after bunch emergence, the time to ripen decreased from 40 to 28 days (Harris et al., 2000). Even though the application of 1-MCP to ethylene-initiated bananas (Bagnato et al., 2003; Golding et al., 1999; Jiang et al., 1999a; Jiang et al., 1999b; Macnish et al., 2000; Macnish et al., 1997; Pelayo et al., 2003) has been investigated the effect of hand position on the bunch on the efficacy of 1-MCP to extend shelf life and improve fruit quality has not been researched. The objectives of this study therefore were to evaluate the effect of time year at harvest and hand position on the bunch on the response of fruit to post-ripening 1-MCP exposure.

3.2 Materials and methods

3.2.1 Plant material and preparation

Mature and green Cavendish banana (cv. Williams) fruits were transported and prepared as outlined in Sections 2.1 and 2.2. In both experiments eighteen bananas were allocated to each treatment. Six fruits from each bunch were placed into a 10 L plastic container as one replicate. The containers were kept at 22 °C and banana ripening was initiated using a determined volume of ethylene gas injected into the containers for two consecutive days. All containers were ventilated for 20 minutes each day until the fruit had ripened to approximately stage 3.5 to 4 (CSIRO, 1972). Thereafter fruit were exposed to 1-MCP for 24 h at 22 °C.

3.2.2 Experimental procedure

3.2.2.1 Stability of 1-MCP levels

A previous review paper suggested that only one third of the initial concentration of 1-MCP remains in the container after 24 h at 5 °C in the presence of plant material, although no experimental data were presented (Blankenship and Dole, 2003). To test this, 1-MCP was released from SmartFresh® powder in the presence of six bananas or without plant material in an empty container repeated three times.

3.2.2.2 Preliminary studies

Green banana fruits (Stage 1) were acquired during July, September and November 2003. They were collected from three hands of the middle section of three bunches. They were prepared and placed into containers as outlined in Sections 2.3 and 2.3.1. After 48 h of ripening initiation with ethylene, containers were ventilated for 20 min and bananas were then treated with 1-MCP at 3, 30, 300, 3000 or 30000 nL L^{-1}. 1-MCP atmosphere was created from SmartFresh® (AgroFresh Inc., Rohm and Haas, Philadelphia, USA). 1-MCP was introduced by placing measured amounts of SmartFresh® into glass vials which were then placed into the containers immediately after mixing the SmartFresh® powder with warm water (40 °C) (Bagnato et al., 2003). The levels of 1-MCP were checked using a Varian gas chromatograph model 3400 as outlined in Section 2.4.

3.2.2.3 Effect of time of year at harvest on 1-MCP application effects

Green banana fruits (Stage 1) were obtained between March and January 2005 at bimonthly intervals (such that six trials were performed). They were collected from the middle section of bunches, prepared, placed into containers and were then treated with ethylene as mentioned in preliminary studies (Section 3.2.2.2). Fruit weight was measured for each finger. A stock 1-MCP atmosphere (300 μL L^{-1}) was created from SmartFresh® as described in Section 2.3 (Able et al., 2002). 1-MCP was introduced for all concentrations by

injection of stock 1-MCP gas into each sealed container. The levels of 1-MCP were checked using a gas chromatograph as per Section 2.4.2.

3.2.2.4 Effect of fruit position on the bunch on 1-MCP application effects

Three bunches of bananas were harvested from the same field in Innisfail, North Queensland in October 2004, February and April 2005. From each bunch, three hands were selected from the top section (basal) and three hands from the bottom section (distal). Fruit weight and diameter were measured for each finger. After preparation individual fruit were placed in containers according to treatment. The experimental procedure was as described for the time of year experiment (Section 3.2.2.3) except that only 0 (control) or 300 nL L^{-1} 1-MCP was applied for 24 h at 22 °C.

3.2.3 Quality assessments

Nine fruit from each treatment were used to measure external parameters, and the remaining nine fruit allocated to each treatment were used to assess internal quality measurable characteristics at colour stage 6 (CSIRO, 1972) as previously described in Section 2.5.

3.2.4 Climate Data Acquisition

Daily minimum and maximum temperatures were recorded at Innisfail Research Station (Qld) (Bureau of Meteorology, 2005) for 2003 to 2005. Both the average daily temperature and number of degree-days (for three months

prior to harvest) were determined using formulae 3.1 and 3.2 respectively. Where the average degree value for a given day was less than zero, it was recorded as zero, not a negative number.

$$\text{Average daily temperature} = \frac{(Max.\,Temp. + Min.\,Temp.)}{2} \qquad \text{(Formula 3.1)}$$

$$\text{Daily Degree day} = Average\ Daily\ Temp. - Base\ Temp.\ (14\ °C) \qquad \text{(Formula 3.2)}$$

The monthly average of recorded daily minimum temperatures at Innisfail Research Station (Qld) (Bureau of Meteorology, 2005) was also calculated for January 2004 to January 2005 (Figure 3.8).

3.2.5 Statistical assessments

A randomised block design was used in experiments, with three replicates. Data were analysed with the Genstat 6 program (Release 6.2, 6th edition, 2002, Lawes Agricultural Trust, VSN International Ltd) using one-way (time of year experiment) or two-way analysis of variance (ANOVA) (fruit position experiment). The least significant difference (LSD) at $P = 0.05$ was used to determine significant differences between means. Total weight loss data were expressed as a percentage of the weight of fruit before ripening was initiated (ripening stage 1). Correlations between fruit firmness, diameter, fresh weight and shelf life were calculated.

3.3 Results

Data of each harvest was analysed individually due to the difference between plant materials among different harvest times.

3.3.1 Stability of 1-MCP levels

In the presence of ethylene-treated fruit, the 1-MCP concentration in the container was reduced by 23% after 90 minutes and by 70% after 24 h. However, there was no reduction in 1-MCP concentration in the container without fruit even after 24 h (Figure 3.1).

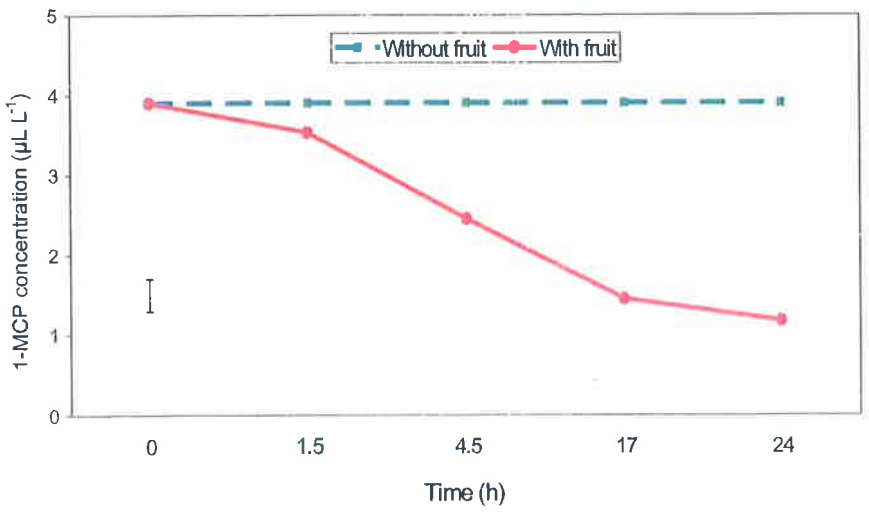


Figure 3.1: 1-MCP concentration in 10 L containers in the presence or absence of six bananas. Vertical bars represent LSD values at the 5% level (n=3).

3.3.2 Preliminary studies

In preliminary trials a very poor response was found with 3 or 30 nL L^{-1} 1-MCP while bananas treated with 30000 nL L^{-1} 1-MCP did not extend shelf life compared with those treated with 3000 nL L^{-1} 1-MCP (Figure 3.2). Bananas treated with 30000 nL L^{-1} 1-MCP also did not degreen properly. As a result, the two lowest and the highest concentrations of 1-MCP were altered, so that 1-MCP at 0 (control), 100, 300, 1000, 3000 or 10000 nL L^{-1} was used in the effect of time of year at harvest experiment during March 2004 to January 2005 (Section 3.3.3).

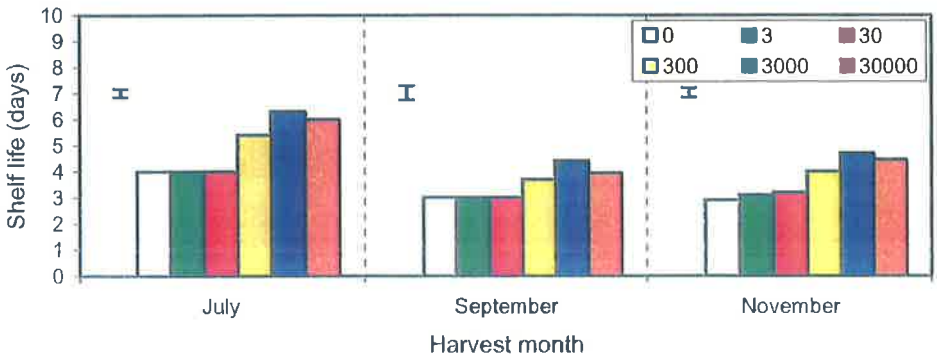


Figure 3.2: Effect of different concentrations of 1-MCP (0, 3, 30, 300, 3000 or 30000 nL L^{-1}) on shelf life of Cavendish bananas (ripening stages 4 to 7) harvested between July to November 2003 at bimonthly intervals and ripened at 22 °C. Bananas were treated with 100 µL L^{-1} ethylene gas for 48 h prior to 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9).

3.3.3 Effect of time of year at harvest on efficacy of 1-MCP

3.3.3.1 Shelf life

Shelf life of control fruit differed throughout the year (Figure 3.3). The lowest shelf life occurred in September (2.7 days). In March, July and January the shelf life of control fruit was similar (3.5 to 3.7 days) and the two highest shelf lives in control fruit were obtained in November (4.0 days) and May (4.3 days).

The most effective concentration of 1-MCP to give the greatest increase in shelf life, changed during the year (Figure 3.3). In March, a 1-MCP concentration of 3000 nL L^{-1} significantly increased shelf life. In May and July, only 300 nL L^{-1} was needed to increase banana shelf life significantly compared with the control. In September, November and January, 100 nL L^{-1} of 1-MCP was enough to extend banana shelf life significantly compared with the control. The highest increase was obtained in May (108%) when 1-MCP was applied at 300 nL L^{-1} followed by September (80%) when 1-MCP was applied at 1000 nL L^{-1}; and November (53%) and January (40%) when 1-MCP was applied at 100 nL L^{-1}. The two lowest increases in fruit shelf life were in March (30%) and July (27%) when 1-MCP was applied at 3000 nL L^{-1}.

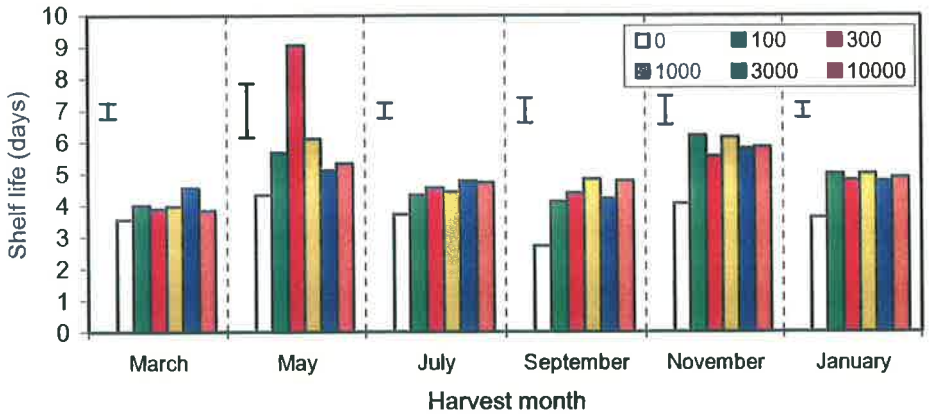


Figure 3.3: Effect of different concentrations of 1-MCP (0, 100, 300, 1000, 3000 or 10000 nL L^{-1}) on shelf life of Cavendish bananas (ripening stages 4 to 7) harvested bimonthly and ripened at 22 °C. Bananas were treated with 100 µL L^{-1} ethylene gas for 48 h prior to 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9).

3.3.3.2 Firmness

Firmness of control fruit was to some extent different among the harvested months throughout the year. The lowest firmness was obtained in March (79 kPa). In January the firmness of control fruit was only slightly higher (85 kPa). In May, July and November the firmness was similar (98 to 100 kPa) and the highest firmness in control fruit was obtained in September (107 kPa) (Figure 3.4).

The effective concentration of 1-MCP, which gave the greatest increase in firmness, changed during the year. Regardless of concentration, 1-MCP increased firmness significantly in March and November. Concentrations of 1000 and 10000 nL L^{-1} were most effective in March and concentrations of

1000 and 3000 nL L^{-1} in November. In July, 1-MCP concentrations at 1000, 3000 and 10000 nL L^{-1} were most effective and produced the greatest firmness in comparison with the control. 1-MCP, regardless of concentration, had no significant effect on firmness in January or September although the trends suggest that higher concentrations of 1-MCP may be necessary before firmness is increased.

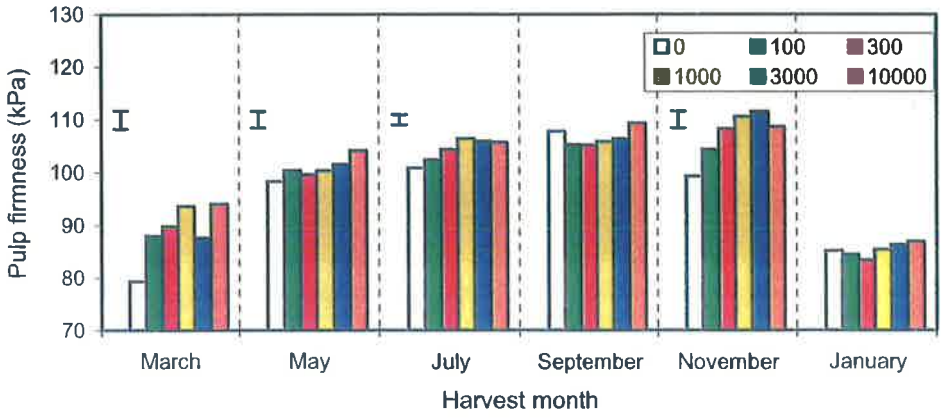


Figure 3.4: Effect of different concentrations of 1-MCP (0, 100, 300, 1000, 3000 or 10000 nL L^{-1}) on pulp firmness of Cavendish bananas (at ripening stage 6) harvested bimonthly and ripened at 22 °C. Bananas were treated with 100 µL L^{-1} ethylene gas for 48 h prior to 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9). Absence of a LSD bar indicates no significant difference between the control and 1-MCP treatment.

The firmness of 1-MCP treated bananas increased significantly and differently compared to the control during the year. The highest increase was obtained in March (18.6%) when 1-MCP was applied at 10000 nL L^{-1}

compared to the control followed by November (12.5%) when 1-MCP was applied at 3000 nL L^{-1}. In May and July, firmness of bananas increased 5.5% and 6% when 1-MCP was applied at 10000 and 1000 nL L^{-1}, respectively.

3.3.3.3 Discolouration

Control banana fruit did not discolour significantly in May, September, November and January. However, some limited discolouration was observed in March and July (Figure 3.5). In general, 1-MCP increased peel discolouration significantly compared with the control. However, the concentrations that caused discolouration varied throughout the year. In March only 1-MCP applied at 10000 nL L^{-1} caused a significant increase in discolouration (Figure 3.5). In May, November and January; 1-MCP at the two highest concentrations significantly increased discolouration compared with the control. 1-MCP at all levels except 100 nL L^{-1} in September and 1-MCP at 3000 and 10000 nL L^{-1} in July increased discolouration significantly compared with the control. The highest increase was obtained in July and September, followed by March and the lowest increases in discolouration were obtained in May, November and January. General observations suggest that although there was a significant difference between the control and 1-MCP treated fruit in discolouration, the amount of discolouration was always less than 1 and so still below the commercially undesirable threshold (1).

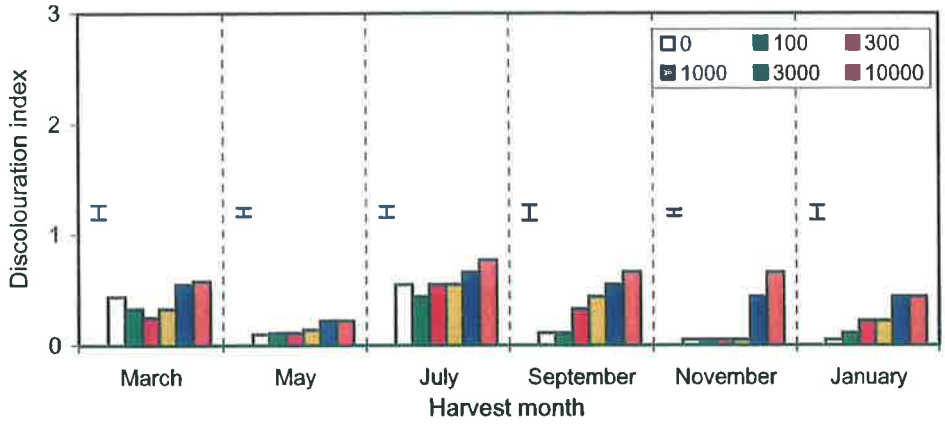


Figure 3.5: Effect of different concentrations of 1-MCP (0, 100, 300, 1000, 3000 or 10000 nL L^{-1}) on discolouration index of Cavendish bananas (at ripening stage 6) harvested bimonthly and ripened at 22 °C. Bananas were treated with 100 µL L^{-1} ethylene gas for 48 h prior to 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9).

3.3.3.4 Weight loss

The percentage of weight loss in control fruit differed among the harvest months throughout the year. Weight loss in control fruit was least in November and January. In March and May the weight losses were similar (3.8%) and the highest weight loss in control fruit was obtained in September (4%).

The concentration of 1-MCP that changed weight loss significantly compared with the control, changed during the year (Figure 3.6). In March, 1-MCP at all concentrations significantly decreased weight loss when compared to the control. In contrast, 1-MCP at all concentrations significantly increased weight loss in September while in January only 1-MCP at either 100 and 1000 nL L^{-1} decreased weight loss significantly.

The highest decrease in weight loss with the application of 1-MCP was obtained in May (28%) when 1-MCP was applied at 3000 nL L^{-1}. The decrease in weight loss was slightly lower in March (22%) when 1-MCP was applied at 300 nL L^{-1}.

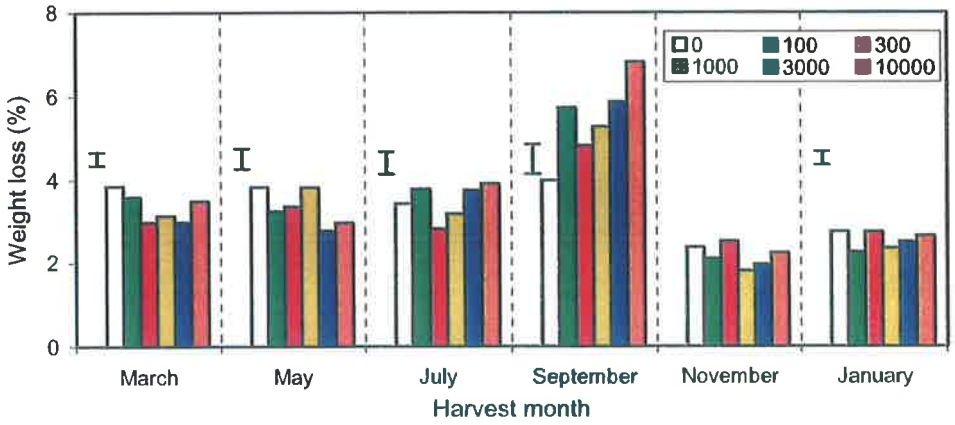


Figure 3.6: Effect of different concentrations of 1-MCP (0, 100, 300, 1000, 3000 or 10000 nL L^{-1}) on weight loss (%) of whole fruit of Cavendish bananas harvested bimonthly and ripened at 22 °C. Bananas were treated with 100 µL L^{-1} ethylene gas for 48 h prior to 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9). Absence of a LSD bar indicates no significant difference between the control and 1-MCP treatment.

3.3.3.5 Total soluble solids

Total soluble solids (TSS) of control fruit were to some extent different among the harvested months throughout the year (Figure 3.7). The lowest TSS was obtained in November (21.1%) and January (21.4%). The two highest TSS of control fruit were recorded in July (24%) and September (24.4%). Monthly trends showed an increase in TSS level during winter (24 to 24.4%) with lower TSS in other months (21.1 to 23.2%).

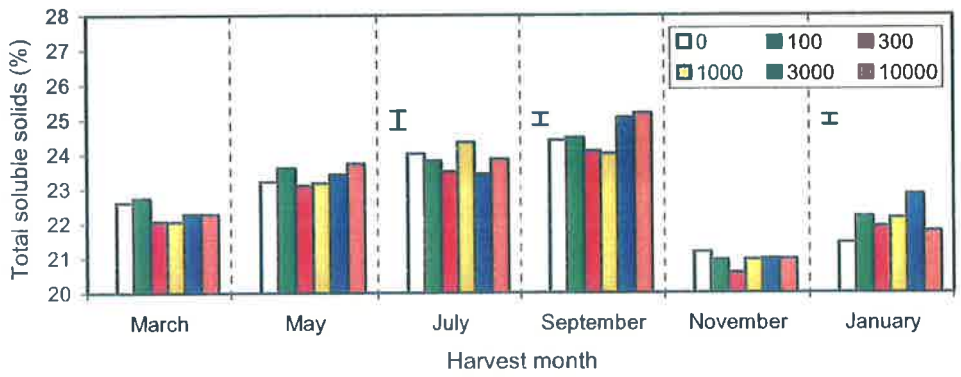


Figure 3.7: Effect of different concentrations of 1-MCP (0, 100, 300, 1000, 3000 or 10000 nL L^{-1}) on total soluble solids (TSS %) of pulp of Cavendish bananas (at ripening stage 6) harvested bimonthly and ripened at 22 °C. Bananas were treated with 100 µL L^{-1} ethylene gas for 48 h prior to 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9). Absence of a LSD bar indicates no significant difference between the control and 1-MCP treatment.

1-MCP at different concentrations did not affect TSS in March, May, and November-harvested fruit. However, 1-MCP significantly decreased TSS when

applied at 300 nL L^{-1} and 3000 nL L^{-1} in July and 300 or 1000 nL L^{-1} in September. In contrast, the level of TSS significantly increased when 1-MCP was applied at 3000 or 10000 nL L^{-1} in September. Additionally, 1-MCP application at all concentrations increased the TSS in January-harvested fruit although this did not increase TSS to similar levels seen in July or September control fruit (Figure 3.7).

3.3.4 Temperature and fruit weight variation

The highest minimum temperatures were recorded during the period leading up to March 2004 and January 2005 harvests while the lowest minimum temperatures were recorded during the periods leading up to the July and September harvests (Figure 3.8).

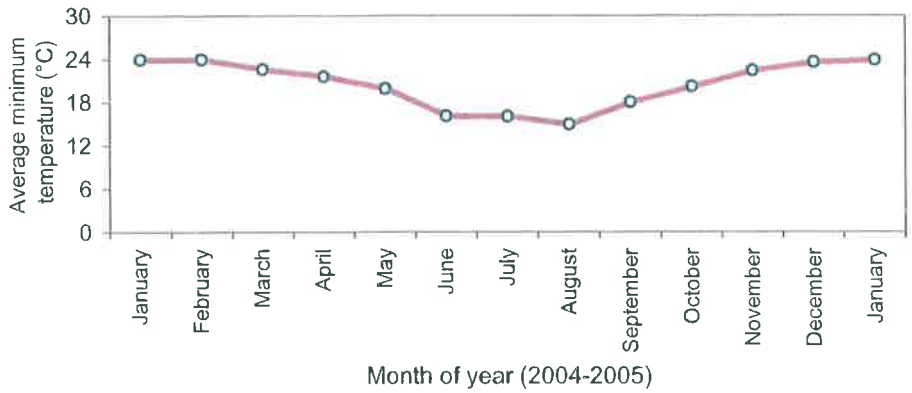


Figure 3.8: The monthly average of daily minimum temperatures at Innisfail Research Station (Qld) (Bureau of Meterology, 2005) for January 2004 to January 2005.

Seasonal variations in temperature expressed in total accumulated growing degree-days (dd) for bananas using 14 °C as a base temperature (Ganry and Sioussaram, 1978) were calculated and revealed that the fruit harvested in different months gained different heat units at each month, with higher degree-days during spring and summer (October to March) and lower degree-days during autumn and winter (April to September). The average degree-days for the three months prior to harvest (bunch emergence to harvest) were 1207 dd in March 2004, 979 dd in May 2004, 670 dd in July 2004, 643 dd in September 2004, 938 dd in November 2004 and 1159 dd in January 2005.

The mean fresh fruit weight of fruit collected from three hands of the middle section of bunches varied among harvested fruit throughout the year, particularly between the warmer months of November through to March compared to cooler months of May to September, with harvested fruit being the heaviest in November and the lightest in September (Table 3.1).

Table 3.1: Fruit weight (g) of Cavendish bananas from the middle section of the bunch. Fruit were harvested in March, May, July, September, November 2004 and January 2005 (each value is the mean ± standard error of 54 samples).

	Harvest month					
	March	May	July	September	November	January
Fruit weight (g)	170.6 ± 13.4	159.8 ± 8.2	173.3 ± 10.3	136.2 ± 7.7	207.1 ± 9.8	183.5 ± 10.4

3.3.5 Within-bunch variability in banana fruit weight and diameter

At all three harvest times fruit at the top of the bunch were significantly heavier and had a greater diameter than fruit from the bottom of the same bunch (Table 3.2).

Table 3.2: Within-bunch variability in fruit weight (g) and diameter (mm) from the top and bottom of the bunch of Cavendish bananas in October 2004, February and April 2005 (Each value is the mean ± standard error of 18 samples).

Harvest month	Fruit weight (g)		Fruit diameter (mm)	
	Top	Bottom	Top	Bottom
October	159.3 ± 14.8	124.2 ± 13.8	34 ± 0.8	31.7 ± 0.3
February	209.4 ± 23.3	151.5 ± 23.9	37.4 ± 0.9	34.7 ± 0.8
April	190.1 ± 19.6	138.8 ± 22.2	35.7 ± 0.9	32.9 ± 0.5

3.3.6 Effect of fruit position on the bunch on 1-MCP application effects

3.3.6.1 Shelf life

The shelf life of control fruit from the top and bottom of bunches did not significantly differ within each trial (Figure 3.9, Figure 3.10). However, 1-MCP treatment at a concentration of 300 nL L^{-1} increased banana shelf life significantly in both fruit from the top and bottom of the bunch in October 2004 and April 2005 but only significantly increased shelf life in fruit from the

top of the bunch in February (42%) (Figure 3.9). 1-MCP treated fruit from the top of the bunch had a greater increase in shelf life than those treated from the bottom of the bunch in October and in February (Figure 3.9 a and b). However, in April shelf life increased more in 1-MCP-treated fruit from the bottom of the bunch than in fruit from the top of the bunch (Figure 3.9 c).

3.3.6.2 Firmness

Although firmness of control fruit from the top and bottom of bunches did not significantly differ within each trial; it was higher in February than other months. However, 1-MCP treatment at a concentration of 300 nL L^{-1} significantly increased firmness in fruit from the top of the bunch in October (12%), February (9%) and April (5%) (Figure 3.11 a, b and c).

3.3.6.3 Discolouration

Hand position on the bunch had no significant impact on the discolouration index of 1-MCP-treated fruit in the three trials (Figure 3.12).

3.3.6.4 Weight loss

Hand position on the bunch had no impact on fruit weight loss of 1-MCP-treated fruit in the three trials (Figure 3.13).

3.3.6.5 Total soluble solids

TSS of control fruit from the top and bottom of bunches did not significantly differ within each trial. However, TSS in 1-MCP treated fruit

decreased significantly in fruit from the bottom of the bunch in October (2.6%) and in April (4%), whereas TSS in 1-MCP-treated fruit from the top of the bunch responded differently (Figure 3.14, a, b and c) regardless of 1-MCP treatment. 1-MCP had no effect in TSS of fruit from the top of the bunch in February, decreased TSS in April and increased TSS in October.

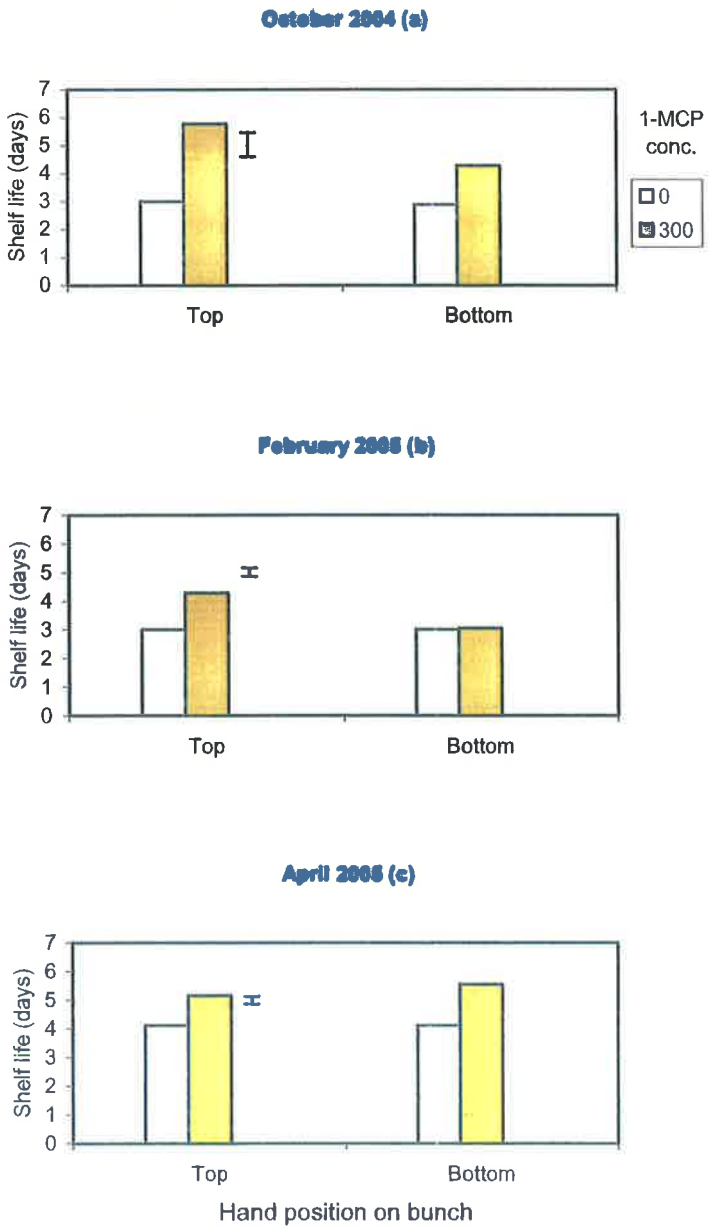
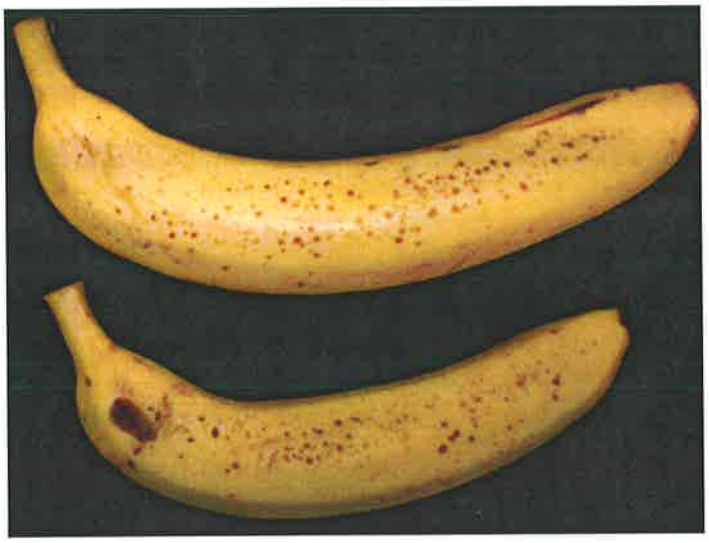


Figure 3.9: Effect of hand position on the bunch on the effect of 1-MCP on shelf life (ripening stages 4 to 7) of Cavendish bananas ripened at 22 °C in October 2004 (a), February 2005 (b), April 2005 (c). Vertical bars represent LSD values at the 5% level (n=9).

Top

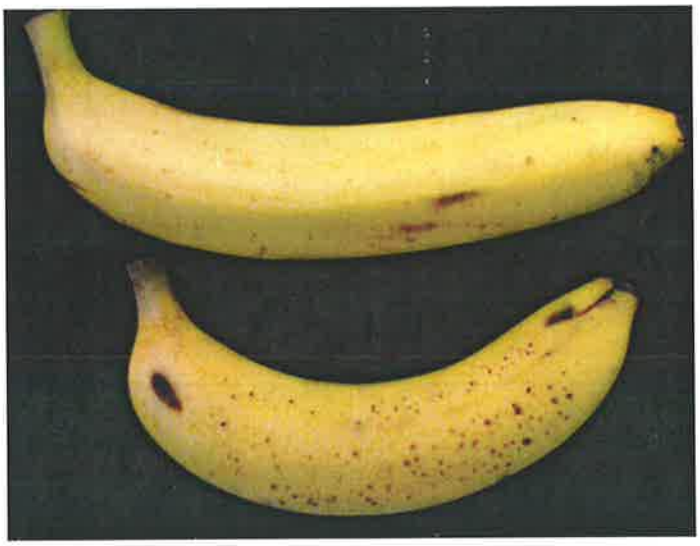
Bottom



a- control

Top

Bottom



b- 1-MCP treated fruit

Figure 3.10: Ripeness stages of control (a) and 1-MCP treated fruit (b) collected from the top and the bottom of the bunch in February harvest after 5 days stored in air at 22 °C.

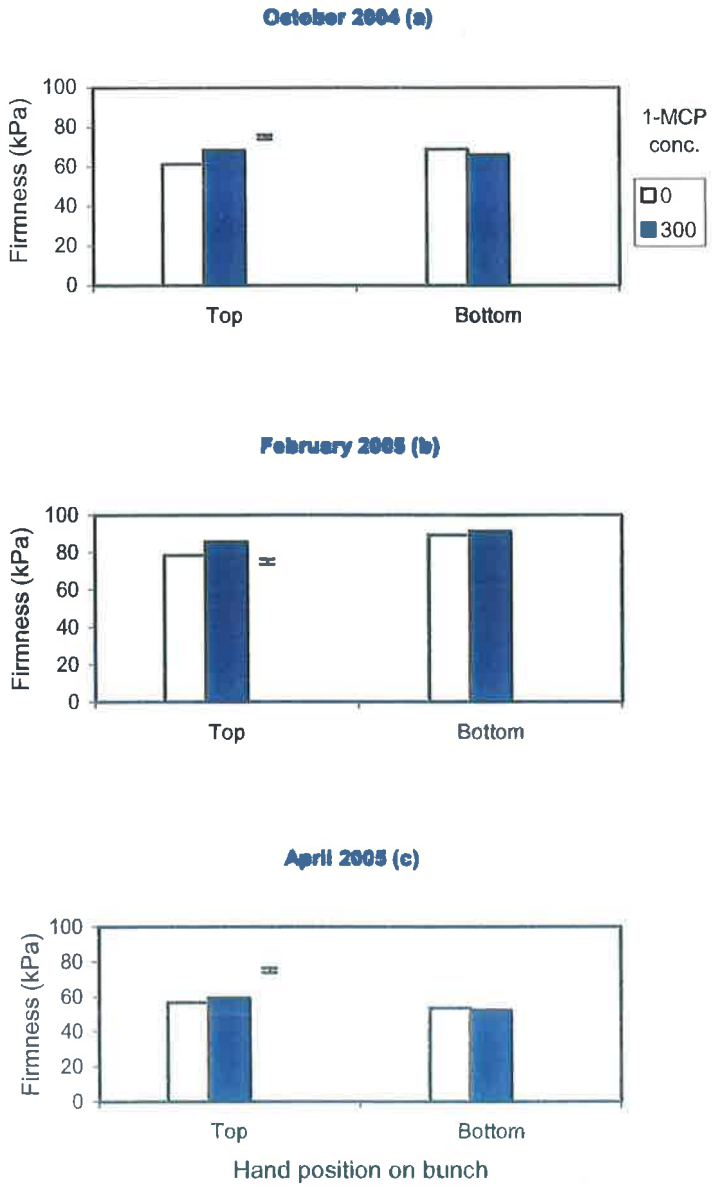


Figure 3.11: Effect of hand position on the bunch on the effect of 1-MCP on pulp firmness of Cavendish bananas ripened at 22 °C in October 2004 (a), February 2005 (b), April 2005 (c) (n=9). Vertical bars represent LSD values at the 5% level.

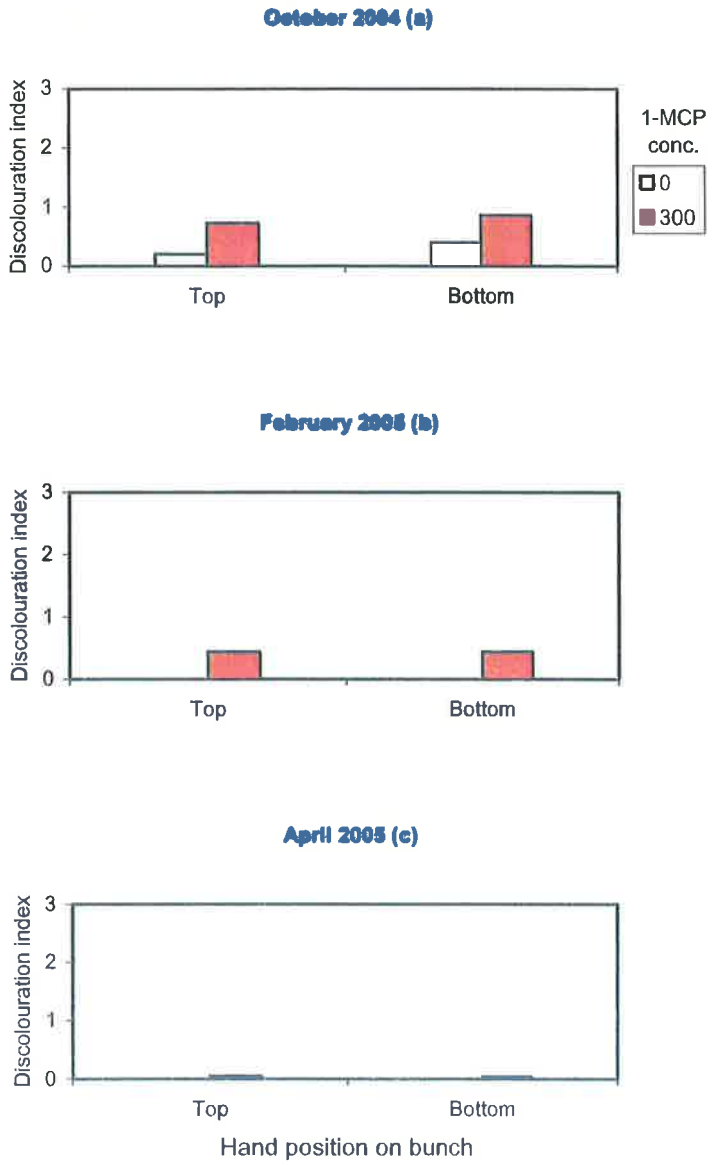


Figure 3.12: Effect of hand position on the bunch on the effect of 1-MCP on discolouration index of Cavendish bananas ripened at 22 °C in October 2004 (a), February 2005 (b), April 2005 (c) (n=9). Absence of a LSD bar indicates no significant difference between control and 1-MCP treatment.

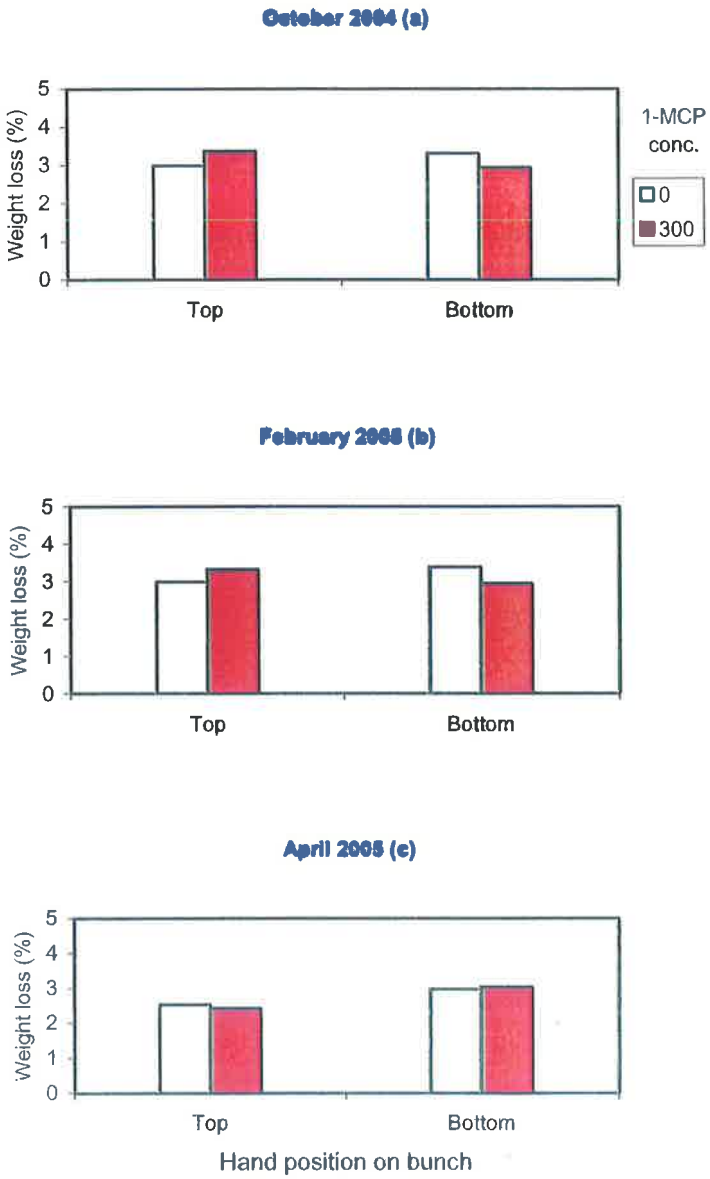


Figure 3.13: Effect of hand position on the bunch on the effect of 1-MCP on weight loss (%) of Cavendish bananas ripened at 22 °C in October 2004 (a), February 2005 (b), April 2005 (c) (n=9). Absence of a LSD bar indicates no significant difference between control and 1-MCP treatment.

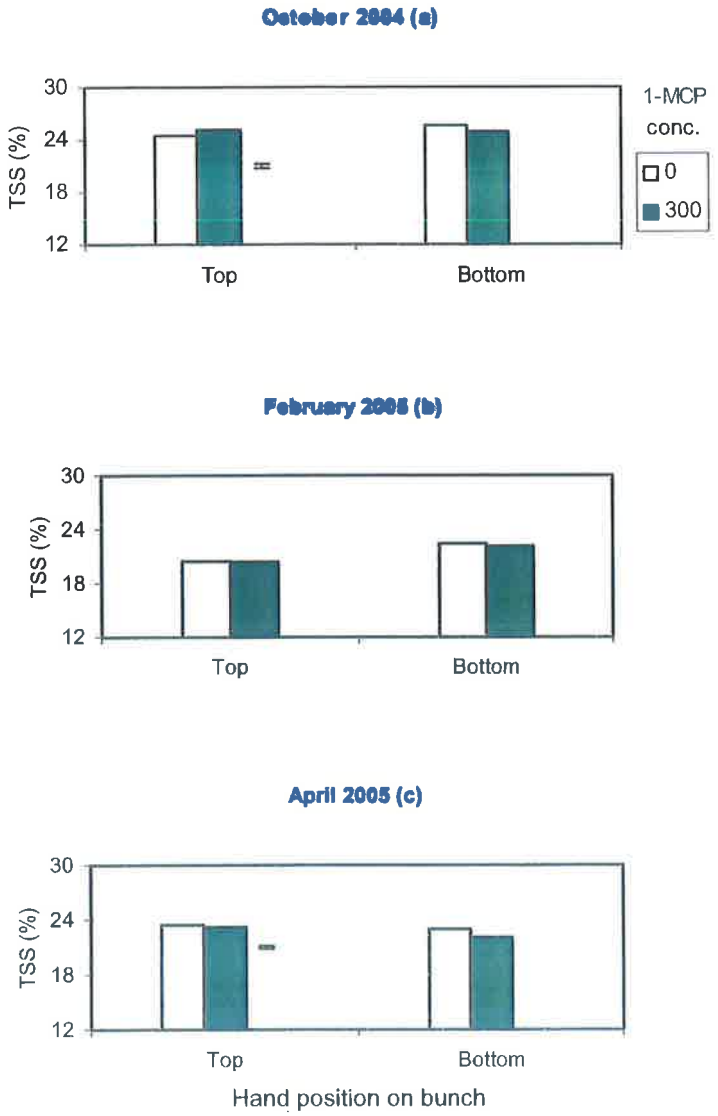


Figure 3.14: Effect of hand position on the bunch on the effect of 1-MCP on total soluble solid (TSS %) of Cavendish bananas ripened at 22 °C in October 2004 (a), February 2005 (b), April 2005 (c). Vertical bars represent LSD values at the 5% level (n=9). Absence of a LSD bar indicates no significant difference between control and 1-MCP treatment.

3.3.7 Data Correlations

No significant correlation between firmness, shelf life and fruit weight loss was found among harvested fruit for the entire year. In most individual months, generally no significant correlations were evident. However, a weak positive correlation between firmness and shelf life of 1-MCP-treated fruit was observed in July (r =0.59). Firmness and shelf life of control fruit also had a moderate positive correlation in September (r =0.66). Additionally, a negative correlation between shelf life and weight loss was observed in control fruit harvested in May (r = -0.71).

Fruit diameter and fresh weight significantly correlated in both fruit from the top (r =0.69) and the bottom (r =0.52) of the bunch. Interestingly, there was a moderate positive correlation between fruit diameter and shelf life of 1-MCP treated fruit from the top (r =0.68) but not in fruit from the bottom of the bunch.

3.4 Discussion

Despite previous research reporting that bananas behave in a similar fashion in response to 1-MCP regardless of the time of year (Bagnato et al., 2003), this research suggests that ethylene-treated fruit harvested in different months responds differently to 1-MCP in terms of its impact on quality and shelf life. The reliance of 1-MCP efficacy on the time of year fruit was harvested is more than likely due to differences in the timing of preharvest

exposure to stresses, how quickly fruit mature and their interaction with the temperature variation seen throughout the year (as per Figure 3.8). This was reflected in fruit from different positions on the bunch.

The shelf life of control fruit was greatest when harvested in autumn followed by fruit harvested in spring, summer and late winter respectively. Interestingly this order changed with the application of 1-MCP such that although the greatest shelf life was in fruit harvested in autumn, this was followed by fruit harvested in late winter, spring and summer respectively. This suggests that 1-MCP is more effective in extending banana shelf life when applied to fruit harvested in late winter. This increased efficacy appears to be strongly related to the thermal units (number of degree-days) received during fruit growth and development before harvest and their possible impact on the size of fruit. Previous studies have reported that the requisite temperature sum between shoot production and harvest was 900 degree-days to arrive at a grade of 34 mm (optimum fruit diameter at harvest) from the time the last hand emerged in bananas (Stover and Simmonds, 1987). Fruit experienced excess degree-days in summer and insufficient degree-days in winter during bunch growth and development, whereas it was adequate (close to ideal) in spring and autumn, resulting in fruit with greater maturity and size at harvest in summer than in winter (Table 3.1). The heavier average fruit weight recorded during the warmer months of November through to March was strongly related to the higher degree-days recorded during this period, with more degree-days in fruit

from the top than the bottom of the bunch. Similarly, the lightest average fruit weight recorded in September had a strong relationship with the lower degree-days experienced during winter. Possible advanced maturity in the summer-harvested fruit is consistent not only with the findings of Marin et al. (1996) who noted that fruit was more advanced in maturity late in spring and early summer than in other months and observations that fruit size is directly correlated to fruit age, or time from bunch emergence because of the associated growth rate (Jullien et al., 2001; Srikul and Turner, 1995) but also agreed with the findings of Liu (1976) who reported that the relative variability in postharvest life of bananas within a bunch was greatest when the bananas were harvested at their most advanced maturity. Srikul and Turner (1995) concluded that the green life of bananas declined exponentially as the fruit grows. Individual bananas weighing 90, 140 and 190 g had a green life of 90, 33 and 15 days respectively. These findings also agree with Jullien et al. (2001) who showed that postharvest life of bananas correlated with the bunch age as expressed in degree-days accumulated since the inflorescence emerged. They reported that after 1030 degree-days, fruit green life decreased with fruit age at harvest exponentially. Given that a reduction in the preclimacteric period leads to a shorter green life and hence shelf life, the observed differences among bunches in maturity and within-bunch maturity and the subsequent impact on shelf life is likely to be the primary reason for differences in the efficacy of 1-MCP.

In this study, fruit harvested in September and May had the lowest average fruit weights (and were subsequently considered less mature at harvest) but the greatest increase in shelf life in response to 1-MCP. The greater firmness of these fruit suggest that they are also less mature in agreement with observations that less mature fruit also tend to have a slightly firmer texture when fully ripe (Thompson, 2003). Although to my knowledge, there has not been any conclusive studies conducted on the impact of 1-MCP on the shelf life of bananas of different maturities, these results do support those of Harris et al. (2000) who indicated that the time to ripen of 1-MCP-treated bananas varied with maturity at harvest, with less mature fruit having a longer green life when treated with 1-MCP. However, even though May-harvested fruit had a smaller size, they experienced the closest to the ideal degree-days (Stover and Simmonds, 1987) and as such this suggests that their maturity and size was optimum in terms of their response to both ethylene and 1-MCP treatments. In contrast, the September-harvested fruit developed during winter, had the longest bunch emergence to harvest interval and as a result a greater age at harvest, and hence a shorter green life (Marriott et al., 1979; Montoya et al., 1984) and subsequent shelf life. This may be indicative of an independent alteration of size and age due to the influence of different environmental conditions (Marin et al., 1996) and in particular, the possible impacts of field chilling as usually evidenced by peel discolouration due to the formation of pigments other than chlorophyll during the ripening of fruit (Kays, 1999). Additionally, 1-MCP had no impact on fruit from the bottom of

the bunch in February (with advanced maturity at harvest). This supports the assumption that maturity impacts 1-MCP efficacy.

Shelf life and firmness are important factors from a postharvest point of view, however, upon the first visual assessment of product quality, colour is critical (Kays, 1999). The colour of the peel is used as an indicator of ripening in many products including bananas. Preharvest factors (Kays, 1999) and postharvest conditions and treatments (Kays, 1991) such as ripening factors and conditions can affect the appearance of bananas. Bananas are subject to chilling damage (as evidenced by peel discolouration) at temperatures below 13 °C (Bagnato et al., 2002; Stover and Simmonds, 1987; Thompson, 2003). Previous studies (Golding et al., 1998; Harris et al., 2000; Jiang et al., 2004) have also reported uneven peel degreening after 1-MCP treatment. Hence, peel discolouration was used to assess not only the effect of preharvest factors (particularly field-chilling injury) but also to evaluate the effect of postharvest conditions and treatments (particularly 1-MCP treatment) on final appearance quality of fruit. In preliminary experimentation from 2003 (data not shown), we observed an increase in peel discoloration in fruit that developed during winter (and fruit were therefore likely to have encountered chilling conditions) as well as uneven peel degreening when fruit were treated with 300 nL L^{-1} MCP. However, in 2004, 1-MCP application at 1000 nL L^{-1} or lower concentrations was sufficient to extend banana shelf life and firmness in most of the harvested months without causing significant peel discolouration (that is,

lower than the commercially acceptable level of 1). The low amount of discolouration probably occurred because the daily minimum air temperatures were above the threshold level for field chilling (13 °C) (Bureau of Meteorology 2005) during these trials. However, chilling or cooler temperatures will affect the ripening physiology of bananas by delaying the climacteric rise (Murata, 1969).

These results strongly support those of Bagnato et al. (2002) who determined the optimal ripening temperatures for Cavendish bananas harvested throughout the year in north Queensland, Australia. We also observed a greater shelf life in May and November-harvested fruit, lower firmness in summer (January and March) and an increase in TSS levels during winter (July and September) months, which was always above the acceptable level (18%) for fully ripe bananas (CSIRO, 1972). This increased TSS in cold seasons (particularly in winter) is undoubtedly due to the fact that a reduction in temperature results in an increase in soluble carbohydrate accumulation in plant tissues (including fruit that acts as a strong sink). This is considered to be either an essential part of the chilling-resistance mechanism or simply a consequence of low temperature stress (Purvis, 1990).

Seasonal differences in the response of bananas to 1-MCP therefore appears to relate strongly to the age of the banana, size of the banana and preharvest conditions, all of which can be related to the responsiveness of the banana to exogenous ethylene and its feedback mechanisms. Exogenous

ethylene exerts a negative feedback regulation on ethylene production in immature climacteric fruits resulting in a reduction in ethylene production (Vendrell and McGlasson, 1971), whereas in mature climacteric fruit, ethylene is autostimulatory and ethylene production increases as a result of ethylene treatment (and hence ripening is faster). In this study, bananas were treated with ethylene before 1-MCP treatment and with their different maturities (among bunches and within-bunch); possibly they will be producing different levels of ethylene at the time of 1-MCP application.

A question that remains is why much higher concentrations of 1-MCP did not consistently give a longer shelf life. The response of climacteric fruits to applied ethylene depends mainly on tissue sensitivity and stage of maturation (Biale and Young, 2000). A possible explanation therefore is that the application of 1-MCP after two days of ripening initiation by 100 $\mu L\ L^{-1}$ ethylene, when autocatalytic ethylene production occurs (Golding et al., 1998), was too late to have a stronger inhibitory effect on progress of the climacteric to give a greater increase in shelf life. This is also consistent with findings of Jiang et al. (1999b) who noted that an extension of ripening is possible only when 1-MCP is applied within 24 hrs of ethylene treatment. Less effectiveness of 1-MCP is consistent with irreversibility of the autocatalytic ripening process once triggered.

The fact that only the highest concentration was effective on fruit harvested in March 2004 or similarly harvested fruit from bottom of the bunch

in February also suggests a difference in ethylene perception capabilities of fruit harvested at different times of the year and also with the greatest within-bunch variability in bananas in most advanced maturity. Any fruit picked either too early or too late in its season (such as those in March) has a shorter storage life than fruit picked at the ideal maturity (Kader, 1999). The amount of 1-MCP may therefore need to be increased to block ethylene action due to an increase in receptors or a decrease in competitive ability (Sisler and Serek, 1997).

3.5 Conclusion

These findings suggest that 1-MCP treatment at 300 or 1000 nL L^{-1} to partially ripened ethylene-treated bananas can be used to extend the shelf life and increase firmness in a reliable manner throughout the year. However, 1-MCP treatment might not always be equally effective in improving shelf life and firmness of partially ripened bananas when applied to fruit picked at advanced maturity at harvest, particularly in summer. 1-MCP efficacy also appears reliant on the hand position on the bunch to some extent.

Chapter 4

Effect of exogenous ethylene and timing of 1-MCP application on postharvest life of bananas

4.1 Introduction

Although ethylene can be deleterious to fresh produce, it can also be used to improve the quality of horticultural products by promoting and ensuring uniform ripening (Saltveit, 1999). Both the concentration and duration of exogenous ethylene significantly impact the response of bananas (Burg and Burg, 1965, Inaba and Nakamura, 1986, Shukor et al., 1988, Wills et al., 2001) as does fruit maturity (Burg and Burg, 1965, Liu, 1976a).

The time of year fruit is harvested and the maturity of the fruit also impact the response of bananas to ethylene and 1-MCP (as described in Chapter 3). It seems likely therefore that the ethylene concentration and the duration of the ethylene treatment prior to 1-MCP application will affect 1-MCP efficacy because of its ability to block ethylene receptors (Sisler and Serek, 1997), consequently preventing ethylene-induced effects such as banana fruit ripening. Ethylene was used at commercial levels within the range of 100

and 1000 μL L^{-1} for either 24, 36 or 48 h in previous studies by various authors prior to 1-MCP application (Macnish et al., 1997, Golding et al., 1999, Bagnato et al., 2003, Pelayo et al., 2003, Macnish et al., 2000a, Jiang et al., 1999a, Jiang et al., 1999b). However, there is limited information about the effect of banana ripening initiation with low ethylene concentrations (less than 100 μL L^{-1}) before 1-MCP application or direct comparisons of different ethylene durations before 1-MCP application.

Although considerable research (Bagnato et al., 2003, Pelayo et al., 2003, Jiang et al., 1999a, Jiang et al., 1999b, Harris et al., 2000, Macnish et al., 2000c, Macnish et al., 2000b) has been devoted to the application of 1-MCP at different concentrations or durations in climacteric fruits including bananas, rather less attention has been paid to the timing of 1-MCP exposure in relation to ethylene application. While 1-MCP application before ethylene initiation of ripening, may be useful in preventing premature ripening during extended periods of banana handling and transport (Macnish et al., 2000b), an extension of shelf life only appears possible when 1-MCP is applied in the earliest phase of banana ripening within 24 to 48 h of ethylene treatment (Bagnato et al., 2003, Jiang et al., 1999a). However, increasing the 1-MCP application time post-ethylene treatment may have some potential (Jiang et al., 1999b) .

Given the climacteric nature of bananas it is likely that time from harvest to 1-MCP application may also influence 1-MCP efficacy. Generally the more perishable the crop, the more quickly after harvest 1-MCP should be applied,

for example it was more effective in banana (Jiang et al., 1999b) or in broccoli (Able et al., 2002) when it was applied quickly after harvest. However, ethylene production and softening in ripening apricots and plums was inhibited when fruit were treated with 1-MCP after storage, but not before storage (Dong et al., 2002). Multiple applications of 1-MCP were also found to be more beneficial than just one application in apple (Mir et al., 2001), but not in broccoli and pak choy (Able et al., 2002).

The response of bananas to the application of 1-MCP and ethylene simultaneously; the effect of pre- and early-climacteric application of 1-MCP (multiple applications); and the effect of time from harvest to 1-MCP treatment have not been extensively examined to my knowledge. Hence, the research outlined in this chapter examines these in more detail. The ultimate aim was to establish whether altering ethylene concentration, duration and timing of 1-MCP application could improve the quality and shelf life of bananas.

4.2 Materials and methods

4.2.1 Plant material and preparation

Mature and green Cavendish banana (cv. Williams) fruits were harvested, transported and prepared as outlined in Sections 2.1 and 2.2. In all three experiments eighteen bananas were allocated to each treatment. Six fruits from each bunch were placed into a 10 L plastic container as one replicate. The containers were kept at 22 °C and banana ripening was initiated using a

determined volume of ethylene gas injected into the containers for two consecutive days as per Section 4.2.2. Prior to 1-MCP treatment (as detailed below) containers were ventilated for 20 minutes. This method was used for ethylene and 1-MCP applications unless otherwise indicated.

4.2.2 Experimental procedure

4.2.2.1 Effect of ethylene concentration on 1-MCP efficacy

Green bananas were acquired in July 2004 and January 2005. After preparation, fruit were randomly assigned to plastic containers. Ethylene concentrations of 2, 20, 50 or 100 μL L^{-1} for two consecutive days or 100 μL L^{-1} for the first day and 2 μL L^{-1} for the second day were applied prior to 1-MCP treatment at 0 or 300 nL L^{-1} for 24 h at 22 °C. 1-MCP was prepared, introduced to the treatments and measured as previously described in Sections 2.3, 2.4 and 3.2.2 respectively.

4.2.2.2 Effect of ethylene duration prior to 1-MCP exposure on 1-MCP efficacy

Green and hard bananas were obtained in February and August 2005. After preparation, fruit were randomly assigned to plastic containers (as previously described in Section 4.2.1). Ethylene was applied at 100 μL L^{-1} and fruit exposed for 30, 40 or 50 h. Containers were ventilated for 20 minutes at 15, 20 and 25 h respectively. After ripening initiation, containers were ventilated for 20 minutes and then bananas were treated with 1-MCP at 0 or

300 nL L^{-1} for 24 h at 22 °C. 1-MCP was used as previously described in Section 4.2.2.1.

4.2.2.3 Simultaneous application of 1-MCP and ethylene

Bananas were harvested and transferred to the laboratory in March, June and December 2004. After preparation, fruit were treated with ethylene at 100 µL L^{-1} for two consecutive days as a control or simultaneously with 1-MCP at different concentrations (30, 100 or 300 nL L^{-1}) on the first or second day. To allow comparison with other experiments and to determine the effect of simultaneous application, a treatment where 1-MCP was applied separately after ethylene treatment was also included. As a result, bananas were treated with 1-MCP at different concentrations, duration and timing in eight different treatments as shown in Table 4.1. Containers were ventilated for 20 minutes each day. After treatment, fruit were removed from containers and placed in plastic bags with the lid slightly open and placed at 22 °C with 90% RH. 1-MCP was created, introduced to the treatments and measured as previously described in Sections 2.3, 2.4 and 3.2.2.

Table 4.1: Treatments used to study the effect of simultaneous application of 1-MCP and ethylene. Fruit were treated with ethylene (E) for two consecutive days (control) or simultaneously with 1-MCP (M) on the first or second day, or treated on the third day.

First day	Second day	Third day
E*	E	
E + M_{300}**	E	
E	E + M_{300}	
E	E + M_{300}	M_{100}
E	E + M_{300}	M_{300}
E	E + M_{100}	M_{100}
E	E + M_{30}	M_{300}
E	E	M_{300}

* E = Ethylene concentration at 100 μL L^{-1}

** M = 1-MCP at subscript concentration (30, 100 or 300 nL L^{-1})

4.2.2.4 Pre- and early-climacteric application of 1-MCP

Two sets of different experiments were conducted during February, March, April and June 2004 to determine which combination of pre- and early-climacteric application and concentration of 1-MCP would improve fruit shelf life and quality. One is using higher concentrations of 1-MCP and the other lower concentrations of 1-MCP in the pre-climacteric stage prior to ethylene treatment. After preparation, green and pre-climacteric fruit were treated with

1-MCP at 0, 30, 300 or 10000 nL L^{-1} for 6 h at 22 °C. Following 1-MCP treatment, the lids were removed from the containers and were ventilated for 20 minutes. All fruit were transferred to a ventilated cool room and stored at 16 ± 0.2 °C and 90 to 95% RH for two weeks. Thereafter, fruit were transferred to the laboratory and held at 22 °C for approximately 3 hrs. All the fruit were then exposed to ethylene at 100 µL L^{-1} for two consecutive days. Fruit from each pre-climacteric 1-MCP application concentration were then treated with 1-MCP at 0 or 300 nL L^{-1} for 24 h at 22 °C (Table 4.2).

Table 4.2: Treatments used to study the effect of pre-climacteric application of high concentrations of 1-MCP prior to 1-MCP application after ethylene initiation.

1-MCP concentration (nL L^{-1}) for 6 h	Storage at 16 °C	Ethylene for 48 h	1-MCP concentration (nL L^{-1}) for 24 h
0	Two weeks	100 µL L^{-1}	0
30			0
300			0
10000			0
0			300
30			300
300			300
10000			300

To study lower concentrations of 1-MCP, green fruit that were selected from the top and the bottom of the bunch were placed into containers (according to fruit position) prior to exposure to very low 1-MCP atmospheres (0, 2, 4, 5, 6 or 10 nL L^{-1}) for 6 h. Containers were then ventilated for 20 minutes. Fruit were treated with ethylene (control) or ethylene followed by early climacteric 1-MCP application (Table 4.3).

Table 4.3: Treatments used to study the effect of pre-climacteric application of low concentrations of 1-MCP prior to 1-MCP application after ethylene initiation.

1-MCP concentration (nL L^{-1}) for 6 h	Ethylene for 48 h	1-MCP concentration (nL L^{-1}) for 24 h
0	100 µL L^{-1}	0
0		300
2		300
4		300
5		300
6		300
10		300

4.2.2.5 Effect of time from harvest on 1-MCP efficacy

Two sets of experiments were conducted during 2004 to compare the effect of time from harvest on 1-MCP efficacy to determine which combination of application time after harvest; length of application and concentration of 1-

MCP would improve fruit shelf life and quality. The first set of experiments examined the effect of different concentrations of 1-MCP in this context using banana fruit obtained during March and April 2004 from the main farm. Banana bunches were labeled and placed into cartons and stored in the cool room at 15 ± 0.2 °C and 90 to 95% RH for 1 week without any treatment. Thereafter, stored fruit were transferred to the laboratory and held at 22 °C for approximately 3 h. They were placed into containers and coincidentally treated with 100 μL L^{-1} ethylene on two consecutive days, followed by 20 minutes ventilation. All the fruit were exposed to 1-MCP at 0, 300 or 3000 nL L^{-1} for 24 h at 22 °C.

In the second set of experiments, the combination effect of timing of 1-MCP application and time from harvest to 1-MCP exposure on fruit from the top and bottom of the bunch was compared using banana fruit acquired during April, May and June 2004. Bananas were labelled as being harvested from the top and the bottom of the bunch. Fruit were divided into two lots. The first lot was treated upon arrival (5 days from harvest) and the second lot treated after 5 days of storage (10 days from harvest) by placing in a refrigerated room at 15 ± 0.2 °C and 90 to 95% RH. The second lot were removed from the cool room and transferred to the laboratory at 22 °C and held for approximately 3 h prior to treatments. Both lots were treated with 100 μL L^{-1} ethylene on two consecutive days, followed by 20 minutes ventilation and then exposed to 1-MCP at 0 or 300 nL L^{-1} for 24 h at 22 °C or treated with 1-MCP at 5 nL L^{-1} for

6 h at 22 °C prior to ethylene gassing with 100 μL L^{-1} followed by exposure to 1-MCP at 300 nL L^{-1} for 24 h at 22 °C.

4.2.3 Quality assessments

Nine fruit from each treatment were used to measure external parameters, and the remaining nine fruit allocated to each treatment were used to assess internal quality measurable characteristics at colour stage 6 (CSIRO, 1972) as described previously in Section 2.5.

4.2.4 Statistical assessments

A completely randomised block experimental design was used in experiments, with three replicates. Data were analysed with the Genstat 6 program (Release 6.2, 6th edition, 2002, Lawes Agricultural Trust, VSN International Ltd) using the general analysis of variance (ANOVA) (simultaneous application of ethylene and 1-MCP, pre- and early-climacteric application of 1-MCP and time from harvest and fruit position trials), or the two-way ANOVA (ethylene concentration and duration, time from harvest and 1-MCP concentration trials). A least significant difference test ($P = 0.05$) was used to determine significant differences between means. Total weight loss data were expressed as a percentage of the weight of fruit before ripening was initiated (ripening stage 1). Data of each harvest was analysed individually.

4.3 Results

4.3.1 Effect of ethylene concentration and 1-MCP exposure

4.3.1.1 Shelf life

At each harvest time in control fruit, the shelf lives (Figures 4.1 and 4.2) were similar where treated with 20 μL L^{-1} ethylene or above. Generally, the shelf life of control fruit was higher when harvested in July (5.5 days) than January (3.5 days) harvest. Regardless of ethylene concentration except where the fruit were treated with ethylene at 100 μL L^{-1} in the July harvest, 1-MCP application significantly increased shelf life.

The highest increase in shelf life of 1-MCP treated fruit was obtained when ethylene was used at 100 μL L^{-1} for the first and 2 μL L^{-1} for the second day in January (39%), followed by July (28%) when ethylene was applied at 50 μL L^{-1} for 2 days or 100 μL L^{-1} for the first and 2 μL L^{-1} for the second day compared to the other concentrations (Figure 4.1, a and b). 1-MCP-treated fruit that were initially treated with 2 μL L^{-1} ethylene did not ripen and remained green (Figure 4.2) and therefore there was no data to present for this treatment in Figure 4.1.

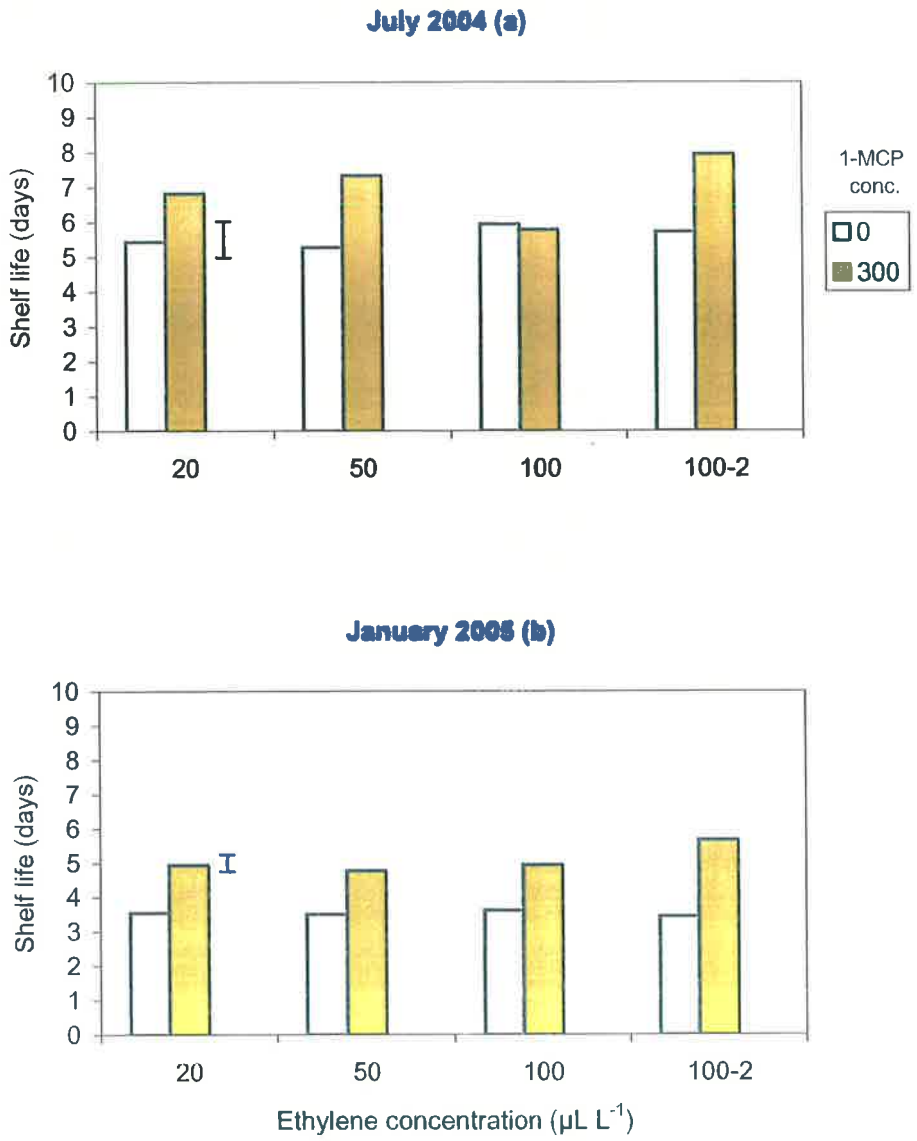


Figure 4.1: Effect of ethylene concentration on the effect of 1-MCP on shelf life of Cavendish bananas ripened at 22 °C in July 2004 (a), January 2005 (b). Bananas were treated with ethylene (20, 50 or 100 µL L^{-1}) for two days or 100 µL L^{-1} for the first day and 2 µL L^{-1} for the second day (100-2) prior to 0 or 300 nL L^{-1} 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9).

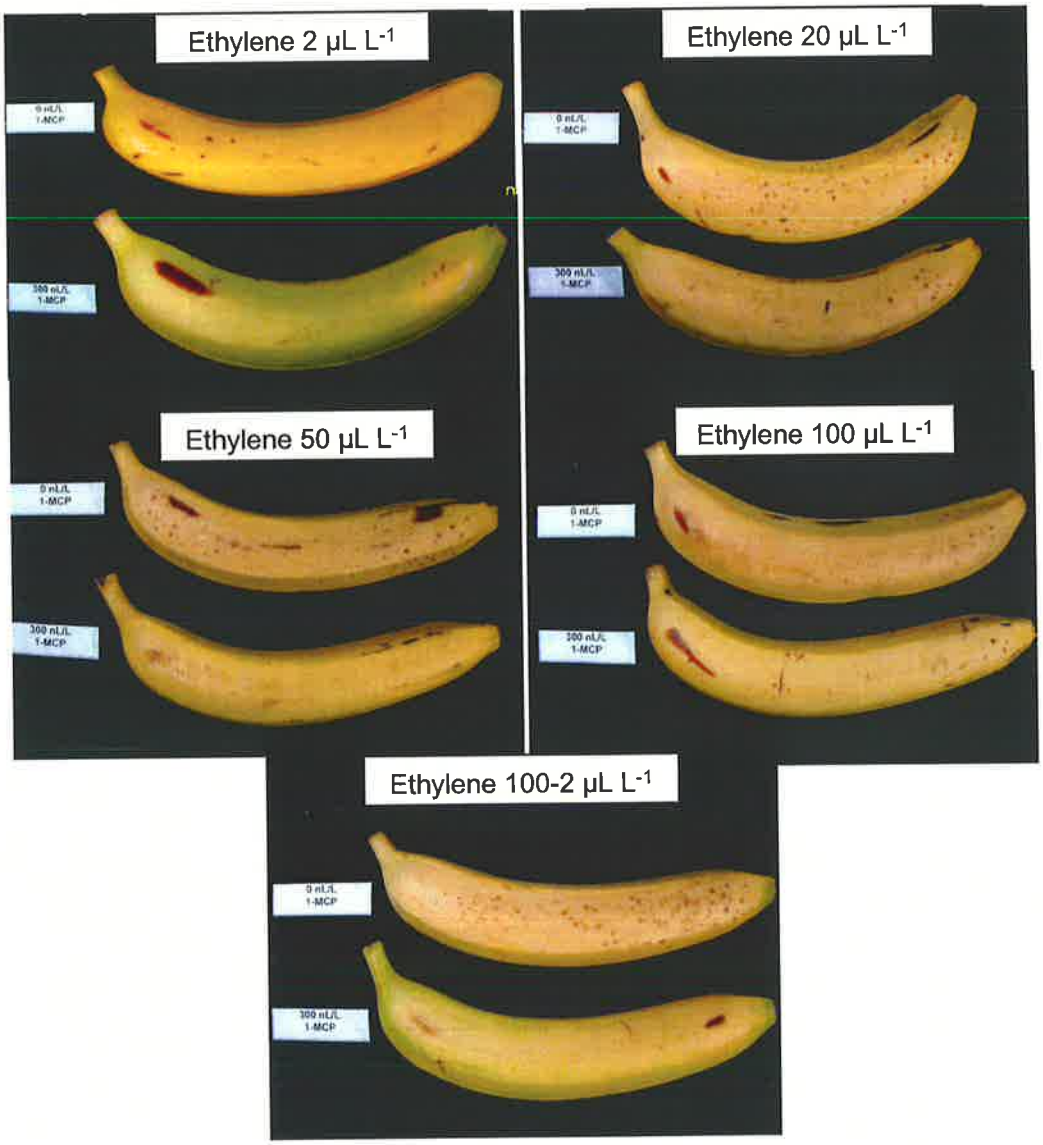


Figure 4.2: Effect of ethylene concentration on the effect of 1-MCP on the ripeness stage of fruit after 1 week of air storage at 22 °C for July-harvested fruit. Bananas were treated with ethylene at 2, 20, 50, 100 µL L^{-1} for two days, or 100 µL L^{-1} for the first day and 2 µL L^{-1} for the second day (100-2) prior to 0 or 300 nL L^{-1} 1-MCP exposure for 24 h at 22 °C.

4.3.1.2 Firmness

Fruit firmness in all control treatments was on average generally higher for fruit harvested in July (90 to 103 kPa) than in January (80 to 90 kPa) while regardless of concentration ethylene treatment did not significantly affect firmness.

1-MCP had minimal effect on firmness in July-harvested fruit and significantly increased firmness in fruit pre-treated with ethylene at 100 μL L^{-1} for the first and 2 μL L^{-1} for the second day (100-2) compared to the control (Figure 4.3 a). However, in January, 1-MCP significantly decreased firmness when applied to fruit that were treated initially with 20 μL L^{-1} ethylene or with 100 μL L^{-1} ethylene for the first and 2 μL L^{-1} for the second day (100-2) compared to the control (Figure 4.3 b).

4.3.1.3 Discolouration

Discolouration of banana peel was minimal across all treatments. However, discolouration was generally greater in January than July. 1-MCP had no effect in July but significantly increased discolouration in January (Figure 4.4), particularly when ethylene used at 100 μL L^{-1} for the first and 2 μL L^{-1} for the second day. However, discolouration was still below the threshold level of (1).

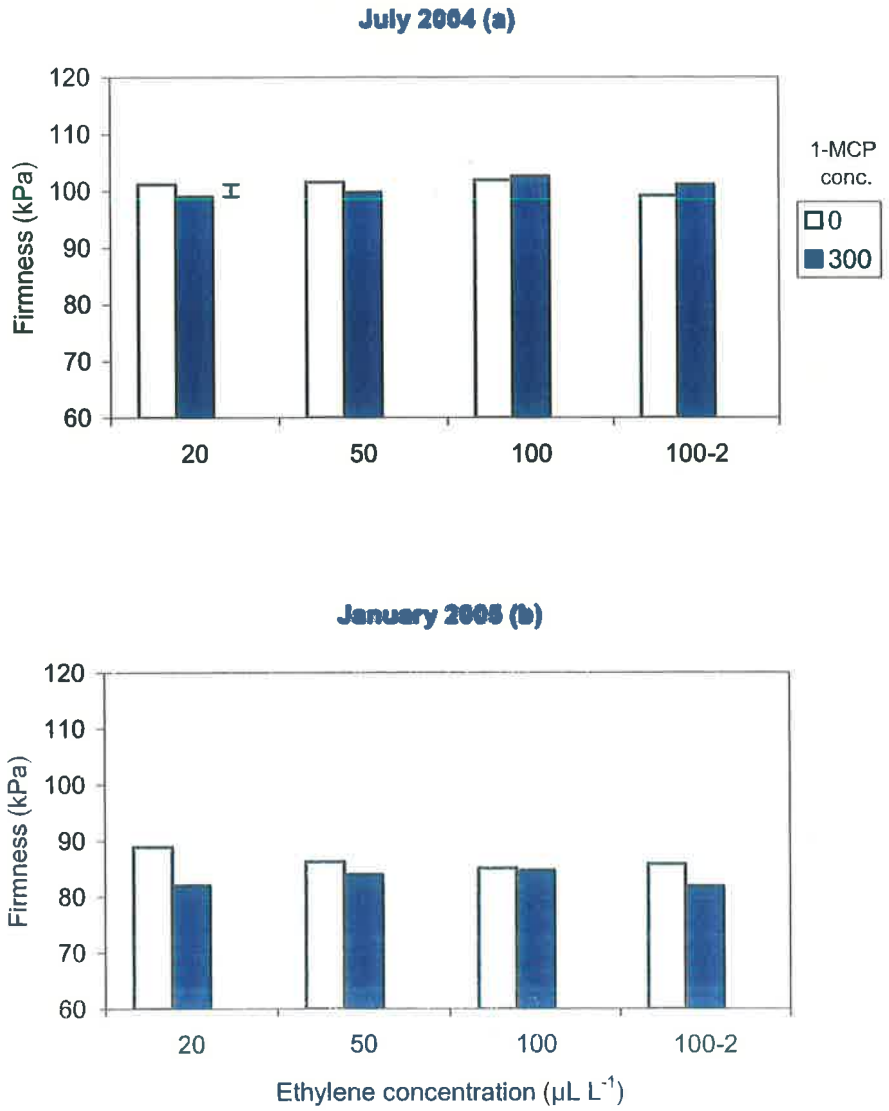


Figure 4.3: Effect of ethylene concentration on the effect of 1-MCP on pulp firmness of Cavendish bananas ripened at 22 °C in July 2004 (a), January 2005 (b). Bananas were treated with ethylene (20, 50 or 100 µL L^{-1}) for two days or 100 µL L^{-1} for the first day and 2 µL L^{-1} for the second day (100-2) prior to 0 or 300 nL L^{-1} 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9). Absence of a LSD bar indicates no significant difference within treatment.

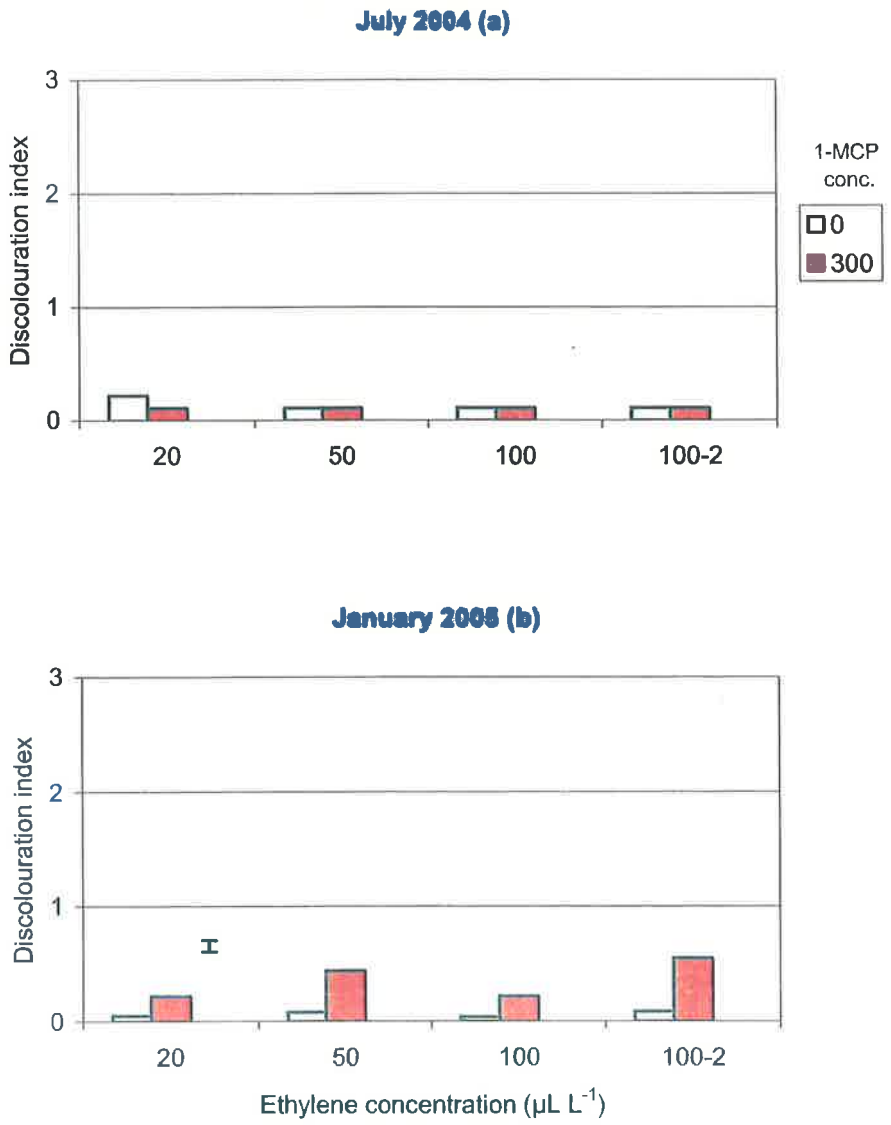


Figure 4.4: Effect of ethylene concentration on the effect of 1-MCP on discolouration index of Cavendish bananas ripened at 22 °C in July 2004 (a), January 2005 (b). Bananas were treated with ethylene (20, 50 or 100 µL L^{-1}) for two days or 100 µL L^{-1} for the first day and 2 µL L^{-1} for the second day (100-2) prior to 0 or 300 nL L^{-1} 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9). Absence of a LSD bar indicates no significant difference within treatment.

4.3.1.4 Weight loss

Weight loss was generally greater in fruit harvested in July than January (Figure 4.5). In July, an ethylene concentration increased from 20 μL L^{-1} to 100 μL L^{-1}, the weight loss of control fruit declined from 4.3% to 3.1%. Weight loss was higher when fruit were treated with ethylene at lower concentrations (20, 50 or 100 μL L^{-1} for the first and 2 μL L^{-1} for the second day) than the highest concentration (100 μL L^{-1}) in July (Figure 4.5 a).

Changes in weight loss did not significantly vary across the ethylene concentration in January-harvested fruit except that fruit with ethylene at 100 μL L^{-1} for the first and 2 μL L^{-1} for the second day did not lose as much weight (Figure 4.5 b). 1-MCP significantly increased weight loss when 20 or 50 μL L^{-1} ethylene were applied in July or when 50 μL L^{-1} or 100 μL L^{-1} for the first and 2 μL L^{-1} for the second day applied in January, but otherwise had no effect.

4.3.1.5 Total soluble solids

Total soluble solids (TSS) of control fruit were generally higher when harvested in July than when harvested in January (Figure 4.6). In July TSS of control fruit decreased as concentration of ethylene increased whereas changes are minimal in January. 1-MCP had no significant effect on TSS of July harvested fruit (Figure 4.6 a), but significantly increased TSS in January when ethylene was applied at 50 μL L^{-1} (Figure 4.6 b).

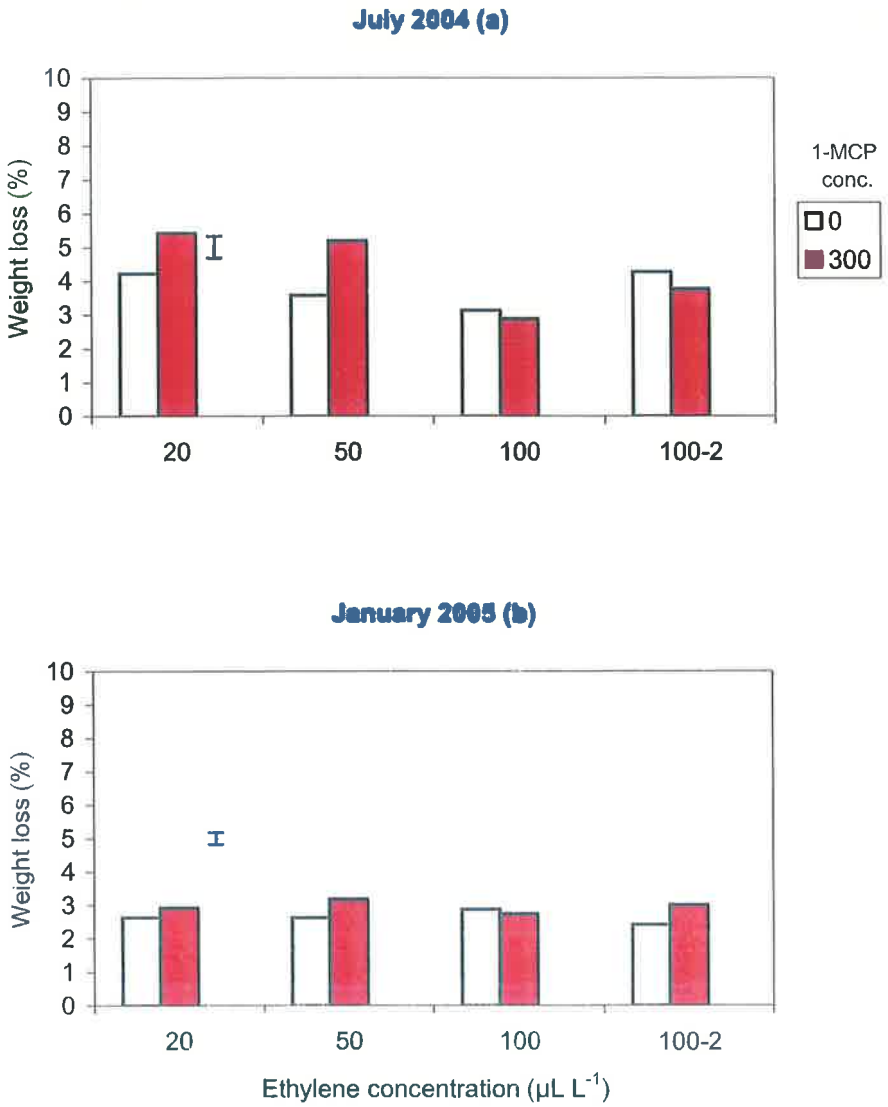


Figure 4.5: Effect of ethylene concentration on the effect of 1-MCP on weight loss (%) of whole fruit of Cavendish bananas ripened at 22 °C in July 2004 (a), January 2005 (b). Bananas were treated with ethylene (20, 50 or 100 µL L^{-1}) for two days or 100 µL L^{-1} for the first day and 2 µL L^{-1} for the second day (100-2) prior to 0 or 300 nL L^{-1} 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9).

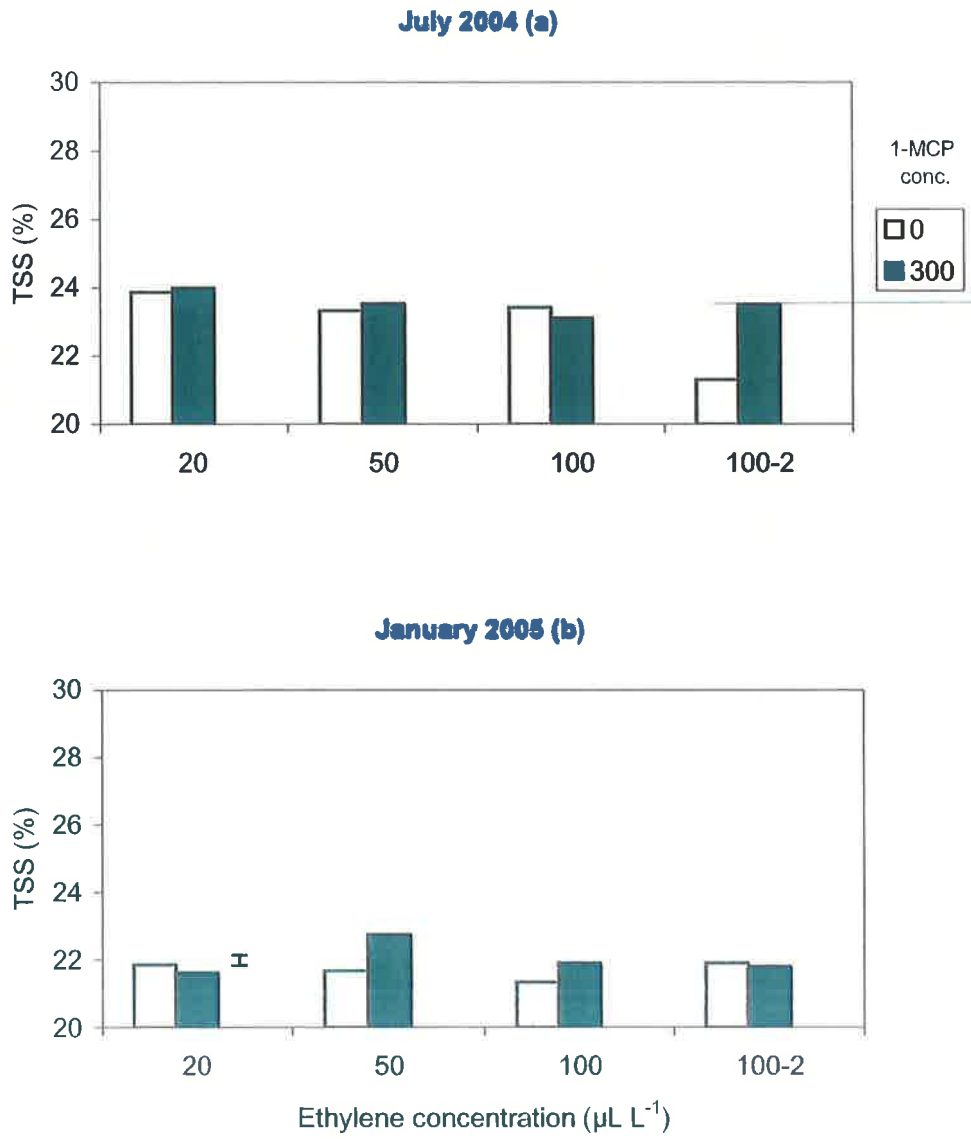


Figure 4.6: Effect of ethylene concentration on the effect of 1-MCP on total soluble solids (TSS %) of Cavendish bananas ripened at 22 °C in July 2004 (a), January 2005 (b). Bananas were treated with ethylene (20, 50 or 100 µL L^{-1}) for two days or 100 µL L^{-1} for the first day and 2 µL L^{-1} for the second day (100-2) prior to 0 or 300 nL L^{-1} 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9). Absence of a LSD bar indicates no significant difference within treatment.

4.3.2 Effect of ethylene duration prior to 1-MCP exposure

4.3.2.1 Shelf life

Shelf life of control fruit was similar when fruit were exposed to the ethylene for different durations in both harvest times in February (3.2 days) and in August (2.2 days).

1-MCP was effective in shelf life extension when applied to the fruit that were exposed to the ethylene for 30 h or 40 h in February compared to the control, but it had no effect in fruit that were ethylene initiated for 50 h (Figure 4.7 a). However, 1-MCP was more effective in extending shelf life when applied to the fruit that were treated with ethylene for 40 h or 50 h in August compared to the control (Figure 4.7 b). In August, 1-MCP treated fruit, which were treated initially with ethylene for 30 h, did not ripen, therefore there was no data to present for shelf life and quality assessments in this treatment.

4.3.2.2 Firmness

Fruit firmness was generally higher in all treatments in August than in February. 1-MCP application to fruit only significantly increased firmness if February-harvested fruit were treated with ethylene for 40 h or August-harvested fruit were treated with ethylene for 50 h (Figure 4.8).

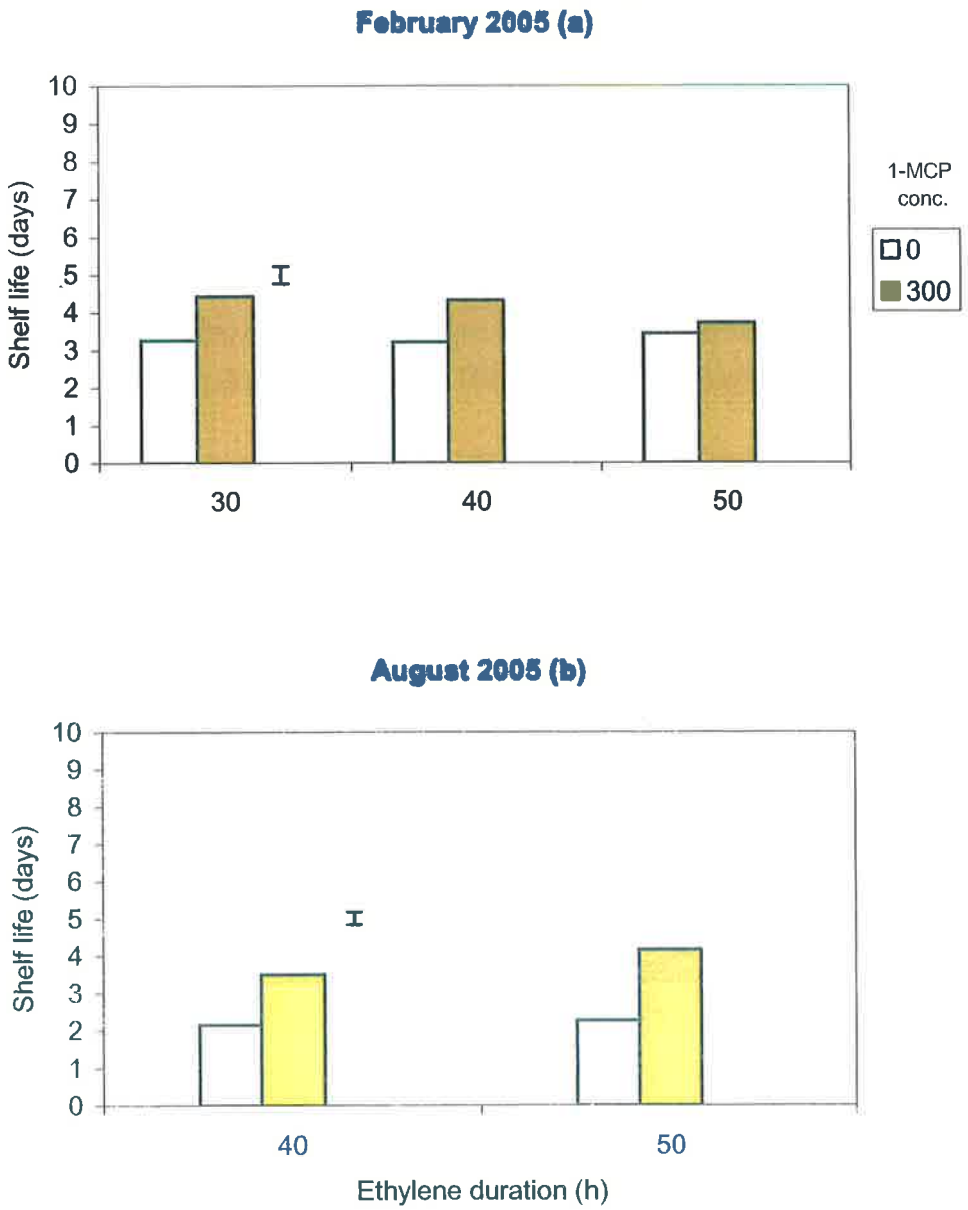


Figure 4.7: Effect of ethylene duration on the effect of 1-MCP on shelf life of Cavendish bananas ripened at 22 °C in February 2005 (a), August 2005 (b). Bananas were treated with ethylene 100 µL L^{-1} for either 30, 40 or 50 h prior to 0 or 300 nL L^{-1} 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9).

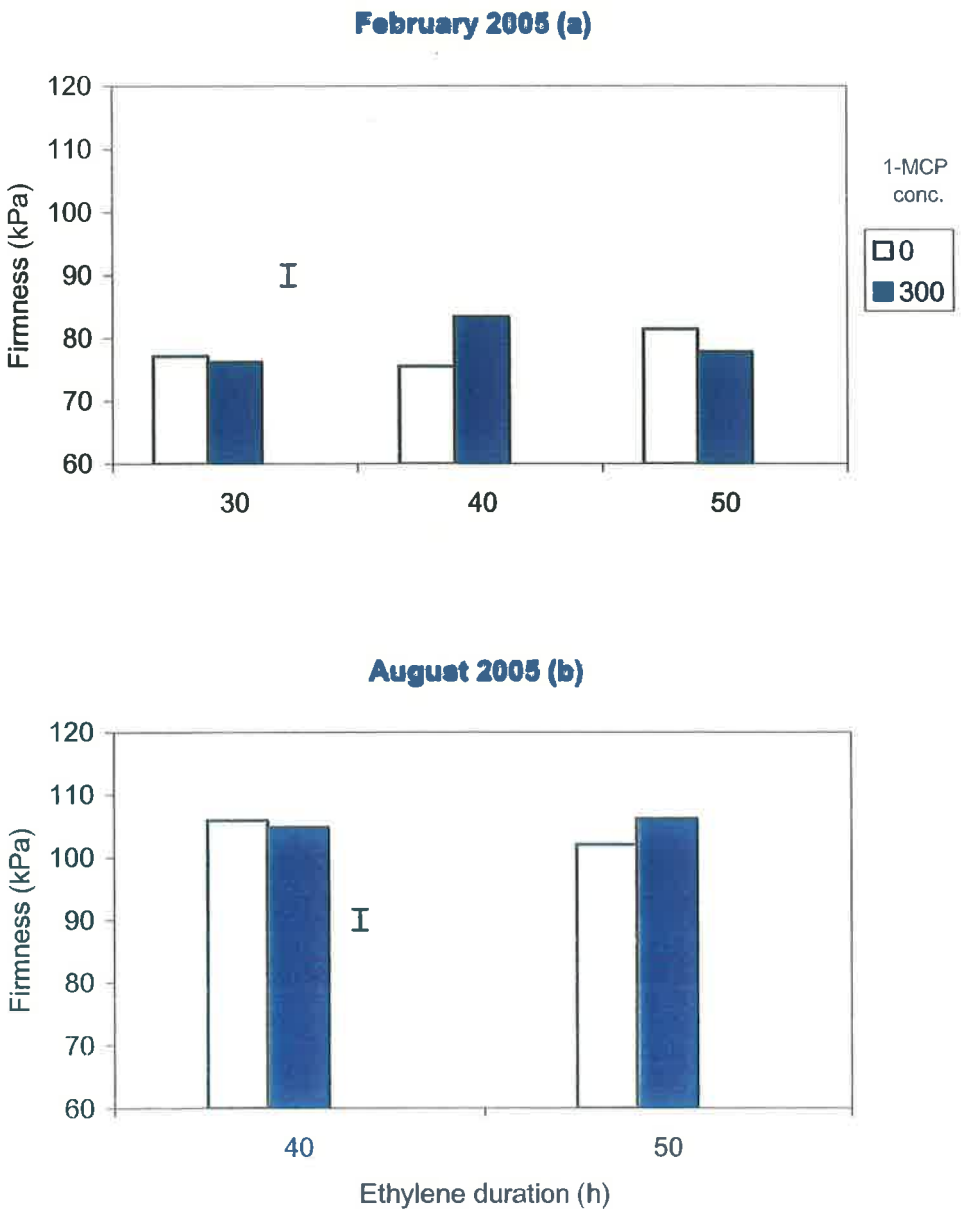


Figure 4.8: Effect of ethylene duration on the effect of 1-MCP on pulp firmness of Cavendish bananas ripened at 22 °C in February 2005 (a), August 2005 (b). Bananas were treated with ethylene 100 μL L^{-1} for either 30, 40 or 50 h prior to 0 or 300 nL L^{-1}1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9).

4.3.2.3 Discolouration

Discolouration of banana peel was generally slightly higher in August-harvested fruit than February-harvested fruit. Fruit exposed to ethylene for 30 h in February and for 40 h in August experienced a greater degree of discolouration when treated with 1-MCP compared to the other ethylene durations (Figure 4.9). Discolouration was above the threshold (1) only for 1-MCP-treated fruit in August regardless of ethylene duration and in February when exposed to ethylene for 30 h.

4.3.2.4 Weight loss

The percentage of weight loss was generally greater when harvested in August than in February (Figure 4.10). Weight loss was significantly lower in 1-MCP treated fruit initially exposed to ethylene for 40 h in February-harvested fruit and for 40 or 50 h in August.

4.3.2.5 Total soluble solids

Total soluble solids (TSS) of bananas were generally higher when harvested in August than in February (Figure 4.11). TSS were significantly lower in 1-MCP treated fruit, regardless of ethylene duration in February-harvested fruit. However, TSS were only lower in 1-MCP treated fruit initially exposed to ethylene for 50 h in August.

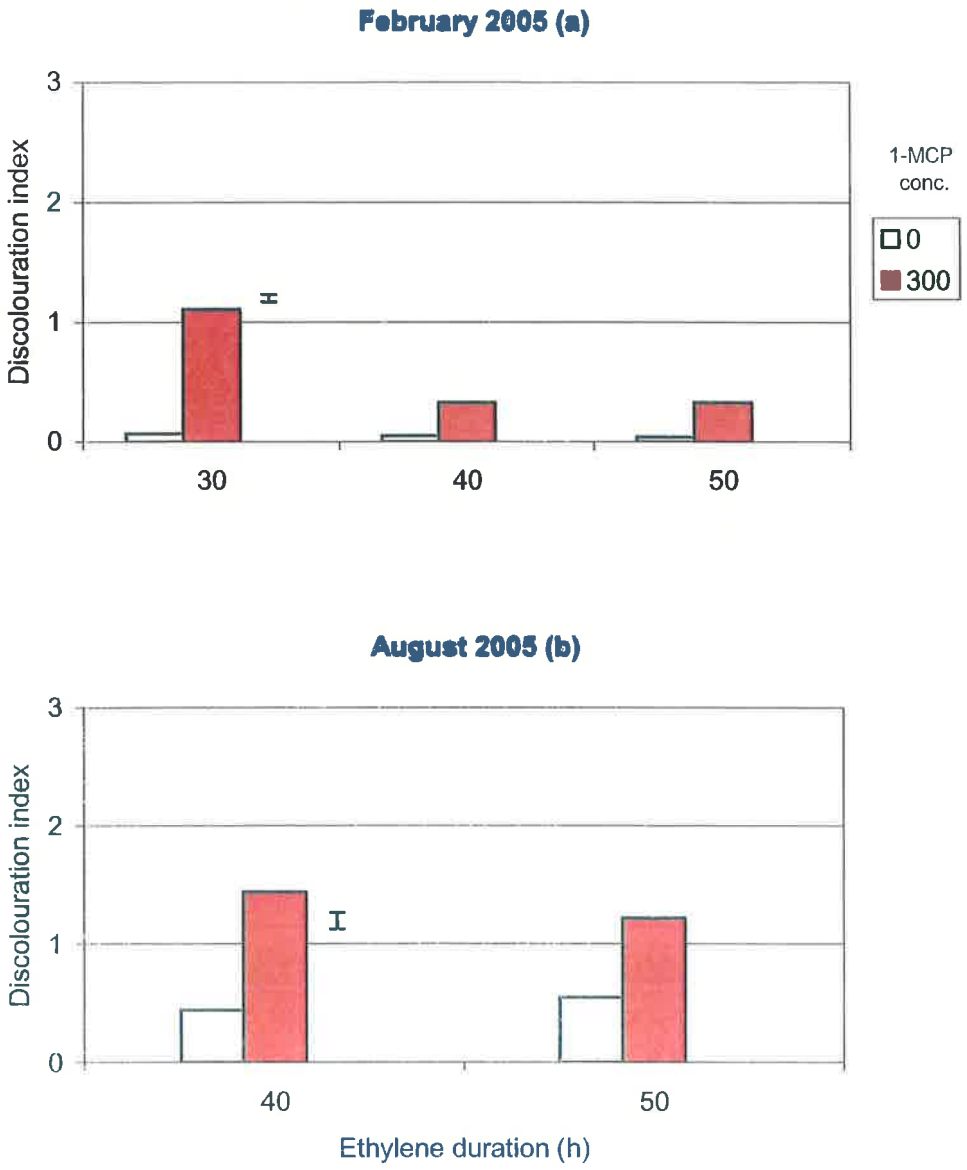


Figure 4.9: Effect of ethylene duration on the effect of 1-MCP on discolouration index of Cavendish bananas ripened at 22 °C in February 2005 (a), August 2005 (b). Bananas were treated with ethylene 100 µL L^{-1} for either 30, 40 or 50 h prior to 0 or 300 nL L^{-1} 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9).

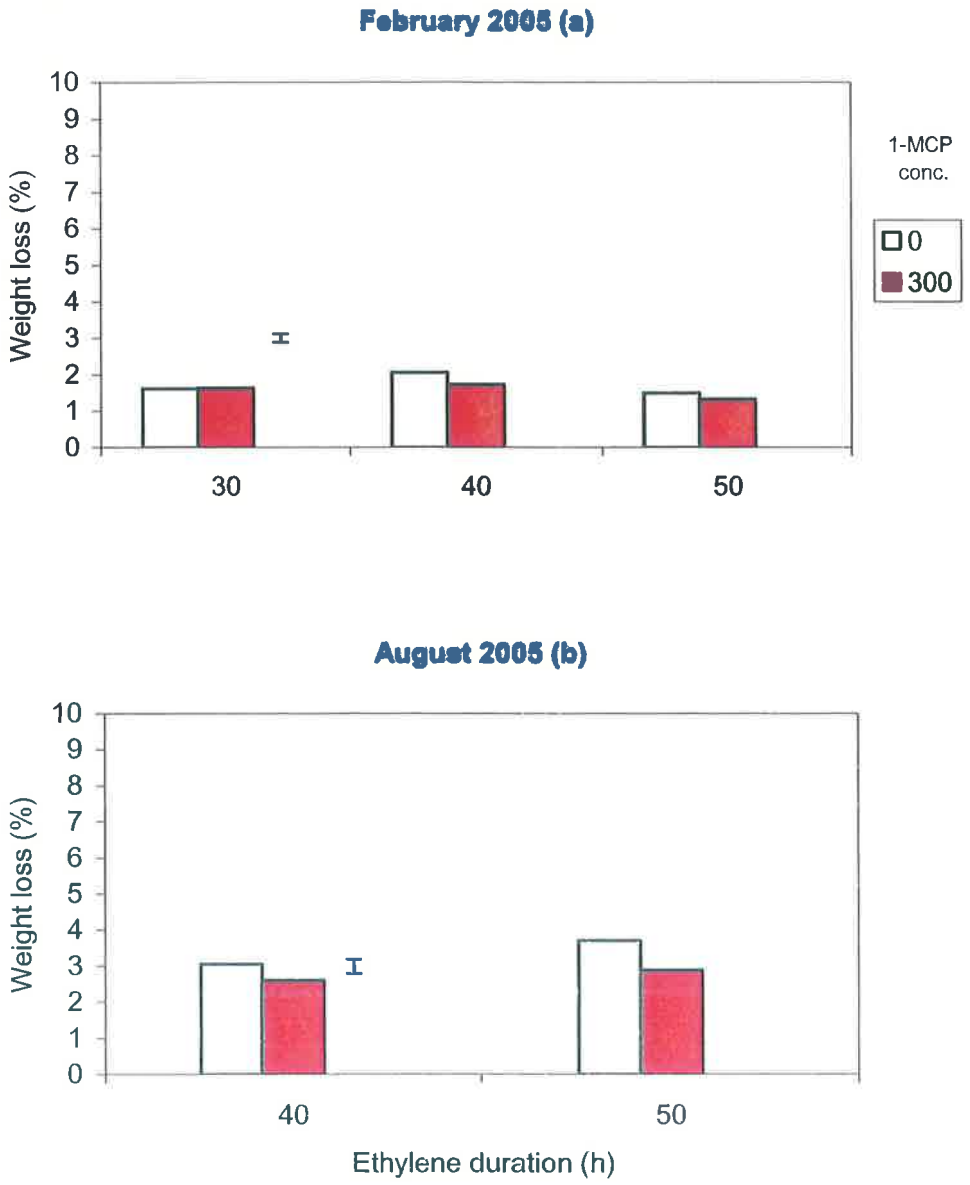


Figure 4.10: Effect of ethylene duration on the effect of 1-MCP on weight loss (%) of whole fruit of Cavendish bananas ripened at 22 °C in February 2005 (a), August 2005 (b). Bananas were treated with ethylene 100 μL L^{-1} for either 30, 40 or 50 h prior to 0 or 300 nL L^{-1} 1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9).

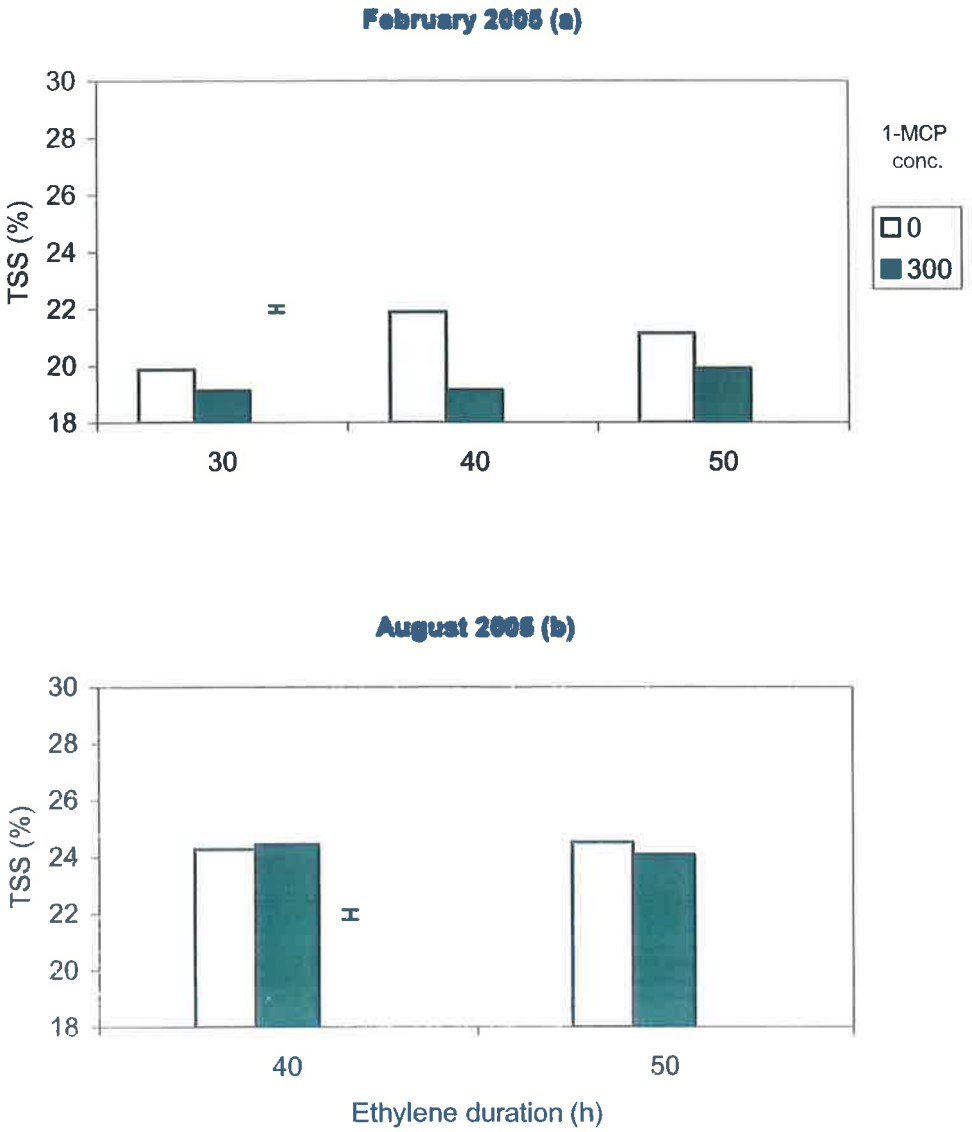


Figure 4.11: Effect of ethylene duration on the effect of 1-MCP on total soluble solids (TSS %) of Cavendish bananas ripened at 22 °C in February 2005 (a), August 2005 (b). Bananas were treated with ethylene 100 μL L^{-1} for either 30, 40 or 50 h prior to 0 or 300 nL L^{-1}1-MCP exposure for 24 h at 22 °C. Vertical bars represent LSD values at the 5% level (n=9).

4.3.3 Simultaneous application of 1-MCP and ethylene

4.3.3.1 Shelf life

Simultaneous application of 1-MCP with ethylene particularly on the second day of ethylene treatment generally increased shelf life regardless of banana harvest month (Figure 4.12). Even though fruit treated with ethylene only (control) were similar regardless of harvest time (that is, 4.2 days in June compared with 4.4 days in December).

Shelf life increased significantly compared to the control when 1-MCP was applied coincidently with ethylene in the second day and reapplied alone in the third day or applied only in the third day, in both harvest times. However, application of 1-MCP at all treatments significantly increased shelf life in December. When 1-MCP was applied at 300 nL L^{-1} in both the second day (simultaneously with ethylene) and third day (alone) fruit did not ripen such that they were still green after one week (Figure 4.13), and therefore there was no data to present for this treatment in Figures.

The greatest increase in shelf life (120%) was obtained when 1-MCP was applied on the second day at 30 nL L^{-1} simultaneously with ethylene and at 300 nL L^{-1} alone on the third day compared to the control in both harvest months.

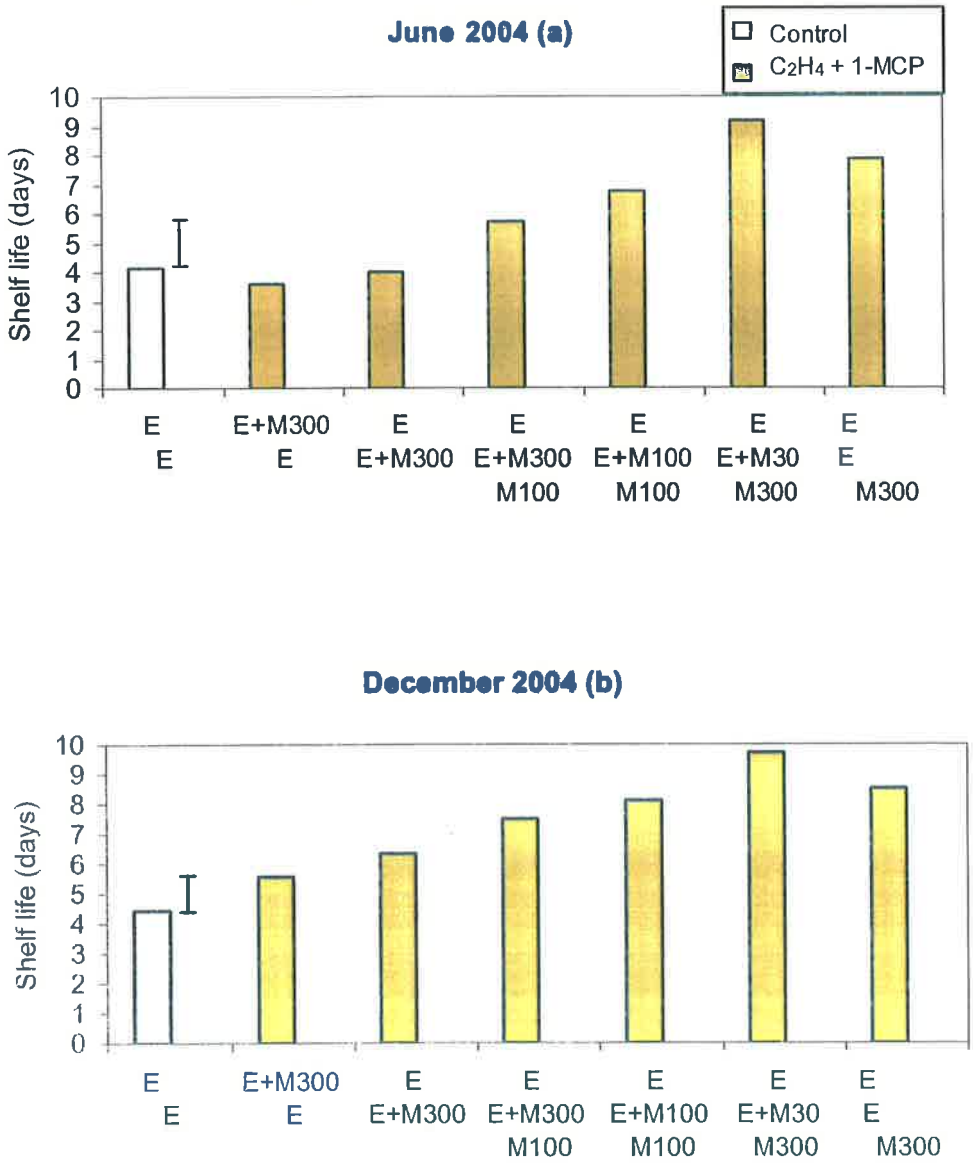


Figure 4.12: Effect of simultaneous application of 1-MCP and ethylene on shelf life of Cavendish bananas ripened at 22 °C in June 2004 (a), December 2004 (b). E = ethylene at 100 µL L^{-1} and M = 1-MCP at subscript concentration (30, 100 or 300 nL L^{-1}). Vertical bars represent LSD values at the 5% level (n=9).

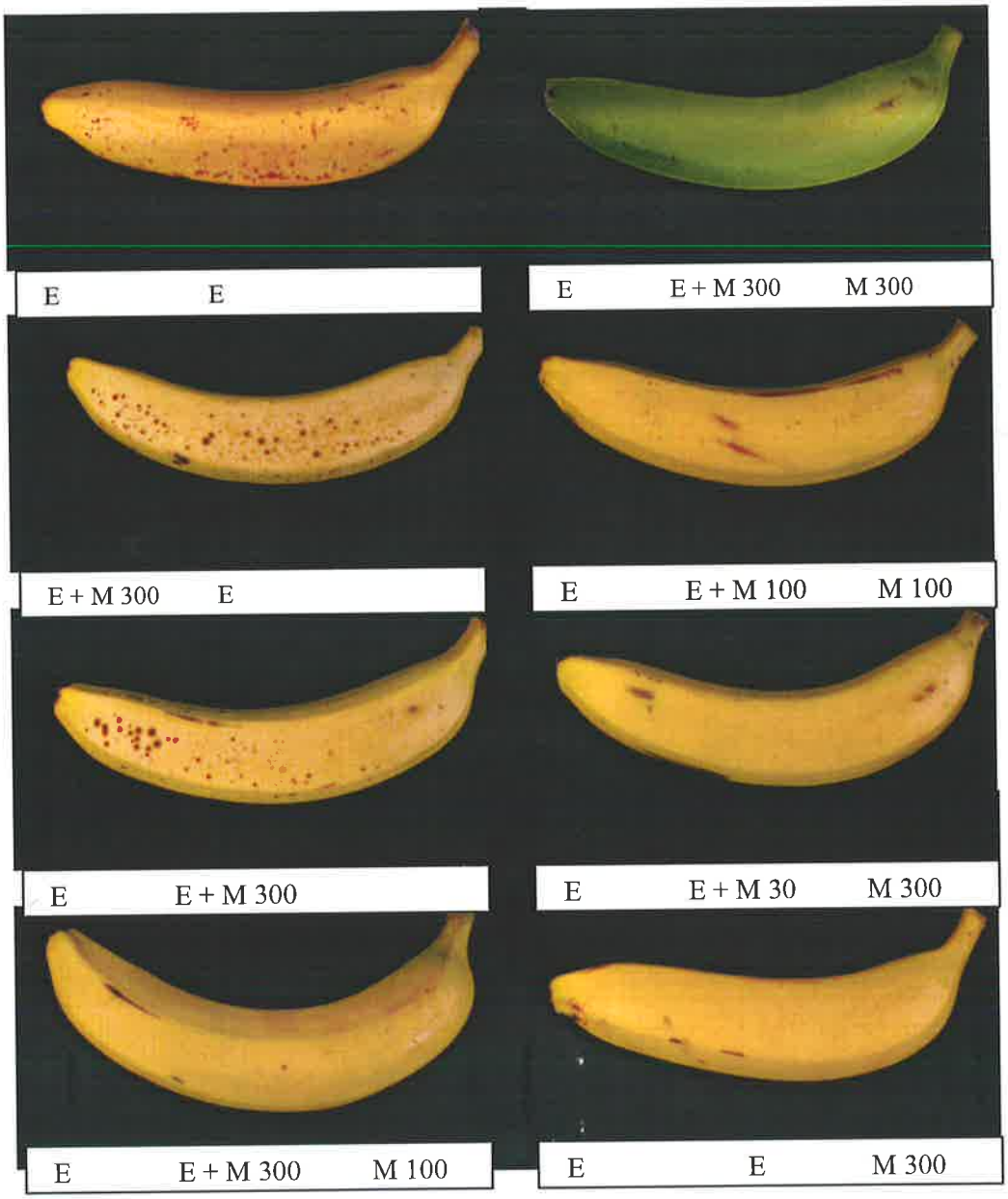


Figure 4.13: Effect of simultaneous application of 1-MCP and ethylene on the ripeness stage of bananas after 1 week of air storage at 22 °C in June-harvested fruit. Bananas were simultaneously treated with ethylene and 1-MCP. E = ethylene at 100 μL L^{-1} and M = 1-MCP at subscript concentration (30, 100 or 300 nL L^{-1}).

4.3.3.2 Firmness

Firmness of control fruit was slightly higher in June-harvested (102 kPa) fruit than in December-harvested (90 kPa) fruit (Figure 4.14). Application of 1-MCP simultaneously with ethylene in the second day followed by 1-MCP treatment in the third day gave lower firmness compared to the control in June but not in the December harvest. 1-MCP increased firmness in December-harvested fruit when 1-MCP was applied at 300 nL L^{-1} simultaneously with ethylene in the first day.

4.3.3.3 Discolouration

Discolouration of control fruit was greater in June (0.66) than December (0), but still below the commercial threshold (1) (Figure 4.15). Simultaneous application of 1-MCP at 300 nL L^{-1} with ethylene on the second day followed by 100 nL L^{-1} on the third day caused greater discolouration just in June.

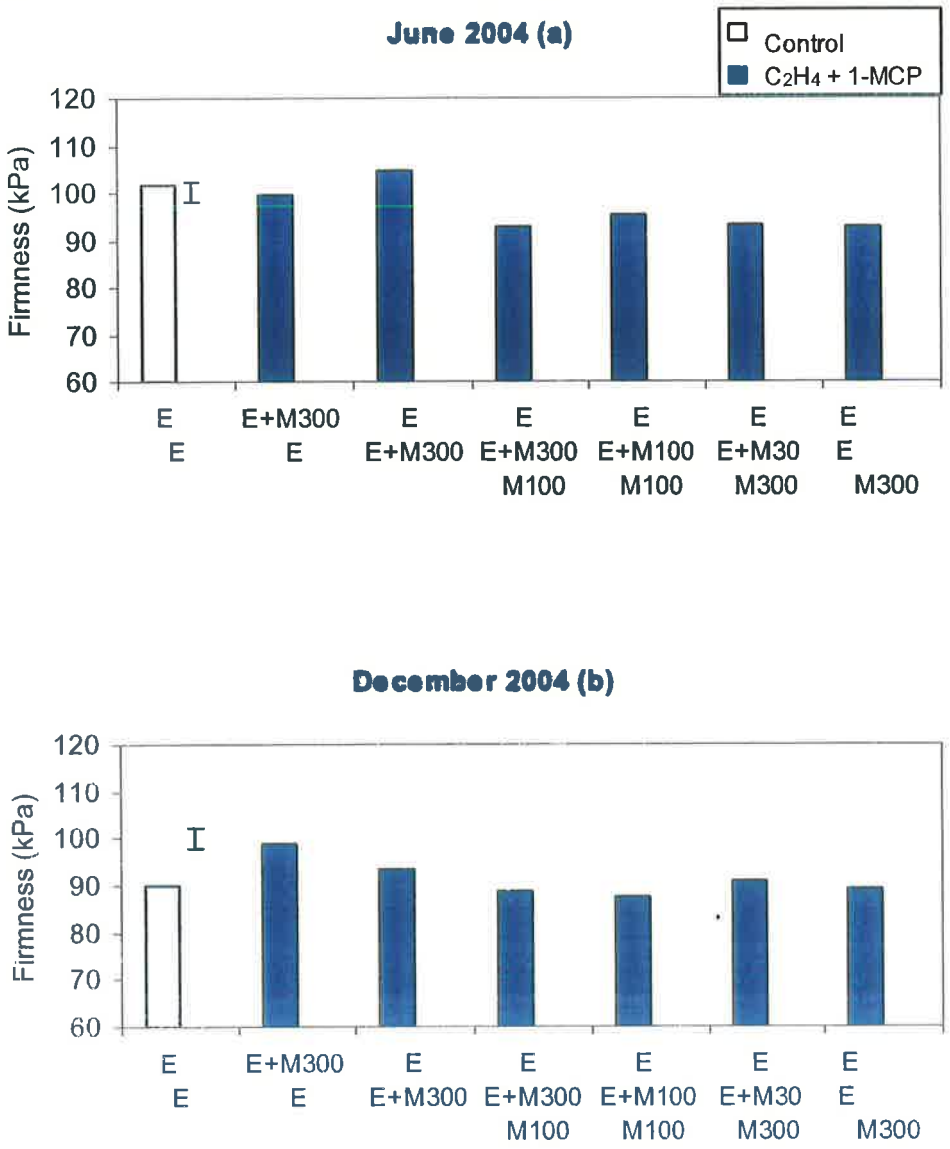


Figure 4.14: Effect of simultaneous application of 1-MCP and ethylene on pulp firmness of Cavendish bananas ripened at 22 °C in June 2004 (a), December 2004 (b). E = ethylene at 100 µL L^{-1} and M = 1-MCP at subscript concentration (30, 100 or 300 nL L^{-1}). Vertical bars represent LSD values at the 5% level (n=9).

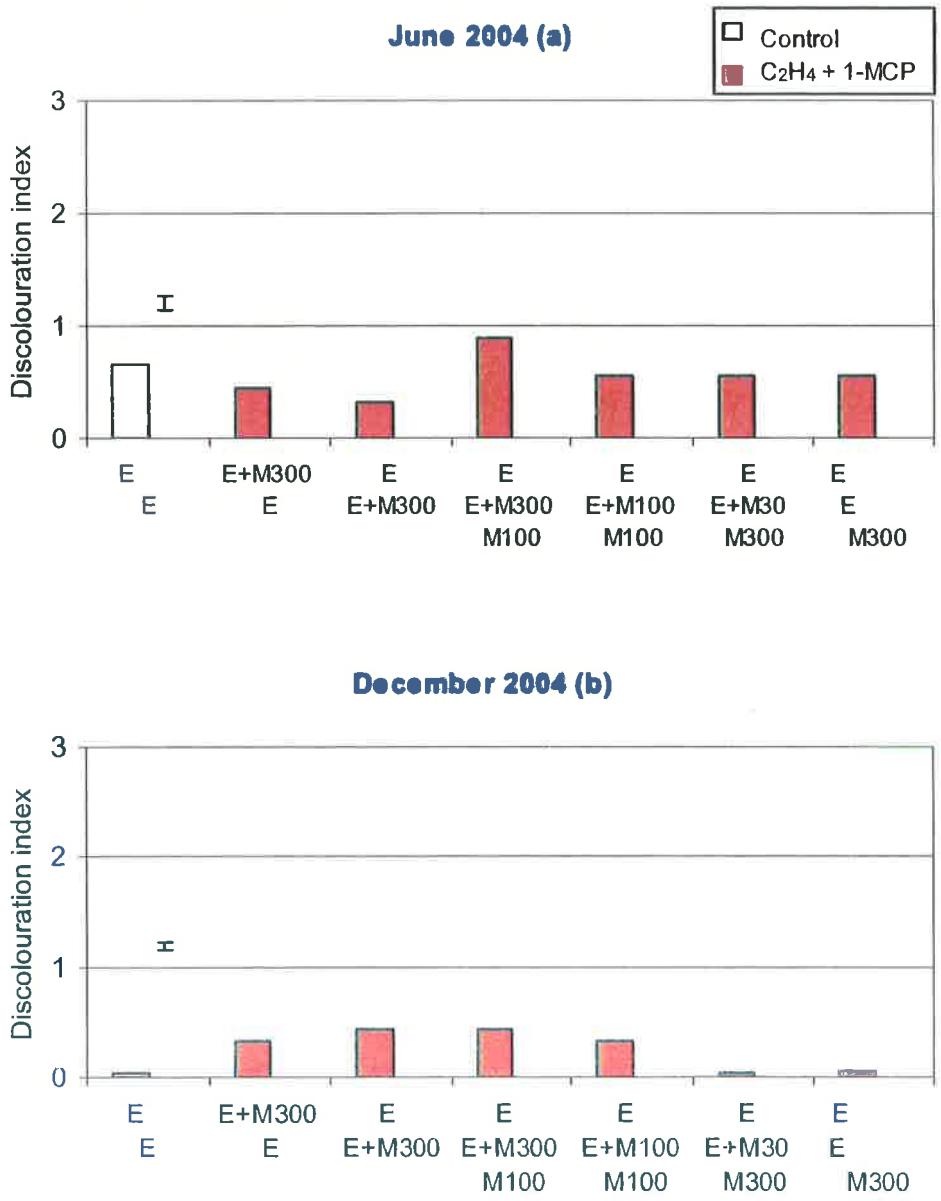


Figure 4.15: Effect of simultaneous application of 1-MCP and ethylene on discolouration index of Cavendish bananas ripened at 22 °C in June 2004 (a), December 2004 (b). E = ethylene at 100 µL L^{-1} and M = 1-MCP at subscript concentration (30, 100 or 300 nL L^{-1}). Vertical bars represent LSD values at the 5% level (n=9).

4.3.3.4 Weight loss

The percentage of weight loss was higher in the control fruit from the June harvest compared to the December harvest. Weight loss of simultaneously-treated fruit with ethylene and 1-MCP increased significantly compared to the control in June. In December weight loss significantly decreased compared to the control except where 1-MCP was applied at 100 nL L^{-1} in the second day with ethylene and alone in the third day (Figure 4.16).

4.3.3.5 Total soluble solids

Total soluble solids of control fruit were similar at both harvest times. Simultaneous application of 1-MCP with ethylene on the first or second day decreased TSS significantly at most treatments, however, there were no similar trends in June and December (Figure 4.17).

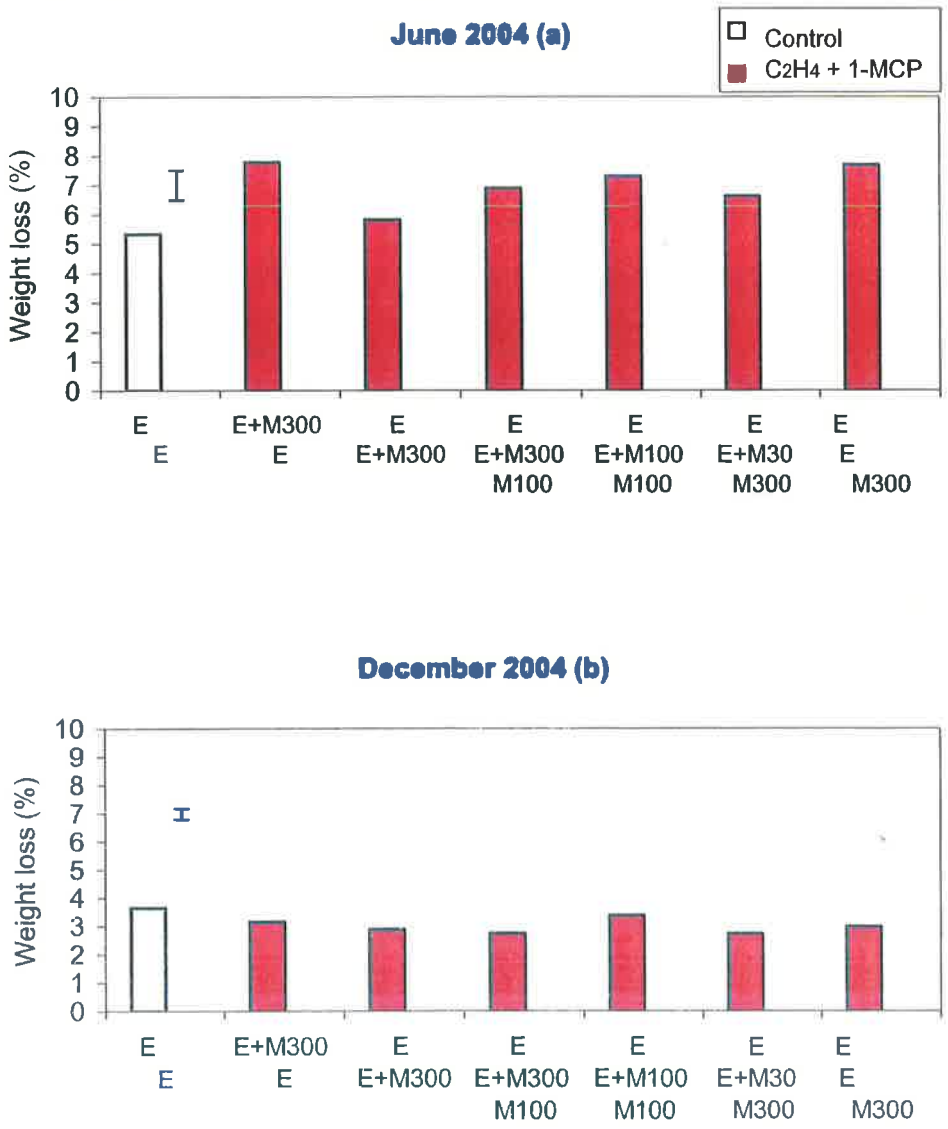


Figure 4.16: Effect of simultaneous application of 1-MCP and ethylene on weight loss (%) of whole fruit of Cavendish bananas ripened at 22 °C in June 2004 (a), December 2004 (b). E = ethylene at 100 μL L^{-1} and M = 1-MCP at subscript concentration (30, 100 or 300 nL L^{-1}). Vertical bars represent LSD values at the 5% level (n=9).

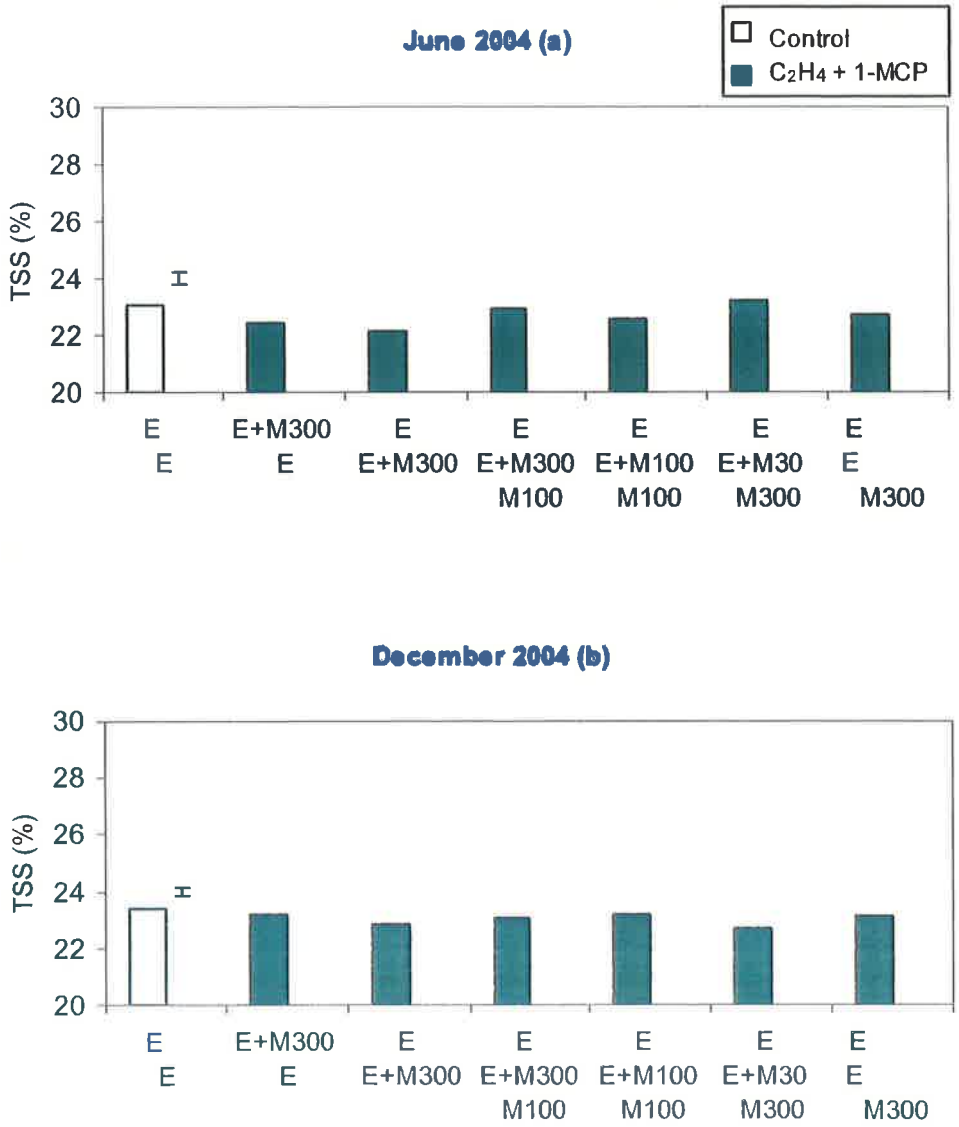


Figure 4.17: Effect of simultaneous application of 1-MCP and ethylene on total soluble solids (TSS %) of Cavendish bananas ripened at 22 °C in June 2004 (a), December 2004 (b). E = ethylene at 100 µL L^{-1} and M = 1-MCP at subscript concentration (30, 100 or 300 nL L^{-1}). Vertical bars represent LSD values at the 5% level (n=9).

4.3.4 Pre- and early-climacteric application of 1-MCP

The data of experiments in both months were not significantly different and therefore they were combined in the figures.

4.3.4.1 Shelf life

Application of 1-MCP at higher concentrations (30 nL L^{-1} and above) for 6 h when fruit was pre-climacteric appeared optimum to extend fruit green life rather than shelf life. As shown in Figure 4.18 the ripeness stage of ethylene treated fruit (control) after 1 week air storage at 22 °C was different than that of 1-MCP treated fruit, as control fruit responded properly to ethylene gassing whereas uneven degreening happened when 1-MCP was applied at 30 nL L^{-1}. 1-MCP-treated fruit at the two highest concentrations (300 or 10000 nL L^{-1}) also did not respond to exogenous ethylene and remained green compared with control fruit. As a result, the effect of 1-MCP application at lower concentrations in the pre-climacteric stage was examined (Figure 4.19).

Application of 1-MCP at lower concentrations (2 to 30 nL L^{-1}) for 6 h when banana was pre-climacteric appeared optimum to extend the fruit shelf life as expected (Figure 4.19). Hence those lower concentrations at pre-climacteric stage were combined with reapplication of 1-MCP in the early-climacteric stage of ripening to examine the effect of timing and reapplication of 1-MCP in banana shelf life and quality.

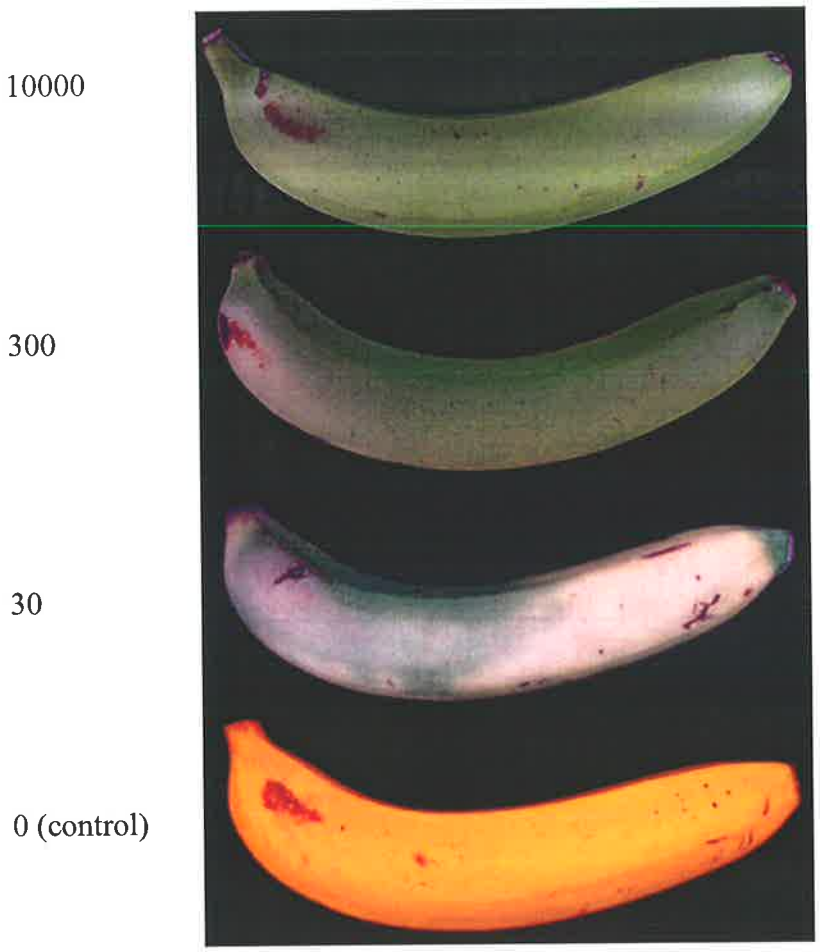


Figure 4.18: Ripeness stage of pre-storage 1-MCP treated bananas after 1 week air storage at 22 °C. Fruit were treated with 1-MCP (0, 30, 300 or 10000 nL L^{-1}) for 6 h, prior to 2 weeks storage at 16 °C followed by 2 days ethylene treatment at 100 µL L^{-1}.

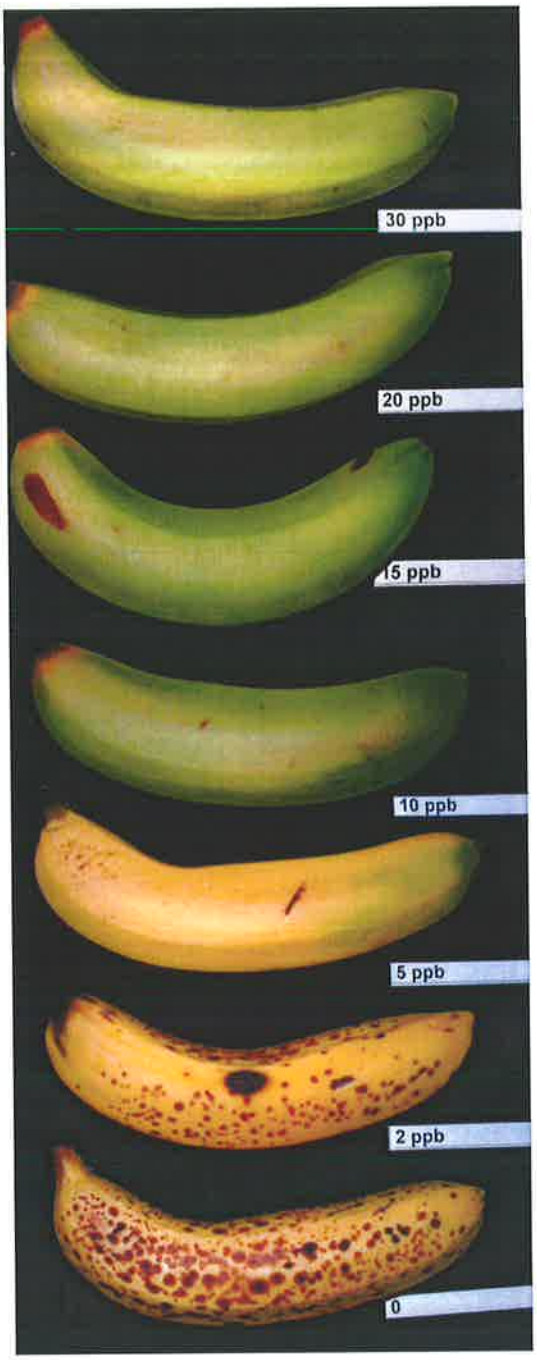


Figure 4.19: Ripeness stage of bananas in a preliminary trial to examine the effect of pre-climacteric 1-MCP application after 1 week of air storage at 22 °C. Fruit were treated with 1-MCP (0, 2, 5, 10, 15, 20 or 30 nL L^{-1}) for 6 h prior to ethylene treatment at 100 µL L^{-1} for two days.

Interestingly, application of 1-MCP at the lower concentrations at the pre-climacteric stage in combination with reapplication of 1-MCP in the early-climacteric stage increased shelf life significantly in both fruit from the top and bottom of the bunch (Figure 4.20). When fruit from the top of the bunch were treated with 6 nL L^{-1} of 1-MCP or higher (Figure 4.21) (during the pre-climacteric stage) and fruit from the bottom of the bunch were treated with 4 nL L^{-1} of 1-MCP or higher concentrations (Figure 4.22), ripening did not occur. Thus, data are not present in the graphical representations for those 1-MCP concentrations.

The highest increase in shelf life was obtained in fruit from the bottom of the bunch when exposed to 2 nL L^{-1} 1-MCP (43%) and in fruit from the top of the bunch when exposed to 5 nL L^{-1} 1-MCP (39%) prior to ethylene treatment and subsequent 1-MCP treatment (300 nL L^{-1}) in the early-climacteric stage.

4.3.4.2 Firmness

Firmness of control fruit from the top and the bottom of the bunch were to some extent similar (Figure 4.23). Application of 1-MCP at both the pre- and early-climacteric stages of ripening increased firmness significantly in both fruit from the top and bottom of the bunch. The highest increase in firmness was obtained in fruit from the top of the bunch (11%) followed by (4%) in fruit from the bottom of the bunch when 1-MCP was applied in the pre-climacteric stages at 5 or 2 nL L^{-1}, respectively.

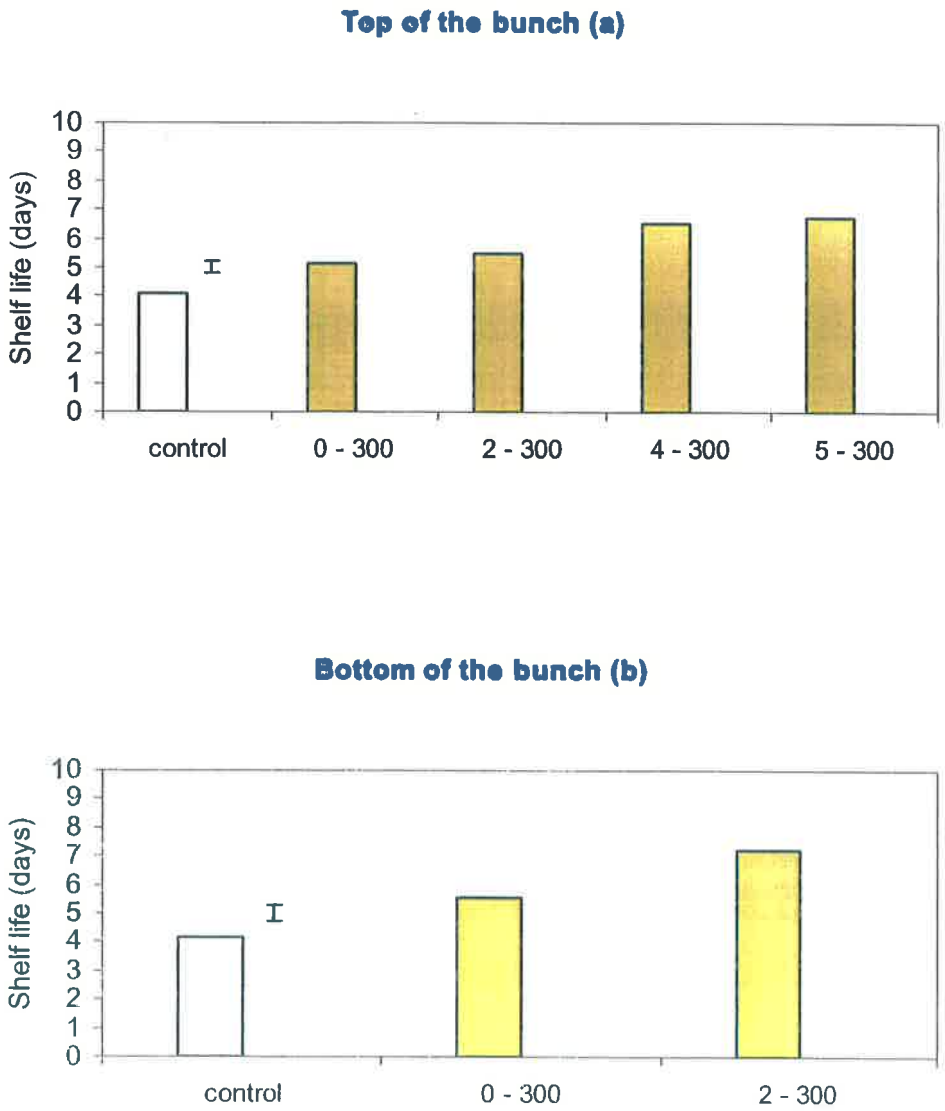


Figure 4.20: Effect of pre- and early-climacteric application of 1-MCP on shelf life of Cavendish bananas in fruit from the top (a) and from the bottom (b) of the bunch ripened at 22 °C. 1-MCP concentrations shown are separated by dash based on stage of application (pre - early climacteric). Vertical bars represent LSD values at the 5% level (n=18).

Figure 4.21: Ripeness stage of pre- and early-climacteric applied 1-MCP bananas from the top of the bunch after 1 week of air storage at 22 °C. 1-MCP (0, 2, 4, 5, 6 or 10 nL L^{-1}) applied at pre-climacteric stage, followed by ethylene treatment (100 µL L^{-1}) for 48 h and then 1-MCP (0 or 300 nL L^{-1}) treatment for 24 h.

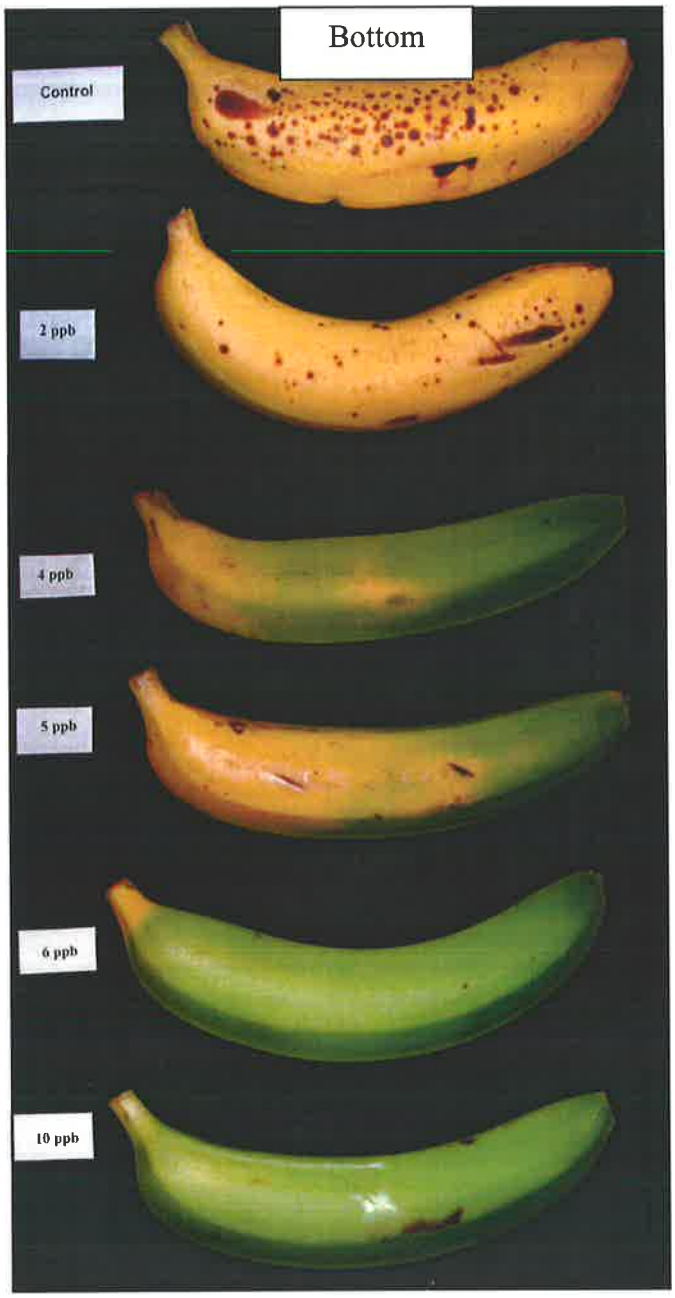


Figure 4.22: Ripeness stage of pre- and early-climacteric applied 1-MCP bananas from the bottom of the bunch after 1 week of air storage at 22 °C. 1-MCP (0, 2, 4, 5, 6 or 10 nL L^{-1}) applied at pre-climacteric stage, followed by ethylene treatment (100 µL L^{-1}) for 48 h and then 1-MCP (0 or 300 nL L^{-1}) treatment for 24 h.

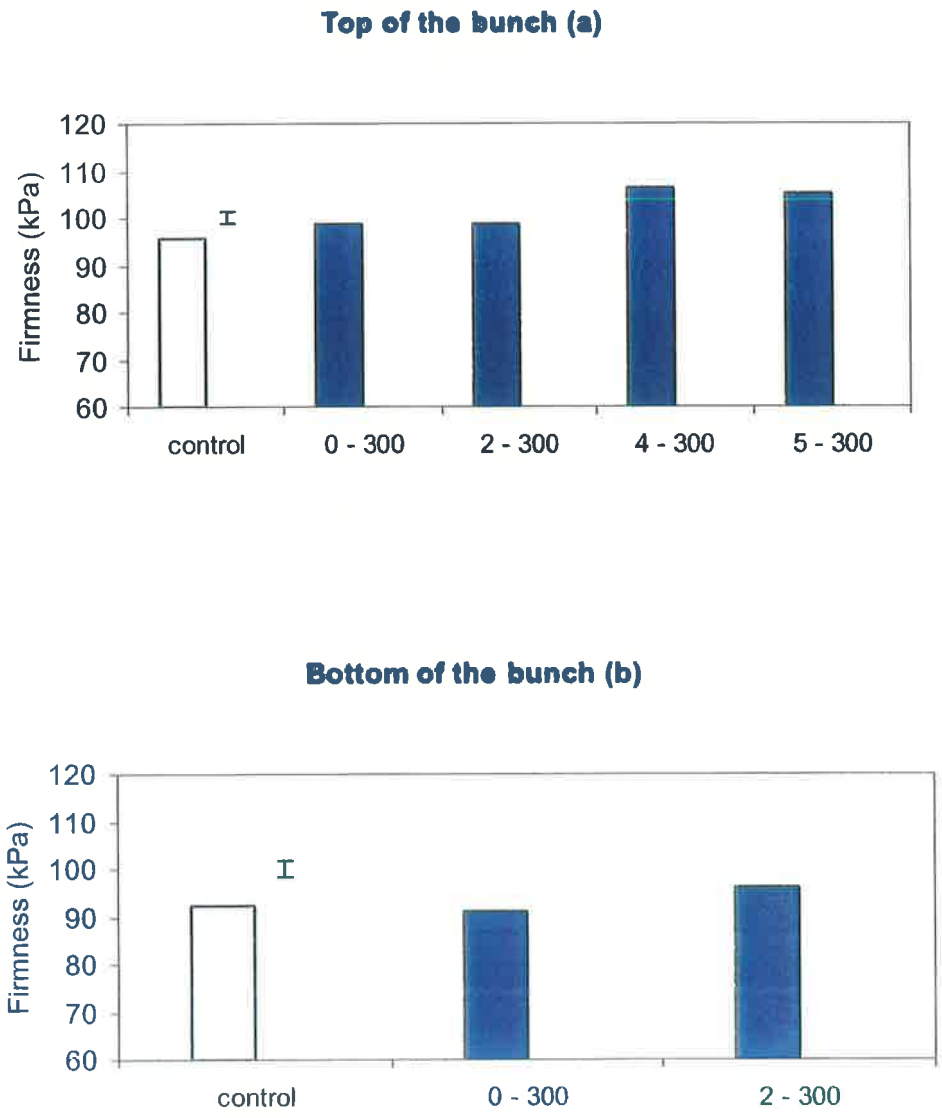


Figure 4.23: Effect of pre- and early-climacteric application of 1-MCP on pulp firmness of Cavendish bananas in fruit from the top (a) and from the bottom (b) of the bunch ripened at 22 °C. 1-MCP concentrations shown are separated by dash based on stage of application (pre - early climacteric). Vertical bars represent LSD values at the 5% level (n=18).

4.3.4.3 Discolouration

Discolouration of peel from bananas treated with 1-MCP at pre- and early-climacteric stages of ripening and also control fruit was too low to record a score. Hence, no data is present for the discolouration.

4.3.4.4 Weight loss

The percentage of weight loss of control fruit was to some extent similar in fruit from the top (2.5%) and from the bottom (2.9%) of the bunch. Application of 1-MCP at 2 nL L^{-1} in the pre-climacteric stage of ripening followed by 300 nL L^{-1} in the early-climacteric stage decreased weight loss significantly in fruit from the bottom (24%) compared to the control but not in fruit from the top of the bunch (Figure 4.24).

4.3.4.5 Total soluble solids

Total soluble solids of control fruit were slightly different in fruit from the top (23.4%) and bottom (23%) of the bunch. The highest decrease (4%) in TSS were obtained when 1-MCP was applied in pre- (2 nL L^{-1}) and in early- (300 nL L^{-1}) climacteric stages in fruit from the bottom of the bunch (Figure 4.25).

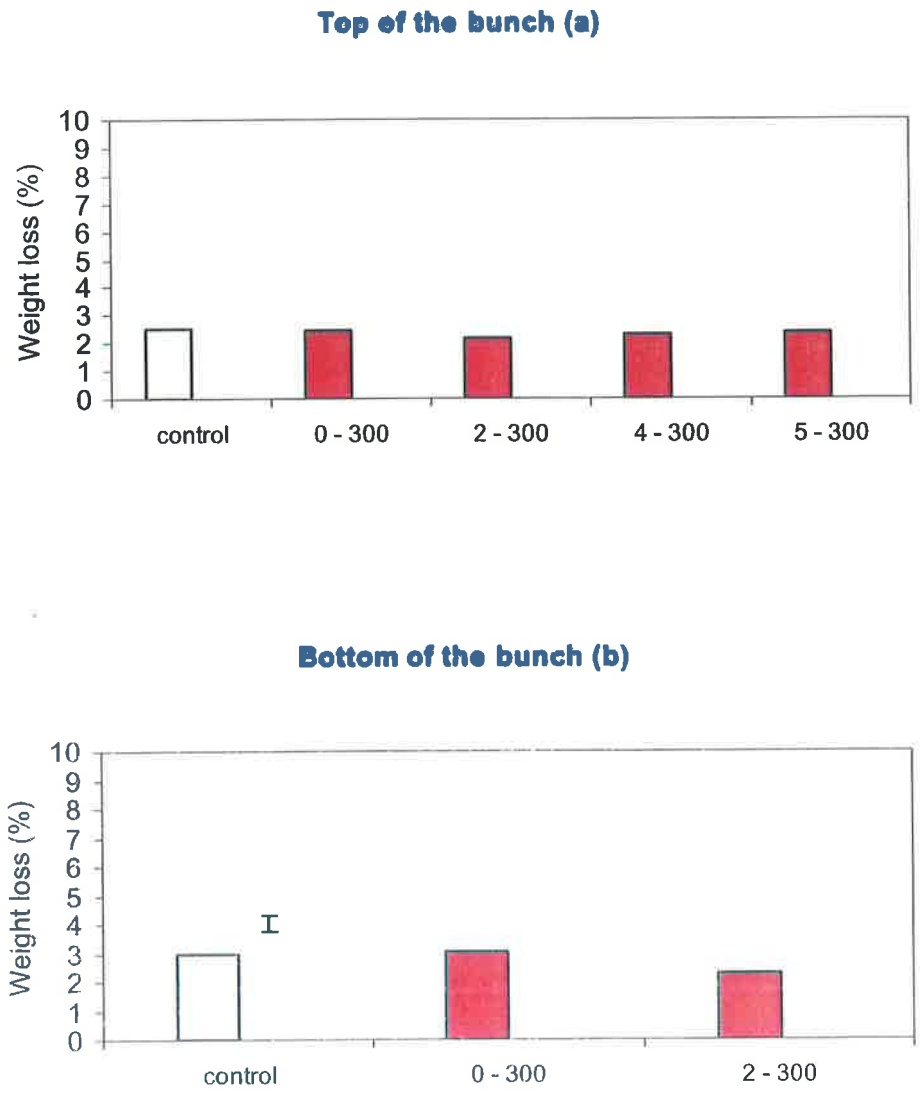


Figure 4.24: Effect of pre- and early-climacteric application of 1-MCP on weight loss (%) of whole fruit of Cavendish bananas in fruit from the top (a) and from the bottom (b) of the bunch ripened at 22 °C. 1-MCP concentrations shown are separated by dash based on stage of application (pre - early climacteric). Vertical bars represent LSD values at the 5% level (n=18). Absence of a LSD bar indicates no significant difference between control and 1-MCP treatment.

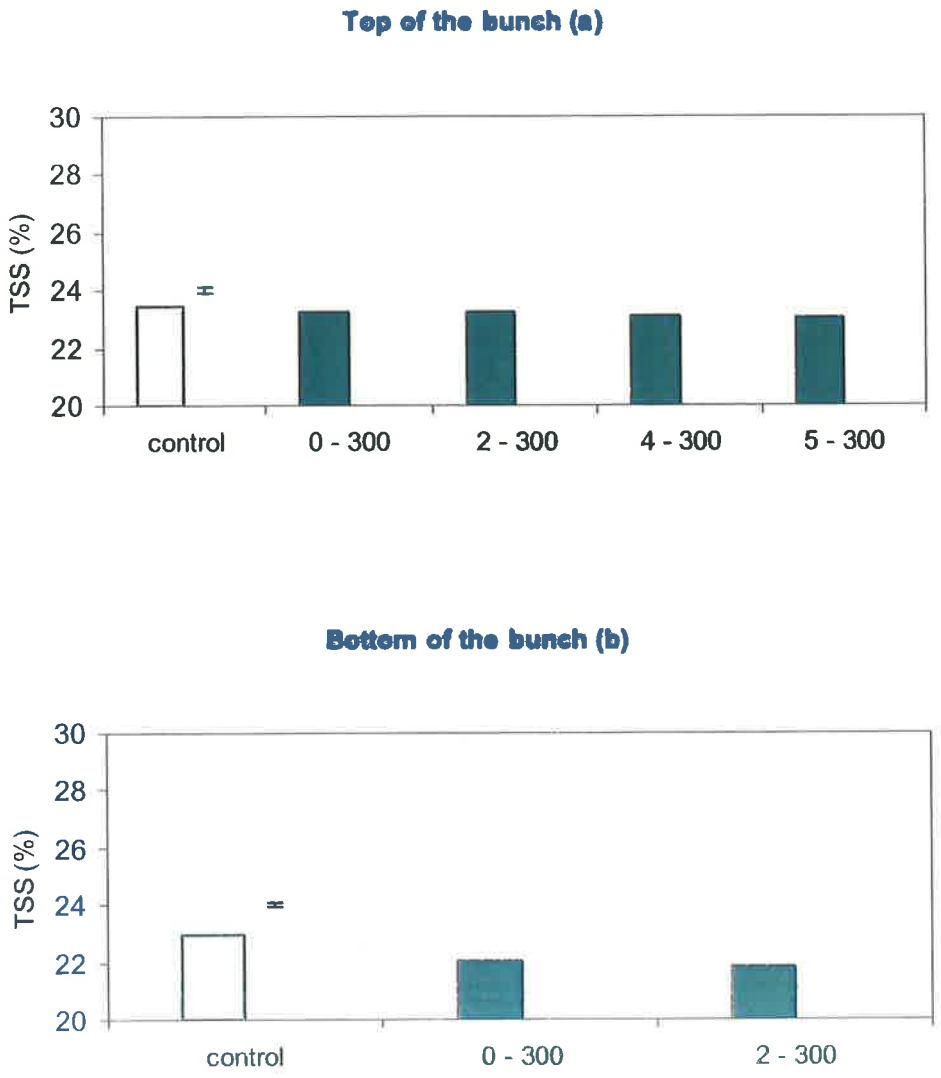


Figure 4.25: Effect of pre- and early-climacteric application of 1-MCP on total soluble solids (TSS %) of Cavendish bananas in fruit from the top (a) and from the bottom (b) of the bunch ripened at 22 °C. 1-MCP concentrations shown are separated by dash based on stage of application (pre - early climacteric). Vertical bars represent LSD values at the 5% level (n=18).

4.3.5 Effect of time from harvest to 1-MCP exposure (1-MCP concentrations)

The data of experiments in both months were not significantly different and therefore they were combined in the figures.

4.3.5.1 Shelf life

Shelf life of ethylene treated fruit (control) was similar when bananas were treated with ethylene on arrival (3.7 days) or after 1 week storage (3.5 days) (Figure 4.26).

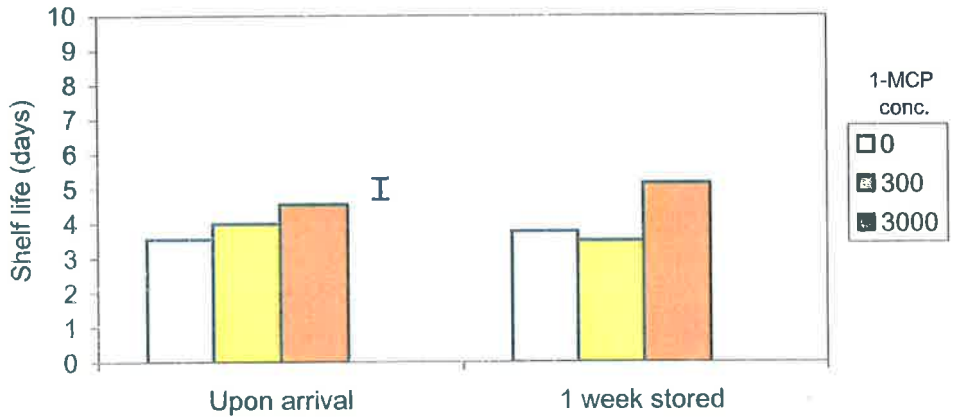


Figure 4.26: Effect of delayed exposure and concentration of 1-MCP (0, 300 or 3000 nL L^{-1}) on shelf life of Cavendish bananas ripened at 22 °C. Vertical bars represent LSD values at the 5% level (n=18).

Shelf life was increased significantly compared to the control when fruit were treated with 3000 nL L^{-1} 1-MCP both on arrival and after 1 week of

storage. However, the increase was greater when the 3000 nL L^{-1} 1-MCP was applied after 1 week. 1-MCP treatment at 300 nL L^{-1} increased significantly the fruit shelf life when it was applied to the fruit on arrival but not in fruit that were stored for 1 week.

4.3.5.2 Firmness

Firmness of control fruit differed from those treated on arrival (91.3 kPa) and from stored fruit (79.3 kPa). Firmness was increased significantly compared to the control when fruit were treated with 1-MCP at 300 or 3000 nL L^{-1} on arrival fruit but not in stored fruit (Figure 4.27). The highest increase in firmness (11%) was obtained when fruit were treated with 1-MCP on arrival at 300 or 3000 nL L^{-1}.

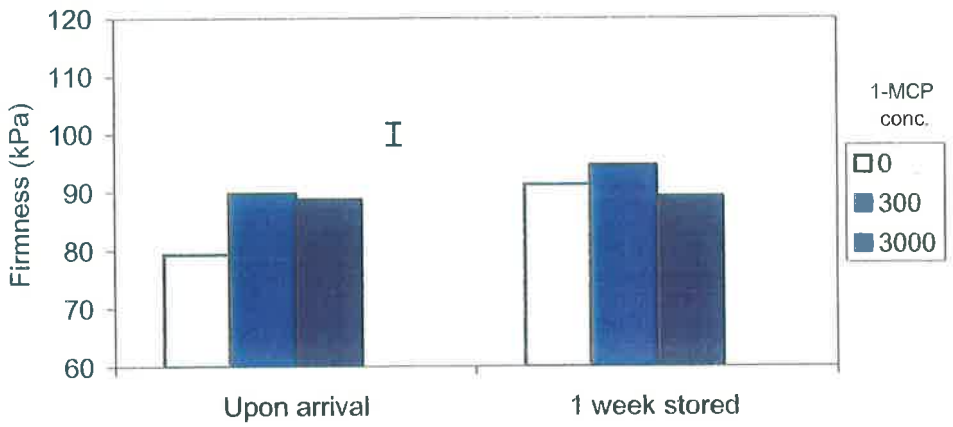


Figure 4.27: Effect of delayed exposure and concentration of 1-MCP (0, 300 or 3000 nL L^{-1}) on pulp firmness of Cavendish bananas ripened at 22 °C. Vertical bars represent LSD values at the 5% level (n=18).

4.3.5.3 Discolouration

Discolouration of stored fruit (0.94) was higher than fruit that had not been stored (0.11). However, discolouration in both treatments was lower than the undesirable level (1). Discolouration of fruit increased significantly when 1-MCP was applied to the fruit immediately upon arrival. The discolouration of 1-MCP treated fruit was lower than the control when 1-MCP was applied to the stored fruit (Figure 4.28).

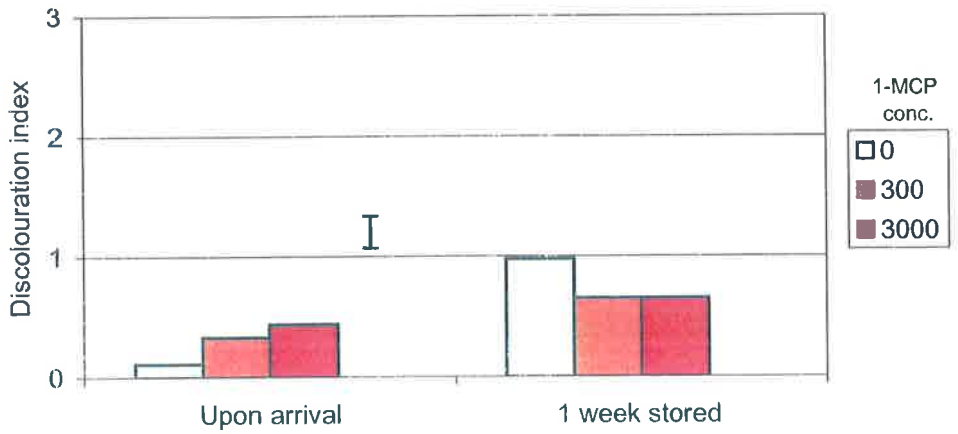


Figure 4.28: Effect of delayed exposure and concentration of 1-MCP (0, 300 or 3000 nL L^{-1}) on discolouration index of Cavendish bananas ripened at 22 °C. Vertical bars represent LSD values at the 5% level (n=18).

4.3.5.4 Weight loss

The percentage of weight loss in control fruit was greater in fruit treated on arrival than in stored fruit. 1-MCP application decreased weight loss in both on arrival and stored fruit compared to the control (Figure 4.29). Weight loss of fruit that were treated on arrival with 1-MCP at both 300 and 3000 nL L^{-1} concentrations decreased significantly, 19% and 22% respectively, compared to the control. Application of 1-MCP at 3000 nL L^{-1} to the stored fruit decreased (16%) weight loss significantly but not at 300 nL L^{-1}.

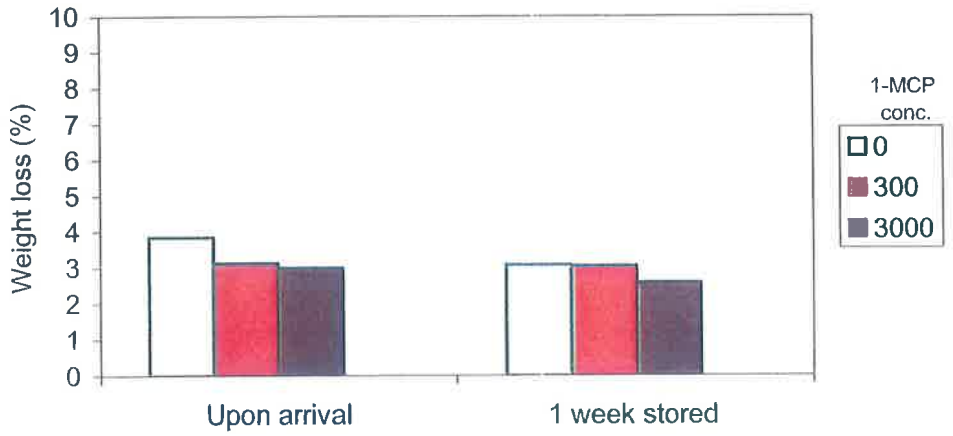


Figure 4.29: Effect of delayed exposure and concentration of 1-MCP (0, 300 or 3000 nL L^{-1}) on percentage weight loss of whole fruit of Cavendish bananas ripened at 22 °C. Vertical bars represent LSD values at the 5% level (n=18). Absence of a LSD bar indicates no significant interaction between upon arrival and stored fruit with 1-MCP treatment.

4.3.5.5 Total soluble solids

Total soluble solids of control fruit were similar in fruit treated on arrival (22.6%) and in stored fruit (22.1%). 1-MCP did not change TSS of either treatment compared to the control (Figure 4.30).

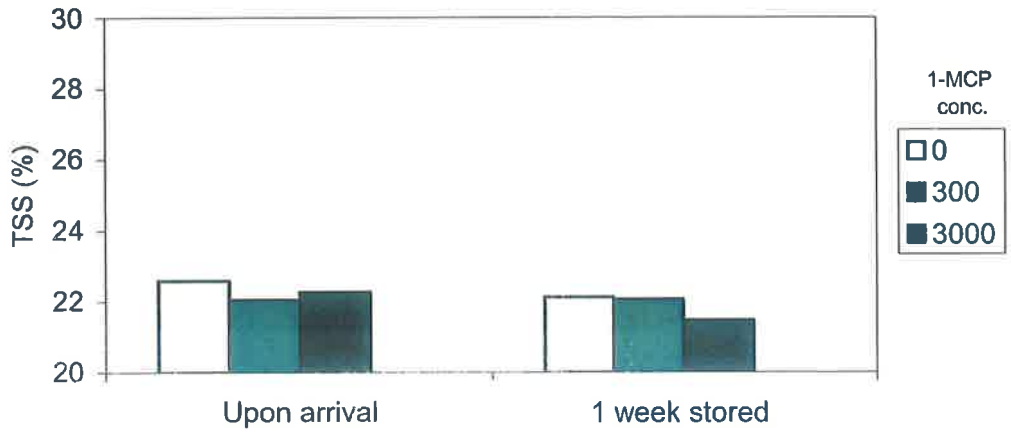


Figure 4.30: Effect of delayed exposure and concentration of 1-MCP (0, 300 or 3000 nL L^{-1}) on total soluble solids (TSS %) of Cavendish bananas ripened at 22 °C. Vertical bars represent LSD values at the 5% level (n=18). Absence of a LSD bar indicates no significant interaction between upon arrival and stored fruit with 1-MCP treatment.

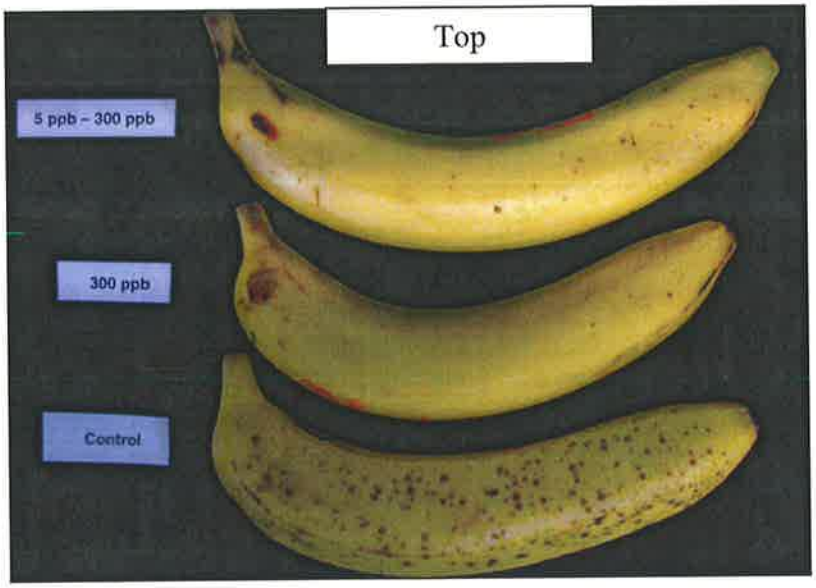
4.3.6 Effect of time from harvest to 1-MCP exposure (Timing of 1-MCP application, time from harvest and comparison of hand position on bunch)

The data of experiments in both months were not significantly different and therefore they were combined in the figures.

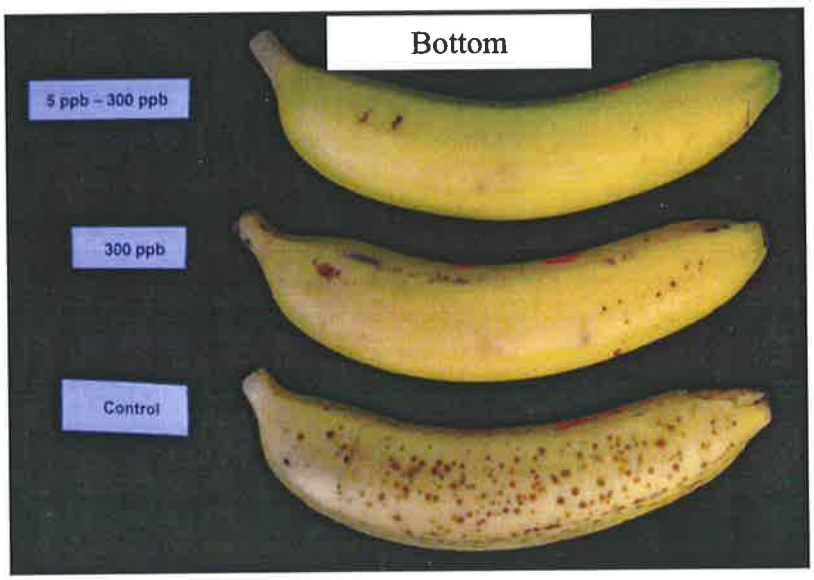
4.3.6.1 Shelf life

Shelf life of ethylene treated fruit (control) was similar in fruit from the top (5.9 to 6 days) and the bottom (4.6 to 4.8 days) of the bunch regardless of the time from harvest to the treatment (Figure 4.31). Shelf life of 1-MCP treated fruit (300 nL L^{-1}) increased significantly in fruit from the top (11%) and from the bottom (26%) of the bunch compared to the control when bananas were treated with 1-MCP 5 days after harvest. Bananas that were exposed to 1-MCP 5 days after harvest in the pre-climacteric stage (5 nL L^{-1}) and then 1-MCP in the early-climacteric stage (300 nL L^{-1}) did not ripen. Thus, no data was obtained for shelf life or quality assessments in this treatment to present in Figures.

Reapplication of 1-MCP was more effective in extending fruit shelf life than only one application after ethylene treatment when bananas were treated with 1-MCP 10 days after harvest, particularly in fruit from the top of the bunch. Shelf life significantly increased in fruit from the top (13%) and bottom (43%) of the bunch compared to the control. Fruit were exposed to 1-MCP in the pre- and early-climacteric stages at 5 and 300 nL L^{-1} (5-300), respectively (Figures 4.31 and 4.32).



a



b

Figure 4.31: Effect of time from harvest and timing of 1-MCP application on the ripeness stage of bananas from the top (a) and the bottom (b) of the bunch after 1 week of air storage at 22 °C. Fruit were treated with 1-MCP (0 or 5 nL L^{-1}) prior to ethylene treatment; or (0 or 300 nL L^{-1}) 1-MCP after ripening initiation with ethylene treatment, at 10 days after harvest, compared to the control.

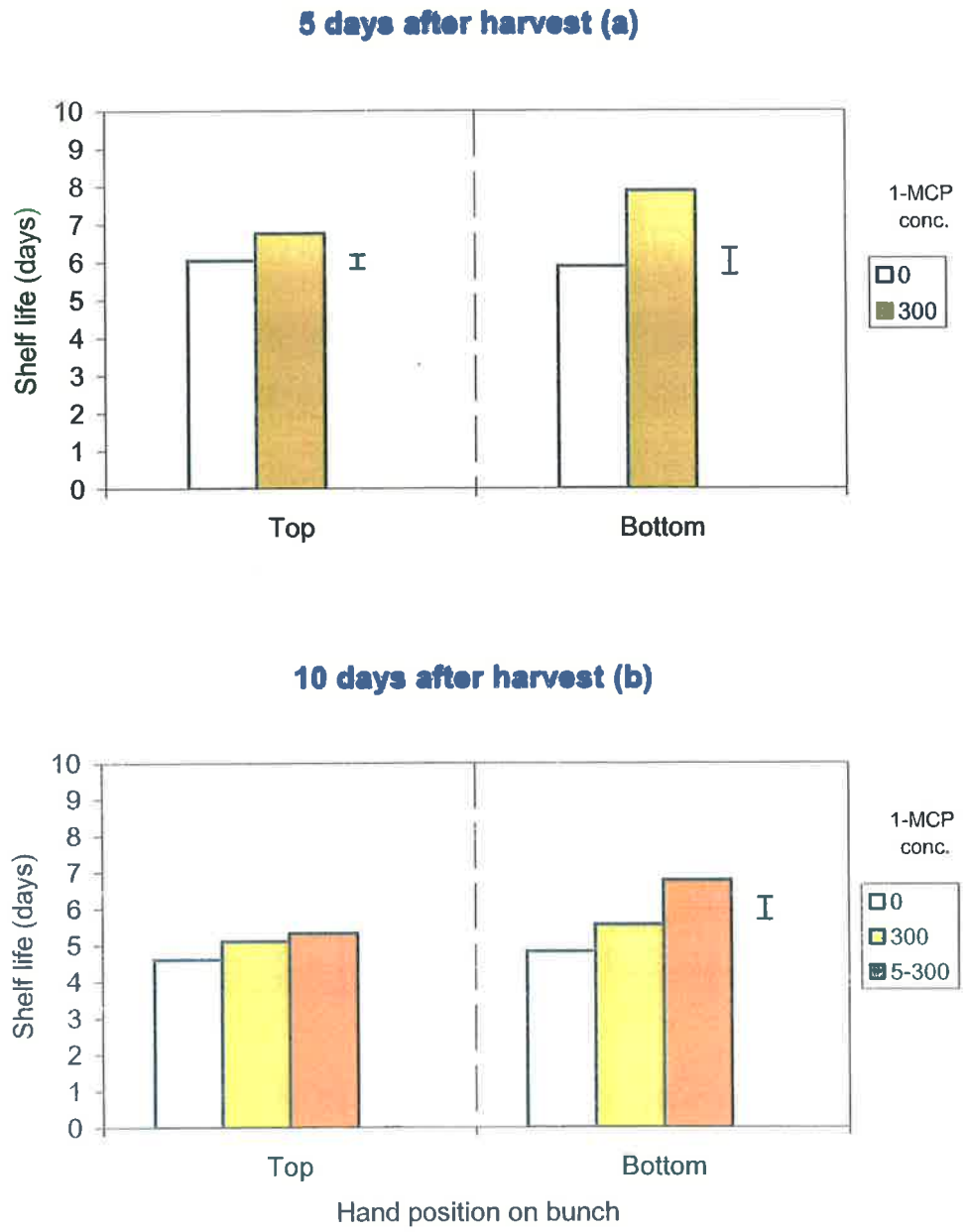


Figure 4.32: Effect of time from harvest and timing of 1-MCP application on shelf life of Cavendish bananas in fruit from the top and bottom of the bunch ripened at 22 °C, 5 days after harvest (a) and 10 days after harvest (b). Vertical bars represent LSD values at the 5% level (n=18). Absence of a LSD bar indicates no significant difference between control and 1-MCP treatment.

4.3.6.2 Firmness

Firmness of control fruit from the bottom of the bunch was slightly higher than from the top of the bunch regardless of the time from harvest to treatment (Figure 4.33 a). Firmness of 1-MCP treated fruit increased significantly in fruit from the top and bottom of the bunch in when fruit were treated at 5 or 10 days after harvest compared to the control.

Treatment of fruit 10 days after harvest with 1-MCP at 300 nL L^{-1} or with 5 nL L^{-1} in pre- and at 300 nL L^{-1} in early-climacteric stage increased fruit firmness significantly in fruit from the top and bottom of the bunch compared to the control (Figure 4.33 b).

4.3.6.3 Discolouration

Discolouration of peel of bananas that were treated with 1-MCP at the pre- and early-climacteric stages of ripening and also control fruit was too low to record a score. Hence, no data are presented for discolouration.

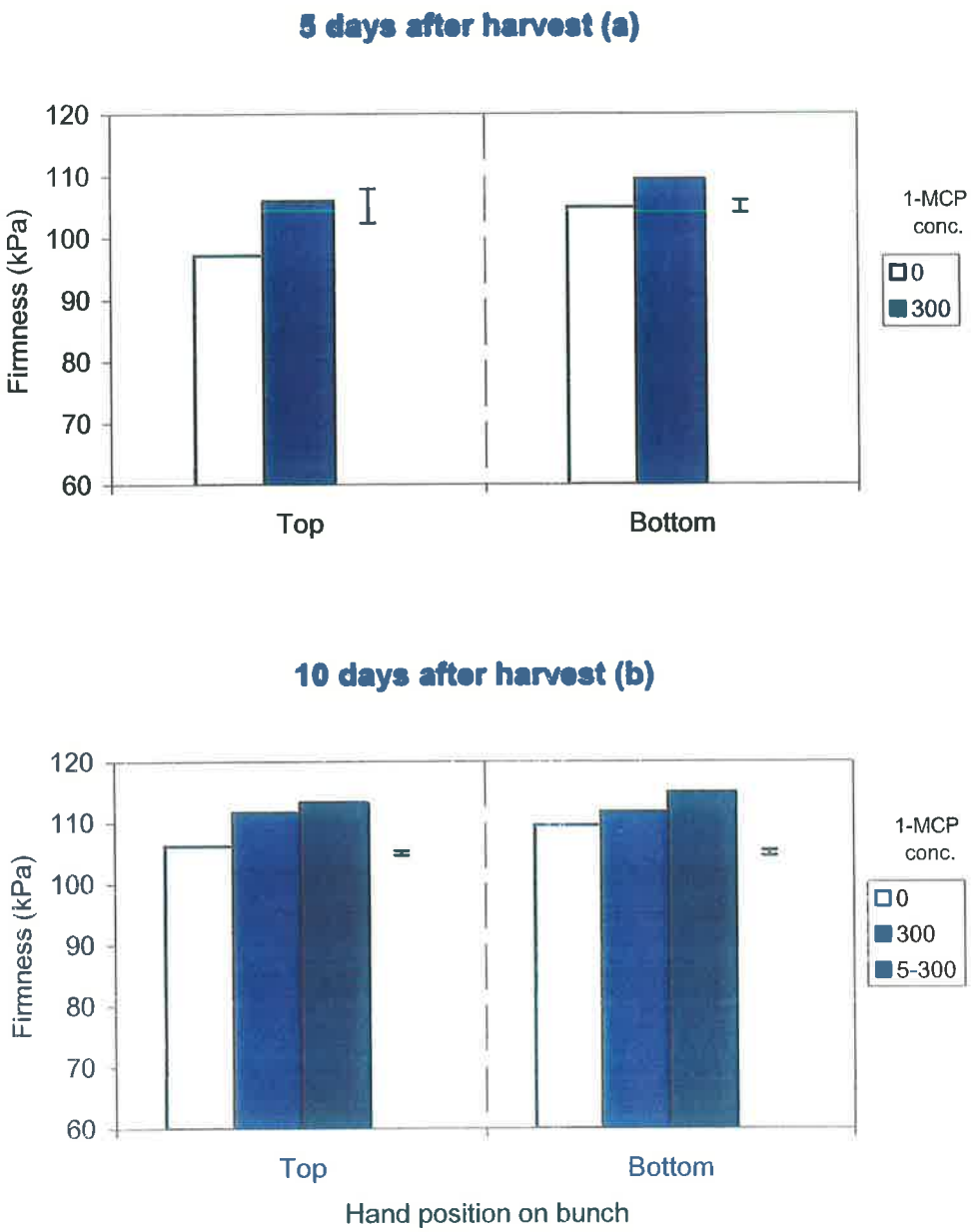


Figure 4.33: Effect of time from harvest and timing of 1-MCP application on pulp firmness of Cavendish bananas in fruit from the top and bottom of the bunch ripened at 22 °C, 5 days after harvest (a) and 10 days after harvest (b). Vertical bars represent LSD values at the 5% level (n=18).

4.3.6.4 Weight loss

Weight loss of control fruit was slightly higher in fruit that were treated at 5 days rather than 10 days after harvest. Weight loss was decreased significantly in 1-MCP treated fruit from the top of the bunch (34%) compared to the control, but not in fruit from the bottom of the bunch when fruit were treated 5 days after harvest (Figure 4.34 a).

Weight loss of fruit from the top of the bunch decreased significantly when fruit were treated with 1-MCP at 300 nL L^{-1}. It was also decreased slightly more when fruit were exposed to 1-MCP at 5 nL L^{-1} in the pre-climacteric stage and than at 300 nL L^{-1} in the early-climacteric stage when fruit were treated 10 days after harvest compared to the control (Figure 4.34 b). Similarly in fruit from the bottom of the bunch, weight loss decreased significantly and was slightly lower in 1-MCP treated fruit, 25% and 32 % respectively, when fruit were exposed to 1-MCP at 5 nL L^{-1} in the pre- and at 300 nL L^{-1} in the early-climacteric stage, at 10 days after harvest compared to the control (Figure 4.34 b).

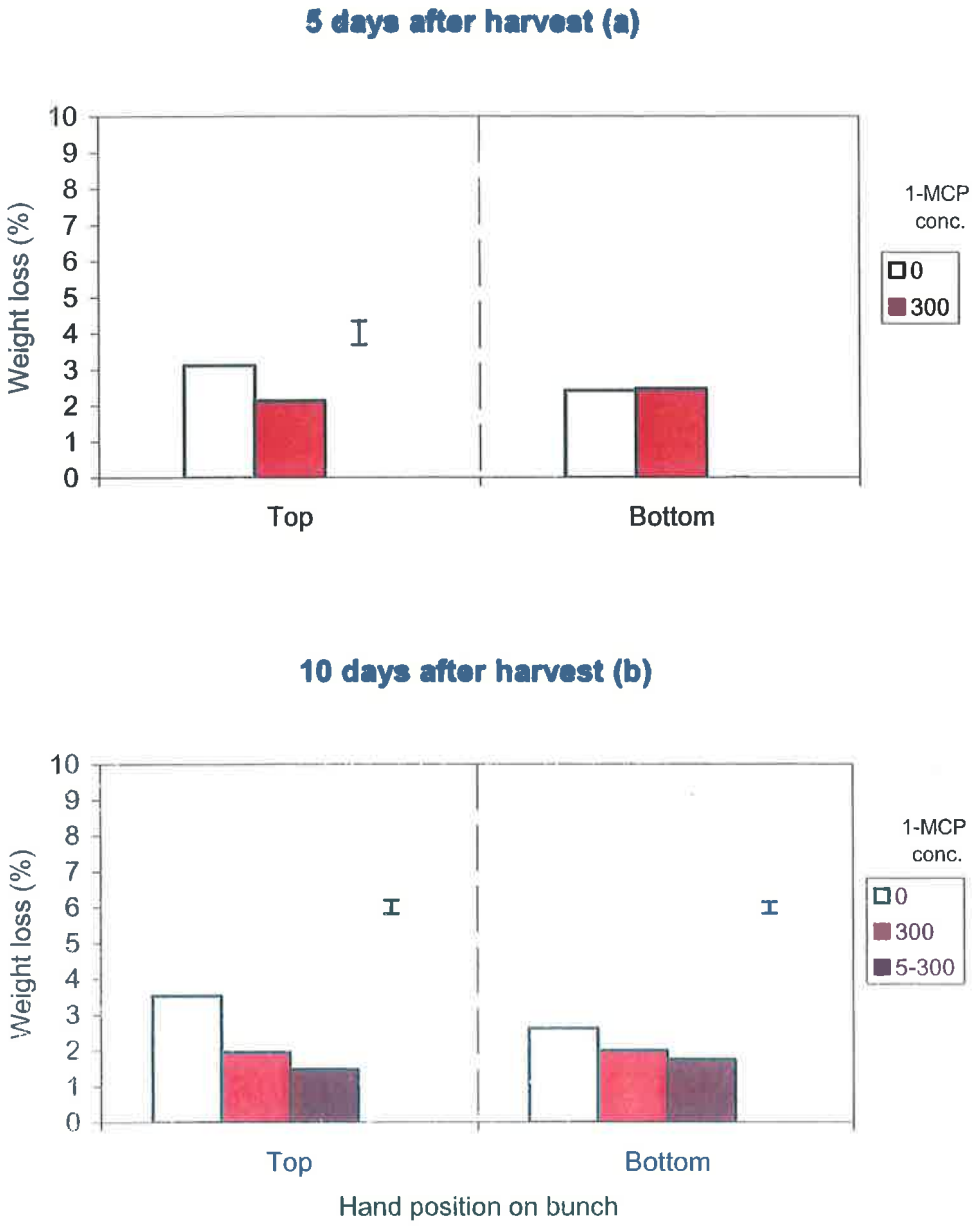


Figure 4.34: Effect of time from harvest and timing of 1-MCP application on weight loss (%) of whole fruit of Cavendish bananas in fruit from the top and bottom of the bunch ripened at 22 °C, 5 days after harvest (a) and 10 days after harvest (b). Vertical bars represent LSD values at the 5% level (n=18). Absence of a LSD bar indicates no significant difference between control and 1-MCP treatment.

4.3.6.5 Total soluble solids

Total soluble solids of control fruit were slightly higher in fruit from the bottom than the top of the bunch regardless of the time from harvest to the treatment. 1-MCP treatment generally increased TSS significantly in fruit from the top and decreased significantly in fruit from the bottom of the bunch regardless of the time from harvest to the treatment (Figure 4.35).

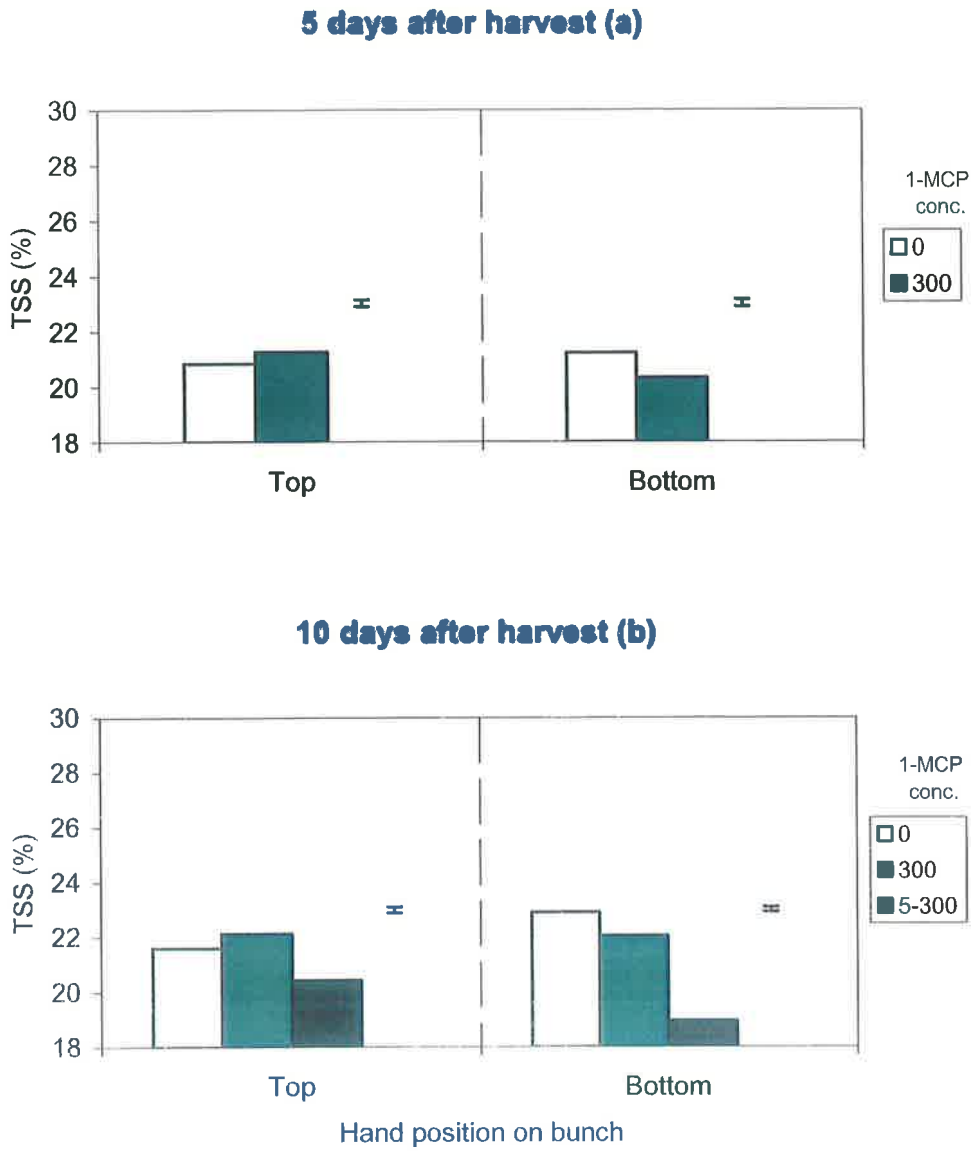


Figure 4.35: Effect of time from harvest and timing of 1-MCP application on total soluble solids (TSS %) of Cavendish bananas in fruit from the top and bottom of the bunch ripened at 22 °C, 5 days after harvest (a) and 10 days after harvest (b). Vertical bars represent LSD values at the 5% level (n=18).

4.4 Discussion

The research detailed in this chapter has confirmed previous research that less than 100 μL L^{-1} of ethylene was sufficient to induce ripening (Burg and Burg, 1965, Inaba and Nakamura, 1986, Liu, 1976a, Liu, 1976b). The time × concentration response to applied ethylene is the basis of recommendations for the commercial ripening of bananas. However, the response of fruit to 1-MCP treatment was highly dependent on not only the exogenous ethylene concentration and duration of application; but also on the timing of 1-MCP treatment and the period that fruit were stored prior to ripening initiation of fruit by ethylene. The results were also consistent with results described in Chapter 3 that indicated time of year at harvest influences the response of bananas to 1-MCP treatment. These effects are more than likely due to differences in the timing of preharvest factors and subsequent alteration in the sensitivity of banana fruit to applied ethylene during the year and their interaction with the timing of 1-MCP application.

The highest increase in the shelf life of 1-MCP treated fruit was obtained when ethylene was applied at 100 μL L^{-1} for the first and 2 μL L^{-1} for the second day compared with other ethylene concentrations (20, 50 or 100 μL L^{-1}). This revealed that although ethylene at an optimum concentration is required for initiation of ripening in bananas, application of ethylene at a lower concentration on the second day is adequate for progression of the ripening process. Consequently, 1-MCP can be more effective in extending the shelf life

than when ethylene was applied at higher concentration (100 nL L^{-1}) for two days. This is more than likely because the time to ripen for climacteric fruits including bananas increases linearly with a logarithmic decrease in ethylene concentration (Wills et al., 2001).

1-MCP did not affect the shelf life, where fruit ripening was initiated with ethylene at the highest concentration (100 µL L^{-1}) in the July (winter) harvest or when fruit were exposed to the ethylene for a longer period of time (50 h) in the February (summer) harvest. This may be due to greater fruit age in winter because of the long bunch emergence to harvest interval and as a result a shorter shelf life (described in Chapter 3). Thus, the amount of ethylene required for initiating ripening of bananas in winter is lower than that required in summer, since sensitivity to ethylene increases with increasing physiological age (Liu, 1976b). It is more than likely that initiation of ripening in bananas following exogenous ethylene application is quicker in the winter harvested fruit as there is more chance of exposure to stress conditions that induce ethylene production before harvest. It seems however, that fruit should be exposed to exogenous ethylene for a longer period of time in winter to ripen properly after application of 1-MCP, because of the negative feedback mechanism of exogenous ethylene in immature harvested bananas in winter (Chapter 3). Similarly, this may be the possible reason for higher discolouration in August than February-harvested fruit when fruit were treated with ethylene for different durations prior to 1-MCP exposure.

Simultaneous application of ethylene and 1-MCP gave a greater increase in shelf life and improved fruit quality than application of 1-MCP to the partially ripened bananas after 2 days ethylene treatment. This may be a result of either using 1-MCP at low concentration at an earlier stage of banana ripening during the second day of treatment and coincidently with ethylene or increasing the 1-MCP treatment exposure duration from 1 to 2 days or both. It has been reported that 1-MCP competes with ethylene for receptors when both are applied simultaneously (Sisler and Serek, 2001). Because 1-MCP has relatively greater affinity than ethylene for ethylene-binding sites (Jiang et al., 1999b), it was hypothesised that application of 1-MCP at low concentration and simultaneously with ethylene will reduce the speed of fruit ripening. The results obtained support the conclusions of Jiang et al. (1999b) who stated that 1-MCP should be applied in the earliest phase of banana ripening initiation to give an extension to ripening. However, this was to some extent in contrast to the findings of Bagnato et al. (2003) who reported that exposure periods from 24 to 72 h did not affect the efficacy of 1-MCP in quality and shelf life of bananas. Application of 1-MCP for a longer duration than 24 h had a significant effect on the assessments. This revealed the importance of timing of 1-MCP application and also continued application of 1-MCP at optimum concentration.

The results also demonstrated that timing of 1-MCP application influences the inhibitory effect of 1-MCP in extending the fruit shelf life. In

addition, the current study supported the findings of Macnish (2000b) who stated that there is considerable potential to manipulate banana ripening by varying the concentration and duration of 1-MCP treatment and altering the relative timing of 1-MCP application. Thus, 1-MCP must be applied at an optimum concentration, duration and time to provide maximum benefit.

Similarly, applications of 1-MCP during both pre- and early-climacteric stages increased fruit shelf life and firmness to a greater extent than if it was applied to ethylene-initiated partially ripened bananas during the early-climacteric stage. Reasons for the greater efficacy of multiple application of 1-MCP include an increase in the number of available binding sites such that 1-MCP can easily bind to the ethylene receptors; increased ability to compete for ethylene binding sites due to greater availability of 1-MCP; or the occupation of ethylene-binding sites by 1-MCP (when applied pre-climacterically) preventing ethylene-binding during ethylene treatment. These may consequently delay the onset of the production of endogenous ethylene (Golding et al., 1998) or decrease the ability of exogenous ethylene applied to promote ripening (Sisler and Serek, 1997) or both. It has also been reported that 1-MCP remains bound to the receptors for longer periods than exogenous ethylene (Sisler and Serek, 1997). The ability of 1-MCP to extend shelf life in the absence of exogenous ethylene implies that it also has the potential to prevent the action of endogenous ethylene, as well as the effects of exogenous ethylene in banana fruit ripening (Macnish et al., 2000a). This was in accord

with a previous report (Serek et al., 1995) that stated 1-MCP should ideally be applied prior to any possible ethylene exposure to prevent some of the damage resulting from exogenous ethylene.

Interestingly, higher concentrations of 1-MCP in multiple applications were required in fruit from the top of the bunch to give a significant increase in fruit shelf life and firmness. This was in accord with the previous results in Chapter 3 that indicated the more mature fruit from the top of the bunch are usually more sensitive to exogenous ethylene.

Time from harvest to 1-MCP treatment had a significant effect on 1-MCP efficacy, as bananas responded differently to reapplication of 1-MCP (5 nL L^{-1} in pre-climacteric and 300 nL L^{-1} in early-climacteric stage) at different intervals from harvest. Reapplication of 1-MCP could delay further the ripening of partially ripened fruit more than when 1-MCP was only applied after 2 days of ethylene treatment. This once again demonstrated the importance of timing of 1-MCP application when fruit are treated with 1-MCP in the pre-climacteric stage. Pre-climacteric application of 1-MCP at 5 nL L^{-1} delayed the ripening and increased the shelf life and firmness of bananas successfully when applied to the fruit 10 days after harvest, but it suppressed the ripening process and the fruit did not ripen when applied to the fruit 5 days after harvest. The reason for this difference can be related to the inhibitory effect of 1-MCP in preventing endogenous ethylene production. As the time from harvest increases, the amount of endogenous ethylene production

increases as a result of autocatalytic production of ethylene in climacteric fruits (Seymour, 1993). When 1-MCP is applied in the pre-climacteric stage to the fruit within a short time frame after harvest, it may block most of the available sites completely, before endogenous / exogenous ethylene is able to bind to them. The results also support findings of Mattheis et al. (2001) cited in Calvo (2002) who stated that the duration between harvest and application of 1-MCP is a critical determinant of 1-MCP response.

Pre-climacteric application of 1-MCP at high concentrations (30, 300 or 10000 nL L^{-1}) blocked the ethylene action for a long time in bananas, as even after 2 weeks storage the fruit did not respond to ethylene treatment and remained green. Failure of the fruit to ripen can be related to lack of responsiveness, as 1-MCP is bound to the ethylene receptors fully, or by limited synthesis of new ethylene receptor binding sites (Jiang et al., 1999b). Ripening of fruit was undesirable and would be unacceptable to industry. This confirms the findings of Macnish et al. (2000b) who noted that 1-MCP has the ability to extend premature ripening periods of green bananas before ethylene initiation of ripening during transport.

4.5 Conclusion

The results in this chapter suggest that exogenous ethylene used for initiation of banana ripening should be applied for a shorter duration in the summer harvest. In winter-harvested fruit, longer exposure duration within the period 30 to 50 h and also lower concentration of ethylene is required prior to

1-MCP treatment to maximise the benefit of 1-MCP. The results also demonstrated that seasonal differences and fruit position on the bunch influence the response of bananas to exogenous ethylene and 1-MCP and consequently alter the efficacy of 1-MCP on shelf life and quality of bananas. In addition both simultaneous application of 1-MCP and ethylene and reapplication of 1-MCP greatly extend the shelf life of partially ripened bananas, and time from harvest to 1-MCP exposure affected the efficacy of 1-MCP.

Chapter 5

Effect of temperature treatments on responses of bananas to 1-MCP

5.1 Introduction

Generally, bananas from north Queensland that are harvested in winter, show higher levels of peel discolouration compared to those harvested in other months of year (as described in Chapter 3), particularly when ripened at lower temperatures (14 and 16 °C), compared with those ripened at higher temperatures (18 and 20 °C) (Bagnato et al., 2002). Temperature therefore, influences the quality of banana fruit not only before harvest but also after harvest, during storage and after ethylene gassing, until the fruit ripen.

Previous studies (Golding et al., 1998; Harris et al., 2000) report that 1-MCP had a significant effect on banana appearance, as 1-MCP-treated fruit showed an unacceptable uneven skin colouration when ripened. Further study (Bagnato et al., 2003) found that application of 1-MCP to the ethylene treated fruit at a high level (30 $\mu L\ L^{-1}$) caused an unacceptable peel colour, but not at lower levels (3 or 300 $nL\ L^{-1}$). Unexpected ripening of banana peel occurred

even following 1-MCP application at a low level (300 nL L^{-1}) to ethylene treated fruit which were harvested in winter (August 2004) as described in Chapter 4. It was therefore hypothesised that application of 1-MCP to the chilling injured fruit may prevent proper degreening as a result of reduction in response to exogenous ethylene in chilled fruit.

Another area that needs to be clarified is the storage temperature used after ethylene and 1-MCP treatments. To understand whether 1-MCP will be commercially practical for bananas, it is also important to determine how it affects the ripening process at different storage temperatures. Previous studies suggested as ideal a range of banana ripening temperatures between 14 to 24 °C including 17 °C in the summer and 19 °C in the winter harvest in Australia (Rippon and Trochoulias, 1976), 16 to 18 °C (Turner, 1997), 18 to 20 °C throughout the year (Bagnato et al., 2002), 14 to 21 °C (Palmer, 1971) or 14 to 24 °C (Peacock, 1980). However, research with 1-MCP has investigated the effect of 1-MCP in preventing or delaying ethylene-induced ripening in bananas at a single temperature (Bagnato et al., 2003; Golding et al., 1999; Jiang et al., 1999a; Jiang et al., 1999b; Jiang et al., 2002; Pelayo et al., 2003). Further studies by Macnish et al. (2000) showed that the response of green bananas to 1-MCP treatment at different temperatures (2.5, 15 or 20 °C) was different when they ripen at 20 °C. Thus, little work has been done on the effect of 1-MCP treatment on ripening, shelf life and quality of partially ripened bananas at different storage temperatures.

The aims of work presented in this Chapter therefore were to examine the effect of ethylene and 1-MCP treatment to stored bananas at chilling temperatures and to determine changes in shelf life and quality in response to 1-MCP-treated bananas ripened in different temperature ranges.

5.2 Materials and methods

5.2.1 Plant material and procedure

Mature and green Cavendish banana (cv. Williams) fruits were transported and prepared as outlined in Sections 2.1 and 2.2. In both experiments eighteen bananas were allocated to each treatment. Six fruits from each bunch were placed into a 10 L plastic container as one replicate. The fruit were then stored at determined temperatures (as detailed in Section 5.2.2) and banana ripening was initiated using a determined volume of ethylene gas injected into the containers, followed by 1-MCP application. All containers were ventilated for 20 minutes each day. 1-MCP was prepared, introduced to the treatments and measured as previously described in Sections 2.3, 2.4 and 3.2.2.

5.2.2 Experimental procedure

5.2.2.1 Effect of pre-ripening chilling temperatures on 1-MCP efficacy

These experiments were conducted with two factors: pre-ripening storage temperature and 1-MCP treatment. Bananas were obtained during November and December 2005 (during the warm season without fruit chilling injury

symptoms). Upon arrival, fruit were stored at three different temperatures (5, 10 or 15 °C) for 6 days and were then removed and transported to the laboratory at 22 °C and held for 4 to 5 h until the fruit surface temperature reached the laboratory temperature. After preparation, fruit were randomly placed in plastic containers. Thereafter, bananas were treated with ethylene at 100 μL L^{-1} for two consecutive days and ventilated for 20 minutes each day. After ripening initiation, bananas were exposed to 1-MCP at 0 or 300 nL L^{-1} for 24 h at 22 °C as previously described (Section 2.3.2). Fruit were then removed from the containers and placed in plastic bags slightly open and placed at 22 °C until the fruit ripened.

5.2.2.2 Effect of ripening storage temperatures and 1-MCP exposure

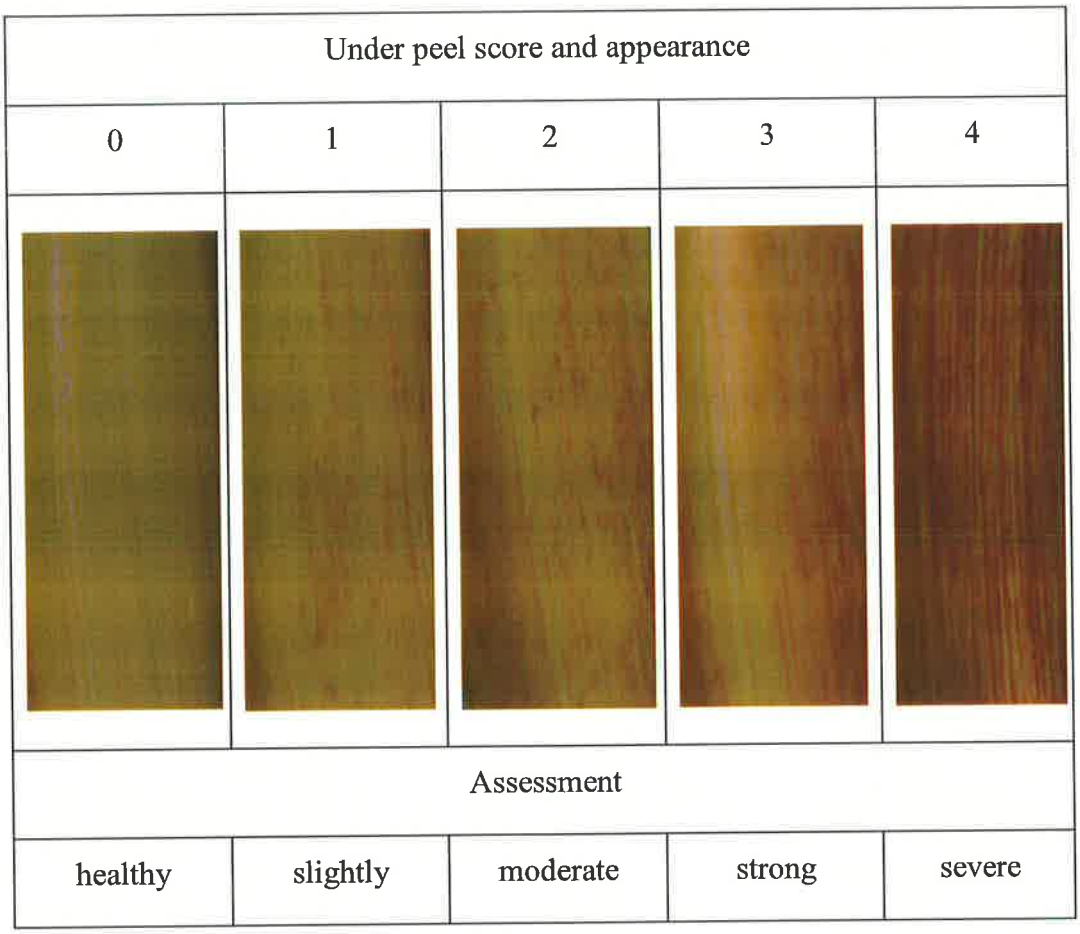
These experiments were conducted with two factors: ripening storage temperature and 1-MCP concentration. Bananas were acquired in December 2004 and May 2005. After preparation, fruit were randomly placed in plastic containers. Fruit were treated with ethylene at 100 μL L^{-1} for two consecutive days and ventilated for 20 minutes each day. After ripening initiation, containers were ventilated for 20 minutes and then bananas were exposed to 1-MCP at 0 or 300 nL L^{-1} for 24 h at 22 °C as previously described (Section 2.3.2). Fruit were then removed from the control and 1-MCP treatment containers and placed in plastic bags slightly open and placed into temperature controlled storage rooms at 16, 19, 22 and 25 °C with approximately 90% RH (provided by South Australian Research and Development Institute, SARDI).

5.2.3 Quality assessments

For the chilling temperature experimentation nine fruit from each treatment were used to assess shelf life and surface discolouration at colour stage 6, nine fruit for under peel chilling injury symptoms and six fruit were used for measuring ethylene production by whole fruit at each sampling time. The under peel chilling injury severities were scored using a discolouration under peel score chart (Figure 5.1). Fruits were classified into five categories, according to the extent to which the under peel surface of the banana was affected such that 0 was healthy; 1, slightly; 2, moderate; 3, strong and 4, severe.

Ethylene production was measured by placing a whole banana fruit in a 1.75 L air-tight plastic container for 3 h at 22 °C after weighing. A 1 mL sample of the head-space gas was withdrawn using a 1 mL syringe and injected into a gas chromatograph (Varian 3400) to measure ethylene concentration as detailed in Section 2.4.1. The rate of ethylene production was expressed as μL kg FW^{-1} h^{-1}. Fruit sampling for measuring ethylene production was done every 48 h in days 0 (before any treatment), 2 (after ethylene treatment), 4, 6, 8 and 10 (after 1-MCP treatment).

Figure 5.1: Scoring index for under peel chilling injury



For ripening storage temperature experimentation, assessments were performed as for previous trials. Nine fruit from each treatment were used to measure external parameters, and the remaining nine fruit allocated to each treatment were used to assess internal quality measurable characteristics at colour stage 6. Methods were described previously in Section 2.5.

5.2.4 Statistical analysis

The chilling temperature experiment was conducted using a split-plot design with time (six levels) in main plot and a factorial arrangement of 1-MCP (two levels) and temperature (three levels) in sub-plots, with three replicates. A randomised block experimental design was used in the ripening storage temperature experiment, using the two-way analysis of variance (ANOVA). Data were analysed with the Genstat 6 program (Release 6.2, 6th edition, 2002, Lawes Agricultural Trust, VSN International Ltd).

A least significant difference test at the 5% level was used to determine significant differences between means. For the ripening storage temperature trial total weight loss data were expressed as a percentage of the weight of fruit before ripening was initiated (ripening stage 1).

5.3 Results

5.3.1 Effect of pre-ripening chilling temperatures on 1-MCP efficacy

The data of experiments in both months were not significantly different and therefore they were combined in the figures.

5.3.1.1 Ethylene production

Although, ethylene production of whole banana fruits held at different pre-ripening storage temperatures in both control and 1-MCP-treated fruit had similar trends to some extent, changes in ethylene production of the fruits in

response to ethylene and 1-MCP treatments at the three temperatures over time was significantly different. Ethylene production of bananas held at 5 °C had not increased after 2 days ethylene exposure, whereas fruit held at 10 or 15 °C had a significant increase in ethylene production by 2 days (Figure 5.2). The amount of ethylene production on day 2 was greater in fruit held at 15 °C than 10 °C (Figure 5.2 b and c). Thereafter, control fruit at each temperature had lower ethylene production than the 1-MCP-treated fruit by day 8. By day 10, ethylene production was greater in control fruit held at 5 °C compared to fruit treated with 1-MCP. 1-MCP treatment had no effect on fruit that had been stored at 10 °C while there was significantly higher ethylene production in 1-MCP-treated fruit of control fruit by day 10 in fruit stored at 15 °C. However, levels of ethylene production were highest for fruit that had been stored at 5 °C followed by 10 and then 15 °C.

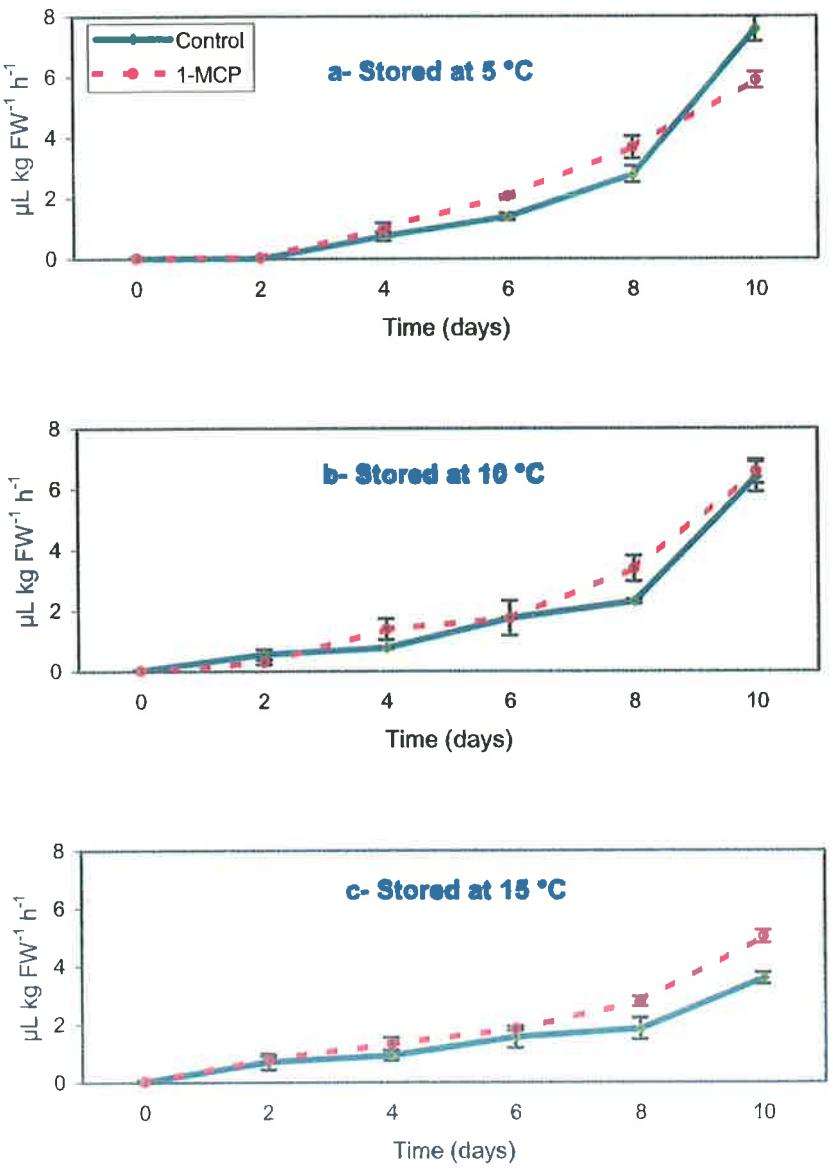


Figure 5.2: Ethylene production of whole banana fruits held at three storage temperatures, 5 (a), 10 (b) and 15 °C (c) for 6 days prior to treatment with ethylene (100 µL L^{-1}) for 48 h (control) or treatment with ethylene (100 µL L^{-1}) for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h and then ripened at 22 °C. Each data point is mean ± S.E. from n=12.

5.3.1.2 Shelf life

Shelf life of ethylene-treated fruit (control) was similar in fruit held at 5 (3.7 days) and 10 °C (3.8 days) respectively, while it was greater in control fruit held at 15 °C (4.6 days) (Figure 5.3). The shelf life of 1-MCP-treated bananas increased significantly compared to the control regardless of storage temperature treatment prior to ethylene and 1-MCP application. However, shelf life of 1-MCP-treated fruit increased more in stored fruit at 10 (135%) or 15 °C (88%) than at 5 °C (25%) compared to the control. No significant difference was found between the shelf life of 1-MCP-treated fruit held at 10 or 15 °C.

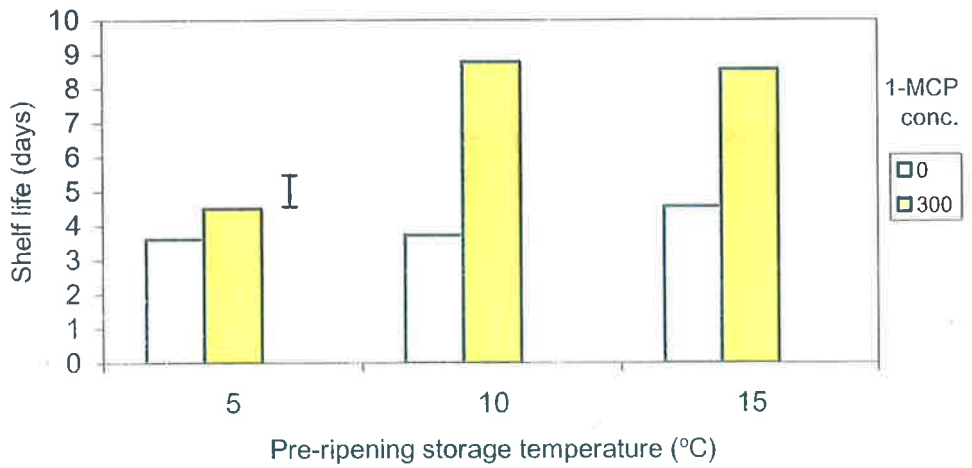


Figure 5.3: Shelf life of bananas held at 5, 10 or 15 °C for 6 days prior to treatment with ethylene (100 μL L^{-1}) for 48 h (control) or treatment with ethylene (100 μL L^{-1}) for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h and then ripened at 22 °C. Vertical bars represent LSD values at the 5% level (n=18).

5.3.1.3 Firmness

Pulp firmness of fruit stored at three pre-ripening temperatures was not significantly different in control fruit, while firmness of 1-MCP-treated fruit decreased significantly compared to the control when fruit were held at 5 (5.5%) or 15 °C (8%), however, 1-MCP had no effect on firmness of fruit stored at 10 °C (Figure 5.4).

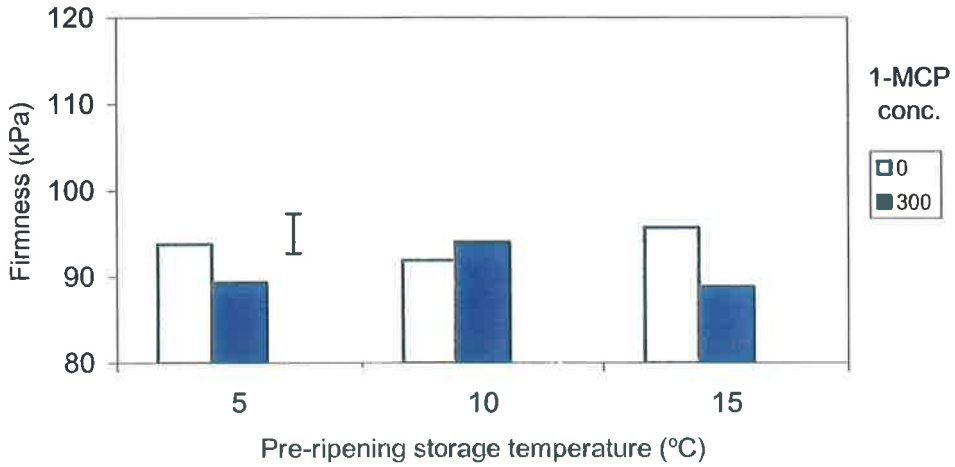


Figure 5.4: Firmness of bananas held at 5, 10 or 15 °C for 6 days prior to treatment with ethylene (100 μL L^{-1}) for 48 h (control) or treatment with ethylene (100 μL L^{-1}) for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h and then ripened at 22 °C. Vertical bars represent LSD values at the 5% level (n=18).

5.3.1.4 Discolouration

Discolouration of banana peel in control and 1-MCP-treated fruit was lower when fruit were stored at 15 °C than 10 or 5 °C (Figure 5.5). 1-MCP application increased discolouration significantly compared to the control

regardless of the storage temperature. However, peel discolouration was significantly increased when the temperature decreased from 15 to 5 °C, in both control and 1-MCP-treated fruit (Figure 5.6).

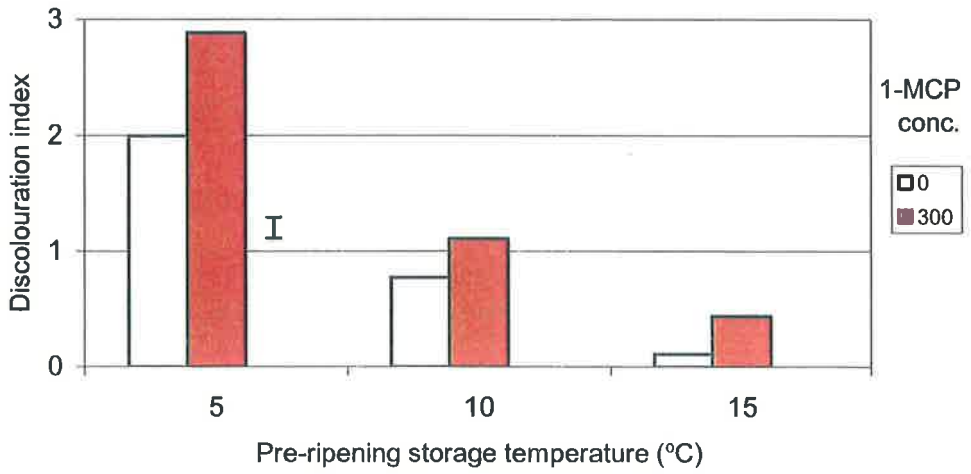


Figure 5.5: Discolouration index of bananas held at 5, 10 or 15 °C for 6 days prior to treatment with ethylene (100 μL L^{-1}) for 48 h (control) or treatment with ethylene (100 μL L^{-1}) for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h and then ripened at 22 °C. Vertical bars represent LSD values at the 5% level (n=18).

a- control

b- 1-MCP treated

Figure 5.6: Ripeness stages and peel appearance of ethylene-treated (control) (a) and ethylene-treated followed by 300 nL L^{-1} 1-MCP exposure (b) in November-harvested fruit after 10 days storage in air at 22 °C. Fruit were held at three storage temperatures in a and b, 5 °C (left), 10 °C (middle) and 15 °C (right) for 6 days prior to the treatments.

5.3.1.5 Under peel chilling injury

Under peel chilling injury symptoms were observed when bananas were stored at 5 °C or 10 °C in both control and 1-MCP-treated bananas (Figure 5.7), but not in fruit stored at 15 °C. 1-MCP had no significant effect on under peel chilling injury across all three temperatures. The greatest under peel chilling injury (3.6) was obtained in fruit pre-stored at 5 °C for 6 days.

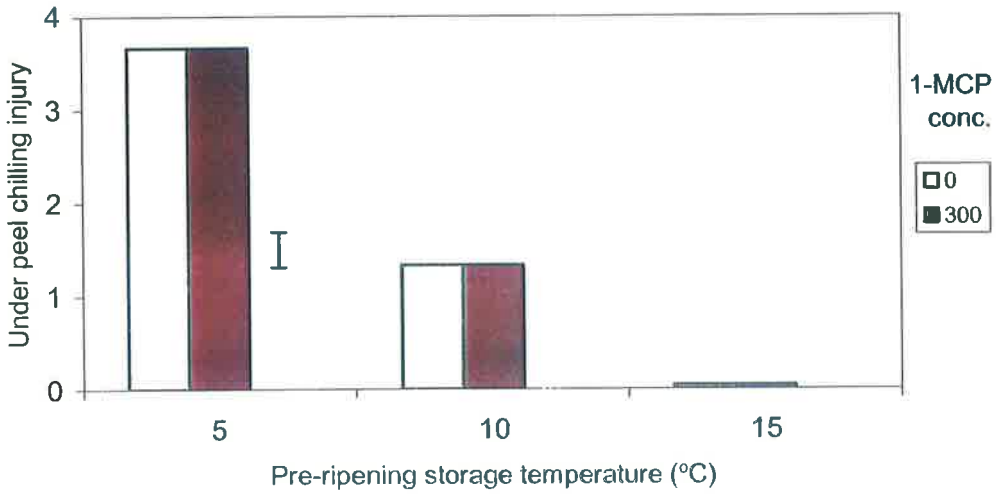


Figure 5.7: Under peel chilling injury of bananas held at 5, 10 or 15 °C for 6 days prior to treatment with ethylene (100 μL L^{-1}) for 48 h (control) or treatment with ethylene (100 μL L^{-1}) for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h and then ripened at 22 °C. Vertical bars represent LSD values at the 5% level (n=18).

5.3.2 Effect of ripening storage temperatures and 1-MCP exposure

The data of experiments in both months were not significantly different and therefore they were combined in the figures.

5.3.2.1 Shelf life

Shelf life of control fruit significantly increased when fruit were ripened at lower storage temperatures (16 or 19 °C) than higher temperatures (22 or 25 °C) (Figure 5.8).

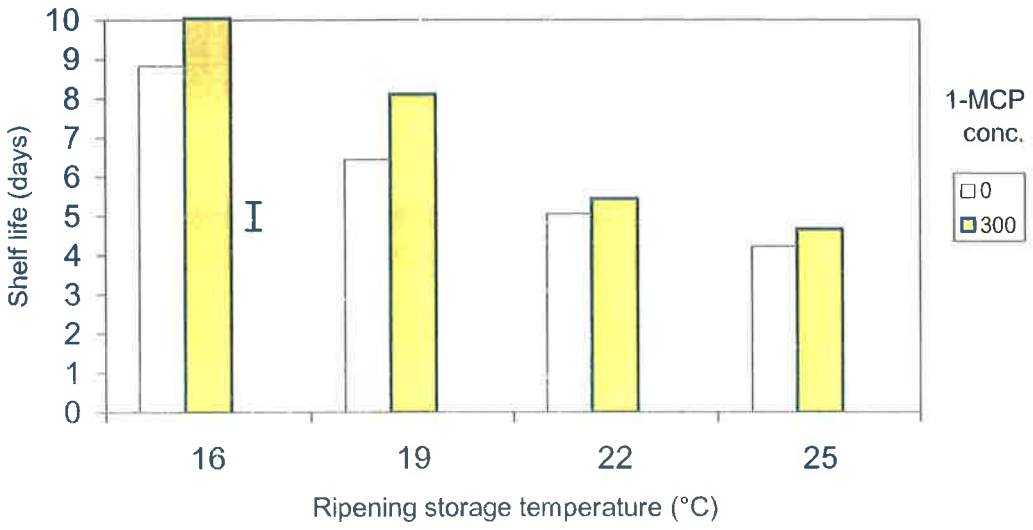


Figure 5.8: Shelf life of bananas ripened at four storage temperatures (16, 19, 22 or 25 °C) following pretreatment with ethylene (100 μL L^{-1}) for 48 h (control) or ethylene treatment (100 μL L^{-1}) for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h at 22 °C in December. Vertical bars represent LSD values at the 5% level (n=18).

Shelf life of control fruit was the highest when fruit were ripened at 16 °C, followed by 19, 22 and 25 °C.

Shelf life of 1-MCP-treated bananas significantly increased compared to the control when fruit were ripened at 16 or 19 °C, but not at 22 or 25 °C. The greatest increase in shelf life of 1-MCP-treated fruit was obtained when fruit were ripened at 19 °C (26%), followed by 16 °C (14%), 25 °C (10.5%) and 22 °C (8%).

5.3.2.2 Firmness

Firmness of control fruit was greater when fruit were ripened at 16 (96.1 kPa) or 19 °C (96.5 kPa) than at 22 (91.5 kPa) or 25 °C (88 kPa) (Figure 5.9). The firmness of 1-MCP-treated bananas increased compared to the control when fruit were ripened at 16 °C, followed by 22 °C, but not at 19 °C and 25 °C.

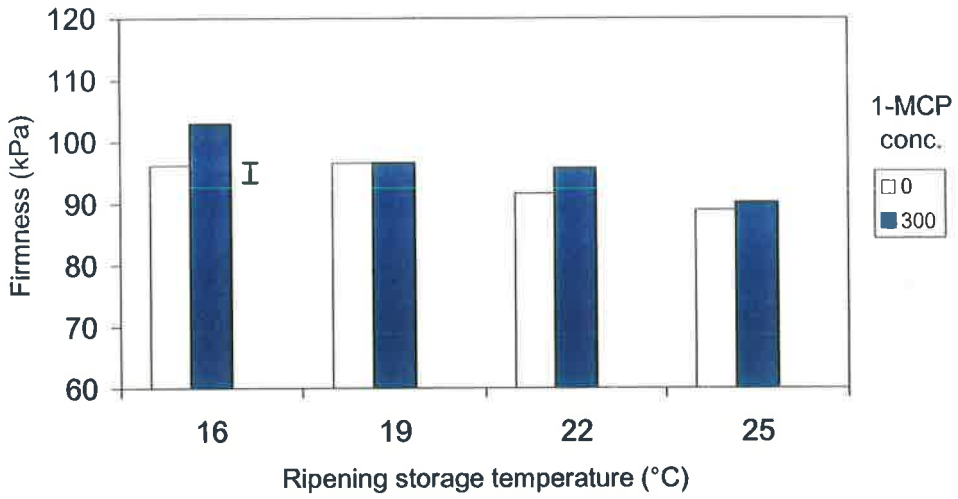


Figure 5.9: Firmness of bananas ripened at four storage temperatures (16, 19, 22 or 25 °C) following pretreatment with ethylene (100 μL L^{-1}) for 48 h (control) or ethylene treatment (100 μL L^{-1}) for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h at 22 °C in December. Vertical bar represents LSD values at the 5% level (n=18).

5.3.2.3 Weight loss

The percentage of weight loss was generally decreased in control fruit when fruit were ripened at lower temperatures within the range of 16 to 25 °C (Figure 5.10). The lowest weight loss in control fruit was obtained at 16 °C (3.5%), followed by 19 °C (4.1%), 22 °C (3.9%) and 25 °C (5.5%).

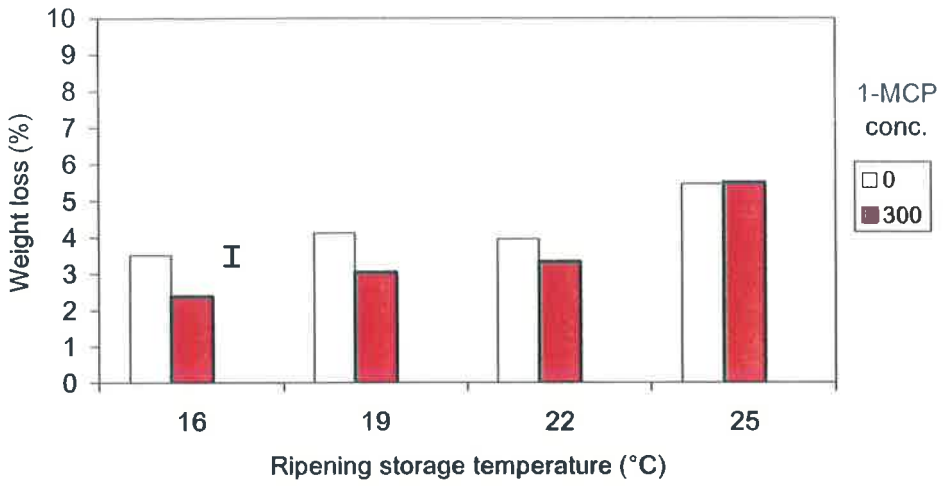


Figure 5.10: Weight loss of bananas ripened at four storage temperatures (16, 19, 22 or 25 °C) following pretreatment with ethylene (100 µL L^{-1}) for 48 h (control) or ethylene treatment (100 µL L^{-1}) for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h at 22 °C in December. Vertical bars represent LSD values at the 5% level (n=18).

1-MCP application significantly declined fruit weight loss compared to the control at all storage temperatures, except 25 °C. The greatest decrease in fruit weight loss was obtained when 1-MCP-treated fruit were ripened at 16 °C (32%), followed by 19 °C (26.2%) and 22 °C (16%) compared to the control.

5.3.2.4 Total soluble solids

Total soluble solids (TSS) were generally higher when fruit were ripened at higher storage temperatures than at lower temperatures (Figure 5.11). 1-MCP increased significantly TSS of fruit ripened at 16 and 25 °C, but not at 19

or 25 °C. However, no similar trend was observed in TSS of control and 1-MCP-treated fruit ripened at different temperatures.

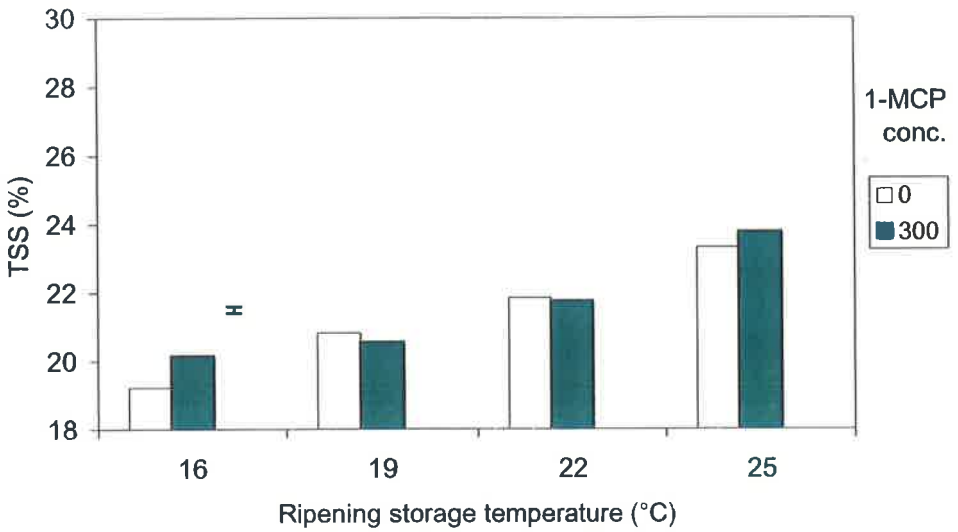


Figure 5.11: Total soluble solids of bananas ripened at four storage temperatures (16, 19, 22 or 25 °C) following pretreatment with ethylene (100 μL L^{-1}) for 48 h (control) or ethylene treatment (100 μL L^{-1}) for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h at 22 °C in December. Vertical bars represent LSD values at the 5% level (n=18).

5.4 Discussion

The results of this chapter demonstrated that both fruit stored at low temperature (5 °C) before ripening and fruit ripened at high temperature (22 or 25 °C) had a significant reduction in fruit shelf life and quality. Responses of bananas to ethylene and 1-MCP treatment in shelf life and peel appearance were similar to some extent when they were stored at chilling temperatures or

when field-chilling was likely to have occurred before harvest, as described in Chapter 3. The response of fruit to 1-MCP treatment was also dependent on storage temperature before ripening or after ripening initiation by ethylene. These effects are more than likely due to slowing down of respiration rate and ethylene production at lower storage temperatures (Wills et al., 1998) and/or becoming less sensitive to ethylene, as effected by chilling temperatures in banana fruit (Jiang et al., 2004).

Similar to exogenous ethylene treatment (as described in Chapter 4), banana ripening can be partially slowed down by lowering temperature during storage or after ethylene gassing. Bananas ripen and deteriorate quickly at ambient temperature. Hence, banana fruit are generally stored after harvest at above 13 °C with the optimum temperature between 13 to 14 °C; otherwise storage life of bananas is limited by development of chilling injury symptoms, commonly darkening of skin during cold storage (Kim and Lee, 1988). In addition, fruit harvested during winter that may be affected by field chilling have been found to have reduced shelf life (Seberry and Harris, 1993). The result was in accord with these previous findings as the peel appearance was undesirable and the shelf life of fruit were significantly decreased when fruit were stored for 6 days before ripening at 5 °C compared to 10 or 15 °C in both control and 1-MCP-treated fruit.

Ethylene application resulted in a rapid peak of ethylene production, which is detrimental for banana ripening (Hulme, 1971), which was delayed

when fruit were held at chilling (5 or 10 °C) compared to optimum (15 °C) temperatures. It is known that endogenous ethylene production is reduced in climacteric fruits at lower temperatures, as well as sensitivity to exogenous ethylene (Knee, 2002). Generally, the lower storage temperature caused more delay in ethylene production with the greatest delay at 5 °C in both control and 1-MCP-treated fruit. However, the greatest ethylene production was obtained in day 10 when fruit were held at 5 °C compared to 10 or 15 °C. This was in accord with previous studies that noted exposure of apple or lemon fruit to temperatures below 10 °C resulted in elevated ethylene production and respiration on returning fruit to higher temperatures (Roger, 1985).

1-MCP treatment significantly increased fruit ethylene production by day 8 in all three temperatures compared to the control. However, at day 10 1-MCP reduced ethylene production in fruit stored at 5 °C or 10 °C, but not at 15 °C. It has been previously suggested that 1-MCP increases the amount of ethylene production of bananas ripened at 20 °C (Pelayo et al., 2003), and the results support these findings. Similar results were also reported by Golding et al., (1999) who stated that 1-MCP may block the normal feedback regulation of ethylene production. In contrast, our results indicated that 1-MCP treatment decreased the over-production of ethylene in chilled fruit. This shows the significance of holding temperature in response of bananas to ethylene and 1-MCP treatment. The magnitude of this effect was dependent on storage temperature.

Fruit that were held at 5 or 10 °C and stressed before ripening at 22 °C were not able to recover from the initial chilling exposure period. This explains the high discolouration index and under peel chilling injury recorded in bananas particularly those stored at 5 °C compared to 10 or 15 °C. Generally, 1-MCP-treated fruit had greater discolouration compared to the control, however, no difference was observed between control and 1-MCP-treated fruit in under peel chilling. Our results were consistent with those of Jiang et al. (2004) who noted that application of 1-MCP prior to cold storage caused more severe chilling injury symptoms in banana peel. They suggested that chilling injury of banana fruit at low temperature is due to reduced ability of tissue to respond to exogenous ethylene and it is reasonable to expect that 1-MCP would increase discolour symptoms given its blocking action. This suggests that exogenous ethylene treatment may inhibit the development of chilling injury in storage in banana fruit to some extent.

The question remaining is why 1-MCP causes uneven skin discolouration as previous authors (Golding et al., 1998; Harris et al., 2000) have reported. This was also the case when 1-MCP was applied to green bananas in the pre-climacteric stage (as described in Chapter 4). Uneven, unacceptable skin discolouration may happen due to nearly complete occupation of ethylene receptors by 1-MCP when 1-MCP is applied at high concentration or for more duration during pre-climacteric stage. This effect is different from the undesirable degreening of peel that generally happens following application of

1-MCP to chilled partially ripened bananas, which is accompanied by light green colour in the peel of ripened fruits.

Shelf life of both control and 1-MCP-treated bananas ripened at 16 °C was greater compared to fruit ripened in higher storage temperatures (19, 22 or 25 °C). This shows that not only ripening temperatures had a significant influence in determining shelf life in ethylene-treated fruit as previously suggested (Bagnato et al., 2002; Rippon and Trochoulias, 1976) but 1-MCP-treated bananas also had greater shelf life than the control. The shelf life and percentage of fruit weight loss were lower in 1-MCP-treated fruit compared with the control at all temperatures except 25 °C. This implies that 1-MCP efficacy was less in higher temperatures probably due to higher respiration and metabolic rates in greater temperatures (Kays, 1991) that accompanies lower shelf life. This suggests that a combination of optimum ripening storage temperature and application of 1-MCP to partially ripened bananas will further extend shelf life.

Although studies by Peacock (1980) suggested a range of 14 to 24 °C had equal effects on ripening, others have indicated there are differences. A ripening temperature of 17 °C for summer-harvested fruit and 19 °C for winter-harvested fruit was recommended by Rippon and Trochoulias (1976). Similar values of 14 to 16 °C for summer-grown fruit and 18 to 20 °C for winter-grown fruit have also been recommended more recently (Bagnato et al., 2002). The

temperature of 16 and 19 °C in partially ripened 1-MCP-treated bananas from this study is consistent with this other research.

The question arises why the results of ripening storage temperature were not in accord with our previous experiments done at 22 °C, that is, 1-MCP efficacy was decreased in the ripening storage temperature experiment in fruit ripened at 22 or 25 °C, in contrast to our previous results that showed the shelf life of 1-MCP-treated fruit increased up to double compared to the control at 22 °C. This difference can be related more than likely to the different flow rate in the laboratory and cool room. The ripening storage temperature experiment was carried out in cool rooms, which had two fans, so higher flow rates may have caused more weight loss from the fruits, as the rise in temperature reduces relative humidity. Relative humidity should be kept at 85 to 90% (Kader et al., 1992), but was reduced to 75 to 80% in higher temperatures (22 or 25 °C). The fruit that were ripened in plastic bags slightly open had 85% relative humidity in the laboratory at 22 °C in all previous experiments. Alternatively, higher flow rates may have increased respiration rate through higher O_2 and / or lower CO_2 concentrations and enhanced speed of ripening.

5.5 Conclusion

The results demonstrated that there is a close interaction between temperature, ethylene production and the ripening and storage characteristics of banana fruit. These results demonstrated that adjusting storage temperature

after 1-MCP treatment could control the speed of ripening of 1-MCP-treated fruit greater than control fruit as it could be significantly retarded by low storage temperature. Pre-ripening storage at chilling temperatures also affected fruit ripening in both ethylene and 1-MCP-treated bananas.

Chapter 6

Impact of 1-MCP treatment on ethylene synthesis

6.1 Introduction

The plant hormone ethylene participates in most stages of plant growth and development including seed germination, fruit ripening, senescence, abscission and various stresses (Abeles, 1985). In climacteric fruits like bananas, ethylene not only induces the ripening process but also regulates its progression (Lelievre et al., 1997). Ethylene production during ripening is highly regulated by two key enzymes, ACC synthase (ACS) and ACC oxidase (ACO), both of which are responsive to exogenous ethylene (Oetiker and Yang, 1995). Exogenous ethylene exerts a negative feedback regulation on ethylene production in immature climacteric fruit such as figs and banana (known as *System 1*), but is autostimulatory in mature climacteric fruit (known as *System 2*) (Lelievre et al., 1997). 1-MCP acts by binding, apparently irreversibly, to the ethylene receptors (Sisler and Serek, 1997) affecting the banana fruit ripening process (Macnish et al., 2000) suggesting that 1-MCP is

potentially an ideal tool to provide a better understanding of ethylene biosynthesis regulation in banana fruit ripening.

The pattern of ethylene production during ripening in banana fruit is different from that in most other climacteric fruits (Mitra, 1997), suggesting that the regulation of ethylene biosynthesis in banana may also be different. In addition, previous reports regarding the role of banana pulp in triggering the ripening of the whole fruit provide conflicting data. Dominguez and Vendrell (1993) suggested that a rise in ACO activity of the pulp before similar activity in the peel was proof that ACO activity of the pulp plays a role in initiating autocatalytic ethylene production during ripening. In contrast, a further study reported that changes in ACO activity during ripening did not support a role for the pulp as a trigger for the ripening of the whole fruit (Moya-Leon and John, 1994).

Previous studies have shown that a 1-MCP concentration of 300 nL L^{-1} (Bagnato et al., 2003) or 1000 nL L^{-1} (Jiang et al., 1999) could be effective in delaying the ripening of bananas. However, there have been limited studies of the effect of 1-MCP on the ethylene biosynthesis enzymes of bananas. While ethylene production and the enzymes responsible for its synthesis have been measured in recent studies with bananas (Pelayo et al., 2003), they have only been examined in bananas treated with 1-MCP in the pre-climacteric stage (when green) and not in partially ripened bananas (Pathak et al., 2003). There also has been no assessment and comparison between banana pulp and peel in

terms of the effect of 1-MCP on the ripening process and ethylene enzyme activities.

The purpose of this study was to evaluate the effect of 1-MCP on ripening-associated changes of bananas after application of ethylene in terms of ethylene production in the whole fruit, and also ethylene biosynthesis enzyme activities both in pulp and peel.

6.2 Materials and methods

6.2.1 Tissue material and experimental procedure

Mature green Cavendish bananas (cv. Williams) were obtained during April and May 2005. Fruits were harvested, transported and prepared as outlined in Sections 2.1 and 2.2. Eighty-four bananas were allocated to each of two treatments, ethylene only and ethylene followed by 1-MCP. Twelve fruits were placed into two 10 L plastic containers (six fingers in each container) for each sampling day. After the first sampling, fruit were treated with 100 $\mu L\ L^{-1}$ ethylene for two consecutive days at 22 °C. Containers were ventilated for 20 minutes each day. Whole banana fruits were then sealed in the same plastic containers and exposed to 1-MCP (0 or 300 $nL\ L^{-1}$) for 24 h at 22 °C. 1-MCP was prepared, applied and measured as previously described in Sections 2.3, 2.4 and 3.2.2. Tissue was harvested before any treatment (day 1), during and after ethylene treatment (days 2 and 3) or after 1-MCP treatment (days 4, 6, 8 and 10). Pulp and peel tissues were separated from the middle section of fruit

(as described in Section 2.5.5), cut into slices, frozen in liquid nitrogen and stored at –80 °C until used.

6.2.2 Quality assessments

Six fruit from each treatment were used to measure ethylene production, and six fruit were used to assess ACS and ACO activities on each sampling day.

6.2.2.1 Ethylene measurement in whole banana fruit

Ethylene production was measured as described in Section 5.2.3 using a gas chromatograph (Varian 3400). The rate of ethylene production was expressed as μL kg FW^{-1} h^{-1}.

6.2.2.2 *In vivo* ACC synthase (ACS) activity in pulp and peel

The ACS activity was measured *in vivo* according to the method of Kato et al. (2000) with some minor changes. Frozen discs of pulp or fragments of banana peel tissue (0.2 g) were homogenised with a pestle and mortar in 3 mL extraction buffer consisting of 0.1 M 4-(2-hydroxethyl)-1-piperazinepropanesulfonic acid (EPPS)-KOH buffer, pH 8.5, 10 mM 2-mercaptoethanol, and 10 μM pyridoxal phosphate at 2 °C (Kato et al., 2000). The homogenate was centrifuged (Eppendorf 5810 R, Eppendorf AG, 22331 Hamburg, Germany) at 4000 × g for 10 minute at 4 °C.

ACS activity was assayed in 12 × 75-mm test tubes in a reaction mixture that consisted of 50 mM EPPS-KOH buffer, pH 8.5, 50 μM S-(5′-adenosyl)-L-methionine chloride (SAM) and the crude extract enzyme in a total volume of 1 mL (Kato et al., 2000). The test tube containing the reaction mixture was sealed with a rubber stopper no. 17 (9.5 mm suba-seal) and incubated for 30 minutes at 30 °C and then the reaction was stopped by adding 0.1 mL of 40 mM $HgCl_2$. Approximately 0.1 mL of a cold mixture of 5% NaOCl and saturated NaOH (2:1, v/v) was injected through the stopper by means of a 1-mL syringe fitted with a 25-gauge needle. The reaction mixture was than agitated on a Vortex mixer for a period of 5 sec before placement on ice. ACC formed in the reaction was assayed by the method of Lizada and Yang (1979) by ethylene measurement using a 1 mL sample of the head-space gas which was withdrawn and injected into a gas chromatograph (Varian 3400) as previously detailed (Section 2.4.1). ACS activity was expressed as mmol ACC formed kg FW^{-1} h^{-1}.

6.2.2.3 *In vivo* ACC oxidase (ACO) activity in pulp and peel

ACO activities were measured *in vivo* according to Pretel et al. (1995) with some minor changes. ACO activity was determined in a test tube (16 mL) containing 2 g banana pulp or peel in a 10 mL volume that was incubated with 25 mM Hepes-Tris buffer (pH 7.5) containing 0.5 M sorbitol and 1 mM 1-aminocyclopropane-1-carboxylic acid (ACC). After 30 minutes, the test tubes containing the reaction mixture were sealed with rubber stoppers no. 25 (12.5

mm suba-seal) and incubated for 1 h at 30 °C with continuous shaking. Then a 1 mL gas sample of the head-space of the test tube was withdrawn and monitored for its ethylene content by gas chromatography as per Section 4.2.1. ACO oxidase activity was expressed as mmol ethylene produced kg FW^{-1} h^{-1}.

6.2.3 Statistical assessments

The enzymatic experiment was conducted using a split-plot design with time (seven levels) in the main plot and 1-MCP (two levels) in sub-plots, with three replicates. Data were analysed with the Genstat 6 program (Release 6.2, 6th edition, 2002, Lawes Agricultural Trust, VSN International Ltd) using the split-plot design. A least significant difference test (P = 0.05) was used to determine significant differences between means.

6.3 Results

The data of experiments in both months were not significantly different and therefore they were combined in the figures.

6.3.1 Ethylene production during ripening in whole banana fruit

Although ethylene production of control and 1-MCP-treated fruit stored in 10 L containers at 22 °C had similar trends, the ethylene production rate by the control fruit was significantly greater than the ethylene production rate by the 1-MCP-treated fruit at days 4, 8 and 10 (Figure 6.1). However, changes in ethylene production were similar in both control and 1-MCP treated bananas at

days 1, 2, 3 and 6. Two ethylene production peaks were observed at day 4 (one day after ethylene treatment) and day 10 for both control and 1-MCP-treated fruit (although these were lower in 1-MCP-treated fruit).

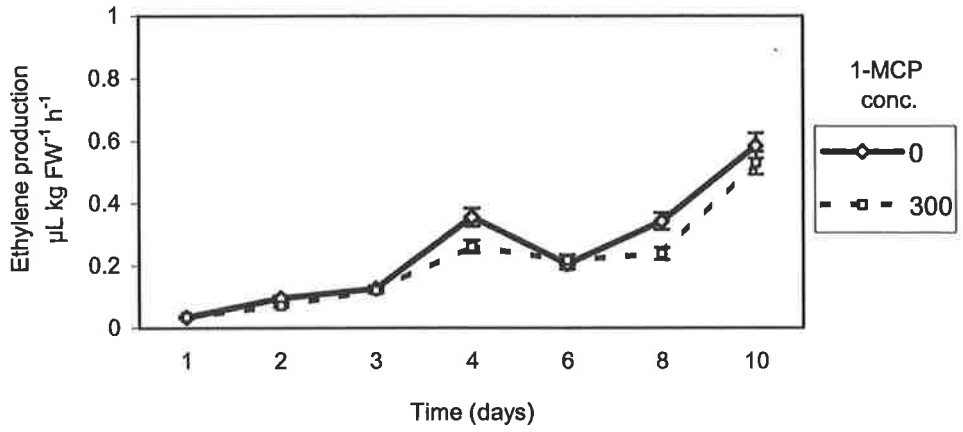


Figure 6.1: Changes in ethylene production of whole banana fruit in fruit treated with 100 µL L^{-1} ethylene for 48 h (control) or treated with 100 µL L^{-1} ethylene for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h and stored at 22 °C. Each data point is mean ± S.E. from n=12.

6.3.2 ACS activity during ripening in pulp and peel

In control fruit, ACS activity of banana pulp increased from the second day of ethylene treatment (day 3) to a peak at day 4 (Figure 6.2 a). ACS activity then declined to day 8 and subsequently increased on day 10. The ACS peak in the pulp was higher in 1-MCP-treated than in control fruit (Figure 6.2). 1-MCP-treated fruit followed a smaller trend except that ACS activity increased at a lower rate between days 2 and 4 but kept increasing until day 6 and then declined.

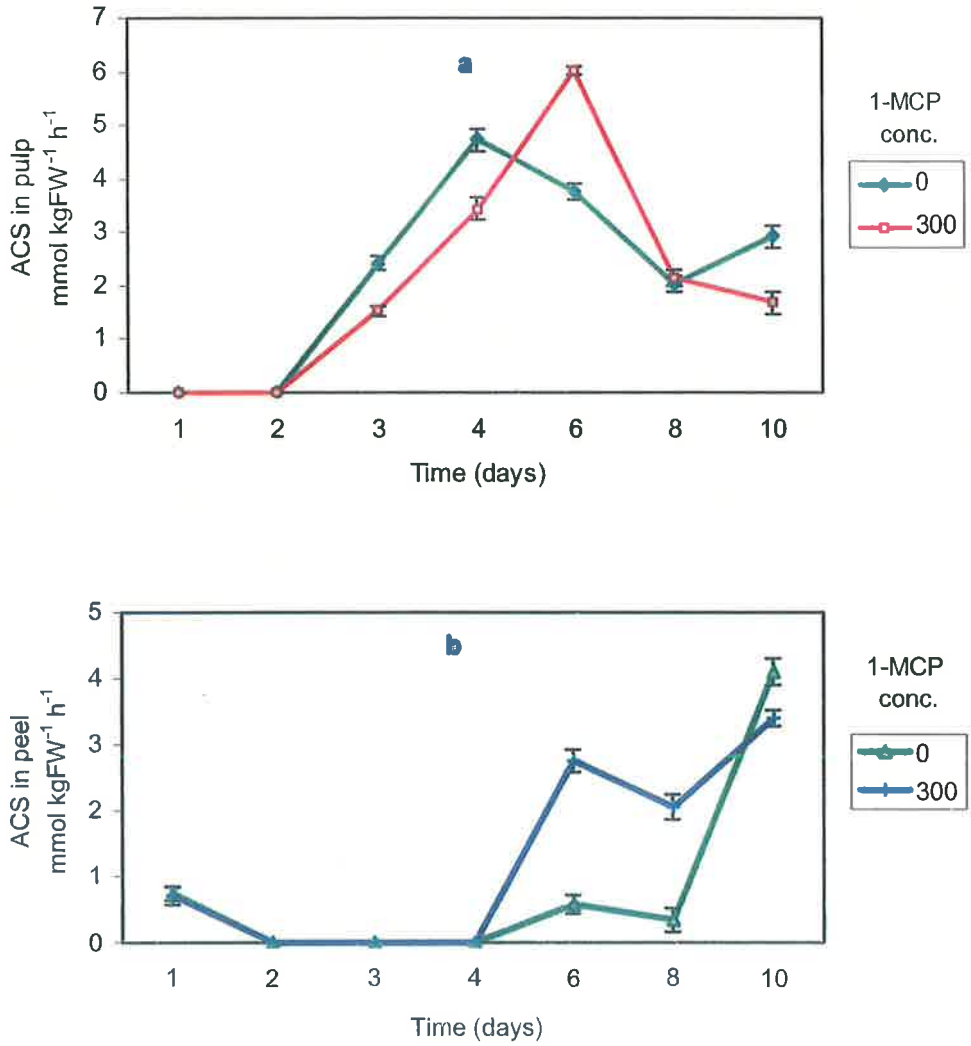


Figure 6.2: Changes in ACS activity of banana pulp (a) and peel (b) in fruit treated with 100 µL L^{-1} ethylene for 48 h (control) or treated with 100 µL L^{-1} ethylene for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h and stored at 22 °C. Each data point is mean ± S.E. from n=12.

Changes in ACS activity in banana peel in both 1-MCP-treated and control fruit was similar up to day 4 (Figure 6.2 b). ACS activity increased much later in the peel with a significant peak from day 8 to day 10 in control

fruit. 1-MCP-treated fruit had a significant increase in ACS activity to day 6 followed by a slight decline to day 8 and then increase to day 10.

6.3.3 ACO activity during ripening in pulp and peel

In control fruit, ACO activity in banana pulp increased during ethylene treatment up to a peak at day 3, however, it declined after ethylene treatment to day 4 and then two more peaks at days 6 and 10 were observed (Figure 6.3 a). 1-MCP-treated bananas had the same trend except that ACO activity did not increase after day 8.

ACO activity in banana peel had the same trend as in pulp except it did not increase after day 8 (Figure 6.3 b). ACO activity in the peel of 1-MCP treated-fruit increased during ethylene treatment up to a peak at day 3, and then steadily declined during and after 1-MCP treatment. The ACO activity was higher in banana peel than pulp, more than 3-fold greater in 1-MCP treated-fruit and approximately 4-fold greater in the control (Figure 6.3). 1-MCP treatment generally decreased the activity of ACO in both banana pulp and peel.

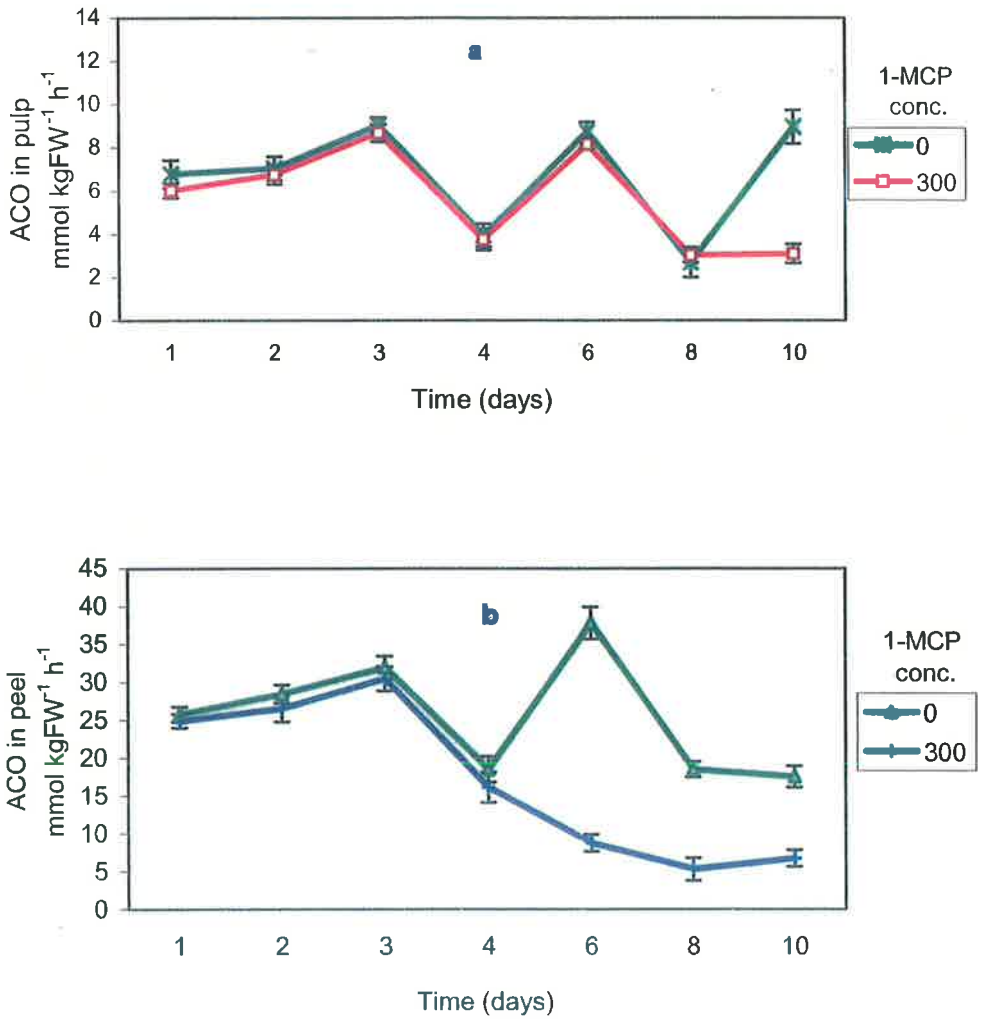


Figure 6.3: Changes in ACO activity of banana pulp (a) and peel (b) in fruit treated with 100 µL L^{-1} ethylene for 48 h (control) or treated with 100 µL L^{-1} ethylene for 48 h followed by 300 nL L^{-1} 1-MCP for 24 h and stored at 22 °C. Each data point is mean ± S.E. from n=12.

6.4 Discussion

The goal of these enzymatic studies was to broaden our knowledge of the physiology of the ripening process of both banana pulp and peel, particularly the inhibitory effect of 1-MCP on these processes. Our results were consistent with the fact that banana peel and pulp show different patterns of ethylene production during ripening (Seymour, 1993), with the pulp tissue reported to be the principal source of ethylene production during ripening (Vendrell and McGlasson, 1971). At the onset of ripening pulp tissues showed higher levels of ACS, and lower levels of ACO activity than peel, as reported previously (Seymour, 1993).

The ethylene evolution pattern in 1-MCP-treated fruits was similar to that of control fruits. A sharp increase in ethylene evolution was observed at day 4 in both control and 1-MCP-treated fruit (the time that 1-MCP was applied to the fruit). This suggests that 1-MCP binds to the ethylene receptors incompletely in partially ripened bananas when applied during the climacteric period and does not suppress endogenous ethylene production, even though the 1-MCP treatment significantly decreased the ethylene production rate.

Application of 1-MCP after two days of ripening initiation, when autocatalytic ethylene production occurs (Golding et al., 1998), was apparently too late to suppress the ripening process, in agreement with the findings of Jiang et al. (1999) who noted that an extension of ripening is possible only when 1-MCP is applied within 24 h of ethylene treatment. This may be due to

the reduced ability of 1-MCP to compete for the receptors (Sisler and Serek, 1997) or there may be fewer available ethylene binding sites.

Assays of ACO and ACS activities in our study of ethylene-treated fruit showed that the peel had higher levels of ACO activity than the pulp (expressed on a g FW basis) and to a greater extent than found previously (Dominguez and Vendrell, 1993; Moya-Leon and John, 1994). However, ACS activity in pulp increased to a greater extent than in the peel and its onset was earlier in the pulp, suggesting that ethylene production in banana fruit pulp triggers banana ripening, which supports the findings of Dominguez and Vendrell (1994) but the findings of Moya-Leon and John (1994).

ACO activity and ethylene production were inhibited by 1-MCP treatment whereas ACS activity increased following 1-MCP application. Because ethylene normally induces the ethylene climacteric in bananas by increasing ACO activity (Turner, 1997), the inhibition implies that 1-MCP also decreased the stimulatory effect of exogenous ethylene on ripening. However, the peel and pulp of Cavendish bananas behave differently in response to ethylene as indicated previously by Dominguez and Vendrell (1994) who stated that the peel is incapable of autocatalytic ethylene production (known as *System* 2) and ripening of peel was affected significantly by exogenous ethylene and the peel ripens earlier than the pulp. The pulp responds irreversibly to ethylene treatment. In addition, it has been reported that treatment with ethylene accelerates the ripening process and the climacteric

peak is reached earlier (Dominguez and Vendrell, 1993; Inaba and Nakamura, 1986).

ACO mRNA is detectable at all times in the pulp but only increases significantly in the peel at climacteric and post-climacteric stages (Lopez-Gomez et al., 1997) suggesting that the ripening process proceeds from the inside outwards (Dominguez and Vendrell, 1993; Tang et al., 1994). Under the conditions of this experiment a sharp peak of control pulp ACS activity during ripening was observed on day 4, that supports the view that pulp ethylene production triggers banana ripening, and subsequently a peak in ACO activity in pulp was obtained at day 6 in control fruit. This shows that pulp may be triggering ripening as previously suggested (Dominguez and Vendrell, 1994). 1-MCP treatment increased ACS activity in both pulp and peel and subsequently the amount of ACO activity and ethylene production is increased.

The increase in ACS activity first in the pulp (day 2) and then in the peel (day 6) in control fruit also support the assumption that banana ripening is from the inside out. While 1-MCP delayed slightly ACS activity in the pulp, its impact is greater in the peel. This supports the findings of Pelayo et al. (2003) who also observed an increase in ACS activity in 1-MCP-treated fruit concurrent with a reduction in respiration rate. This is also consistent with findings of Dominguez and Vendrell (1994) who found that exogenous ethylene treatment increased respiration and endogenous ethylene production of banana fruit. It could be concluded that this decrease in respiration rate is a

result of 1-MCP blocking normal feedback regulation and increasing transcription. It has been previously shown that transcript accumulation and activity of ACS in tomato fruits is affected by 1-MCP treatment (Nakatsuka et al., 1997). Similar results were reported by Golding et al. (1999) who suggested that 1-MCP may block the normal feedback regulation of ethylene production and that the transcription of ACS in bananas may possibly be enhanced. By day 10, fruit treated with 1-MCP had much lower levels of ACS and ACO activity. This reduction in ACS and ACO activity suggests that transcription has decreased and normal feedback is re-established. This would be possible if the fruit has synthesised new receptors (Jiang et al., 1999).

6.5 Conclusion

The results of this research support previous studies that noted the ripening of peel was affected significantly by exogenous ethylene and that the peel ripened earlier than the pulp. In addition, it is concluded that the fruit pulp has a more important role in triggering banana ripening than the peel, as peel and pulp respond differently to exogenous ethylene in terms of ACO and ACS activity and to some extent in ethylene production.

Chapter 7

General discussion

7.1 Introduction

1-methylcyclopropene (1-MCP) is a postharvest chemical tool that is attracting the attention of postharvest scientists and the horticulture industry worldwide. Prospects for commercialisation of 1-MCP for several products including fruits and vegetables appear high (Watkins, 2006). Postharvest researchers attempt to provide more data as they aid horticultural industries to realise the full potential of 1-MCP. Although it has been demonstrated that 1-MCP can delay ripening of bananas (Bagnato et al., 2003; Jiang et al., 1999a), there is little information about the effect of preharvest and postharvest factors on the efficacy of 1-MCP on bananas, particularly in Australian conditions, and the few studies performed have reported some variability in the results (Harris et al., 2000; Pelayo et al., 2003). Bananas are a perishable, climacteric fruit with variable quality and a shelf life often insufficient for marketing. Hence, an increase in the shelf life and improvement of fruit quality would be useful and economical for the producer, wholesaler, retailer and also the consumer. Given that Cavendish bananas (cv. Williams) account for 47% of bananas traded worldwide (Arias et al., 2003) and 95% of the banana

production in Australia (Daniells, 1986); the development of tools such as 1-MCP appear essential for banana production and postharvest management to meet consumer market demands (such as extended shelf life and ideal yellow peel) throughout the year.

The general aim of this thesis was to gain more insight into the determinants and causes of differences in the ability of 1-MCP to improve the quality and shelf life of Cavendish bananas. Three main questions guided this research. Whether preharvest factors and postharvest conditions affect 1-MCP efficacy; whether 1-MCP can be applied reliably throughout the year; and the effect of 1-MCP on ethylene synthesis. The introductory Chapter (Chapter 1) provided a general overview of theoretical notions and empirical findings relevant to these research questions. The subsequent empirical Chapters (Chapters 3 to 6) dealt with these questions in more detail. This final chapter includes an overview and discussion of the main conclusions that can be drawn from the empirical studies.

7.2 Summary of main conclusions

The main contributions of the research presented in this thesis can be summarised in five conclusions. The *first conclusion* is that time of year at harvest and fruit position on the bunch influence the responses of bananas to 1-MCP in shelf life and quality. The *second conclusion* is that the response of partially ripened bananas to 1-MCP treatment was also dependent on both the exogenous ethylene concentration and duration of application. The *third*

conclusion is that the timing of 1-MCP application has a significant impact on the response of bananas in shelf life and quality. The *fourth conclusion* is that the pre-ripening and ripening storage temperatures significantly influence the efficacy of 1-MCP in banana shelf life and quality. The *fifth conclusion* is that the response of peel to exogenous ethylene and 1-MCP treatment is different than the pulp.

7.3 Main findings, implications and commercial benefits

Time of year at harvest and hand position influences 1-MCP efficacy

The studies presented in this thesis particularly in Chapters 3 and 4 consistently showed that time of year at harvest (seasonal effects) is a major source of variation in responses of partially ripened bananas to 1-MCP treatment, which also affected differently the efficacy of 1-MCP in fruit collected from different positions on the bunch. 1-MCP treatment at low concentrations to partially ripened ethylene-treated bananas can be used to extend the shelf life and increase firmness in a reliable manner throughout the year. However, the efficacy of 1-MCP treatment varies among harvested fruit in different months and also among fruit from different positions on the bunch mainly due to the variation in fruit maturity (size) and age at harvest, which displayed a strong relationship with different degree-days experienced by the fruit before harvest. 1-MCP did not have a negative impact on shelf life and quality assessments except that there was an onset of more peel discolouration in winter-harvested fruit when 1-MCP was applied at higher concentrations.

Seasonal effects have been previously shown to influence banana ripening quality (Bagnato et al., 2002; Rippon and Trochoulias, 1976). However, as indicated in the review of literature the effects of preharvest factors have not been considered in studies investigating the application of 1-MCP to bananas. Only one study has suggested that preharvest factors may be an important factor underlying variation in response of bananas to 1-MCP application (Pelayo et al., 2003). In addition, a recent review (Watkins, 2006) noted that there is still some limitation for commercial application of 1-MCP for bananas and concluded that ethylene, timing and concentration of 1-MCP application, and fruit maturity are significant factors affecting commercial application of 1-MCP for bananas. Watkins (2006) also noted that the data obtained thus far are insufficient for using 1-MCP on many horticultural products (including bananas) under commercial conditions. This is the main reason that post-ripening 1-MCP exposure was investigated throughout the year to determine whether preharvest factors have an effect on the efficacy of 1-MCP on shelf life and quality of bananas. The research detailed in the present thesis provides a first empirical demonstration of the links between preharvest factors and conditions (such as seasonal effects) and variations in responses of bananas to 1-MCP treatment in shelf life and quality. One strategy for the use of 1-MCP on partially ripened bananas is that 1-MCP treatment of banana fruit should be considered once the time of the year at harvest is considered, particularly in summer-harvested fruit where 1-MCP has a more

pronounced effect on extending the shelf life and improving the quality of bananas.

Another practical implication of these findings is that bananas to be treated with 1-MCP also must be sorted according to the hand size before treatment, as the response of green or partially ripened bananas collected from the top and bottom of the bunch to the 1-MCP application vary to some extent because of variation in maturity within a bunch, particularly when 1-MCP is applied at very low concentrations at the pre-climacteric stage, or when 1-MCP is applied to the more mature summer-harvested fruit. In addition, based on the concentration of applied 1-MCP in the pre-climacteric stage to green fruit it may have two commercial benefits. First, to prolong storage periods of green banana handling and transport as previously suggested (Macnish et al., 2000). Second, extend the shelf life of partially ripened bananas significantly if 1-MCP is also reapplied at higher concentration (300 nL L^{-1}) after ethylene initiation. These findings also suggest that fruit should be picked at optimum size (maturity) and as such, in summer-harvested fruit an earlier harvest is recommended.

The conclusion that seasonal effects influence responses of bananas to 1-MCP in shelf life and quality is consistent with theoretical and practical explanations of these differences in terms of the ripening of ethylene-initiated bananas (Biale and Young, 2000; Liu, 1976a; Liu, 1976b; Vendrell and McGlasson, 1971). According to these explanations, storage life of bananas has

a strong correlation with fruit maturity, size, tissue sensitivity and minimum treatment time required for ethylene response. Bananas are harvested throughout the year and therefore they are picked with different maturity, size, bunch-emergence to harvest time and also affected by other preharvest conditions. These factors alter the minimum treatment time required for ethylene response in harvested fruit during the year and consequently the exact time for 1-MCP application for maximum benefit. Each of these factors therefore can affect the 1-MCP concentrations that should be applied to fruit. This has increased the need for focus on research into the effect of ripening treatment factors and also in relation to preharvest elements and timing of 1-MCP application on efficacy of 1-MCP.

Ethylene concentration and duration affects 1-MCP efficacy

The research in the present thesis also casts some doubt on the assumption that ethylene has an effect on ripening of 1-MCP-treated bananas. In this study, bananas were treated with ethylene before 1-MCP treatment or 1-MCP applied at preclimacteric stage to green bananas and with their different maturities (among bunches and within-bunches), different bunch-emergence to harvest time and possible chilling injury. The results (Chapters 4 to 6) indicated that responses of both ethylene-treated partially ripened fruit (ripening stage 3 to 3.5) and green bananas (ripening stage 1) to 1-MCP treatment differed. Although a previous study (Bagnato, 2002) suggests that different concentrations of ethylene (50, 300 or 1000 $\mu L\ L^{-1}$) had no impact on

shelf life of Cavendish bananas, others (Biale and Young, 2000; Liu, 1976a; Liu, 1976b; Vendrell and McGlasson, 1971) have shown that the treatment time required for ethylene response varies and is dependent upon the fruit maturity, size and tissue sensitivity. All of these elements are variable in harvested fruit at different times of the year as described in Chapter 3. Thus, an important theoretical implication of these explanations is that the amount of endogenous ethylene production after ethylene treatment is different among harvested fruit during the year due to differences in time required to induce autocatalytic ethylene production. This is consistent with previous studies in terms of ethylene and banana maturity (Vendrell and McGlasson, 1971), and consequently harvested fruit in different seasons will respond differently to 1-MCP treatment.

Timing of 1-MCP application affects responsiveness of bananas

The results presented in Chapters 4 to 6 also strongly support this assumption that harvested fruit in different times of the year will produce different levels of ethylene at the time of 1-MCP application as a result of different responsiveness of the banana to the exogenous ethylene and its feedback mechanism (Vendrell and McGlasson, 1971). Another strategy for use of 1-MCP on bananas is that the concentration and duration of ethylene applied must be determined with seasonal differences in mind to ensure the consistency in response of partially ripened bananas to 1-MCP treatment.

Results presented in Chapter 4 not only confirmed the results of Chapter 3 that there is a strong relationship between preharvest factors and the response of bananas to ethylene and 1-MCP treatment, but also highlighted the advantages of ensuring the timing of 1-MCP application, duration of exposure is optimal and the significance of ripening treatment factors. Shelf life was increased more than two-fold in 1-MCP-treated fruit compared to the control in both summer and winter-harvested fruit when bananas were treated simultaneously with ethylene or by reapplication of 1-MCP. The theoretical implication of these results is that in these cases the concentration of 1-MCP is a significant factor, as 1-MCP application at higher concentration and at earlier stage has a negative impact on peel discolouration and also may prevent proper degreening, while a low concentration of 1-MCP has no effect on shelf life extension. An important strategy for commercial use of 1-MCP is therefore to apply to partially ripened fruit as soon as possible after harvest to allow maximum beneficial delays in ripening. The efficacy of 1-MCP increased when applied at an earlier stage (after one day ethylene gassing), whereas it decreased when applied to the fruit stored for a long period. In the case that bananas are stored longer than one week (time from harvest to 1-MCP), a combination of pre-and early-climacteric application of 1-MCP will have a greater effect on fruit quality and shelf life. When 1-MCP was applied for a longer period either simultaneously with ethylene or applied in both the pre- and early-climacteric stages, shelf life increased significantly. This is in contrast to previous findings (Bagnato, 2002) that had showed extended

exposure 24 to 72 hrs did not affect the efficacy of the 1-MCP treatment. However, the results were consistent with Jiang et al. (1999b) who found the effectiveness of 1-MCP was dependent on the length of 1-MCP exposure and concentration.

Pre-ripening and ripening storage temperatures influences 1-MCP efficacy

The studies presented in Chapter 5 demonstrated that there is a close interaction between temperature, ethylene production and the ripening and storage characteristics of banana fruit in accord with previous findings (Bagnato et al., 2002; Rippon and Trochoulias, 1976) that indicated peel appearance can be negatively affected by climate changes such as field-chilled temperatures less than threshold (13 °C). Results in Chapter 5 suggest that 1-MCP-treated fruit that were chilled had greater peel discolouration, and peel had some green colour when fruit ripened, which is not desirable to the industry. However, the results of Chapter 5 revealed that chilling temperatures before ripening have a significant effect on the response of fruit to ethylene treatment. The magnitude of this effect was dependent on the chilling temperature and the duration of exposure (Bagnato, 2002). The main goal to study the effect of pre-ripening (optimum and chilling) temperatures allowed establishment that uneven degreening in winter-harvested fruit was accounted for by the combination of chilling temperatures and fruit maturity. Although in this study the chilling temperatures and the duration of exposure to chilling temperatures were not exactly similar conditions to the field and fruit were also

detached from the plant, undesirable degreening was more intensive in winter-harvested field-chilled bananas than summer-harvested fruit that were stored artificially in chilled temperatures. Additionally, chilling temperatures had a greater impact on the ripening of peel than the pulp. The pulp ripened more quickly than the peel in both ethylene and 1-MCP-treated fruit. The peel did not degreen properly in fruit stored at chilling temperatures before ripening, whereas in field-chilled winter-harvested bananas both pulp and the peel did not ripen properly. Although the effect of field-chilling injury was not investigated comprehensively in this thesis, an important implication of this explanation is that both chilling temperatures and fruit maturity influence the ripening of 1-MCP-treated fruit. Fruit maturity at harvest also significantly impacts the response of bananas to ethylene treatment and particularly to a greater extent in peel than the pulp. Again, this supports the previous studies by Vendrell and McGlasson (1971), who stated that less mature bananas show negative feedback mechanisms to ethylene; and by Dominguez and Vendrell (1994) who noted the ripening of peel was affected significantly by exogenous ethylene and that the peel ripens earlier than the pulp. The ripening of banana peel strongly relates not only to presence of ethylene but also to the duration and concentration of ethylene with more impact on more mature fruit than less mature winter-harvested fruit.

To understand whether 1-MCP will be commercially practical for bananas, it was also important to determine how it affects the fruit ripening

when they are stored in different storage temperatures. The results (Chapter 5) also indicated that adjusting ripening storage temperature after 1-MCP treatment could control the speed of ripening of 1-MCP-treated fruit better than control fruit as it could be significantly retarded by low storage temperature.

Physiological responses to 1-MCP differ in peel and pulp

In order to understand how 1-MCP and exogenous ethylene act on the ripening of bananas and whether their impact will be similar in both pulp and peel, activity of ethylene enzymes in both pulp and peel, and ethylene production in whole fruit were investigated in both ethylene and 1-MCP-treated fruit. Results in Chapter 6 also showed that the fruit pulp has a more important role in triggering banana ripening than the peel, as peel and pulp respond differently to exogenous ethylene in terms of ACO and ACS activity and to some extent in ethylene production. 1-MCP had a significant effect on ripening of both pulp and peel. However, as the peel reacts like a non-climacteric fruit therefore an optimum concentration and duration of ethylene gassing is required before 1-MCP treatment to have a desirable and reliable effect of 1-MCP with less negative impact on peel degreening. 1-MCP reduced the activity of ACO significantly in an earlier stage after application in peel, but not in pulp. This study therefore revealed that application of 1-MCP on partially ripened bananas may cause a negative impact on peel degreening, but not on pulp ripening because of the potentially different impacts on ethylene biosynthesis of the subsequent climacteric in pulp and peel.

7.4 Directions for future research

Even though this research has allowed an insight into the physiology of bananas and technological application of 1-MCP on bananas, further research into the following areas is required.

Continued research into the effect of ripening treatment factors on 1-MCP-treated fruit throughout the year is required to determine a standard length of exposure and suitable timing of 1-MCP application for commercial enterprises in relation to ripening factors before commercial markets can use this ethylene antagonist properly and reliably with maximum benefit. It appears therefore that to have a reliable and consistent response of bananas to 1-MCP, different protocols should be designed for use on bananas, which are harvested in different seasons, focusing on 1-MCP concentration, duration and proper timing of 1-MCP application in relation to ethylene gassing. However, when fruit are exposed to 1-MCP at optimum concentration, for a longer duration and coincident with ethylene (as in this thesis), simultaneous application of ethylene with 1-MCP tackled this issue successfully. Thus, not only the speed of fruit ripening and firmness increased more but also the peel degreening occurred in an even manner. However, as these low concentrations, durations and simultaneous application with ethylene have not been examined in conditions exactly like those found in commercial situations, further focus on this issue is required to recommend a more reliable protocol for industry. This is because under commercial conditions, handling practices and many factors

including maturity or ripeness stage at harvest, time between harvest and 1-MCP treatment, treatment temperature and desired effects on quality and shelf life will need to be taken into account.

Further analysis into the optimisation of ripening storage temperatures between 13 to 20 °C throughout the year (particularly in winter) in 1-MCP-treated banana is required especially with a focus on the effect of different chilling storage temperatures between 5 to 12 °C for different durations on 1-MCP efficacy on ethylene-treated fruit. In addition, research into intermittent storage temperatures is recommended in order to have similar situations to field temperature variations. This would determine the threshold level of chilling temperatures in the field, or after harvest in storage, and also the duration of exposure that causes the appearance of undesirable discolouration when 1-MCP is applied to the ethylene-treated fruit, so that it could be predictable through constant monitoring of climatic conditions during the banana growing period in winter, as well as during postharvest handling, transportation and ripening processes.

Finally, sensory assessments of partially ripened 1-MCP-treated bananas at optimum concentrations (300 to 1000 nL L^{-1}) would be of benefit to consumers.

All of these areas will provide the banana industry with extra data that will ultimately lead to better management practices in the field, at harvest, as well as postharvest.

7.5 Concluding remarks

This study has allowed a better understanding of 1-MCP application on ripening of bananas in relation to shelf life, visual appearance and internal quality when grown under various climate conditions. The results presented in this thesis demonstrated that preharvest and ripening treatment factors influence on shelf life and quality of 1-MCP treated bananas. To maximise the efficacy of 1-MCP on extending banana shelf life with desirable appearance quality and firmness for postharvest industry throughout the year it is essential to keep in mind the following issues.

First, bananas should be picked at optimum size and maturity, particularly for summer-harvested fruit. Second, an attempt should be made to monitor the time from harvest to 1-MCP application. Third, 1-MCP should be applied at higher concentrations (1000 or 3000 nL L^{-1}) to partially ripened summer-harvested fruit. Fourth, concentration and the duration of ethylene gassing of bananas varies with seasonal differences. Finally, field-chilling injury in winter-harvested fruit in north Queensland, needs to be monitored.

Improved quality and shelf life of Cavendish bananas will ensure bananas with the best appearance taste and a longer shelf life are distributed to

consumers worldwide as well as will reducing the postharvest losses of bananas, to prevent the economical impacts on the postharvest chain from producer to final consumer.

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